ANTIBIOTIC USE IN THE TREATMENT OF CHRONIC WOUNDS

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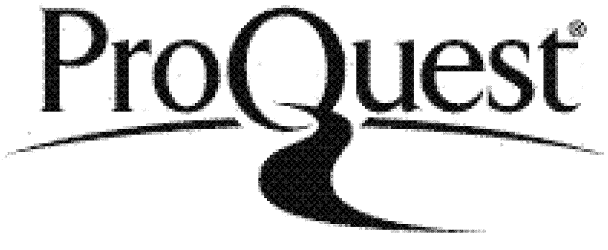

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Summary

Chronic wounds cause substantial morbidity and healthcare costs and prevalence is rising as the population ages and diabetes increases. Microbes are ubiquitous in chronic wounds, with *Staphylococcus aureus* and *Pseudomonas aeruginosa* commonplace. Antibiotic resistance is also widespread and increasing. Patients with chronic wounds are exposed to many antibiotic resistance risk factors.

This study investigated antibiotic consumption by patients with chronic wounds and the prevalence of and risk factors for antibiotic resistant organisms in such wounds. Finally, the impact of resistance on the cost of treatment was investigated.

Antibiotic consumption by patients with chronic wounds treated in primary care was significantly higher than matched patients without chronic wounds. This included greater quantities of flucloxacillin, co-amoxiclav, metronidazole, and ciprofloxacin.

The prevalence of antibiotic resistant organisms in chronic wounds of patients attending a specialist wound-healing clinic was investigated. No patients carried vancomycin-resistant enterococci in their wounds. The prevalence of methicillin-resistant *S. aureus* (MRSA) was 10%. No wound characteristics were associated with MRSA. Carriage was associated with previous MRSA and 'other' systemic antibiotics. The prevalence of ciprofloxacin-resistant *P. aeruginosa* was 11%. Exploratory analysis identified previous antibiotics (specifically ciprofloxacin, 'other' topical antimicrobials and 'other' systemic antibiotics) and wound aetiology as risk factors. Healing wounds were less likely to carry ciprofloxacin-resistant *P. aeruginosa.*

Treatment costs for venous leg ulcers were explored using Markov models: one year's treatment, following presentation, cost £1008. Antibiotic resistance prevalence had little impact on cost. The frequency of nursing visits (for healed and active ulcers), cost of hospital appointments and cost of nurses had the greatest impact.

In summary, antibiotics are commonly used in primary care management of chronic wounds. However ciprofloxacin and 'other' systemic antibiotics may be associated with carriage of antibiotic resistant organisms. The impact of resistance on treatment costs of venous ulcers is small, provided effective alternatives are available.

Publications

Chapter 1 has, in part, been published:

Howell-Jones RS, Wilson MJ, Hill KE, Howard AJ, Price PE and Thomas DW. A review of the microbiology, antibiotic usage and resistance in chronic skin wounds. *Journal of Antimicrobial Chemotherapy* 2005;**55**(2):143-149.

Chapter 2 has been published:

Howell-Jones RS, Price PE, Howard AJ and Thomas DW. Antibiotic prescribing for chronic skin wounds in primary care. *Wound Repair and Regeneration* 2006;**14**(4):387-393

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Aims

This Thesis aims to bridge some of the gaps in our knowledge of chronic wounds, antibiotics, and antibiotic resistance. In the proceeding chapters a narrative review of the literature is presented, followed by investigations to address the following three aims:

i. To describe and quantify antibiotic prescribing for chronic wounds in the primary care setting and compare this with antibiotic usage to patients without chronic wounds
ii. To determine whether antibiotic resistance poses a significant barrier to effective patient care in one specialist wound healing clinic
iii. To investigate which factors are associated with the carriage of resistant organisms in chronic wounds
iv. To model and explore the economic aspects of chronic wound care, particularly in relation to changing levels of antibiotic use and antibiotic resistance.

Chapter 1 . Introduction

This chapter presents a narrative literature review of the issues surrounding antibiotic treatment and resistance in the management of chronic skin wounds. There are three broad components: firstly an outline of chronic wounds, secondly antibiotic resistance and finally economic implications of both chronic wounds and antibiotic resistance. The first component outlines the burden of chronic wounds and discusses the micro-organisms present in chronic wounds and the definition of infection in such wounds. This leads on to a discussion of the role of antibiotics in chronic wound treatment, including evidence, guidelines, current usage and topical antimicrobial products. The second component of this review discusses antibiotic resistance, looking in detail at antibiotic resistance in *Staphylococcus aureus* and *Pseudomonas aeruginosa* before considering antibiotic resistant organisms in chronic wounds. Finally the economic impact of chronic wounds and antibiotic resistance are discussed.

1.1 Chronic wounds

1.1.1 Burden of chronic wounds

Chronic skin wounds have diverse underlying causes with the common theme of refractory healing. In a healthy adult, acute wounds can be expected to heal within 12 to 15 days,[1] followed by a period of scar tissue remodelling which can continue for several months.[2] In contrast, chronic skin wounds may take several years to heal and in some instances healing will not occur.

Chronic wounds are debilitating, distressing and may be painful for the patient; pain has been consistently highlighted as a major factor in studies investigating the impact of leg ulceration on quality of life.[3] In addition to personal morbidity, this condition generates a substantial societal burden. Financial implications arise not only from the demand on health care systems but also to a lesser extent the loss of economic productivity by

affected patients.[4] Estimates of the financial cost to the health care system alone have generated figures in excess of £400 million per annum.[5] These wounds are principally managed in the primary care setting. Although conducted some years ago, Callam *et al.*[6] demonstrated that in the UK setting the vast majority (83%) of patients were managed entirely in the community, with only 5% being hospital inpatients and 12% jointly managed by the primary care team and outpatient departments. More recent evidence from Campbell *et al.*[7] suggests specialist venous ulcer services are becoming widespread in the UK (63% of areas). Although they report that many of these are managed from acute trusts, their results are very likely to suffer from selection bias as only vascular surgeons at hospitals were invited to complete their questionnaire.

Epidemiological data suggest that the burden of chronic wounds will increase in the future. The UK population is ageing and chronic wounds are much more prevalent in the elderly. Callam *et al.*[6] stated 20 years ago, "*as people's life span increases the size of the problem of leg ulcers will probably increase*". In addition to an ageing society, diseases which cause or contribute towards chronic wounds have also increased. The prevalence of diabetes has increased (recently exceeding two-million people in the UK) and therefore the number of those at risk of complications such as diabetic foot ulcers is also rising.

The most common aetiologies of chronic wounds include venous leg ulcers, arterial ulcers, mixed leg ulcers, diabetic foot ulcers, decubitus wounds and non-healing surgical wounds. The underlying causes, contributory factors as well as estimated prevalence and the population at risk are summarised in Appendix 1.1. Studies in the UK have estimated the point prevalence of leg and foot ulcers to be in the region of 1.48/1000 population[6] and there is clear evidence that prevalence increases with advancing age[6,8,9] with up to 36/1000 population in those over 65 years old.[10] The

annual prevalence (proportion of population affected over a one-year period) of venous leg ulcers alone has been estimated to be 1.7% in those aged 65 years and over.[11]

In addition to the underlying pathology of chronic wounds, there are many additional factors that prevent healing. These act at the cellular-level, wound level, person-level through to the social environment level. For example reactive oxidative stress can delay healing at a cellular level, while in the wound environment contributory factors include micro-organisms and blood circulation. At the person-level important factors include nutrition (obesity or malnutrition), diabetes, smoking, age, co-morbidities (e.g. diabetes, arteriosclerosis), medication (e.g. immunosuppressants, cytostatics, anti-inflamatory drugs and anticoagulants), self-harm (e.g. wound trauma) and compliance with treatment (e.g. compression). While at the social environment level stress and social support may play a role in wound healing.[12]

The precise mechanisms by which many of these factors interfere with the healing process are still to be fully elicited, for example the mechanisms of fibroblast senescence are only beginning to be determined. With regards to this thesis, however, it is of particular interest to further explore the relationship between micro-organisms and chronic wounds.

1.1.2 The role of microbes in chronic wounds

The impact of micro-organisms on chronic wounds has been extensively studied and reviewed using different approaches to elicit their possible role in non-healing. These have ranged from highlighting the occurrence of particular species[13] or groups of organisms,[14] to assessing the impact of microbial populations on clinical outcomes.[15] In many cases, studies are difficult to compare due to the use of different methods of

specimen collection and microbial analysis as well as differences in patient demographics and in the aetiology and infection status of ulcers.[16] In addition, clinical analyses tend to be limited in scope and based on assumptions regarding relative pathogenicity.

The microflora of leg and foot ulcers is usually polymicrobial and recent studies using molecular techniques have emphasised the complex ecology of these wounds.[17,18] Using conventional techniques, the mean number of bacterial species per ulcer has been found to range from 1.6 up to 4.4.[19-22] Davies *et al.*[23] found no association between the presence of any individual bacteria in non-infected venous leg ulcers and healing, but they did find an inverse relationship between the presence of ≥ 4 bacteria genera and wound healing in six months. It may be that the presence of bacteria and non-healing are not cause and effect but are both consequences of other factors.

Staphylococcus aureus and coagulase negative staphylococci have been the predominant organisms isolated from both prospective, purpose-collected samples and retrospective analysis of clinical investigations. *S. aureus* has been reported in frequencies varying from 43% of infected leg ulcers[24] to 88% of non-infected leg ulcers[25] while *S. epidermidis* has been reported in 14% of venous ulcer specimens[26] and 20.6% of diabetic foot ulcers (DFUs).[21] *Pseudomonas aeruginosa* is another frequently identified organism and has been found in 7% to 33% of ulcers.[20,25,27] A number of other aerobic species have also been reported, including *Escherichia coli*,[21,24-28] *Enterobacter cloacae*,[24-26,28] *Klebsiella* species,[21,24-26,28] *Streptococcus* species,[13,15,21,22,24-28] *Enterococcus* species[21,22,25,27,28] and *Proteus* species.[22,24-28] This is by no means an exhaustive list, but is illustrative of the range of aerobic bacteria that exist in chronic wounds.

In addition to aerobes, anaerobic organisms are frequently identified in wounds, albeit with considerable variation. Trengove *et al.*[29] found obligate anaerobes in one quarter of chronic leg ulcer samples, whilst Ge *et al.*,[28] in possibly the largest study of microflora in diabetic foot ulcers, which included 825 patients with mild or moderate infection (not treated with antibiotics in the previous two weeks) recruited into two Phase 3 trials in the US, found obligate anaerobes constituted only 6% of DFU wound isolates. However, a focused study by Bowler and Davies[24] found anaerobes in 73% of non-infected leg ulcers and 82% of infected leg ulcers. The most common isolates found in both the infected and non-infected leg ulcers were *Peptostreptococcus* species and pigmented and non-pigmented *Prevotella/Porphyromonas* species.[24] *Finegoldia magna* (previously classified as *Peptostreptococcus magnus*) was found by Hansson *et al.*[25] to be present in 19.6%, and *Peptoniphilus asaccharolyticus* in 9.8% of non-infected venous leg ulcers. Kontiainen and Rinne[22] found that clinical swabs sent for analysis, presumably from infected, or assumed infected wounds, yielded obligate anaerobic rods (mainly *Bacteroides* species) from 12% of ulcers and anaerobic cocci (peptostreptococci) from 8%. Ge *et al.*[28] found *Bacteroides*, *Peptostreptococcus* and *Prevotella* species to be the most frequently isolated obligate anaerobes in mild or moderately infected DFUs.

While some of the issues associated with these microbiological studies include single-site studies, small sample size and limited description of the study population, the consistent message from all the studies is that chronic wounds are virtually inevitably colonised by microbes, of which the most commonly isolated include *Staphylococcus* species and Pseudomonads as well as anaerobes, where the right conditions are used.

The continuity of the microbial profile of chronic wounds over time is unclear from the limited literature that has examined this issue. Hansson *et al.*[25] considered the

microflora of venous leg ulcers to be a relatively stable entity having found that 90% of ulcers that were followed for four months, or until healing, contained at least one resident organism that was isolated from all monthly swabs. Furthermore, Gilchrist and Reed[30] considered chronic wounds to have stable microbial populations, following the observation that once a species was present it generally remained so under hydrocolloid dressings, with the exception of the transient appearance of *P. aeruginosa*. However, closer examination of their data shows that 85% of wounds acquired new aerobes (and 45% new anaerobes) over the 8-week study period. Furthermore, this study included only 20 patients. In a prospective study, Trengove *et al.*[29] monitored the occurrence of new bacterial groups appearing in wounds after initial swabs had been taken. They found at least one new bacterial group present in subsequent swabs in 82% of patients, and thus concluded that the microbial populations of chronic wounds alter over time. Each of these studies suggests that although there may be a degree of stability for some microbial populations, the chronic wound is in fact a dynamic environment. However, there are to date no definitive studies of bacterial succession within chronic wounds, the influence of antibiotics on this succession, or of the interactions between bacterial succession and healing. Recently, using molecular techniques, unculturable bacteria present in chronic wounds have been identified, suggesting the presence of latent bacteria within these wounds.[18]

1.1.3 Infection status of chronic wounds

The interaction between ulcer and bacteria has been stratified into four levels: contamination, colonisation, critical colonisation and infection.[31] Contamination and colonisation by microbes are not generally considered to inhibit healing, whereas infection is considered to have a negative impact on healing. The term 'critical colonisation' has been used to describe the stage at which bacteria begin to adversely

affect wound healing.[31] However, this line between colonisation and infection can be difficult to define. Furthermore, it may be that wound healing in the presence of bacteria may be dependent on ulcer aetioglogy and other factors.[27,32]

A range of clinical criteria have been used to define infection in chronic wounds. The American Diabetes Association developed a Consensus Development Conference on Diabetic Foot Wound Care,[33] that stated a DFU should be considered infected when there are purulent secretions or the presence of two or more signs of inflammation (erythema, warmth, tenderness, heat, induration). These guidelines were based on a multidisciplinary eight-member panel that heard presentations from 25 experts and audience contributions and then developed consensus opinion on six questions around treatments for diabetic foot ulcers (the report does not state that any consensus gaining techniques, such as the Delphi techinique, were used).

In the UK, guidelines have been published in the Drug and Therapeutics Bulletin (DTB). The DTB publishes anonymous, evidence-based (without explicit search strategies) articles that are aimed at non-specialist practitioners, that undergo extensive peer review and revision. This report also recommends that infection in the diabetic foot should be suspected in patients with purulent discharge from an ulcer, at least two features of inflammation (redness, induration, pain, tenderness, warmth) or systemic features (e.g. fever, malaise).[34]

Guidelines for the management of chronic venous leg ulcers produced by the British Association of Dermatologists and the Royal College of Physicians,[35] recommend that infection should be considered if one of the following is present: pyrexia, increased pain, increasing erythema of surrounding skin, lymphanagitis or rapid increase in ulcer size. These guidelines were the result of a multidisplinary meeting held in 1991, which included dermatologists, vascular surgeons, general practitioners, community nurses

and physicians involved in the care of the elderly all based in the UK. In total there were 22 participants in the workshop and contributors to the guidelines which were based on text presented and discussed at the workshop and on comments received in response to the wide dissemination of the proceedings of the meeting to speciality associations. No information is given in any of these three papers, regarding potential conflicts of interest for those practitioners contributing to the guidelines.

It is accepted that chronic wounds by their very nature may not always display the classic symptoms of infection (pain, erythema, oedema, heat and purulence) and it has been suggested that an expanded list, including signs specific to secondary wounds (such as serous exudate plus concurrent inflammation, delayed healing, discolouration of granulation tissue, friable granulation tissue, foul odour and wound breakdown) be employed to identify infection.[36] However, in a systematic review by Nelson *et al.*[37] the question regarding which clinical criteria should be used to diagnose infection in chronic wounds was addressed. The authors identified only the above study, by Gardner *et al.*[36] The predictive value of each sign or symptom was calculated and it was demonstrated that, with the exception of increased pain and wound breakdown, neither the classic signs and symptoms of infection, nor those specific to secondary wounds, are useful for identifying infection. The results of this study should be interpreted with caution as it involved only a small number of patients (n=36), with a variety of wound types (19 patients had pressure ulcers, 7 venous leg ulcers, 6 secondary incision wounds and 2 non-healing traumatic wounds). The patients were recruited from 3 Veteran Affairs facilities and one chronic wound clinic at a university medical centre in the US (86% were male). All patients were required not to have arterial disease. Infection was defined as $\geq 10^5$ organisms per gram of tissue or the presence of β-haemolytic streptococci.

Microbiologically, a critical bacterial load, synergistic relationships between bacterial species and the presence of specific pathogens have all been proposed as indicators of infection. The presence of microbes *per se* is not indicative of wound infection. However, the possibility that a critical microbial load might directly affect the healing outcome in both acute and chronic wounds has been considered for several decades, with a direct relationship first being demonstrated by Bendy *et al.*[38] in 1964. Since then, work carried out by Robson[39] and others has led to the widely held opinion that non-healing is associated with (although not necessarily caused by) bacterial load of more than 10^5 bacteria per gram of tissue. Davies *et al.* identified bacterial load, determined by both swab and biopsy sampling, to be predictive of healing in 66 non-infected chronic venous leg ulcers followed for 6 months in a UK specialist wound healing clinic.[23]

The concept of bacterial synergism which recognises the importance of interspecies interactions has been purported to occur in chronic wounds through studies such as that by Bowler and Davies.[24] They found the growth and pigmentation of some Gram-negative anaerobes to be enhanced by some facultative bacteria through the provision of an essential, unidentified growth factor. Furthermore, they found significantly greater numbers of anaerobes in infected ulcers compared with non-infected ones. The authors went on to argue that although enhanced virulence due to synergism between bacterial species has not been directly demonstrated in these wounds, there is evidence of it in other infections such as acute necrotising soft tissue infections and hence it is likely to occur in the wound environment.

With regards to specific pathogens, beta-haemolytic streptococci,[13,39] *S. aureus*,[27] enterobacteriaceae[27], and *Pseudomonas* species[27,29] have all been implicated as having potentially adverse effects on wound healing. The impact of these species may vary in

different settings, for example, over 60% of arterial and diabetic ulcers colonised with *S. aureus* went on to develop an infection compared with only 20% of venous ulcers similarly colonised.[27] This study, which included a total of 63 patients recruited at initial presentation at an outpatient wound healing clinic in Germany over a four month period. The number of patients in each group was small: 14 arterial ulcers, 14 diabetic and arterial ulcers, 17 diabetic ulcers and 18 venous leg ulcers. No sample size calculations were reported. Wounds were assessed weekly and infection determined on local signs of clinical infection, but the exact signs necessary are not clarified.

In summary, micro-organisms are identified in the deep tissue of all chronic wounds, yet despite numerous publications on this topic, due to the differences in the types and characteristics of the wounds being studied, sampling methods used, patient characteristics (including treatment history) and microbiology methods, the role they play and the impact of specific species on wound longevity is unclear. It is clear however that the presence of bacteria complicates the management of chronic wounds. Organisms frequently implicated in non-healing and infection include *S. aureus*, β-haemolytic streptococci, and *P. aeruginosa*. The diagnosis of infection is (currently) clinical: microbiological studies cannot distinguish between infection and colonisation due to the universal colonisation of chronic wounds.[23,40] Microbial analysis can be of benefit when considered in concert with clinical observations to confirm causative organisms and their sensitivities,[41] and so enable refinement of antibiotic regimens.[40] Clinical diagnosis of infection can, however, also be problematic due to the nature of the wounds and this uncertainty may lead to the unnecessary use of antibiotics, which shall be discussed in the following sections.

1.1.4 Antimicrobial treatment of chronic wounds

1.1.4.1 The evidence for antibiotic treatment

Whilst the mainstay of treatment for chronic wounds is designed to address the underlying causes, e.g. the use of compression bandages for venous leg ulcers, anecdotal evidence suggests antibiotics are frequently prescribed in these patients. In 2000, O'Meara and colleagues[42] published a systematic review of wound care management, including the use of antimicrobial agents, with the objective of systematically assessing 'the clinical- and cost- effectiveness of systemic and topical antimicrobial agents in the prevention and healing of chronic wounds'. Therefore the authors conclude that there was no existing evidence to support antibiotic use for chronic wound healing but that more rigorous studies are required. The review itself is comprehensive in nature and well-conducted from a group well-known for their systematic reviews on wound treatments (NHS Centre for Reviews and Dissemination, University of York), however it is by its nature limited by the studies which have previously been published. The review addressed clear questions using clearly defined criteria and employed a thorough literature search strategy that used 18 electronic databases, extensive, defined search terms and searched conference proceedings and bibliographies of included studies and hand-searched specified journals. There was no restriction for inclusion according to language or date. Furthermore the quality of studies was assessed and only those reaching minimum quality markers were included. These quality markers included an objective measure of wound healing as the outcome (i.e. measured changes in the size of a wound or complete healing), randomised controlled trials (RCT) or controlled clinical trials (CCT) and the unit of allocation had to be the patient, limbs or lesion. Those studies that were included in the review were assessed for overall quality by the following criteria: clear inclusion and exclusion criteria, overall sample size, *a priori* sample size calculation, true randomisation,

comparability of groups reported at baseline, blinded outcome assessment, objective outcome measures, reporting of withdrawals, and intention to treat analysis. Only objective outcome measures were attained in all included studies (as it was an inclusion criteria) which demonstrated the low quality of research in this area. The authors comment that a major problem with many of the included studies is inadequate sample size, with some studies including as few as 10 patients. Despite the extensive effort the reviewers went to to identify studies, of which they located 400 of potential interest that were assessed by title and abstracts where available, 150 were reviewed in hard-copy and only 30 were included in the review (25 RCTs, 5 CCTs). Only six studies examining the use of systemic antibiotics for chronic foot or leg ulcers met the review's inclusion criteria. Moreover, there were still many methodological problems with the included studies, for example only two had undertaken *a priori* sample size calculations. The six included studies also differed with regards to the aetiology of ulcers and their infection status. Two studies included only venous leg ulcers and two only diabetic foot ulcers, while the remaining two included ulcers of mixed aetiology. The inclusion/exclusion policy for wounds with clinical signs of infection was only clearly defined in two studies; as an inclusion criteria for one study and an exclusion criteria for the other. The outcome measure of all studies was, however, an objective measure of wound healing and not the resolution of infection.

An updated systematic search for patients on antibiotic use in chronic wounds was conducted as part of this review (by RH-J). The literature generated since the publication of the O'Meara *et al.* report in 2000,[42] was searched and nine further relevant studies on systemic antibiotics in chronic wounds were identified (eight trials, one systematic review). Although this systematic review was not as extensive as that by O'Meara *et al.*,[42] nevertheless extended search-terms were used covering systemic antibiotics, including generic names, and chronic wound terms of specific and non-

specific aetiology. MESH headings and text were used, where appropriate, and the following databases searched: Medline, ISI Web of Science, EMBASE, CINAHL, Cochrane Database of Systematic Reviews, ACP Journal Club, Database of Abstracts of Reviews of Effects, Cochrane Central Register of Controlled Trials, British Nursing Index and SIGLE for publications from 2000 to 2006. Only English language, randomised studies with a concurrent control group, or systematic reviews, with a clinical outcome were included. This search differed from O'Meara *et al.* in that an objective measure of outcome was not an inclusion criteria. All studies identified investigated the impact of antibiotic regimens on diabetic foot infections or skin and soft-tissue infections including infected ulcers as one sub-group. The trials identified explored the relative efficacy of antibiotic treatment regimens in diabetic foot infections and complicated skin and soft-tissue infections, and frequently aimed to show non-inferiority, rather than improved outcome. The studies are summarised below:

1. Nelson *et al.*[43] Systematic review of antimicrobial treatments for diabetic foot ulcers.

Nelson *et al.*[43] conducted a systematic review of antimicrobial treatment for diabetic foot ulcers. This extensive review found no evidence to support use of any particular type antibacterial agent for the prevention of amputation, resolution of infection or ulcer healing in diabetic foot ulcers. They conclude that the strength of the evidence is poor due to the low quality of many of the studies and the lack of repeated comparisons of the same agents. This review itself was of a high standard with extensive and well-defined search strategy by a well-known group. The main limitation of this review was the poor quality of the available literature.

2. Siami *et al.*[44] Clinafloxacin versus piperacillin-tazobactam in the treatment of severe skin and soft tissue infection.

Siami *et al.*[44] investigated the efficacy of clinafloxacin versus piperacillin-tazobactam in patients with a range of skin and soft tissue infections. This RCT of antibiotic treatment, which included 76 patients with infected diabetic foot ulcerations, failed to demonstrate any difference in the clinical cure rates 6-14 days post-therapy between treatments. For evaluable diabetic foot patients (n=54) the clinical cure rates were 51.7% Vs 48.0% for clinafloxacin and piperacillin-tazobactam respectively. The sample size on this study was however estimated to show equivalence between the two treatments for patients with severe SSTIs, and power was not calculated to be able to look at the individual types of SSTI. The study recruited 400 patients from multiple centres, however, no figures are given on the size of the source population, the number of eligible patients, or the recruitment rate. Patients were only eligible for this study if they had severe SSTI that required hospitalisation and IV antibiotics. Results are presented as clinical cure rates (defined as cure or failure) that were assessed by a blinded assessor but were, necessarily, subjective. Cure required remission of signs and symptoms of baseline infection, and receipt of no more than one dose of non-protocol antibiotics. The clinical cure rates were presented for the clinically evaluable population which consisted of patients who had the correct diagnosis, did not take prior antibiotics (as per protocol), completed specified clinical assessments and received medication as prescribed. In other words, this was a per-protocol analysis and while it shows efficacy of the treatment drug it does not indicate effectiveness in the real-world population. Intention-to-treat (ITT) results are only presented for patients at the long-term follow up data point (21-35 days post treatment) when no difference was seen between the two treatment arms. Therefore, while this RCT appears to be a reasonably well conducted, with considered sample sizes and blinded evaluation, it does not provide strong evidence regarding antibiotic use in chronic wounds as it was powered to show non-

inferiority and included only a small number of patients with diabetic foot ulcers, and due to the lack of ITT analysis in this specific population.

3. Lipsky *et al.*[45] Linezolid versus ampicillin-sulbactam/ amoxicillin-clavulanate in foot infections in diabetic patients.

Lipsky *et al.*[45] investigated the efficacy of linezolid compared with ampicillin-sulbactam/ co-amoxiclav in the treatment of diabetic foot infections including infected ulcers. In this open randomised study, no difference in efficacy between treatments was seen for all diabetic foot infections. However, when analysed by diagnosis, infected ulcers (n=245) had significantly higher clinical cure 15-21 days after completion of treatment with linezolid compared to aminopenicillin/beta-lactamase inhibitor (81% Vs 68%, p=0.018). The main criticism of this study is that clinical cure was a subjective measure and assessors were not blinded as to the antibiotic treatment patients were receiving; assessment could very likely therefore have been biased. Clinical cure was defined as resolution of all clinical signs and symptoms of infection and a healing wound after $\geq$ 5 days of therapy. Included patients had cellulitis, paronychia, infected ulcer (not further defined), deep soft tissue infection, abscess or osteomyelitis. However, no details are reported as to the location or success of recruitment, other than 45 sites in 8 countries from April 2001 to April 2002. The main analysis conducted in this study was intention to treat analysis whereby all patients who were enrolled in a study group were included in the analyses even if they did not complete the course or comply with the study protocol. In this way, the efficacy seen in RCTs more closely represents effectiveness that may be seen in the real world. This is of particular importance if patients are more likely to discontinue treatment in one arm of the study, for example due to the side effects of treatment. Therefore, to give a true reflection of the potential value of a drug, these patients need to be included in analyses. In this

study, more patients had an adverse event and discontinued therapy in the linezolid treatment arm than the aminopenicillin/beta-lactamase inhibitor arm (64% compared with 12% respectively for any adverse event) and 18 compared with 4 patients discontinued therapy in the two arms respectively due to adverse events.

4. Weigelt *et al.*[46] Linezolid versus vancomycin in treatment of complicated skin and soft tissue infections.

Weigelt *et al.*[46] compared linezolid treatment with vancomycin for patients with skin and soft tissue infections, including infected ulcers. Infected ulcers contributed 6.7% of infections but results are not presented separately for this population. A multinational, multicentred, randomised, controlled trial was conducted. The study was open-label and furthermore the outcome measure was subjective, which could result in the introduction of assessor bias.

A total population of 1200 were recruited and 1180 randomised and received study drug (the intention to treat population): 592 received linezolid and 588 vancomycin. Clinical response was assessed by resolution of signs and symptoms of infection identified at baseline. Patients were considered to be treatment failures if they "exhibited persistence or progression of baseline clinical signs and symptoms of infection, development of new clinical findings consistent with active infection, or an inability to complete the study because of adverse events". In the intention to treat population, 7 days after the end of treatment, 92.2% of patients in the linezolid group and 88.5% of patients in the vancomycin group had achieved clinical cure (p=0.057). While this difference was not significant, further analyses by the authors in the modified intention to treat population (those with culture-confirmed gram-positive pathogen at baseline) and in the clinical evaluable population (patients with more than four days of antibiotic treatment and who returned for the 7 day post-treatment visit) were both significantly different between the

two study arms. Linezolid was also found to be superior in the treatment of confirmed MRSA infection. This study was powered to show the superiority of linezolid compared to vancomycin. The major issue with this study is that it was an open-label study with outcome measures that were not entirely objective. The results of the intention to treat analysis have however been presented.

5. Giordano *et al.*[47] Intravenous/oral moxifloxacin versus intravenous piperacillin-tazobactam followed by oral amoxicillin-clavulanate for the treatment of complicated skin and skin structure infections.

Giordano *et al.*[47] compared intravenous/oral moxiflocacin with intravenous piperacillin-tazobactam followed by oral co-amoxiclav in the treatment of skin and soft-tissue infections and found no difference in clinical cure rates or bacteriological eradication. The study looked at treatment regimens that switched from intravenous to oral treatment, with at least 3 days intravenous therapy, and a total duration of treatment of 7 to 14 days. The decision to switch was made by an assessor (blinded as to study group) and was based on clinical condition and tolerability of oral antibiotics. The primary outcome measure was clinical response at the test of cure visit (10 to 42 days post treatment) in the population that had no protocol violations and had received all and only those antibiotics allowed by the study (termed efficacy valid population). Results for the intention to treat population were not presented. Clinical response was defined as the "disappearance of acute signs and symptoms related to the infection or sufficient improvement such that additional antibiotic therapy was not required". Six-hundred and one patients constituted the intention to treat population, but results were only reported on the 367 who were defined as efficacy valid (180 in moxifloxacin group and 187 in the piperacillin/tazobactam group). The overall clinical response in this population was 79% and 82% in the moxifloxacin and piperacillin/tazobactam groups

respectively. Data are presented separately for patients with diabetic foot infections (n=37, 21% of the moxifloxacin group and n=41, 22% in the piperacillin/tazobactam group) and infected ischaemic or decubitis ulcers (n=13, 7% in the moxifloxacin group and n=10, 5% of the piperacillin/tazobactam group). The clinical response in diabetic foot infections was 68% in the moxifloxacin group and 60% in the piperacillin/tazobactam group, while in the ischaemic/decubitis ulcers the response was 77% and 61% respectively. The study was only powered to demonstrate non-inferiority. The results were not objective measures but the assessors were blind to the study group of the participant so bias should have been minimised.

6. Lipsky *et al.*[48] Ertapenem versus piperacillin/tazobactam for diabetic foot infections.

Lipsky *et al.*,[48] in a multicentred, double-blind RCT, found ertapenem to be equivalent to piperacillin-tazobactam in the treatment of diabetic foot infections, with outcome measured as a favourable clinical response. Five hundred and seventy-six patients were enrolled and randomised in the study, of whom 289 were treated with ertapenem and 285 were treated with piperacillin-tazobactam (the modified intention to treat population). From these, 226 and 219 in the ertapenem and piperacillin-tazobactam groups respectively did not have any protocol violations and were described as the clinically evaluable population at discontinuation of intravenous antibiotics (DCIV); 206 and 196 in the ertapenem and piperacillin-tazobactam groups respectively were clinically evaluable at 10 days post DCIV. The modified intention to treat population consisted of the intention to treat population with the exception of those patients that did not receive any treatment (n=10) or those who did not meet the disease definition (n=2) and therefore is very likely to have the same generalisability as other intention to treat populations.

Intravenous drugs were prescribed for a minimum of five days, after which patients could be switched on to oral antibiotic therapy. The maximum total duration of antibiotic treatment with study drugs was 28 days. A favourable response to treatment was determined clinically (in the results presented here), and defined as "resolution of all or most pretherapy signs and symptoms of infection (and specifically of fever, lymphangitis, and purulent drainage), and if the patient had no need for additional intravenous antibiotic therapy (at the end of intravenous therapy assessment) or any antibiotic therapy (at the 10-day follow up after end of intravenous therapy)". The outcome measure was therefore subjective but it was determined by an assessor blinded to study group.

The clinical response at DCIV was described as favourable for 94% and 92% for those receiving ertapenem and piperacillin-tazobactam respectively in the clinically evaluable populations. Ten days after DCIV, the response rate in the clinically evaluable population was 87% and 83% for the two groups respectively. The modified intention to treat population at 10 days after DCIV was 71% in the ertapenem group and 66% in the piperacillin/tazobactam group. The authors did not find any significant difference in favourable clinical response between the two groups in any of these analyses. The study was powered as a non-inferiority trial.

7. Lipsky and Stoutenburgh,[49] Daptomycin versus vancomycin or semi-synthetic penicillins for treating infected diabetic foot ulcers.

Lipsky and Stoutenburgh,[49] in a sub-group analysis of Phase III trials, found daptomycin not to differ significantly from the comparator antibiotics (vancomycin or semi-synthetic penicillin) in the resolution of diabetic ulcer infection. This study was powered to test for non-inferiority of daptomycin to the comparator antibiotic (i.e. that the difference in outcome between daptomycin and the comparator was <10%). From a

total of 1092 trial participants, 133 had suspected Gram positive diabetic foot infections sufficiently severe to require hospitalisation and were thereby eligible for inclusion in the sub-analysis: 103 were clinically evaluable (no definition given) and included in the analysis. Infection was defined as 3 or more of the following: >38C temperature, leucocytosis, local pain, tenderness to palpation, erythema, induration or purulent secretions. Firstly patients were assigned to either semi-synthetic penicillin or vancomycin treatment according to clinical history and the likelihood of MRSA infection by the investigating clinician. Following this allocation, patients were randomised to receive either the comparator drug or daptomycin. The outcome measures used in this study were subjective: the primary outcome measure was improvement or resolution of clinical infection (6-20 days after the completion of antibiotic treatment). The clinical cure rate was 66% in the daptomycin group and 70.0% in the comparator group. Although subjective, the outcomes were determined by investigators blinded to study group. As with many clinical trials, drug efficacy does not necessary equate with effectiveness, however this study does show that daptomycin is as effective in clinical resolution of infection (as determined subjectively) at assessment 6 to 20 days post-treatment as vancomycin and semi-synthetic penicillin. Whether this translates to improved wound healing was not however determined in the study.

8. Clay *et al.*[50] Metronidazole plus ceftriaxone (once daily) versus ticarcillin/clavulanate (every 6 hours) as empirical treatment for diabetic lower extremity infections.

Clay *et al.*[50] found metronidazole with ceftriaxone once daily to be as clinically effective, and cheaper, than ticarcillin with clavulanic acid six-hourly in the treatment of diabetic lower extremity infections. Success rates at 96 hours and end of treatment were

72% (n=26) and 81% (n=36) respectively in the metronidazole with ceftriaxone group and 76% (n=26) and 82% (n=28) respectively in the ticarcillin with clavulanic acid group. This study recruited hospitalised males with diabetes and a lower extremity infection from a Veteran's Affairs Medical Center in the US. Recruited patients (n=70) were randomised by a computer generate schedule to a treatment group and outcome was assessed at 96 hours and at the end of treatment. Clinical stability was the outcome measure and this was defined as at least one of the following: body temperature <38.3C, normalised finger-stick blood sugar concentration, improved wound staging or a white blood count $<10^5$ per mm^3. While these are, for the most part, objective, the assessors were not blinded to the study group of the patient. This was a small trial and no indication of a sample size calculation are given.

9. Embil *et al.*[51] Meropenem versus imipenem/cilastatin in complicated skin and skin-structure infections in patients with diabetes mellutis.

Embil *et al.*[51] in a sub-group analysis of patients with skin and soft tissue infection and diabetes found the clinically evaluable cure rate for those treated with meropenem to be 85.6% and those treated with imipenem-cilastatin to be 72.4%, while for patients without diabetes the cure rate with meropenem was 86.6% and with imipenem-cilastatin 89.0%. The clinically evaluable population consisted of patients who met all inclusion and exclusion criteria, had received the study drug for >72 hours, had not missed two doses within the first 48 hours and not missed two consecutive doses at any time, had not received any other systemic antibacterials and had a follow up evaluation at 7-14 days (or if necessary 28 days) after discontinuing treatment. The intention to treat cure rates (including all patients that had more than one dose of study drug) were 51.5% and 51.5% for diabetic patients treated with meropenem and imipenem-cilastatin respectively and 62.1% and 67.6% for non-diabetic patients treated with meropenem

and imipenem-cilastatin respectively. This was a post hoc analysis comparing efficacy tolerability of meropenem in patients with diabetes with that in patients without diabetes. Due to the post hoc nature, no statistical comparisons were made. The original study was powered to show equivalence. The most common infections in both populations were complex abscess (30.0% and 48.8% of infections in patients with and without diabetes respectively), cellulitis (24.6% and 12.4% of infections in patients with and without diabetes respectively) and ischaemic/diabetic ulcers (20.9% and 1.9% of infections in patients with and without diabetes). The study was a multicentred, international, double-blind and randomised-controlled. Outcome was determined clinically and defined as cured if "all signs and symptoms of the skin and skin-structure infection had adequately resolved or improved to such an extent that no further antibacterial therapy was necessary". Failure was therefore defined when signs and symptoms had worsened or not adequately resolved, as were patients who required more than 14 days of intravenous antibiotics (or >21 days of intravenous and oral antibiotics).

Following this systematic review, it appears that the conclusions drawn by O'Meara *et al.*[42] in their review conducted three years previously are still valid: there exists insufficient evidence concerning the use of systemic antibiotics in wound healing, and until such data exist, other criteria may be used to guide the use of antibiotics, such as cost minimisation. The studies identified in this systematic review (by RH-J) focus for the most part focus on showing non-inferiority of one antibacterial treatment against an established treatment for severe skin and soft tissue infections, including infected chronic wounds. The general quality of the trials was reasonable however in only five out of the eight trials described above were assessors blind as to the treatment arm of subjects and intention to treat analyses were not presented for all studies. Furthermore,

none of these studies investigated wound-healing as an end point but focussed on the resolution of infection, frequently within a relatively short time period.

While systematic reviews and meta-analyses are considered to be the highest standard of evidence they are still subject to biases and are limited by the standard of the literature available for inclusion. As discussed by O'Meara *et al.*,[42] many studies of antibiotics are small, or have been powered only to show non-inferiority, may have methodological problems such as non-blinded outcome assessment, subjective outcome assessment, outcome related to microbiological outcome rather than wound-healing and so forth.

Furthermore, there is also the potential for publication bias as many studies are conducted or sponsored by industry, for example linezolid and daptomycin trials identified above. One problem arising from the strong presence of industry in research in this area is that they will frequently only be interested in showing efficacy for their latest antibiotics (or other wound treatments) and will not be concerned with addressing basic treatment questions, such as whether flucloxacillin or co-amoxiclav is a more successful empirical treatment for wound infection. In the field of wound healing, these limitations are not confined to studies of antibiotics but exist for many other treatment options too, such as optimal wound dressing. Therefore a lack of evidence does not necessarily equate with a lack of effectiveness – it is simply that the studies to answer the question have not been conducted (or have not been conducted to a sufficiently high standard) for inclusion in a systematic review.

In the light of poor evidence appropriate antibiotic use can only therefore based on expert opinion. The following section reviews published guidelines on antibiotic treatment for chronic wounds.

1.1.4.2 Recommendations for antibiotic treatment

Numerous recommendations (based on expert opinion) regarding the use or avoidance of antibiotics for chronic skin wounds exist, and these differ according to ulcer aetiology. Importantly, there is a much lower tolerance of suspected infection in diabetic foot ulcers due to the risk of amputation and subsequent morbidity and mortality. Thus, the early use of antibiotics at signs of infection is generally advocated,[32,52] and occasionally use in uninfected ulcers is advocated.[53] In contrast, recommendations with regard to venous ulcers advocate antibiotic use solely in the presence of clinical signs or symptoms of infection.[35,54]

Highlighting the difficulties for the clinician, the International Working Group on the Diabetic Foot[55] recommends a complex antibiotic strategy which involves intravenous and/or possibly oral use of empirical broad-spectrum antibiotics in the presence of deep foot infections. The list of regimens suggested includes ampicillin/sulbactam, ticaracillin/clavulanate, amoxicillin/clavulanate, clindamycin and a quinolone, second or third generation cephalosporin and a quinolone, and metronidazole with a quinolone.[55] These guidelines are clearly difficult to interpret and implement in practice due to the broad nature of the recommendations.

The clinical guidelines on Type 2 diabetes by Hutchinson *et al.*[56] recommend only that ulcers with extensive cellulitis and/or osteomyelitis should be treated with intensive, systemic antibiotics. They comment that the polymicrobial nature of diabetic foot wounds would suggest use of a broad spectrum antibiotic, but conclude that there is insufficient evidence to distinguish between the relative effectiveness of different antibiotic regimens. They were also unable to find sufficient information to determine whether antibiotics are more effective than placebo for superficial or skin deep ulcers. These recommendations are included in an extensive evidence based guideline on the

treatment of Type 2 diabetes. A clear and defined literature search was undertaken to identify evidence that included systematic reviews, meta-analyses, RCTs and other comparable studies (as well as QoL studies and economic analyses). Only English language evidence from 1983 onwards was included, however, 8 databases were searched and attempts made to access grey literature, identify unpublished trials and search conference proceedings. The authors evaluated the strength of studies around the general principles of internal, external and construct validity. Once the quality of the evidence had been assessed it was then graded, and through this, the final guideline recommendation was also graded according to the supporting evidence.

An update of Hutchinson *et al.*'s[56] guidelines by the UK National Institute for Clinical Excellence in 2004, is no more specific, recommending only that patients with non-healing or progressive ulcers with clinical signs of active infection receive intensive, systemic antibiotics.[57] Again, this guideline was based on sound reviewing methodology but was hindered by the lack of quality evidence.

The SIGN guidelines for chronic leg ulcers are equally general, again recommending that systemic antibiotics only be instituted when there is clinical evidence of infection.[54] Guidelines from the British Association of Dermatologists and The Royal College of Physicians on the management of chronic venous leg ulcers recommend treatment with systemic penicillin upon ulcer infection with beta-haemolytic streptococci, while cellulitis caused by other organisms should be treated according to bacteriological sensitivity.[35]

Clinical Knowledge Services and the Health Protection Agency guidelines recommend first line treatment of infection in venous leg ulcers with flucloxacillin.[58,59] This recommendation is based on the predominance of *S. aureus* as an infecting agent. CKS also recommend flucloxacillin (or erythromycin) for superficial or non-limb threatening

diabetic foot infections, but state that the choice of antibiotic is less clear for deep infections. Whether superficial or deep infection, CKS strongly recommend that patients are urgently referred to specialists (or admitted to hospital in the case of limb or life threatening infection) or, if that is not possible, primary care practitioners should seek specialist advice.[60] Where flucloxacillin is recommended, a 7-day course is advised, although it is recommended that treatment be reviewed in the light of microbiology results. There is a lack of evidence regarding the optimal duration of treatment.[40]

1.1.4.3 Antibiotic use in clinical practice

Despite the scarcity of evidence supporting the effectiveness of antibiotics, they are still widely used in the treatment of chronic wounds. A Swedish audit showed 26.6% of chronic wound patients (leg and foot ulcers, pressure ulcers, post-operative and traumatic wounds which had not healed in six weeks) were receiving systemic antibiotics at the time of the study while a further 33.5% not receiving antibiotics at the time of the study, had done so in the previous six months. In total, therefore, 60.1% of chronic wound patients had received at least one antibiotic in the six month period.[61]

The extent and type of antibiotic use for chronic wounds in the UK is unknown. Overall outpatient antibiotic usage is similar in Sweden and the UK (based on defined daily doses per 1000 inhabitants), but it is not known whether this overall similarity reflects similar prescribing patterns for different diseases. In the light of the lack of evidence, it will be important to identify which antibiotics are being used for chronic wounds and how frequently they are used.

1.1.4.4 Topical antimicrobials in wound care

Topical antimicrobial preparations include both topical antibacterials (for example, silver sulfadiazine, fusidic acid and metronidazole) and topical antiseptics (such as sodium chloride, chlorhexidine and povidone-iodine).[62]

Previous authors have commented that the use of topical preparations outweighs the available evidence and that this is an area which requires further study.[63] The systematic review by O'Meara *et al.*[42] previously discussed (Section 1.1.4.1 The Evidence for antibiotic treatment) also addressed the question of evidence to support topical antimicrobials. As discussed previously, this was a high quality systematic review limited by the available evidence. This was also found to be the case with topical antimicrobials, however research of acceptable (although limited) methodological standard was identified that indicated the following may be beneficial to wound healing: allopurinol, dimethyl sulphoxide, silver sulphadiazine and silver zinc allantoinate cream. An initial improvement was also seen with both povidone-iodine hydrocolloid and silver-impregnated charcoal dressings, but neither of these maintained their advantage until the end of the study periods. The systematic review conducted as part of this review (by RH-J; described in Section 1.1.4.1) also searched for evidence on topical antimicrobial treatment. This was conducted as previously described for systemic antibiotics but using appropriate topical antibacterial and antiseptic terms. Only one study was identified that provides further evidence in this area. Fumal *et al.*[64] investigated the effect of povidone-iodine, silver-sulphadiazine and chlorhexidine digluconate on both the healing rate and histological properties of clinically non-infected chronic leg ulcers (n=51). This open, randomised trial in which patients had two similar ulcers and acted as their own control found povidone-iodine solution to significantly increase ulcer healing rate at six weeks and significantly decrease time-to-

healing compared with control. Silver sulphadiazine and chlorhexidine digluconate both showed slightly increased healing rates and decreased time-to-healing but neither showed significant improvement compared to the controls. There are however some methodological issues with this research. It is difficult to tell which population of patients this study represent as no details are given regarding the source population for patients or the number eligible to participate: in total 51 patients were recruited over a five year period. Of greater impact on determining the validity of the study is the lack of details given on the patients and wounds included in the study. For example no information is given on the average duration of wounds and baseline comparability in the intervention compared to the control ulcers, although the authors do state that the inclusion criteria specified that the two ulcers 'looked similar' at study entry. It is not known how many of the patients had both wounds on the same leg and how many were on separate legs. Wounds were randomly allocated to either the intervention group or the control group, and once in the intervention group, wounds were consecutively assigned to one of the three antimicrobial intervention treatment groups. There were therefore 17 wounds in each intervention group. This is a small sample and no indication was given that sample size calculations were carried out to indicate that this provided sufficient power to detect a difference. A further methodological problem with this study was that assessors were not blinded to the study group, however, the outcome measure were objective and therefore there should have been limited scope for researcher bias at this point. The analyses were non-parametric and compared median time to healing and healing rates however it is not clear for how long wounds were followed to ascertain time to healing. The study therefore has several limitations, however, it does appear to suggest that povidone-iodine can improve healing rates when used as a topical antimicrobial compared to no topical antimicrobial, in ulcers without clinical signs of infection, however, this may have been a chance finding and needs

further clarification in a well constructed, reported and suitably powered study of topical antimicrobials.

The use of patients with more than one ulcer to act as their own control has been used in other studies.[65,66] This study design allows for ultimate matching in terms of many patient characteristics, for example activity levels, diet and nutrition, smoking and so forth. Depending on the study design, this may also include matching on leg-factors such as venous sufficiency. However, many wound characteristics might be quite different despite being on the same patient, for example duration, infection status, healing status and perfusion factors might all differ from ulcer to ulcer. Furthermore there is the question of how representative these patients are of the general population of patients with chronic wounds, as it may be that they have greater wound-associated morbidity, such as venous insufficiency, to result in more than one wound in the first place. Representativeness, with regards to general morbidity may not be of that great importance if the aim of the study is to show the effect of one topical treatment compared to another. Representativeness would however be a concern if there were potential for interaction between morbidity levels and treatment success, for example for systemic treatments and level of venous insufficiency or diabetes control.

The benefit of topical therapy may, theoretically, be due to their ability to deliver high local concentrations of antibiotic irrespective of vascular supply.[40] Further benefits which have been cited include the avoidance of adverse systemic effects,[40,67] and a low incidence of resistance.[67] However, others argue that topical antibiotics are a major driving force behind the development of antibiotic resistance.[68,69] There are also concerns regarding toxicity to human cells,[70] and sensitisation,[71] the incidence of which varies considerably between substances.[72]

Overall, published guidelines on the treatment of chronic wounds do not recommend the use of topical antimicrobials: guidelines for DFU's recommend only systemic antibiotics for infections,[55,57] and SIGN guidelines on the care of chronic leg ulcers specifically advise against the use of topical antimicrobials, as they are frequent sensitizers and have no effect on healing.[54] They do, however, state that short course metronidazole gel for odoriferous ulcers might be a possible exception.[54]

In summary, wounds cause great morbidity particularly for the elderly. The role of microbes in the non-healing of such wounds is debated but it is suggested that high-bacterial load or a greater number of species might impact on healing. Infection itself should be defined on clinical criteria due to the virtual omnipresence of microbes in chronic wounds, however, the evidence regarding which clinical criteria should be used is weak and many such criteria are of limited sensitivity and specificity. The evidence regarding the choice of antibiotic regimens is also very weak and therefore guidelines and recommendations are most frequently based on expert opinion. The range of antibiotics, and frequency of their use, for chronic wounds in the UK is however unknown.

Given the important role of micro-organisms in non-healing, and the use of antibiotics in the management of chronic wounds, it is of importance to discuss antibiotic resistance both in general and in chronic wounds specifically.

1.2 Antibiotic resistance

Microbial resistance to antibiotics has consistently evolved to every antibiotic that has been produced since the beginning of the antibiotic era and is associated with an increase in mortality, morbidity and cost in the order of 1.3 -2 fold for patients with resistant versus susceptible infections.[73] In this section, antibiotic resistance in respect to

those common microbes that are important in the pathology of chronic wounds, namely *S. aureus* and *P. aeruginosa* will be discussed extensively.

1.2.1 Resistance in *Staphylococcus aureus*

The most important antimicrobial resistant phenotype of *S. aureus* is meticillin-resistant *S. aureus*: MRSA (methicillin and meticillin are equivalent terms, but convention is now to use the term meticillin). Meticillin, originally called celbenine, was specifically created as a derivative of penicillin that could withstand the action of penicillinase. It was introduced as a therapeutic agent in 1959-1960[74] and meticillin resistant *S. aureus* (MRSA) was first reported just one year later.[75]

The gene responsible for inferring resistance to meticillin is the *mecA* gene. This gene together with additional DNA is referred to as the *mec* element or staphylococcal chromosomal cassette (SCC*mec*).[76] *Mec*A codes for a penicillin-binding protein (PBP2a) with very limited affinity for beta-lactam antibiotics.[77] This gene is foreign to *S. aureus* and its exact origin is unknown. It is possible that multiple donors, possibly coagulase negative staphylococci, were involved.[74] A close homologue has been found in *Staphylococcus sciuri* (an animal commensal, most strains of which are susceptible to meticillin), and an insertion sequence present in *SCCmec* types I and IV is found in *Staphylococcus haemolyticus.*[74]

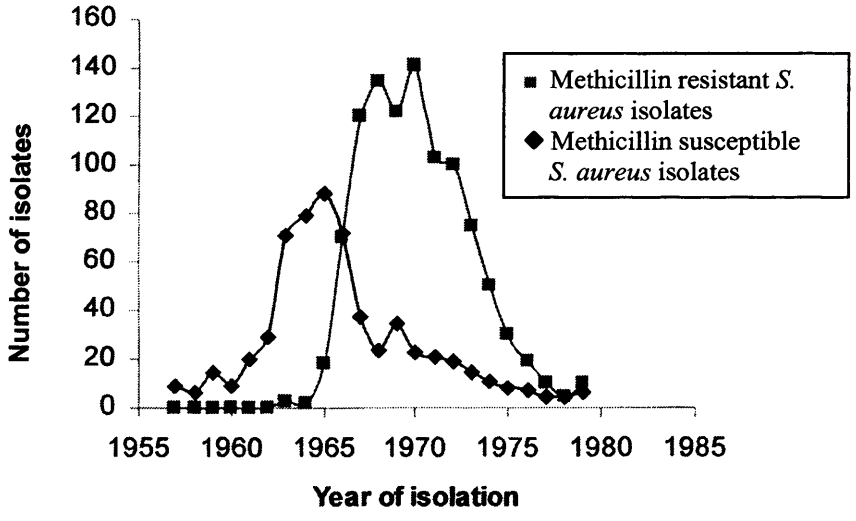
MRSA is now a ubiquitous problem due to the worldwide spread of only a small number of clonal types. These include the epidemic Iberian, Brazilian, New York/Japan, Paediatric and Hungarian clones. These clones have been named after the geographical location in which they were first located and/or some unique epidemiological property.[74] In the UK, the predominant circulating strains are E-MRSA 15 and E-MRSA 16 (defined by phage-type).[78]

The nomenclature used for MRSA types has been described as 'irrational' by Enright *et al.*[79] They, and others, argue that the molecular techniques (generally pulsed-field gel electrophoresis and phage-typing) that have been used to investigate outbreaks are not suited to long-term global epidemiological studies.[78,79] This requires the use of a technique that is highly discriminatory of variation that accumulates slowly over time. Here Enright *et al.* propose the use of multi-locus sequence typing (MLST).[80] Using this technique several of the classical epidemic strains have been found to be indistinguishable. The authors argue that the MLST group (termed ST in their proposed nomenclature) should be used, together with the susceptibility profile of the isolate (i.e. MRSA, MSSA, GISA (glycopeptide-intermediate resistant *S.* aureus) and the SCC*mec* type (I-IV) to name specific strains (eg. ST5-MSSA, ST5-MRSA-I).[79]

This molecular work has enabled the evolution and spread of MRSA clones to be postulated. The major epidemic clones are considered to be either descendents of other epidemic clones or to have arisen by the horizontal transfer of the *mec* element.[79]. The present circulating strains of MRSA are thought to have come from very few ancestral strains,[81] although it is thought that successful MSSA clones gave rise to successful MRSA clones on multiple occasions (due to the occurrence of isolates with the same ST but different SCC*mec* types). Horizontal transmission is most likely to have happened in the commonly circulating MSSA clones.[79] Indeed, Crisóstomo *et al.*[82] showed using historical samples collected in the Danish surveillance system (a collection of all Danish *S. aureus* bacteraemia isolates from 1957 onwards), that the genetic backgrounds of early MRSA isolates and MSSA isolates of the same time had closely related, or identical, genetic profiles. In addition, they showed very neatly the transition between predominating MSSA and MRSA isolates following the introduction of meticillin into clinical practice (Figure 1.1).

Interestingly, in addition to the transition from predominance of MSSA to MRSA, following the introduction of meticillin, it has also been shown that there can be transition between the predominant clonal types. This has been shown in a surveillance study in a Portugese hospital which identified the introduction of the Brazilian clone and a subsequent change in the prevalence of the previously predominant Iberian clone, from 89% of isolates to 55%, while the prevalence of the Brazilian clone rose from just 5% to 38% of isolates.[83] While a surveillance study of a Spanish Hospital, from 1998 to 2002, saw the predominant clonal type change from the Iberian clone (ST247-MRSA-1A MLST-sensitivity-SCC*mec* type) to EMRSA-16 (ST36-MRSA-II); a strain previously only associated with the UK.[84] Such transitions may occur due to the greater fitness of one strain compared to another which may arise due to either a genetic mutation conferring an advantageous phenotype or due to local changes in clinical practice which favour one strain (or a combination of these factors).

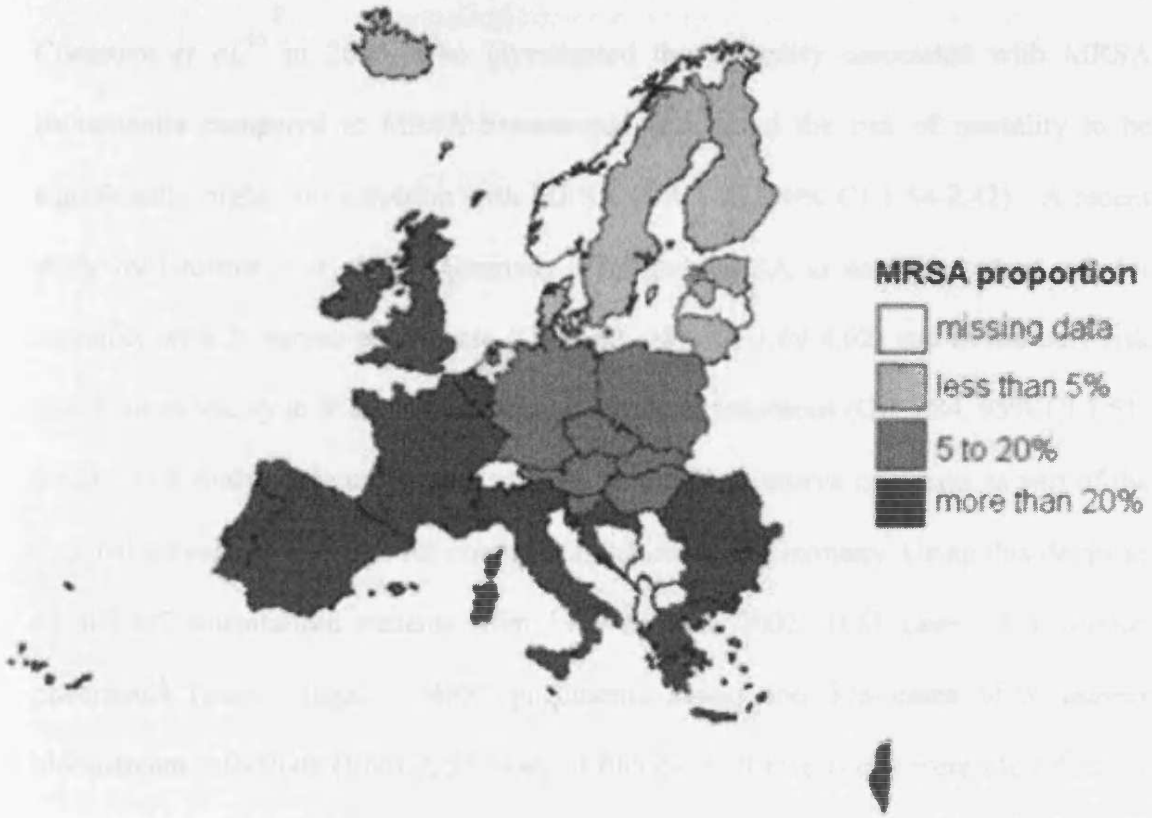
Figure 1.1 Sequential appearance of meticillin-susceptible and meticillin-resistant blood isolates of *S. aureus*. From Crisóstomo *et al.*[82]



In Europe, there is a clear divide in the prevalence of MRSA between northern and southern countries. An EARSS study of blood isolates from across Europe identified this difference (Figure 1.2). The lowest prevalence of MRSA was found in Iceland,

0.5% of *S. aureus* isolates, while the highest prevalence was found in Greece where the equivalent proportion was 44%.[85] Worldwide, high levels of MRSA (>40% of *S. aureus* isolates) have been reported from countries as diverse as Chile, Argentina, Japan, Singapore, South Africa and Taiwan.[86] There are several possible explanations for the variation between countries, and these include differences in virulence or colonization properties of the circulating MRSA strains, or country differences such as levels of antibiotic use and hospital hygiene.[87]

Figure 1.2 Geographical variation in proportions of meticillin-resistant *Staphylococcus aureus* (MRSA) (1999-2002). From Tiemersma *et al.*[85]



In the UK, the proportion of *S. aureus* isolates that were MRSA in 2002 was 44.5%; this was a significant increase from 30.5% seen in 1998.[85] Although in Wales, surveillance data suggests that levels of MRSA bacteraemia increased from 1993, but reached a

plateau around 1997, while the same occurred for all laboratory reports of MRSA infection or colonisation around 2000.[88]

In addition to overall prevalence, the mortality associated with *S. aureus* has increased and this is thought to be entirely due to the rise in MRSA. Griffiths *et al.*[89] found that MRSA accounted for 66% of death certificates that mentioned *S. aureus* in 2002 compared with only 12% in 1993. The number of deaths involving MRSA increased from 51 in 1993 to 800 in 2002.[89] While it is likely that this does demonstrate an increase in mortality associated with *S. aureus* it is also likely to reflect increased awareness and thereby reporting on death certificates. More substantial evidence of the increased mortality associated with MRSA comes from a meta-analysis undertaken by Cosgrove *et al.*[90] in 2003, who investigated the mortality associated with MRSA bacteraemia compared to MSSA bacteraemia and found the risk of mortality to be significantly higher for infection with MRSA (OR 1.93, 90% CI 1.54-2.42). A recent study by Gastmeier *et al.*[91] in Germany identified MRSA as an independent risk for mortality with *S. aureus* pneumonia (OR 2.62, 95% CI 1.69-4.02) and as the only risk factor for mortality in *S. aureus* primary bloodstream infections (OR 3.84, 95% CI 1.51-10.2). This study made use of data reported from 273 intensive care units as part of the national surveillance system for nosocomial infections in Germany. Using this database of 505,487 hospitalised patients from 1997 to June 2002, 1851 cases of *S. aureus* pneumonia (from a total of 6888 pneumonia cases) and 378 cases of *S. aureus* bloodstream infections (from 2357 cases of bloodstream infections) were identified, of which 18.9% and 25.1% respectively were MRSA. The patients included in this study are therefore likely to be highly representative of the ICU population in Germany. There were however limitations with the study, in particular the database did not include antibiotic usage variables or severity of disease, and only variables included in the database could be investigated as risk factors. Besides MRSA, the other factors found

to be associated with mortality from pneumonia in ICU were teaching hospital (other than university hospital), age above the median and identification of *Stenotrophomonas maltophilia* from a patient specimen. This study therefore provides clear evidence of the mortality in German intensive care units associated with MRSA, and these findings are likely to be true in other European settings.

It is very difficult to distinguish true hospital-acquired MRSA (HA-MRSA) from true community-acquired MRSA (CA-MRSA). The Centers for Disease Control and Prevention[92] has stated that MRSA is likely to be CA-MRSA if all of the following criteria are met:

- MRSA diagnosis in outpatient setting or MRSA culture positive within 48 hours after hospital admission.
- No medical history of MRSA infection or colonization.
- No medical history in the past year of:
    - Hospitalisation
    - Admission to a nursing home, skilled nursing facility, or hospice
    - Dialysis
    - Surgery
- No permanent indwelling catheters or medical devices that pass through the skin into the body.[92]

Frequently, however studies use deviations from this definition, or investigate community-identified rather than community-acquired MRSA, for example when including patients identified as MRSA carriers in the first 24 hours of hospital admission, or residents of long-term care facilities respectively. Salgado *et al.*[93] undertook a meta analysis to investigate the prevalence of community acquired MRSA and its risk factors. The pooled prevalence was found to be 1.3%. However, there was considerable heterogeneity between populations, in particular, subjects who were sampled from a health care facility were at greater risk of carrying MRSA than subjects

sampled in the community (RR 2.35, 95% CI 1.56-3.53). The authors consider that this difference is likely to stem from unidentified or non-excluded healthcare associations and therefore the prevalence amongst persons without the typical risk factors remains low ($\leq$ 0.24%). Furthermore, Lesens *et al.*[94] looked at MRSA bacteraemia and tried to identify those that were truly community-acquired compared with those that had a health care associated risk factor (in three tertiary care hospitals in Ireland and France). They found that 56% of those defined as community acquired when the first positive blood culture was performed more than 48 hours after admission. actually had healthcare-associated bacteraemia, as defined by Friedman *et al.*;[95] including such factors as receipt of intravenous therapy, attendance at hospital or hemodialysis clinic in previous 30 days and hospitalisation in previous 3 months.

Recently the spread of Panton Valentine Leukocidin (PVL) producing strains of *S. aureus* has caused great concern. PVL *S. aureus* strains can be MSSA or MRSA and are usually associated with community (as opposed to hospital) settings (and therefore in the case of MRSA are considered to be true CA-MRSA). The PVL toxin is associated with an increased ability to cause disease and can be highly virulent in otherwise healthy persons. In the UK, PVL has so far only been seen in a couple of areas, most notably in the South West but more recently the West Midlands. It has caused the rapid death of seven persons over two years (2004-2006) in the UK (children and adults).[96] More commonly PVL *S. aureus i*s associated with skin infections such as cellulitis and abscesses. As with other strains of *S. aureus* some people do not suffer from disesase but will be carriers. In the UK, the strains from Plymouth were relatively easy for the microbiology laboratory to identify due to the unique anti-biogram. It may be that PVL producing strains occur in other parts of the UK but have not been identified as routine PVL detection is not undertaken. Work is however underway to investigate how widespread PVL strains are in the UK.[96] It is possible that PVL *S.*

aureus will become of greater significance unless efforts to limit its spread are successful.

MRSA has therefore been shown to be widespread and a growing problem; media coverage of the bacterium has often been sensationalist,[97] with headlines such as "Superbug stopped my mother walking"[98] and "MRSA 'kills 10,000".[99] Hamour *et al.*[97] investigated patients' perceptions of MRSA in 2000, and found that despite widespread media coverage less than half of patients had prior knowledge of "superbugs" or MRSA. Perhaps not surprisingly, of those patients that had prior knowledge of MRSA, the media was the most frequent source of information. It was also found that the possibility of infection provoked high levels of anxiety amongst patients.[97] In relation to the patients' understanding of the methods of transmission, 70% thought MRSA could be acquired from the "hospital environment", 34% from operations or other procedures, 8% from hospital staff and 18% were unsure.

Studies investigating risk factors associated with colonisation and infection with MRSA are abundant in the scientific literature. The following section summarises these risk factors and outlines the possible mechanisms by which they function.

1.2.1.1 MRSA risk factors

i) Previous Hospitalisation

Previous hospitalisation is a strong risk factor for carriage and infection with MRSA, irrespective of whether that MRSA is subsequently identified in a hospital or a community setting. Many studies have explored hospitalisation within various time frames and found it to be associated with both infection and colonisation. In the hospital setting, Graffunder and Venezia[100] investigated nosocomial MRSA infection and found the risk of MRSA infection to be nearly twice as much in patients who had

been previously hospitalised (Odds Ratio (OR) 1.95, 95% CI 1.02-3.76). In addition, this risk has been identified in many different populations. The following references outline the risk associated with prior hospitalisation found for patients with *S. aureus* bacteraemia.

Lodise *et al.*[101] have found previous hospitalisation to be associated with current MRSA in their study to identify institute-specific prediction factors for identifying patients with MRSA as opposed to MSSA bacteraemia. In this study, they identified a history of hospitalisation as one of the main risk factors for MRSA bacteraemia (OR 2.5, 95% CI 1.5-3.8). Furthermore, hospital onset bacteraemia (defined as a positive blood-culture more than 72 hours after admission) was also a significant risk factor for MRSA compared to MSSA bacteraemia (OR 3.0, 95% CI 1.9-4.9). The other significant risk factors in this study were prior antibiotic exposure (OR 9.2, 95% CI 4.8-17.9) and presence of a decubitus ulcer (OR 2.5, 95% CI 1.2-4.9). The authors investigated the impact of interaction terms but found they did not improve the predictive capability of the model. The model did not explain all MRSA cases, and the estimated prevalence in patients without any of these risk factors was 15%. This cohort study, based in one 279-bed hospital in Detroit, USA, was a retrospective review of patients attending over a 2½ year period from January 1999. During this time 494 patients with *S. aureus* bacteraemia were identified, 45.5% of whom had MRSA bacteraemia. The analysis conducted aimed to provide local data on the likelihood of MRSA infection in patients with *S. aureus* bacteraemia. While it is likely, and has been shown elsewhere, that the risk factors associated with MRSA and MRSA *S. aureus* bacteraemia are similar across healthcare institutions and even countries, the exact contribution, and predictive ability, of each factor is likely to change with setting.

Tacconelli *et al.*[102] investigated hospital inpatients (at one hospital in the US) with MRSA bacteraemia that was identified within 24 hours of admission over 5.5 year study period, from January 1997. One-hundred and twenty-seven of these 130 patients were defined as health-care associated, by four criteria defined by the authors to describe exposure to a healthcare setting or intervention: i) patients who had required IV therapy, chemotherapy, specialized nursing or wound care at home or an ambulatory visit in the 30 days prior to bacteraemia, ii) required chronic haemodialysis, iii) had been hospitalised for more than 2 days in the previous 6 months or iv) were resident in a long-term care facility or nursing home. Data were determined for patients from a retrospective analysis of medical records. Three patients excluded from analysis as they did not have healthcare associated bacteraemia defined by these criteria, however they all had health-care associated factors not covered by this definition. MRSA bacteraemia cases were matched with patients who were admitted on the same day and also met the healthcare exposure criteria. Control patients were therefore considered to have come from the same population as the cases. However, they had neither MRSA nor bacteraemia and therefore it may be that the risk factors identified reflect those for bacteraemia and not specifically for MRSA bacteraemia. The data were analysed in two logistic regression models, the first included all variables of interest, while the second excluded previous MRSA on the basis that this information might not be available on admission. In the first analysis, previous MRSA infection or colonisation, cellulitis at hospital admission, presence of a central venous catheter and skin ulcers at hospital admission were all significantly associated with MRSA bacteraemia. The factors found to be significant in the second model were presence of a central venous catheter, hospitalisation in the previous 6 months, quinolone therapy in previous 30 days and diabetes mellitus. When previous MRSA infection or colonisation was included in the model, prior hospitalisation was not associated with increased risk of MRSA, however,

when this variable was excluded, prior hospitalisation was associated with increased risk of MRSA bacteraemia (OR 2.01, 95% CI 1.76-5.97). It is possible in this model, that prior hospitalisation was working as a surrogate marker for previous known MRSA. Prior hospitalisation was included in this model as the number of hospitalisations in the previous 6 months. This study does however show that even in patients known to have healthcare associated risk factors previous hospitalisation itself is an important factor (at least when previous MRSA status is unknown).

A similar study by McHugh and Riley[103] that investigated *S. aureus* bacteraemia identified in hospitalised patients in a 640-bed hospital in Seattle US, found previous hospitalisation to increase the risk of MRSA compared to MSSA by an OR of 7.9 (95% CI 1.9-33.1). Patients with *S. aureus* bloodstream infection identified from laboratory records over a three-year period were included in this retrospective case-control study of patients with MRSA compared with MSSA bloodstream infections. Investigated risk factors included antibiotic use in the previous two-weeks, prior hospital admission in the previous month and surgery in the past five years. Only 46 patients with MRSA bloodstream infections were identified and only 20 of whom were included in the study as cases (20 were outpatients and 6 had incomplete or missing charts), 108 patients with MSSA bloodstream infections were identified, 83 of whom were inpatients and 40 of which were randomly selected as controls. All risk factors suggest that patients obtained their MRSA in a healthcare setting. The study should be considered in context of the small number of patients from one hospital using retrospective data and possible over-fitting of the logistic regression model due to the large number of variables investigated for the small number of cases and indicated by wide confidence intervals.

Studies in other populations of patients have also found previous hospitalisation to be significantly associated with MRSA. For example, Warren *et al.*[104] investigated

patients in intensive care and found prior hospitalisation to be the only independent risk factor associated with colonisation or infection with MRSA (OR 7.35, 95% CI 3.96-13.67).

Patients identified in the community setting have also identified prior hospitalisation as one of the strongest risk factors for MRSA carriage and/or infection. For example, Grundmann *et al.*[105] investigated the prevalence of MRSA carriage in elderly persons living in the community in the UK (n=962, of whom 8 had nasal carriage of MRSA). Hospital admission (yes or no) in the previous 6 months was found to be a significant independent risk factor for the carriage of MRSA (OR 13.0, 95% CI 2.5-68.2). The only other factor identified as associated with MRSA was diabetes (OR 6.8, 95% CI 1.33-34.3). The wide confidence intervals in of these estimates are likely due to the small number of MRSA cases, despite the large sample size.

Furthermore, even in an outbreak situation in young healthy males at a military training camp in the US, one of the two risk factors for acquisition of MRSA was a family member or friend who worked in a health care setting (OR 2.9, 95% CI 1.09-7.15). The only other significant risk factor was a room-mate with a skin infection prior to training (OR 3.44, 95% CI 1.34-8.85

There are studies which have not found prior hospitalisation to be of significance in determining which patients have MRSA. Hori *et al.*[106] however, undertook a study of nasal colonisation of inpatients with a minimum hospital stay of 21 days and did not find hospitalisation to be associated with increased risk of MRSA carriage but this is likely to be due to the fact that by virtue of the study design, all patients have three weeks hospitalisation history, after which time factors associated with this, rather than previous hospitalisation episodes, are of greater importance.

Therefore, in conclusion for many populations prior hospitalisation has been shown to be a significant risk factor for carriage or infection with MRSA. As previously discussed the prevalence of MRSA in the hospital setting is very much higher than that in the community. It many settings MRSA has gained a foot hold and is fairly commonly spread from person to person (possibly via an intermediary object or person). Previous hospitalisation is therefore likely to represent a higher probability of previous (unidentified) MRSA. The reasons why some patients are more likely to acquire MRSA carriage or infection (in the hospital setting or elsewhere) may be due to factors such as antibiotic use, invasive devices for example. These potential risk factors are discussed below.

ii) Residential or nursing home residency

Residency in a nursing home or other long-term care facility is another healthcare associated risk factor. Residency in either a nursing or residential home has been identified as a strong risk factor for carriage or infection with MRSA.

Studies investigating patients on admission to hospital who have identified nursing home residency as a significant risk factor for MRSA include McHugh and Riley,[103] in their previously mentioned study investigating risk factors associated with MRSA bloodstream infections, found residency in a group home to be strongly associated with MRSA infection (OR 15.66, 95% CI 2.38-103.1). Although the confidence intervals on this estimated risk are extremely large, and likely due to the small number of patients involved and over-fitting of the logistic regression models. In addition, Jernigan *et al.*[107] identified the significant, independent risks for MRSA carriage upon admission to hospital to be admission to a nursing home in the previous year (OR 16.5, 95% CI 1.4-192.1) and at least one hospitalisation, of 5 days duration, in the previous year (OR 3.91, 95% CI 1.1-13.9).

Other studies have focussed on patients identified as MRSA positive in nursing or residential homes, and found specific factors associated with homes to increase the probability of MRSA in residents. For example, in a point prevalence study by O'Sullivan and Keane,[108] investigating residents in six long-term care facilities in Ireland, residents in one particular nursing home were found to be at greater risk of MRSA colonisation (OR2.69, 95% CI 1.01-7.16). Swabs were collected from multiple sites including the nares, throat, axilla, groin or perineum and any wounds present and a total of 910 individuals were screened and 786 included in the risk factor analysis. The participating homes varied in size from 33 to 255 beds and the prevalence rate varied from 1 to 27%. Interestingly, in this study previous hospitalisation was not a significant risk factor in the multivariable analysis; the final model included only males, pressure sores and one particular nursing home. Varicose ulcers and diabetes were investigated in univariable analyses and found to be non-significant.

The reasons why patients in one nursing home were more at risk that other patients was not specifically explored further by O'Sullivan and Keane. However, a study investigating MRSA carriage in nursing homes in Germany found the size of the nursing home to be important. The greatest risk of colonisation occurred in middle-sized homes (41 to 100 beds) (OR 3.06, 95% CI 1.39-6.76 compared with residents in large homes, >100 beds), while the risk to residents in small care homes ($\leq$ 40 beds) was OR 1.87 (95% CI 0.62 to 5.63).[109]

The logic to explain the finding that patients from nursing homes are more likely than patients not from nursing homes to carry or be infected with MRSA, is likely to be the same as previously described for patients with previous hospitalisation. Nursing homes residents are more likely to have unrecognised colonisation due to a combination of cross-infection, high-prevalence and other factors both relating to the individuals and to

nursing homes. The impact of these factors may differ between homes, depending on their characteristics and procedures, and that may explain the different risk of MRSA in different care homes.

iii) Antibiotic usage

Several studies have investigated the impact of different antibiotic classes on the risk of MRSA infection or colonisation. Particularly strong associations have been identified with fluoroquinolones. The impact of antibiotic consumption at the individual level was investigated by Graffunder and Venezia,[100] in their study of MRSA nosocomial infection in one tertiary care facility in the US. They found that, in addition to previous hospitalisation, antibiotic usage was independently associated with increased risk of MRSA compared to MSSA. The authors investigated the relationships using three models of antibiotic usage: i) the significant beta-lactam antibiotics grouped in classes, ii) all beta-lactam antibiotic combined together and iii) number of grams of antibiotics (to investigate any dose-response relationship). In all three models, levofloxacin was found to be a significant factor in the risk of MRSA (models i) and ii) OR 8.01, 95% CI 3.15-20.3; model iii) OR 1.76, 95% CI 1.21-2.56). Macrolides, however, were only found to increase the risk of MRSA using models i) and ii) (OR 4.06, 95% CI 1.15-14.4) with no association being identified with number of grams of macrolides, suggesting a less robust association.

Furthermore, at the individual level Weber *et al.*[110] undertook a case-case-control study specifically to investigate the effect of fluoroquinolones on MRSA. They found levofloxacin and ciprofloxacin to both be independently associated with an increased risk of MRSA (OR 3.38 95% CI 1.94-5.90 and OR 2.48, 95% CI 1.32-4.67 respectively compared to patients with no infection). In addition, by modelling the impact of antibiotics on MSSA patients compared to control (non-infected) patients, they showed

this associated was not due to an increased risk of *S. aureus* infection but was specifically associated with meticillin-resisitant organisms. Fluoroquinolones have also been identified as a risk factor in the persistent carriage of MRSA.[111]

An ecological study by Muller *et al.*[112] investigating hospital acquired MRSA identified in clinical and screening samples >48 hours after admission to a 1228-bed French university hospital (October 2000 to September 2001). Using different models for each antibiotic class found the following antibiotics to be associated with an increased risk of MRSA: beta-lactams (especially penicillins), fluoroquinolones, cephalosporins, macrolides and aminoglycosides (antibiotic usage was determined from pharmacy records of the type and quantity of antibiotics distributed to each unit and recorded as DDD). Furthermore, they found this relationship to persist after accounting for colonisation pressure and type of hospital unit. Colonisation pressure was defined as the number of MRSA patient days (patients in whom MRSA had been identified through either clinical or surveillance samples) divided by the total number of patient days for each unit. Unfortunately, because different antibiotic classes were investigated in separate models, which included colonisation pressure and type of hospital unit, the relative impact of each antibiotic class could not be determined. This method may have also introduced error as the impact of other antibiotics were not accounted for in the analysis and therefore, if there was any co-linearity between variables (such that units that used more of an antibiotic associated with MRSA also used more of an antibiotic not associated with MRSA, the non-associated antibiotic may have spuriously been found to be associated with MRSA). The study identified 234 patients with MRSA (from October 2000 to September 2001), of whom 124 had acquired MRSA >48 hours after admission. While the study design does not allow for direct implication of risk factors, and although conducted over a one-year period in one hospital, this study does add to the body of evidence suggesting an association between antibiotic use and

MRSA prevalence and furthermore suggests that this exists over and above colonisation pressure and type of hospital unit

The ecological effect of antibiotic use on the prevalence of MRSA was identified as quantifiable and temporal in a study by Monnet *et al.*[113] in an outbreak situation in a tertiary care facility. Changes in macrolide, third-generation cephalosporin and fluoroquinolone usage were followed, after a lag period, by changes in percentage of MRSA isolates, both increases and decreases. This study, which took place in a 1,200 bed hospital in Aberdeen, Scotland identified 9441 non-duplicate, non-surveillance *S. aureus* cultures, from 6412 patients, over a 5 year period from beginning of 1996. During this period there was an outbreak of MRSA, with the percentage of *S. aureus* isolates identified as MRSA rising from 0.6%, 5.0%, 14.9%, 24.1% to 31.9% in the years from 1996 to 2000. The denominator for MRSA prevalence was the total number of *S. aureus* tested for meticillin resistance. Antibiotic use at drug and class level was measured monthly using the quantity delivered to each hospital ward from the hospital pharmacy, and expressed as Defined Daily Doses (DDD). Through modelling the authors were able to see a strong relationship between lag antibiotic usage and MRSA prevalence. The final model included the variables previous monthly prevalence of MRSA, and previous months use of macrolides, third-generation cephalosporins and fluoroquinolones and accounted for 90.2% of the variation in the model. It is also of note that the change in quantity of antibiotics used was of greater impact at the beginning of the outbreak (i.e. 1997) than later on when MRSA had become endemic (i.e. 2000). This means that large decreases in antibiotic usage would be necessary to have any impact on endemic MRSA.

The biological mechanism by which antibiotic usage influences MRSA carriage or infection is considered to be, at least in part, down to antibiotics killing susceptible flora

and creating a niche for MRSA to inhabit. There are considered to be three distinguishable groups with regards to carriage of *S. aureus*: i) persistent carriers – approximately 20% of the population that almost always carry one type of strain; ii) intermittent carriers – approximately 60% of the population that harbour *S. aureus* intermittently and the strains vary over time; and iii) non-carriers – approximately 20% of the population that never carry *S. aureus*.[114] Persistent carriage is thought to have a protective effect against the acquisition of other strains, at least during hospitalisation, however, this barrier can be broken down by antibiotic therapy.[115] This suggests that the acquisition and transmission of antibiotic resistant *S. aureus*, in the hospital at least, may involve mainly persistent and intermittent carriers treated with antibiotics.[116] This theory gives biological plausibility to prior antibiotic usage as a risk factor for infection or carriage of MRSA, whereby antibiotics do not directly induce antibiotic resistance in the colonising strains, but instead clear the existing *S. aureus* and create a niche in which antibiotic-tolerant strains can thrive.

However, Weber *et al.*[110] consider the creation of a niche for antibiotic-resistant bacteria to only be part of the process by which antibiotics, particularly fluoroquinolones, promote antibiotic-resistant organisms. A partial role is also played by the selection of fluoroquinolone-resistant strains from heteroresistant *S. aureus* populations and the linkage of resistance genes, which means these strains are also likely to carry resistance to meticillin.[117] Weber *et al.*[110] argue, however, that these theories cannot explain the greater impact that is seen from fluoroquinolones and not by other antimicrobial agents. They support work undertaken by Bisognano *et al.*[118] who demonstrated that fluoroquinolones increase the adhesion of *S. aureus*, particularly resistant strains, and propose a combined mechanism by which fluoroquinolones simultaneously improve the binding ability of *S. aureus* and select for MRSA strains (due to the increased susceptibility to fluoroquinolones of MSSA strains compared with MRSA).

iv) Health-care interventions

A range of specific health-care interventions have been associated with increased carriage or infection with MRSA. Such interventions include enteric feeding,[100] central venous lines,[102] urinary catheters[109] and intensity of care.[119]

The study by Dziekan *et al.*[119] investigated hospital transmission routes through a case-control study implemented at one hospital in Germany after interventions were put in place to deal with an MRSA outbreak within the hospital. The study identified the intensity of care, defined by the degree of diagnostic monitoring of vital functions, to be significantly associated with nosocomial MRSA acquisition (OR 8.7, 95% CI 2.17-34.49). The authors also constructed a model to determine which nursing and treatment activities and procedures were most associated with MRSA transmission and found naso-gastric tube, central venous catheter and fluoroquinolones were identified as the significant independent variables (OR (CI) 7.6 (2.08-27.8), 11.1 (2.67-46.5) and 6.5 (1.43-28.82) respectively).

It is perhaps not surprising that those activities that require multiple manipulations are associated with MRSA, presumably due to the increased risk of cross-contamination with increased contact from health care staff. Furthermore, patients with indwelling devices may be more vulnerable to both colonisation (of the device which is not protected by the body's immune system) and infection (through devices that go from the outside to the inside of the body).

v) Co-morbidity

A variety of co-morbidities have been associated with increased risk of MRSA. Some examples include lung disease in hospitalised patients (US),[110] cellulitis in patients being admitted with *S. aureus* bacteraemia (US),[120] gastroenteritis in community

residents (Taiwan),[121] prior endocarditis in the homeless and urban poor (US),[122] diabetes in community residents (UK) [105] and chronic wounds (in hospitalised patients in the US and nursing home residents in Ireland).[101,123] This list is clearly not exhaustive but shows the range of morbidities that have been linked with MRSA. In some circumstances it is difficult to establish whether these factors represent truly independent risk factors for MRSA or whether they are somehow confounders in the relationship between a true risk factor and MRSA. For example, Lu *et al.*[121] undertook a large (n=1838), community point prevalence study in Taiwan to investigate prevalence of (nasal swabs) and risk-factors for (self-completed questionnaire) MRSA. The authors used multivariable analysis with stepwise logistic regression to explore the risk factors associated with nasal carriage but do not state whether interaction terms or co-linearity were explored. Because these factors were not explored it is not possible to tell whether gastroenteritis is indeed a true risk factor for MRSA carriage in the community, or whether it is a confounder in the relationship between MRSA carriage and previous antibiotic consumption.

It is of interest to this Thesis to further discuss studies that have identified chronic wounds as risk factors for MRSA. Wounds, of various aetiologies, have been investigated in several risk factor studies of MRSA with conflicting results. Lodise *et al.*[101] in their cohort studies of patients with bacteraemia at one US hospital found pressure ulcers to be a significant factor in their prediction model of MRSA in patients with *S. aureus* bacteraemia. Tacconelli *et al.*[102] also found skin ulcers at hospital admission to be significantly associated with hospital onset MRSA bacteraemia in one US hospital. However, as discussed above the control patients for this study were patients without bacteraemia and therefore it is possible that skin ulcers were associated with bacteraemia itself rather than specifically MRSA bacteraemia.

In the non-hospital setting, O'Sullivan and Keane[123] also found pressure ulcers to be significant in the risk of MRSA among nursing home residents in six long-term care facilities in Ireland.

Other studies however have not found a convincing relationship between MRSA and wounds. Whilst, carriage of MRSA in nursing homes in Germany has been associated with the presence of wounds but not pressure ulcers.[109] Furthermore Hori *et al.*[106] investigating patients, who had been hospitalised for at least 21 days, found no association between chronic wounds and MRSA even in univariable analysis. Interestingly, Grundmann *et al.*[105] found chronic wounds to be a confounding factor, being associated with both carriage of resistance and hospital admission.

The role of co-morbidities in acquisition of MRSA is interesting and it is unlikely that the same biological mechanism is working in all cases. While in some cases there will be a true association between co-morbidity and MRSA, for others the relationship might be spurious (and due to chance). Furthermore, confounding between co-morbidities and other significant risk factors and MRSA should be investigated. For example, it is possible that diabetes (with the associated increased probability of infection) is associated both with increase risk of MRSA but also increased risk of antibiotic consumption or prior hospitalisation.

vi) Other factors

Several other factors have been associated with MRSA in different populations and settings. Examples cited in the literature include male sex,[123] social deprivation score,[124] and staff deficit.[125] Other factors (which are discussed in more detail below), such as intravenous drug taking, may be very specific for the population under study, while processes which impact on transmission have been shown to be important in other

studies (for example MRSA associated with non-use of antibacterial soap in care homes).

A very specific population was explored by Charlebois *et al.*[122] who investigated MRSA carriage in the urban poor. In this population, self-reported injecting drug use was found to be a significant factor in the risk of MRSA, along with prior endocarditis and previous hospitalisation within one year. This study sampled the urban poor population by systematically selecting people from homeless shelters and free meal programs (60.2% of study population) and from low-income single room occupancy hotels (39.7% of study population), in San Francisco, US. The study included 833 persons from whom nasal samples were taken from August 1999 to April 2000. Interviews were undertaken but the interview schedule and details were not presented nor were *a priori* sample size calculations. The study was conducted to see whether the incidence of *S. aureus* and MRSA was different in this population that may occupy more crowded living conditions and have poor access to sanitation facilities (compared to the general population).

Loeb *et al.*[126] in their investigation of MRSA in nursing home residents found factors such as staffing levels and antimicrobial soap use by staff, at the nursing home level to be important. This large, North American prospective cohort study recruited nursing homes (with more than 100 beds) from Canada and the USA in 1998-99. Two hundred homes were invited to participate, of which 50, with 9156 residents, agreed (participating homes had significantly greater number of beds than non-participating homes). The study included antibiotic resistance and antibiotic usage data at the individual level and institutional level factors related to infection control. Over a 12-month period, 353 *S. aureus* isolates were cultured from residents and 115 found to be MRSA. Residents in homes in the US were more likely to receive antibiotics than

residents in Canada. With regards to MRSA, the authors investigated the impact of penicillin and fluoroquinolones using two separate statistical models. However, in both models, the risk of MRSA was significantly decreased by the use of antibacterial soap by staff (OR 0.24, 95% CI 0.12-0.47 in the model investigating the impact of penicillin and OR 0.22, 95% CI 0.13-0.36 in the model investigating the impact of fluoroquinolones). In the model investigating the impact of fluoroquinolones this was the only risk factor remaining in the model, however in the model assessing the impact of penicillin, use of antimicrobial soap in the nursing home for both patients and staff and the number of registered nurses per 100 residents were also found to be protective against MRSA and intravenous therapy in the nursing home was a risk factor for MRSA (OR 8.55, 95% CI 3.6-20.0). It may be that the number of nurses per 100 residents affects the time available for consistent and complete hand-washing. It is a weakness of the study by Loeb *et al.*[126] that they were not able to include individual covariates such as underlying illnesses or prior hospitalisation, however the focus on facility-level risk factors gives an insight into the effect of infection control measures that have an impact on resistant infections in nursing homes.

MRSA is clearly a very successful pathogen that has spread across the world and has a large impact on modern medicine, not least in the UK. Many factors can be seen to predispose patients to carriage or infection with MRSA, including antibiotic usage, previous hospitalisation or residency in a nursing home. Co-morbidities have also been associated with MRSA, including chronic wounds.

1.2.2 Resistance in *Pseudomonas aeruginosa*

Concern has grown in recent years over the development and spread of antibiotic resistance in *P. aeruginosa*, and particularly multidrug resistance. The prevalence of resistance in *P. aeruginosa* isolates in a study in 1999 was found to be generally low: <12% of clinical isolates submitted from 25 hospitals across the UK (each hospital submitted 100 consecutive clinical samples). Resistance rates were significantly higher in patients with cystic fibrosis, but were still below 15%.[127] However, multi-resistance has been associated with adverse outcomes.[128] Importantly, in contrast to *S. aureus*, resistance in *P. aeruginosa* is not due to the spread of a few clonal types, but emerges in a step-wise manner following exposure to anti-pseudomonal agents.[128]

P. aeruginosa is intrinsically resistant to many antibiotics. This is in large part due to the low permeability of the outer membrane, which works in combination with the secondary resistance mechanisms of efflux pumps and antibiotic specific enzymes.[129] Resistance to beta-lactams in *P. aeruginosa* is usually mediated by derepression of chromosomal beta-lactamases, which results in resistance to all susceptible beta-lactams and even beta-lactamase-resistant beta-lactams (such as co-amoxiclav), with the exception of the carbapenems.[129] *P. aeruginosa* can attain high levels of resistance to some cephalosporins (e.g. ceftazidime), which are poorly hydrolyzed by beta-lactamases, through the combination of such secondary resistance mechanisms as the low permeability of the outer membrane. The fourth generation cephalosporins (e.g. cefpirome, cefepime and cefaclidine), offer more effective treatment mainly due to their higher outer membrane permeability, as well as their lower affinity for beta-lactamases.[129] Other mechanisms that confer resistance to beta-lactams have been described, such as plasmid-encoded beta-lactamases and permeability changes, but these are quite rare in the clinical setting. The major resistance mechanism for

imipenem and meropenem is however mediated by a change in membrane permeability by the loss of a specific porin. This has been reported to occur in as many as 50% of *P. aeruginosa* infections treated for more than a week with imipenem.[130] Resistance to aminoglycosides can be mediated by acquisition of plasmids (which tends to induce resistance to a specific aminoglycoside) or by a resistance gene in the chromosome, although this latter form is thought to have little clinical relevance.[129] In addition, low level resistance to all aminoglycosides can result from decreased uptake across either the inner or outer membrane.[129]

P. aeruginosa can induce quinolone resistance by either mutations in the target site DNA gyrase, or through efflux mechanisms. It is thought that lower level, quinolone-specific resistance is induced following step-wise selection with increasing levels of quinolones through target site mutation of DNA gyrase. While selection resulting in changes in the efflux mechanism affords resistance to a wide range of structurally unrelated antimicrobial agents in addition to the fluoroquinolones.[129] Through this mechanism, induction of fluoroquinolone resistance is at risk of driving an increase in the development of multi-resistant *P. aeruginosa*.[131]

1.2.2.1 Risk factors associated with resistance in P. aeruginosa

The major risk factor associated with antibiotic resistance in *P. aeruginosa* is the use of antibiotics. Hsu *et al.*[132] undertook a case-control study of patients at one hospital in the US, comparing ciprofloxacin resistant *P. aeruginosa* colonisation or infection (n=91) with ciprofloxacin-susceptible *P. aeruginosa* colonisation or infection (n=86) in the US. Patients who had received any of the investigated agents (including fluoroquinolones) in the previous 10 days were excluded, as were patients with cystic fibrosis. *P. aeruginosa* infection and medical histories were obtained by review of the laboratory and medical records respectively. Hsu *et al.* found the independent risk factors associated with

fluoroquinolone resistance to be fluoroquinolone exposure within 30 days (OR 12.6, 95% CI 4.9-32.2), nosocomial residency before isolation of *P. aeruginosa* (OR 8.6, 95% CI 3.5-20.7) and diabetes (OR 6.4, CI 2.1-19.3). Sixty-four percent of cases had received fluoroquinolone treatment, compared with 10% of controls. They did not investigate the impact of any other antibiotics, but did include a number of co-morbidities, APACHE II score and demographic data.

Troillet *et al.*[133] investigated those risk factors specifically associated with imipenem resistance using a case-control study in a hospital where cases (n=40) had clinical infection with a resistant organism and controls (n=387) had a clinical *P. aeruginosa* infection that was susceptible to imipenem. In this study, in the US, the authors found imipenem resistance to be significantly associated with imipenem treatment (OR 23.2, 95% CI 4.1-132.7), but not with other beta-lactam drugs. The authors argue that their results show that cross-infection or the hospital environment do not have an important role in the epidemiology of imipenem-resistant *P. aeruginosa*. The authors investigated 25 variables in univariable analysis, including variables to describe possible exposures in the hospital environment. The other variable found to be independently associated with imipenem resistance was being a transplant recipient (although the authors state that the reason for this association was unclear). Length of hospital stay prior to isolation of *P. aeruginosa* negatively confounded the effect of imipenem consumption. Although this study shows a strong association between imipenem exposure and imipenem resistance, the odds ratio was found to have very wide confidence intervals and the imipenem exposure only accounted for 15% of imipenem resistance in the model (the authors suggest that this may be due to the inclusion only of antibiotic exposures after admission).

Zavascki *et al.*[134] also investigated risk factors associated with imipenem-resistant *P.aeruginosa* in Brazil. In this study, case patients were compared with two control groups. Control group one comprised randomly selected patients from the same unit, while control group two consisted of patients with imipenem-susceptible *P. aeruginosa*. The authors identified carbapenem consumption as a significant risk factor for imipenem-resistance in *P. aeruginosa* in both studies. In addition, when comparing patients with imipenem-resistant *P. aeruginosa* with control patients from the same unit, an interaction was found between carbapenem use and vancomycin use such that carbapenem use without vancomycin resulted in an OR of 3.57 (95% CI 1.38-9.19) whilst the risk associated with carbapenem and vancomycin use was 43.71 (95% CI 4.46-428.53). Other risk factors identified in this study included mechanical ventilation and at least one hospital admission in the previous year. Neither of these factors was significant in the model comparing resistant and susceptible isolates and therefore may represent risks associated with *P. aeruginosa* rather than with imipenem resistance. In the comparison of patients with susceptible and resistant *P. aeruginosa* the only factor of significance, other than carbapenem, was renal failure, which the authors suggest may be associated with severity of illness.

A similar study was conducted by Defez *et al.*[135] to investigate multidrug resistance in *P. aeruginosa* infection, in a university hospital in France. Eighty cases were included in the study and there were two control groups. The first control group comprised of matched hospital patients, and three control cases (n=240), who were present on the day the bacteria was isolated from the case, were chosen for each study case. The control group were matched with the cases on type of hospital unit and time at risk (i.e. period between admission and isolation of the bacteria in the case (required to be at least equal and not more than 7 days more than the case)). The second control group (n=75) included patients with non-multiple resistant *P. aeruginosa* nosocomial infection.

These patients could not be matched to cases due to the small numbers but the inclusion criteria (with the exception of resistance status) were the same. In their comparison of patients with resistant *P. aeruginosa* compared to matched patients (patients without *P.* aeruginosa) they found exposure to beta-lactams (OR 2.5, 1.0-6.3) or fluoroquinolones (OR 4.1, 1.5-11.7) to be linked to multidrug resistant *P. aeruginosa* infection. The other significant risk factors were advancing age, transfer from another unit, being bedridden, urinary catheterisation and nasogastric feeding. While in their investigation of patients infected with *P. aeruginosa* only fluoroquinolones and surgery (protective) were significantly independent factors in the risk of multidrug resistance in *P. aeruginosa* compared to sensitive infections (OR 4.7, 95% CI 1.8-12.0 and OR 0.5, 95% CI 0.20.98 respectively). The authors suggest that the protective nature of surgery (frequency of surgical intervention was lower in those with multidrug resistant *P. aeruginosa* than non mutlidrug resistant infection) may be because patients with multidrug resistant infections were older and had a worse prognosis (and therefore might be less likely to be offered surgery, or less likely to survive it). Interestingly diabetes was investigated but not found to be significantly in either model. This study design allows for the identification of both those risk factors associated with antibiotic resistance organisms in the whole patient environment as well as looking at the risk factors associated with resistance in those with an infection.

Carmeli *et al.*[136] compared the risk associated with different antibiotics on the emergence of antibiotic-resistant *P. aeruginosa* (identified through clinical specimens) in a historical cohort of inpatients in the US. Two-hundred and seventy one patients were included in the study which investigated the risk of emergence of resistance to four study drugs: ceftazidime, ciprofloxacin, imipenem and piperacillin. The study also included other risk factors covering underlying conditions, exposures during admission, baseline isolate descriptors and demographics. The number of isolates resistant to each

study antibiotic was 19 (7%), 58 (21%), 36 (13%) and 5% (n=15) respectively. Imipenem was found to be the only drug significantly associated with the risk of emergence to any of the study drugs (hazard ratio (HR) 2.8, 95% CI 1.2 to 6.6). Imipenem was also very strongly associated with resistance to itself (HR 44, p=0.001). Ciprofloxacin however was not found to be associated with the emergence of resistance to any of the study antibiotics, but was strongly associated with resistance to itself (HR 9.2, p=0.04). Similarly, piperacillin was associated with resistance to itself (HR 5.2, p-0.01) but not to any study antibiotic. The relative impact of different classes of antibiotic is difficult to interpret in many studies. This study, although based on inpatients attending one hospital, gives an indication that imipenem might have greater impact on the prevalence of fluoroquinolone and other resistance than ciprofloxacin.

With respect to chronic wounds there is only very limited evidence. Troillet *et al*[133] found that isolation of *P. aeruginosa* from a wound (unspecified as to whether acute or chronic) was not a significant factor in terms of imipenem resistance This was also found to be the case when Carmeli *et al.*[136] investigated the risk of resistance to a selection of antibiotics. No studies were identified that explicitly investigated chronic wounds as a potential risk factors for antibiotic resistant *P. aeruginosa.*

In summary, the use of antibiotics can be seen to directly impact on the development of *P. aeruginosa* resistant isolates. Antibiotic resistant *P. aeruginosa* are known to colonise chronic wounds but the significance of such wounds on the development of resistant infection has not been investigated,.

1.2.3 Antibiotic resistance and chronic wounds

Chronic wounds have been seen to be variably associated with an increased risk of carriage or infection with resistant microbes. The polymicrobial nature of chronic

wounds is, however, likely to provide an ideal environment for genetic exchange between bacteria. Indeed, the importance of this environment to the world of antibiotic resistance was highlighted by the isolation from chronic wound patients of the first two cases of vancomycin-resistant *S. aureus* (VRSA) in the United States.[137,138]

It is hardly surprising that antibiotic resistant organisms have been found to colonize and infect chronic wounds. Colsky *et al.*[139] in a retrospective review of ongoing antibiotic surveillance of patients admitted to one tertiary care dermatology unit in the US found as many as half of all *S. aureus* isolates from hospitalised dermatology patients with leg ulcers to be meticillin resistant *S. aureus* (MRSA) and more than one third of *P. aeruginosa* isolates to be resistant to ciprofloxacin. A study by Tentolouris *et al.*[19] in an outpatient diabetic foot clinic in the UK found 40% of *S. aureus* isolated from non-limb threatening infected foot ulcers to be MRSA; giving MRSA a prevalence of 15% in all DFU patients with clinical evidence of ulcer infection. Furthermore, there were significantly more MRSA isolates from patients who had received prior antibiotic therapy, compared with those that had not in this retrospective review of wound swabs taken from patients with non-limb or life threatening DFU infections. A follow-up study, in the same clinic, identified a similar proportion of meticillin resistance in the *S. aureus* isolates, but showed that the prevalence of MRSA in foot ulcers had almost doubled over a three year period to 30% of all DFU patients with ulcer infection.[140] Ge *et al.*[28] investigated resistance in bacterial isolates from infected DFUs, from patients who had not received antibiotics during the previous fortnight, and found 12% of *S. aureus*, 46% of *S. epidermidis* and 45% of *S. haemolyticus* to be meticillin resistant. This large study of 825 Phase 3 RCT participants in the US also found high levels of resistance to erythromycin in most species of Gram-positive organisms. The previously mentioned Swedish audit of all chronic wounds by Tammelin *et al.*[61] also found 12.5%

of *S. aureus* isolates and 21.7% of *Pseudomonas* species isolates to be resistant to a clinically relevant antibiotic.

Different populations of wound patients can show wide variations in the levels of antibiotic resistance encountered. For example, a prospective study of the chronic venous leg ulcers (displaying no clinical signs of infection) from 66 patients who had received no antibiotics in the previous month identified very low levels of antibiotic resistance; only two patients were found to have MRSA (7.7% of those patients colonised with *S. aureus* (n=26)).[17] In contrast, a separate, retrospective investigation of leg and foot ulcer swabs sent for analysis at the PHLS from the same out-patient clinic in Cardiff (from wounds presumed to be infected or displaying prolonged non-healing) demonstrated much higher levels of MRSA: 36% of patients with *S. aureus* (unpublished data). The underlying reason for these differences are unknown and could be multi-factorial, including such factors as infection status, prior antibiotic therapy and the level of contact with healthcare institutions.

Chronic wound patients are clearly a high risk group for the acquisition, carriage and dissemination of antibiotic resistant organisms. For patients with chronic wounds, those factors associated with increased risk of carriage or infection with antibiotic resistance are not well elucidated. Day and Armstrong[141] reviewed the limited evidence on risk factors for the carriage of MRSA in diabetic foot wounds. While they found no studies that had directly addressed this issue, suggested risks include cross-contamination of wounds from the patient themselves, inanimate objects or health care personnel, long-term use of antibiotics, prior hospitalisation and severity of illness (which may itself increase exposure to MRSA endemic environments, such as hospitals and nursing homes). The review was not systematic (narrative review) and no search strategy is

reported. Therefore information on the databases searched, search terms used, language and date limits are not available.

Some studies suggest that the carriage of antibiotic resistant organisms in chronic wounds could lead to increased likelihood of MRSA infection. Coello *et al*,[142] investigated the risk factors for MRSA disease in inpatients initially only colonised by MRSA in one hospital in Spain. They followed patients prospectively during their period of hospitalisation and identified the presence of MRSA from clinical specimens and reviewed medical notes to determine whether the patient had an infection or colonisation. They found surgical wounds and pressure ulcers to significantly increase the risk of MRSA infection with the only other significant risk arising from intravenous catheters, although it should be noted that MRSA infection included infection of wounds or ulcers. Vascular ulcers were not found to be associated with MRSA infection in crude hazard ratio analysis.

Persistent carriage of MRSA following discharge from hospital may also be increased by the presence of a wound.[143] Later work by Scanvic *et al.*[144] supports this finding. They investigated the duration of MRSA colonisation after hospital discharge through MRSA status at readmission and found the only significant variable, in the multi-variable analysis, to be a break in the skin. This study (in a 1200 bed hospital in France conducted for 10 months commencing at the beginning of 1998) investigated previous MRSA positive patients on re-admission for current MRSA carriage. The median duration of MRSA persistence was 8.5 months, although this estimate is clearly biased by the lack of regular prospective sampling and the inclusion of only those patients who required readmission to hospital (for any reason, not specifically wound-related). The study is based on 78 patients who were initially MRSA positive and were re-admitted to hospital during the study period; 31 were MRSA positive on readmission and 47

negative. Furthermore in only 17 (55%) of the persistent carriers did both MRSA strains have the same antibiotic susceptibility pattern (some PFGE patterns were investigated but it is not clear to which samples these relate). Despite its weaknesses, the study does suggest that breaks in the skin (which included skin ulcers) can be a risk factor for prolonged MRSA carriage in patients that have previously had MRSA and are being re-admitted to hospital.

The risk that wound patients carrying antibiotic resistant organisms pose to others is also unknown. However, dressing changes alone have been shown to disperse significant numbers of bacteria into the air in patients.[145,146] The extent of this dispersal varies according to the type of dressing involved (with hydrocolloid dressings dispersing fewer organisms than traditional absorbent cotton wool and gauze dressings) and is slow to decline.[145,146] Wound patients are also clearly a group of patients who have a high level of contact with health care staff and could themselves act as a reservoir for cross-contamination. High prevalence of antibiotic resistance, especially MRSA, affects treatment decisions concerning wounds and raises the question of whether and when empirical regimens should cover these resistant organisms.[147]

It is clear from the literature that expert opinion suggests that antibiotics have an important role to play in the treatment of clinically infected chronic wounds. However, there are no conclusive scientific studies to support antibiotic use, let alone those that might definitively guide antibiotic choice, dose and duration. The use of antibiotics is not risk-free for the individual with both the immediate risk associated with anaphylactic reactions[148] and the longer term prospect of antibiotic use making co-morbidities more difficult to treat. For example, the use of macrolides and metronidazole up to 10 years previously have, respectively, been associated with clarithromycin and metronidazole resistance in *Helicobacter pylori* isolates.[149] In

addition, antibiotic resistance in the general population is a continuing and growing concern. The contribution made to the development, maintenance and dissemination of resistance by those antibiotics issued for chronic wounds is not yet known. However, it is known that antibiotics, especially fluoroquinolones, pose a significant risk for the promotion of both MRSA and resistant strains of *P. aeruginosa.*

There is reason to believe that the chronic wound patient population may be of importance due to the high levels of antibiotic prescribing to these patients, the degree of microbial load associated with their lesions and the potential they provide for dissemination of resistant organisms to others. The presence of chronic wounds has been found to be associated with MRSA colonisation, MRSA infection and persistent carriage of MRSA. The factors that make some wounds more likely to be colonised or infected with antibiotic resistant organisms would be of great value in determining prevention strategies, but these have not yet been elicited.

1.3 Economic implications of chronic wounds and antibiotics

Increasingly attention is being paid to the costs associated with illnesses and their management. Clinical interventions are frequently required to demonstrate cost-effectiveness, as well as clinical effectiveness. The management of chronic wounds has not escaped this examination. Studies have ranged from investigations into the overall cost of chronic wounds, down to establishing which is the most cost-effective compression bandaging regimen for venous leg ulcers.

1.3.1 Economics of chronic wounds

Economic studies in the field of wound care have been used to establish the cost associated with chronic wounds, identify the most cost-effective treatments and compare costs in different health care settings.[150-155] In the early 1990's Bosanquet *et*

al.[5] estimated that £400 million was spent annually on the care of leg ulcers in the UK. More recently, the cost to the National Health Service (NHS) of treating venous leg ulcers has been estimated by Tennvall and Hjelmgren[150] to range from €814 to €1994 (2002 Euros) per ulcer, equivalent to £506 to £1240 in 2002 UK Sterling.[156] Tennvall and Hjelmgren [150] modelled costs associated with wound care in Sweden and the UK. UK experts were used to advise on practices in the UK, but data from a Swedish database of venous leg ulcer patients was used to construct the model. The study included only tangible costs to the healthcare provided and did not estimate the quality of life gained by treating such wounds. However, they did estimate the costs associated with wounds of different sizes and duration, although wound infection and antibiotic treatment was not considered.

Cost-effectiveness studies have explored several distinct areas of wound care such as the selection of dressings and bandages. Iglesias *et al.* compared four-layer bandages with short-stretch bandages for the treatment of venous leg ulcers and, following adjustment for confounders, found four-layer bandaging to be the dominant strategy being both more effective and cheaper (although not statistically significant). The difference in costs was mostly attributable to more frequent nurse visits in the short-stretch bandage arm of the study.[151] This was a UK based RCT of bandages which assessed the cost-effectiveness alongside the effectiveness. Data were collected from the RCT to inform the cost-effectiveness study, for example effectiveness and resource use data. This might lead to concerns that the study is not generalisable to real-life and the treatment of patients in primary care or wound clinics. While these concerns are valid to a certain extent, efforts were made to increase the external validity of the study. For example, this was a multi-centred trial in which, once patients had been randomly allocated to a treatment group, clinicians were able to choose from a number of products when applying dressings and bandages to ulcers.

Harding *et al.* conducted a cost-effectiveness analysis of three wound management protocols (sponsored by the manufacturer of one product). The model was populated with data from a review of the literature and consensus of expert opinion. Hydrocolloid dressings were found to be more cost-effective than gauze and a replacement skin in the treatment of venous leg ulcers.[152]

The cost effectiveness of community leg ulcer clinics compared to the usual care provided by district nurses (in a randomised controlled trial) was investigated by Morrell *et al.*[153] in the UK. This study in effect introduced both the Charing Cross method of four layer compression bandaging and the use of community clinics compared to usual care as not all the components of four-layer bandaging were available on prescription in the community. Nonetheless, the study identified the incremental cost effectiveness ratio (ICER) to be £2.46 (-£31.94 to £99.12) per ulcer free week. This was calculated from the additional costs for clinic treatment group (£14.51 per week) compared with the benefit of 5.9 ulcer free weeks in the clinic treatment group. The authors used modelling to identify under what circumstances the use of community clinics would dominate, in economic terms, by being both cheaper and more effective and found increased throughput at clinics and altered grades of nurses to be the most influential factors.[153]

Cost-effectiveness analyses of wound healing products, treatments and clinics are extremely useful for clinical practitioners, formulary composers and healthcare commissioners amongst others. They enable costs to be explicitly examined, and can show the gain in health against cost of the intervention, be that a new dressing or way of delivering healthcare.

The perspective taken in many economic models is that of the healthcare provider which do not include the wider costs to society or individual patients. For patients

themselves the costs can be more complex, involving both tangible costs (e.g. loss of earnings, travel costs) and intangible costs (e.g. quality of life, pain). The cost to society of leg ulcers may, although controversial, include the loss of productivity. There is the lack of value attributed to those that do not work or undertake paid work, for example the retired, when considering loss of productivity. Some even consider its inclusion to be double-counting, as it should be captured by the effectiveness measure, such as quality adjusted life years.[157] While leg ulcers are a disease of the elderly, one survey in the US found 42% of patients with leg ulcers who were not working at the time stated that their leg ulcer was a factor in their decision to stop working. Furthermore, those patients who were working all stated that their leg ulcer limited what they were able to do at work.[4]

The health economics associated with treatments and healthcare of chronic wounds other than venous leg ulcers have also been investigated. Oretegon *et al.*[154] investigated the cost-effectiveness of prevention and treatment in diabetic foot ulcers, comparing two guideline-based care regimens with standard practice. The model was based on a Dutch diabetic population (although data to populate the model were, by necessity, taken from other countries as well). Guideline-based care consisted followed either intensive glycaemic control or optimal foot care. These guidelines were proven to decrease incidence of foot disease, but due to uncertainty in the exact amount, a reduction in foot disease incidence of 10% to 90% for the guideline groups was assessed. Using a Markov model of lifetime costs associated with diabetic foot ulcers they found the cost of gaining one QALY by following guidelines to be less than $25,000.

The additional cost incurred by diabetic patients due to the development of a foot ulcer were identified by Ramsey *et al.*[158] to be $27,987 in the two years following incidence of foot ulcer (in 1995 dollar values). Ramsey *et al.*[158] undertook a 3-year retrospective

study of a large health maintenance organisation in the US and compared the costs incurred by foot ulcer patients compared to control patients (each foot ulcer patient was matched to four control patients with diabetes but no recorded history of ulceration, osteomyelitis or amputation over the study period.

The cost-effectiveness of pressure-relieving devices in the prevention and treatment of pressure ulcers at different stages were investigated by Fleurence.[155] It was found that alternating pressure mattress overlays could be cost-effective for the prevention of pressure ulcers, while alternating pressure mattress replacements could be cost-effective for the treatment of severe and superficial pressure ulcers.[155]

It can be seen therefore that there has been a range of studies, which have addressed questions regarding the costs associated with the treatment of chronic wounds. Many of these studies have focussed on certain products or dressings, but some have taken a wider view of costs.

1.3.2 Economics of antibiotic resistance

Antimicrobials, in economic terms, are a scarce resource: consumption (current use) decreases effectiveness (future value).[159] Antimicrobial resistance can be considered as a negative externality associated with the use of antibiotics for treating infections, whereby the effects of resistance are not felt by either the consumer or the supplier of antibiotics but on the welfare of the community.[160] The incentive to decrease antibiotic use does not exist due to the lack of direct impact on the supplier or consumer.[161] Economic studies have looked at the cost to society of resistant infections,[162] economic policies for reducing antibiotic consumption as a mean of tackling resistance,[161] costs associated with infection control measures to combat resistance,[163] and the optimum

choice of empirical antibiotics given different prevalences of resistance.[164,165] Frequently, however, costs associated with antibiotic resistance have been ignored.

Where included, the cost of antibiotic resistance from the hospital perspective is the most frequently studied with costs including in-hospital mortality, length of hospital stays, and increased drug, investigation and personnel costs.[73,160] One example of such studies include Rubin *et al.* who investigated the economic impact of *S. aureus* infections in New York hospitals and found the attributable cost per patients of MRSA infection compared to MSSA infection to be $2,500 ($34,000 compared to $31,500 in 1995 US dollars).[166] There are however many limitations with their study, which demonstrate the difficulties of obtaining data on the costs associated with MRSA infections. Although the authors claim to consider costs from the societal perspective, only post-discharge costs have been included in addition to hospital costs (no costs of dying or productivity loss, or post-discharge complications that do not result in hospitalisation are incorporated). While the study is based on discharge data from the State of New York Department of Health, which collects data from all hospitals in the State, these data were used only to give the number of cases of bacteraemia, pneumonia, endocarditis, SSI, osteomyelitis and septic arthritis. Research or expert clinical opinion was then used to estimate the proportion of these infections (apart from pneumonia) that were attributable to *S. aureus*. The expert panel was then used again to estimate the proportion that were MRSA (assumed to be 29%) and finally the expert panel estimated the additional costs MRSA infection would incur above that of MSSA infection. The average cost of disease (e.g. bacteraemia) was taken as the cost for MSSA infection. Therefore, it can be seen that these estimates are based on expert opinion and many assumptions regarding the available data. This is perhaps, an extreme example but in many economic models assumptions and opinion are necessary as data are frequently

unavailable for all parameters. This is particularly an issue with studies of antibiotic treatment and resistance.

Costs to patients and society which are much less frequently included in economic analyses of the impact of antibiotic resistance include, for patients with resistant infections, the on-going precautionary costs for future episodes of illness, long-term effects on health and the loss of work and family time associated with increased hospitalisation. There is also a cost for patients who do not have resistant infections, including the use of broader spectrum empirical antibiotics (frequently more expensive, with more adverse effects and lower efficacy against susceptible strains) to cover increasing prevalence of antibiotic resistance. Furthermore there is the cost to society as a whole, such as increased surveillance, investment in novel antibiotics, infection control measures and premature deaths.[160] Coast *et al.* consider that due to the number of decision makers involved in prescribing antibiotics and the diffuse nature of the externality of antibiotic resistance that economic evaluation alone is unlikely to ensure that the costs of resistance are incorporated into the decision making process.[160]

There are many difficulties associated with the inclusion and assessment of costs associated with antibiotic resistance, not least the uncertainty surrounding what costs might be incurred. Coast *et al.*[161] consider the likely consequences to be that patients infected with resistant organisms will be less likely than patients with susceptible infections to respond to first-line antibiotic treatment, will require both extra investigations and extra treatments (usually more expensive) and potentially longer hospital stays and periods away from work and will have a greater likelihood of premature death.

Separating the true cost of resistant infection from that of susceptible infection and interventions to control and prevent spread of resistance is very difficult.[163] Studies have

however identified higher costs associated with the treatment of MRSA compared with MSSA infections in case-control studies. The cost and impact associated with MRSA varies with the setting, for example MRSA is likely to be most severe in settings such as ICU, while isolation from superficial sites and in long-stay patients in the community may have little impact.[163]

Furthermore there are many uncertainties associated with resistance mechanisms, the effect of increasing use of particular antibiotics, development of new antibiotics and any spread of resistance from other areas.[160,161] It is not clear how a change in any of these would impact on the costs associated with antibiotic treatment of infections.

The impact of discounting is also unknown. Discounting is a commonly used economic method, which takes into account the preference for incurring benefits now and costs in the future. For example, £1 or one healthy life year now is perceived to be of greater value to us now than in 10 years time. In studies that investigate outcomes over a period of years, the practice of discounting future costs in economic studies therefore affects the perceived impact of antibiotic resistance. Many of the costs of resistance are likely to be incurred by future generations and therefore cost analyses will be greatly influenced by the time frame and discounting applied. Short time frames and/or high discount rates are likely to identify a cost to society of reducing antibiotic usage, while long time frames and/or low discount values are likely to identify an overall benefit to society.[161]

Studies that have in some way overcome (or by-passed) these difficulties to apply economic analysis to antibiotic usage and resistance are outlined below. These include Le and Miller[164] who explored the most cost-effective treatment that should be chosen for empirical therapy (trimethoprim-sulfamethoxazole or fluoroquinolone) for uncomplicated urinary tract infection, given different prevalences of trimethoprim-

sulfamethoxazole resistant organisms. Costs included in this analysis were costs associated with antibiotics, hospitalisation and medical doctor visits. This study identified the threshold value for a change in strategy from empirical trimethoprim-sulfamethoxazole treatment to empirical fluoroquinolone treatment at a resistance prevalence of 22%, although this was raised to 43% when the cure rate (i.e. susceptibility) to ciprofloxacin was 90%. They, perhaps unsurprisingly, did not include in their analysis the potential costs associated with increased use of ciprofloxacin in terms of perpetuating resistance to itself and other antibiotics.[164]

Eandi and Zara[167] have used a simulated decision model to explore the impact of antibiotic resistance on the costs associated with amoxicillin resistance in lower respiratory tract infection from both the health care providers perspective and that of society. Data for this model were not real but were 'common knowledge and common sense' data. The aim of the model was to show the link between changes in clinical outcome as a result of antibiotic resistance and costs on the cost-effectiveness of treatment regimens. They state that the main problem when considering the economic impact of antimicrobial resistance is the scarcity of data on the consequences for mortality and morbidity from infectious diseases. Costs associated with resistance were explored from the societal perspective (including loss of earning from death as well as costs to patients or their relatives while ill) and from that of the local health care organisation. The use of tornado diagrams identified the loss of earnings per year to have the greatest influence on the treatment choice.[167] It should be remembered that the data for this study are not real, and that the availability of data is one of the main limitations in economic studies of antibiotic resistance. Nonetheless, their model shows that it is possible to investigate the costs of antibiotic resistance to the healthcare organisation and society (although many societal costs are excluded such as the development of new antibiotics, surveillance systems and so forth).

Vinken *et al.*[165] explored the economic evaluation of linezolid, flucloxacillin and vancomycin in the empirical treatment of cellulitis in UK hospitals using a decision analysis model. The model looks at costs from the hospital perspective but includes post-discharge costs. They investigated the choice of empirical treatment given differing MRSA prevalence in cellulitis caused by *S. aureus* and suggest that use of linezolid as the empirical treatment for cellulitis would result in a greater success rate than first-line treatment with flucloxacillin and once the prevalence of flucloxacillin-resistance exceeded 24.1%, empirical linezolid treatment would be more cost-effective.[165] Data on probabilities of clinical events were collected from the literature, while data on healthcare utilisation for first line antibiotic treatment and length of stay for patients successfully treated with first line antibiotics were derived from clinical trials. The search strategy for data was not stated and neither were inclusion criteria given. Data regarding treatment failures and the probability of switching antibiotics were derived from expert opinion using the Delphi technique (five surgeons and five infectious diseases doctors). Cost estimates were from 1997-99 tariff lists (BNF for drugs, 1998 tariffs for trust hospitals for NHS procedures, hospitalisation and consultation fees). The study was sponsored by Pharmacia. This study shows that it is possible to investigate the cost-effectiveness of different treatment regimens considering different prevalences of antibiotic resistance.

Shah *et al.*[168] explored the direct medical costs associated with inpatient use of vancomycin for MRSA in skin and soft tissue infections (amongst other infections) using a decision analytic model. This was US-based model from the hospital perspective that looked at the costs associated with four MRSA infections. Based on estimates from the literature (only Medline searches 1995-2003) with Medicare costs. The costs associated with drug acquisition, administration and monitoring were $31 per dose and $779 per course. The inclusion of hospitalisation costs put the total cost for

vancomycin treatment at $23,616. In sensitivity analyses, the factor that affected the cost of vancomycin most significantly was per diem hospital costs. This study emphasises that drug acquisition can be only a fraction of the true cost of treatment.[168]

It can be seen from these studies that antibiotic resistance has been incorporated into a variety of economic studies. These have ranged from investigating the cost associated with resistance, to looking for the most cost-effective treatment in the light of changing prevalence of resistance. The costs associated with chronic wound management, antibiotic use and antibiotic resistance have been separately addressed. Common to both chronic wound treatment and antibiotic use is that the cost associated with the clinical material (e.g. dressings, bandages, or antibiotics) is frequently not one of the most important factors in the overall cost. More important factors include the time of healthcare professionals and duration of treatment. However, the impact of antibiotic resistance on the cost of chronic wound treatment has not been explicitly explored.

Antibiotic use and antibiotic resistance have been seen to be integral to the treatment of infected chronic wounds. Data on the cost implications associated with increasing antibiotic resistance do not however exist. Such data would be of value to inform preventative strategies as well as to potentially plan for healthcare needs.

Chapter 2 . Antibiotic use for chronic wounds in primary care

2.1 Abstract

The aim of this study was to describe and quantify systemic antibiotic prescribing for patients with chronic skin wounds presenting at primary care and to compare this with antibiotic prescribing for patients without chronic wounds. Data for one year were extracted from a morbidity database comprising approximately 185,000 patients attending general practices in Wales. Patients with chronic wounds were identified using Read Codes and compared with randomly selected age-band, sex and general practice matched non-wound patients. To address known variability in the capture of prescribing data in the database, only practices with Read Coded prescribing data were included. Patients with chronic wounds received a significantly greater number of antibiotic courses than non-wound patients ($p<0.001$). Increased prescribing was evident for flucloxacillin, co-amoxiclav, cefaclor, cefalexin, erythromycin, trimethoprim, metronidazole and ciprofloxacin ($p<0.01$ for all). In single-diagnosis visits (where only chronic wound related morbidities were recorded) 11% of antibiotics prescribed were of duration ≥8 days. Patients with chronic wounds also had a higher prevalence of diabetes (16.5% Vs 6.6%, $p<0.001$) and attended at general practice more frequently than non-wound patients (median (inter-quartile range) of 25 (17-40) visits per year Vs 12 (4-20), $p<0.001$). Importantly however, exclusion of diabetic patients and analysis of the proportion of visits on which patients received antibiotics did not affect the significance of the difference in antibiotic consumption. These data, therefore, demonstrate a strong association between the occurrence of chronic wounds and antibiotic prescribing in primary care, and wide variation in type and duration of antibiotic therapy for chronic wounds.

2.2 Introduction

Chronic skin wounds cause significant morbidity and generate considerable healthcare costs. The treatment of these wounds focuses on the underlying causes and prevention,[54,169] however, the wound microflora play an important role, not only in mediating the impaired healing but also in clinical infection which may supervene.[24,170] The concept of bacterial burden in these wounds has been established over a number of years,[39] and modulation of the wound microflora and bacterial biofilm is an important treatment-aim in the management of patients with chronic skin wounds. This treatment may be affected by antimicrobial dressings (e.g. silver), antiseptics and the prescription of topical or systemic antibiotics.

Antibiotic resistance is a major world health problem from which patients with chronic wounds are certainly not exempt. Antibiotic resistant organisms have been found to both colonise and infect chronic wounds, for example, 50% of *S. aureus* isolates from hospitalised dermatology patients with diabetic foot ulcers were found to be meticillin resistant (MRSA).[139] In addition, the first two cases of vancomycin-resistant *S. aureus* in the United States were both isolated from patients with chronic wounds.[137,138] The potential impact of a population with increasing numbers of individuals at risk of developing chronic wounds (due to increased lifespan and type II diabetes), together with the opportunity the chronic wound environment affords for genetic exchange between bacteria is an important issue.

Antibiotic use, both at an individual and group level, is one of the main risk factors associated with antibiotic resistance.[106,136,171,172] Antibiotic therapy has important and clear indications in chronic skin ulcers showing signs and symptoms of infection, such as cellulitis or osteomyelitis,[54,56] however, evidence supporting the effectiveness of

systemic antibiotics in the absence of such complications does not exist.[42] Despite this, they are still widely employed in the treatment of chronic wounds.[61]

The frequency of antibiotic prescription for patients with chronic wounds has been demonstrated in a previous study in Sweden.[61] Although this study included antibiotics prescribed in both the primary and secondary care settings, it revealed that 26.6% of chronic wound patients were receiving systemic antibiotics at the time of the study while 60.1% of patients had received them during the previous six months.[61] The main burden of the treatment for chronic wounds, however, falls on the primary care sector of the health services and is delivered by nurse practitioners and family doctors,[6] therefore, it is clearly important to determine, in detail, the pattern of prescribing in this setting alone.

In this study, we sought to determine the quantity and pattern of antibiotic prescribing that is occurring for patients with chronic wounds in the primary care setting, in the UK and to compare this with antibiotic prescribing in patients without chronic wounds.

2.3 Methods

2.3.1 Study Population

Data from 19 practices from the General Practice Morbidity Database for Wales (GPMD) were included in this study over a 12-month period, for the year 2000. The practices had comprehensive, validated, and interpretable prescribing data (generated using Read Codes) and represented a total of 184852 patients (source population, approximately 6% of the Welsh population).

Patients with chronic wounds (PCW) were identified and defined as cases if they had one or more diagnostic or treatment Read Codes specific for chronic wounds. All patients identified as cases were included in the study. Read Codes form a

comprehensive list of health-care terms used in computer-based systems.[173] For these patients only, any attendance for which a specific or non-specific wound code had been recorded was classified as a wound-related visit. The non-specific wound Read Codes were not considered sufficiently precise to differentiate between the many non-chronic wounds recorded in the database. They were, however, used to identify attendances for chronic wounds by those patients previously identified as having such wounds using the specific codes. Attendances for PCWs identified in this way, were classified as *single-diagnosis* visits when there was an absence of any other Read Codes for the same patient on the same date relating to other diseases or actions. Codes relating to administration or visit information (such as location) did not affect this classification. Ulcer aetiology was identified using the most specific chronic wound code available for each patient. Wound infection was considered to be present for a patient when a Read Code explicitly referring to skin, wound or bone infection, or a microbiology investigation, was present.

A control group was selected to enable antibiotic prescribing for PCW (cases) to be compared with non-wound patients. Controls were patients without any wound diagnostic or treatment Read codes recorded during the year, who were selected by individually matching for age-band, sex and general practice at a ratio of four matched patients to each PCW. For each case, all potential control patients for each patient were identified and each assigned a random number (between 0 and 1, generated using Excel). For each case, the four matched potential control patients with the lowest randomly generated numbers were selected as the controls. Patients with and without chronic wounds were identified as diabetic if they had any diabetic-related Read Codes. The duration of antibiotic courses was determined by using the number of tablets or capsules given on prescription as a proxy marker.

Comparison of the antibiotic prescribing was made between the cases and the controls. Exposure for each case and control was defined as identification of an antibiotic Read Code associated with the patient's record during the one-year period (year 2000) and thereby antibiotics identified for cases both before and after any indication that a patient had a chronic wound were included.

2.3.2 Data analysis

The extracted data were analysed using SPSS version 10. Differences between the groups were investigated using non-parametric tests (Mann-Whitney U and Chi-squared) due to the skewed nature of the data. Unless otherwise stated, the *p*-values reported were calculated using the Mann-Whitney U test. To explore the impact of the number of visits on the quantity of antibiotic prescriptions, the average of the proportion of visits on which patients were prescribed antibiotics (expressed per 100 visits) were compared for the two patient groups. The number of courses of different types of antibiotic prescribed are presented per 1000 patients, derived by factoring up for PCWs and down for non-wound patients. A descriptive analysis of the duration of antibiotic courses for cases and controls was undertaken and presented (as a percentage of all the antibiotic prescriptions for which there were data on the number of tablets) for courses of <5 days, 5-7 days and ≥ 8 days duration.

2.4 Results

The general practices included in the GPMD with comprehensive prescribing data, represented a total patient population of 184852 and ranged in size from 4961 to 17107 (mean 9729) patients. Four hundred and fifty-five PCWs were identified from the GPMD. Age and sex data were available for 400 of these (Figure 2.1) (cases), who were subsequently matched with 1600 non-wound patients (controls). The PCWs

contributed a total of 386.32 person-years of data, and the 1600 non-wound matched patients 1558.71 person-years.

The breakdown of chronic wounds by aetiology is shown in Table 2.1. The most common aetiology recorded was venous ulceration but it can be seen that aetiology was not clearly indicated in over 60% of PCWs, although in nearly 40% of these cases the ulcer was known to be located on the leg. None of the patients had Read Codes indicating more than one aetiology. Whilst only 11 patients were directly coded as having diabetic foot ulcers, a further 55 PCWs were identified as having diabetes using non-wound codes. Diabetes was found to be significantly more prevalent in PCWs than matched patients (16.5% and 6.6% respectively, χ^2=40.42, p<0.001).

Figure 2.1 Demographics of the population of patients with chronic wounds

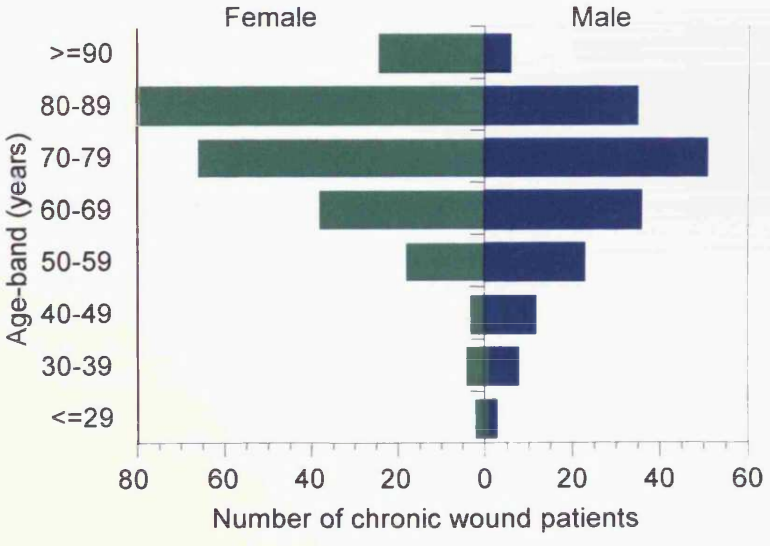


Table 2.1 Breakdown of wound aetiologies (determined using the most precise Read Code available).

Wound aetiology	Number of patients (with age and sex data)	% of all patients with chronic wounds	% of study population
Venous ulcer	79 (68)	17.4	0.043
Arterial ulcer	4 (4)	0.9	0.002
Diabetic ulcer	11 (11)	2.4	0.006
Pressure ulcer	68 (49)	14.9	0.037
Unspecified leg ulcer	113 (106)	24.8	0.061
Unspecified ulcer	104 (89)	22.9	0.056
Ulcers identified by nursing or laboratory codes (therefore unspecified aetiology)	76 (73)	16.7	0.041
Total	455 (400)	100	0.246

Over two thirds of PCWs received at least one course of antibiotics during the year, compared to less than one third of non-wound patients (χ^2=207.12, p<0.001). Table 2.2 summarises the quantities of antibiotic prescribing in the two groups, and shows that the number of systemic antibiotic courses received during the year was significantly higher for PCWs, compared to matched patients. In addition, including only those patients who were prescribed antibiotics, PCWs were again shown to have received significantly more antibiotic courses compared to patients without wounds.

Table 2.2 Summary table of the parameters of antibiotic prescribing for PCW and non-wound patients.

Parameter		PCW	Pts without CWs	Statistic
Number of patients		400	1600	-
Number of antibiotic courses per patient per year	mean (SE)	2.32(0.141)	0.63(0.036)	-
	median (IQR)	1 (0-3)	0 (0-1)	*p*<0.001 (MWU)
	min-max	0-22	0-14	-
% of patients who received at least one antibiotic course		68.3	29.4	*P*<0.001 (Chi)
No. of antibiotic courses of those patients that did receive antibiotics	mean (SE)	3.39 (0.171)	2.15 (0.088)	-
	median (IQR)	3 (1-5)	2 (1-3)	*p*<0.001 (MWU)

PCW: Patients with chronic wounds; CW: Chronic wounds; SE: Standard error; IQR: Inter-quartile range; MWU: Mann-Whitney U test; Chi: Chi-squared test

PCWs were found to attend at general practice significantly more frequently than matched patients: median (inter-quartile range (IQR)) of 25 (17-40) visits during the year for PCWs compared to a median (IQR) of 12 (4-20) visits for non-wound patients ($p<0.001$). The total number of visits by the 400 PCWs was 12070; the number of attendances per year ranged from one to 73 for matched patients and from two to 140 for PCWs. Wound infection was specifically indicated by Read Codes on 258 visits by PCWs (2.14% of all visits). Overall, PCWs received systemic antibiotics in 7.01% of visits (n=846), however, wound infection was specifically indicated in only 12.29% (n=104) of these visits. It is also of interest to note that in 8.94% (n=210) of the 2348 visits classified as single-diagnosis visits, antibiotics were prescribed. However, wound infection was specified in only 15.72% (n=33) of these single-diagnosis visits that generated an antibiotic prescription.

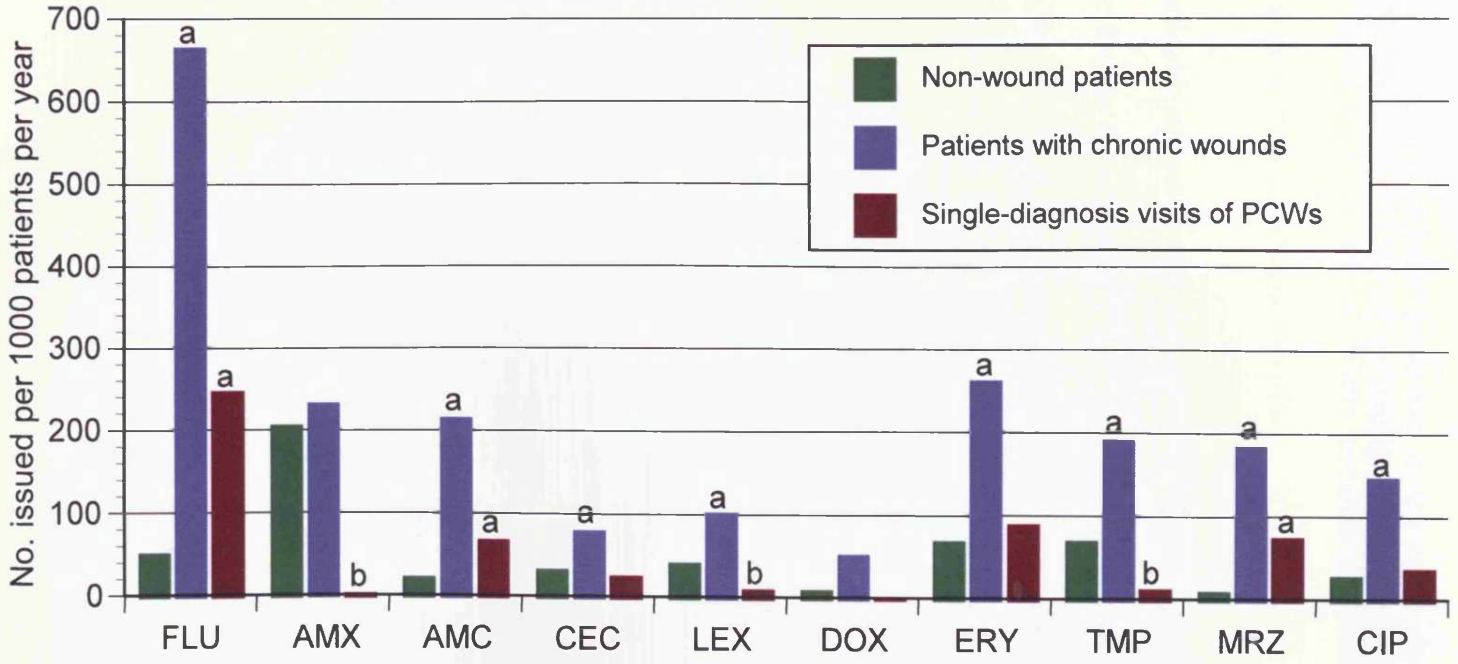
There was a significant correlation between the frequency of attendance and the number of systemic antibiotics received per year for both PCWs and matched patients ($r=0.416$, $p=0.01$ and $r=0.346$, $p=0.01$ respectively). Importantly, however, analysis of the proportion of visits on which antibiotics were received by each patient, showed that the higher frequency of attendance by PCWs did not account fully for the increased antibiotic consumption. Antibiotics were prescribed to PCWs on a median (IQR) of 4.8 (0.0-11.5) occasions per 100 visits. This was significantly higher than patients without chronic wounds who received antibiotics on a median (IQR) of 0.0 (0.0-4.3) occasions per 100 visits ($p<0.001$).

The greater prevalence of diabetes found in PCWs compared to non-wound patients was shown not to significantly affect the relationship between wound status and antibiotic consumption. Notably, exclusion of all diabetic patients from the analysis showed PCWs to still receive significantly more antibiotics than non-wound patients (median

(IQR) of 2.0 (0-4) and 0.0 (0-1) for PCWs and non-wound patients respectively, $p<0.001$). Moreover, further investigation showed there to be no significant difference in the number of antibiotics prescribed between patients with diabetic foot ulcers (DFUs) and patients with other ulcers (median (IQR) of 2 (1-3.25) and 2 (0-4) for DFU and other ulcers respectively, $p=0.121$).

A breakdown of those antibiotics prescribed in the greatest quantities in this study population is shown in Figure 2.2. The antibiotics included are those for which >50 courses per 1000 patients were prescribed in at least one patient group. A significantly greater number of prescriptions were issued to PCWs compared to non-wound patients for flucloxacillin ($p<0.001$), co-amoxiclav ($p<0.001$), cefaclor ($p=0.008$), cefalexin ($p<0.001$), erythromycin ($p<0.001$), trimethoprim ($p<0.001$), metronidazole ($p<0.001$) and ciprofloxacin ($p<0.001$). In addition to this overall greater consumption, PCWs received significantly more flucloxacillin ($p<0.001$), co-amoxiclav ($p=0.001$) and metronidazole ($p<0.001$) courses in those visits by PCWs classified as single-diagnosis visits than non-wound patients received in all visits. In single-diagnosis visits patients with chronic wounds received significantly fewer amoxicillin ($p<0.001$), cefalexin ($p=0.013$) and trimethoprim ($p<0.001$) prescriptions than non-wound patients received in all visits. Although more than 50 doxycycline prescriptions were issued per 1000 PCWs, this figure was derived from the large quantity of doxycycline issued to only four patients.

Figure 2.2 The number of antibiotics issued per 1000 patients per year for patients with chronic wounds, non-wound matched patients and in the single-diagnosis visits of 1000 patients with chronic wounds.



FLU: flucloxacillin; AMX: amoxicillin; AMC: co-amoxiclav; CEC: cefaclor; LEX: cefalexin; DOX: doxycycline; ERY: erythromycin; TMP: trimethoprim; MRZ: metronidazole; CIP: ciprofloxacin; a: significantly greater than non-wound patients, $p<0.01$; b: $p<0.05$ significantly less than non-wound patients.

The duration of antibiotic prescriptions was determined using the number of tablets or capsules as a proxy marker. The percentage of prescriptions for which the quantity of tablets or capsules was recorded was 78.9% for non-wound patients, 75.1% for PCWs and 83.1% for those antibiotics prescribed in the *single-diagnosis* visits by PCWs. Table 2.3 shows the duration of antibiotic prescriptions issued to non-wound patients and to PCWs in their *single-diagnosis* visits.

It is of particular interest to note that more than 10% of prescriptions were issued for duration ≥ 8 days for erythromycin, ciprofloxacin and co-amoxiclav. Erythromycin courses of duration ≥ 8 days accounted for 15.4% of non-wound patients', 19% of PCWs' and 27.3% of *single-diagnosis* visits' erythromycin prescriptions. While ciprofloxacin courses of duration ≥ 8 days constituted 13.2% of the ciprofloxacin prescriptions of non-wound patients compared with 30% of those of PCWs. Co-amoxiclav courses of ≥ 8 days accounted for 20.6% of co-amoxiclav prescriptions issued to PCWs and 15% of those issued on *single-diagnosis* visits, however, strikingly, they accounted for less than 4% of co-amoxiclav prescriptions to non-wound patients.

Table 2.3 Duration of antibiotic prescriptions for non-wound patients, patients with chronic wounds and the single-diagnosis visits of patients with chronic wounds.

Duration	Percentage of antibiotics *	
	Patients without chronic wounds	Single-diagnosis visits of PCW
< 5 days	3.4	0
5-7 days	89.3	88.8
≥ 8 days	7.3	11.2

* Percentage of the antibiotic prescriptions for which data on the number of tablets or capsules prescribed were available. PCW: Patients with chronic wounds

2.5 Discussion

2.5.1 Main findings

This study has demonstrated the quantity of antibiotic prescription in the primary care setting for the treatment of chronic wounds. This study has also found that patients with chronic wounds receive significantly more antibiotics than patients without chronic wounds of the same age and sex, and registered at the same general practice. This antibiotic consumption represents both an increased number of prescriptions and increased duration of antibiotic courses in patients with chronic wounds than that observed in the control population. The necessity for antibiotic use in the treatment of uninfected chronic wounds is, however, an area of debate in the literature. A limited number of studies have investigated the effectiveness and relative efficacy of antibiotic regimens,[42] but no previous study has quantified the use of antibiotics for chronic wounds in the primary care setting in the UK.

Patients with chronic wounds received significantly greater numbers of a broad range of antibiotics than did non-wound patients. The only antibiotic found to be prescribed in similar quantities to both wound and non-wound patients was amoxicillin; a popular broad-spectrum, penicillinase-sensitive antibiotic which is commonly prescribed for respiratory infections.[62] Those antibiotics which were prescribed in significantly greater quantities to patients with chronic wounds on *single-diagnosis* visits (visits by patients with chronic wounds with only treatment or diagnostic codes relating to a wound) compared to all visits by non-wound patients were flucloxacillin, co-amoxiclav and metronidazole.

The finding that amoxicillin, cefalexin and trimethoprim were all prescribed significantly less in the *single-diagnosis* visits of PCWs compared to all visits by non-wound patients is suggestive that these antibiotics were not being prescribed in large

numbers specifically for chronic wounds. However, this comparison compares a proportion of the visits made by PCW with all the visits made by patients without chronic wounds, and therefore, the difference might be expected. Verification of the extent to which these antibiotics are prescribed for chronic wounds would require the use of a methodology whereby the reason for an antibiotic prescription is stated and not implied by other factors recorded at the visit.

The possibility that the observed increase in antibiotic consumption in patients with chronic wounds, compared to non-wound patients, was simply the result of higher co-morbidity was investigated. The data, however, suggest that the presence of a chronic wound increases antibiotic consumption, irrespective of other factors, following the investigation of two potentially confounding or interacting factors: the frequency of visits and diabetes.

2.5.2 Relationship to other studies

Over the one-year period, two-thirds of patients with chronic wounds were seen to receive at least one antibiotic. The common use of antibiotics in the management of patients with chronic wounds has been demonstrated in Sweden by Tammelin *et al.*[61] Tammelin *et al.*[61] investigated antibiotics prescribed in both the primary and secondary care setting and found 60% of patients to have received antibiotics in the previous six months. Leistevuo *et al.*[174] also highlighted the level of antibiotic prescribing when they identified chronic skin ulcers as one of the seven most common reasons for antibiotic prescription in the elderly in Finland. In this study the most frequently prescribed antibiotic treatment against skin infections, of which chronic skin ulcer was the most common problem, was first-generation cephalosporins. In this study, second-generation cephalosporins were prescribed significantly more frequently for patients with chronic wounds but were not the most frequently used group of antibiotics.

Those antibiotics most frequently prescribed to patients with chronic wounds in *single-diagnosis visits* were flucloxacillin, co-amoxiclav and metronidazole. All three of these antibiotics appear appropriate for infections caused by the bacterial species most commonly isolated from chronic wounds. Flucloxacillin is a penicillinase-resistant penicillin, commonly used for the treatment of skin and soft tissue infections.[62] Co-amoxiclav, on the other hand, is a broad-spectrum β-lactamase-resistant penicillin and metronidazole is highly active against anaerobic bacteria.[62] The predominant species found in both infected and non-infected chronic wounds are staphylococcal species including both *Staphylococcus aureus* and coagulase negative staphylococci.[24] Other common aerobes found include coliform bacteria, pseudomonads and streptococci.[24] Anaerobes have also been implicated as potentially pathogenic organisms in chronic wounds and although they may be missed by routine analysis of clinical samples, they have been found in a high proportion of leg ulcers when specifically investigated.[24] The majority of antibiotic regimens used in the treatment of chronic wounds are chosen empirically,[40] and should be based on the most common pathogens and local antibiotic sensitivity data.[175] The scientific evidence supporting the use of antibiotics for chronic wounds is however limited. In 2000, O'Meara *et al.*[42] published a systematic review investigating the use of antimicrobials in leg and foot ulcers and found little evidence to support the use of systemic antimicrobials in this situation. This conclusion was reinforced by a more recent literature search.[176]

The prevalence of chronic wounds identified in this study was 0.25%. A review of the literature by Briggs and Closs[177] estimated the prevalence of open chronic leg and foot ulcers receiving treatment from health professionals to be in the range of 0.11% to 0.18%. Venous leg ulcers were estimated to contribute 40-80% of such wounds, giving an estimated prevalence of venous leg ulcers to range from 0.04% and 0.14%. In this study, the prevalence of all chronic wounds identified was 0.25% and the prevalence of

venous leg ulcers to be 0.04%. The number of venous leg ulcers identified in this study was therefore at the lower end of population estimates.

Patients in this study were frequent attenders at general practice. Data reported by the Office for National Statistics from the General Household Survey in 2000, found the average GP consultation rate to be four consultations per year: a figure which has remained fairly stable from 1972 to the latest figures in 2005.[178,179] Within the older age groups the consultation rate was higher: with an average of 5, 6, and 7 consultations per year for 45-64 year olds, 65-74 year olds and 75 years and over respectively. This survey conducted personal interviews (face-to-face, with telephone interviews where necessary) with adults aged 16 years or over, resident at addresses selected using a probability, stratified two-stage sample design. The addresses were sampled to be representative of area (based on postcode sectors) and certain indicators from the 1991 Census. In 2000, 12,393 addresses were identified (excluding ineligible addresses) and at each address the survey attempted to interview all adults and a good response rate was achieved (70%; of the 30% who were selected for interview but lost to the sample, 26% refused to participate and 4% could not be contacted).[180] While the survey methodology is extensive in selecting a sample representative of the UK population there are several reasons why the consultation rate could have been underestimated. Firstly, the survey would have included persons who were registered and persons who were not registered with a general practitioner, and therefore the attendance of persons registered with a general practitioner may be slightly higher than the general population as a whole. Secondly, consultation history was based on recall. While this is unlikely to introduce bias it may again have lead to under or over estimation of the true consultation rate. Thirdly, all reasons for refusal to participate are not given, it is suggested to range from a dislike of surveys to ill-health. Again, this could have caused the estimated consultation rate to be lower than the true consultation rate due to the lack

of participation by persons in ill-health, who would be the persons most likely to have consulted recently. Finally, the General Household Survey only interviews residents of private households and therefore does not include those living in institutions and residential homes, which again may lead to underestimating the true consultation rate. Despite the potential for slight underestimation of the population consultation rate in the General Household Survey, it is clear that the patients included in this study, both those with and without chronic wounds, consulted more frequently than older age-groups of the general population.

This study found that patients with chronic wounds made a significantly greater number of visits than non-wound patients and that frequency of visits correlated with the number of antibiotics prescribed for both patient groups. Previous research by Heywood *et al.*[181] has shown that the frequency of visits can affect the consumption of antibiotics as well as reflect chronic health problems: a finding that appears to be echoed by our results. Heywood *et al.*[181] compared frequent attenders (defined as those with 12 or more visits in one year) with non-frequent attenders (those with less than 12 visits). In our study, the mean number of visits per year for both PCWs and non-wound patients was above this arbitrary threshold of 12 visits per year. However, Heywood *et al.*[181] looked only at patients aged 20-64 years, while in this study >80% of the study population were aged 60 years or older. Furthermore, the study by Heywood *et al.* conducted in 1991/2, was based in one UK teaching general practice with a patient population of 12,400 patients, therefore it may be that this study group are not representative of the general population. Two researchers searched patient case-notes to identify consultations. Control patients, defined as those who consulted <12 times in one year, had a median of 3 visits (range 0 to 11) and were matched to patients who consulted ≥ 12 times on age and sex. Overall, the all-age doctor-patient consultation rate was 3.4 consultations per patient per year in 1991. In this study, the analysis of the

proportion of visits on which patients received antibiotics showed that while the greater consumption of antibiotics by patients with chronic wounds is partially explained by their greater number of attendances at general practice, this does not account for all of the additional antibiotic prescriptions they received.

2.5.3 Strengths and Weaknesses

A strength of this study was that it used anonymous routinely collected data from 19 general practices across Wales. The quality of the data obtained from the GPMD is however dependent on the accuracy of the data entered on to the system. Initial data collection for the GPMD is by general practitioners who record patient data onto their computer systems, this is then aggregated for all practices by Health Solutions Wales, who check the accuracy and completeness of the data collection.

The GPMD incorporated 38 practices in 2000 and covered 10% of the population of Wales, with an age and sex distribution similar to that of Wales. It is recognised however that the database may be subject to sampling error, that the quality of the data included are dependent on the quality of coding in practices and that the participating practices are not geographically representative of Wales.[182] Furthermore, previous studies that have attempted to validate the quality of prescribing data in the database from 1995-6 found only 45% of prescription items that were dispensed to be included in the database. Wide variation in the percentage of prescription items included in the database was seen (practices ranging from 0.2% to 99% of prescription items recorded in the database).[183]

In this study, using data from the year 2000, the quality of prescribing data included in the database was considered from the outset. The accuracy of the analysis was maximised by including only those practices known to have Read Coded prescribing

data as well as by matching PCWs with non-wound patients from the same general practice. Data from 19 practices were excluded from the analysis and only data from 19 practices considered by Health Solutions Wales to have comprehensive, validated and interpretable prescribing data recorded using Read Codes was analysed. However, despite these efforts it may be that antibiotic prescribing was underreported in the database and therefore antibiotic usage is underestimated, for both cases and controls, in this analysis. Furthermore, exclusion of half the practices involved in the GPMD may have compromised the representativeness of the database. It is not known how much nursing activity is recorded on to practice computer systems, however, nurses are unable to prescribe antibiotics and therefore a GP must be consulted for this event to occur. However, the reliance on routine databases is known to be potentially problematic due to diagnostic accuracy.[184]

A further strength was the study design: a case-control study. Identifying patients with chronic wounds and selecting matched patients from the same practice, age-band and sex with which to compare them allowed for any antibiotic prescribing attributable to chronic wounds to be determined over and above that prescribing likely due to morbidity associated with old-age, or physician threshold for prescribing. The source population for this case-control study was the patients attending general practices that participated in the GPMD. Cases were selected according to diagnostic and treatment Read codes. Controls were carefully selected to represent the source population, without disease (i.e. chronic wounds). Although a case-control study design was used here, together with the associated terminology indicating exposure as antibiotic consumption and cases as patients with chronic wounds, it was not the intention of the study to suggest that exposure (antibiotic consumption) leads to disease (chronic wounds) as is the aim of many case-control studies. Furthermore, in the main analyses, all antibiotic prescribing for patients during the year was included. Therefore, for cases, antibiotics

prescribed both before and after the insertion of a chronic wound Read Code were included in the analysis. A case-control design was used to enable comparison between the antibiotic consumption in patients with chronic wounds (diseased population) compared with that of patients without chronic wounds (non-diseased population).

In this study, subjects were matched at a ratio of four controls (non-wound patients) to each case (wound patient) within the parameters of age-band, sex and general practice. It has been shown that there is little gain in statistical terms from including more than four matched subjects for each case.[185] Indeed, in many cases the most efficient study design matches cases and controls on a 1:1 ratio. However, where the number of cases is limited, increasing the number of controls can increase the power of the study.[186] In this study, using an established database, no additional cost was incurred through increasing the number of control patients per case and therefore this maximum value of four control patients for each case was chosen. This method of choosing multiple control patients for cases is commonly employed in database studies.[187,188] This study was therefore of reasonable size and included 400 patients with chronic wounds and 1600 control patients. This was the maximum number of cases that it was possible to include in this study using the GPMD as all patients with chronic wounds were identified (n=455) and all those with demographic data were included (n=400). No *a priori* sample size calculations were undertaken.

The criteria for matching were chosen to enable antibiotic prescribing over and above the level that would be expected for similar patients without chronic wounds, therefore age and sex were chosen as the basic demographics on which to match. Furthermore, it is well known that general practitioners vary greatly in their propensity for prescribing antibiotics. It was not possible, with the data available, to match for individual practitioner, however it was possible to match for the general practice, thereby negating

some of the influence of individual prescribing tendencies. Furthermore, matching on general practice effectively matches for GP catchment area. Thus, some of the potential variation in prescribing due to different socio-economic factors between cases and controls may have been controlled for by selecting cases and controls from the same geographical areas.

Patients were not matched on the presence of any morbidities, including diabetes, which is a weakness of the study. Diabetes can increase the exposure opportunity (i.e. consumption of antibiotics) but is also a risk factor for disease and therefore is a potential confounding factor.[186] The effect of confounding is to alter the strength of an apparent relationship between two variables.[184] Previous studies have shown patients with diabetes to have greater risk of infection than patients without diabetes. For example, Shah and Hux[189] found the risk ratio for at least one infectious disease associated hospitalisation or physician claim to be 1.21 (95% CI 1.20 to 1.22) in patients with diabetes compared with those without, in a large retrospective case-control study using records from the universal healthcare system in Ontario, Canada. It is possible that the differences seen may not represent a true difference in infectious disease burden but reflect a difference in healthcare seeking behaviour, care-giver vigilance or lower threshold for hospital admission between diabetic and non-diabetic populations.[189]

Due to the potential confounding effect of diabetes, which was not controlled for in the study design, and the significantly greater number of cases compared to controls with diabetes, stratified analyses were undertaken. The population was stratified and only those patients without diabetes investigated. Furthermore, antibiotic prescribing in cases with diabetes was compared with cases without diabetes. Analysis excluding diabetic patients showed that wound patients still received significantly more antibiotic courses

than non-wound patients and, furthermore, wound patients with diabetes did not receive a greater number of antibiotic courses than wound patients without diabetes. Matching on diabetes at the outset of the study would have minimised the impact of diabetes as a confounder, however this may have influenced the ability to allocate suitable control patients.

A further weakness of the study was that the wound status of patients selected from the database could not be independently verified and therefore sensitivity and specificity values of the selection process could not be calculated. In theory, patients with wounds could have been missed and patients without wounds may have been falsely included as patients with chronic wounds. It was assumed that the greater risk lay in classifying patients without chronic wounds as patients with chronic wounds by one of the many acute wound codes in the database, and attempts were made to limit this through the use of only the most specific Read Codes. In contrast to the literature, the definition of a chronic wound in this analysis was therefore dependent only on the presence of particular Read Codes. Elsewhere, definitions frequently include specification of the site of the ulcer (i.e. below the knee), the duration of non-healing (i.e. one month, six weeks), and clinical indications such as outcomes of Doppler assessments.[8,10,54,190] However, the use of such definitions in the retrospective analysis of a large database was not possible. Our definition, like that used by Margolis *et al.*[11,191] to investigate the prevalence of leg ulcers and pressure ulcers using the General Practice Research Database, depended only on one visit by a patient for which a wound-related code was recorded.

Many of the chronic wounds identified in our study were of unspecified aetiology. This may reflect a lack of detailed clinical assessment in primary care. Cornwall *et al.*[192] found that clinical assessment of ulcer patients in the community was frequently

lacking, and that this led to long periods of ineffective and often inappropriate treatment. More recently, in Norway, a structured, validated questionnaire survey of community nurses in Oslo between September 1999 and March 2000 (with 59% response rate, including a total of 145 patients with wounds) found 34% of leg and foot ulcers being treated by nurses in the community to have no diagnosed aetiology, with the cause of the ulcer reported as mechanical damage or other, unknown diagnosis.[193] The authors consider this a major problem as it can lead to the ulcer being incorrectly treated. In our study, the most common classification of chronic wound was leg ulcer, followed by venous ulcer. Although the underlying cause of the majority of leg ulcers is venous disease,[177] best-practice patient care requires investigation of the causative factors.[54]

As stated above the prevalence of venous leg ulcers identified in the database was at the lower end of population estimates of chronic wound prevalence. This is consistent with arguments that some patients with venous leg ulcers, or other chronic wounds, would have been missed or could not be classified due to the reliance on a wound-associated Read Code within the time period to identify such patients.

Classification of wounds as showing clinical signs of infection in this study was problematic. Infection was specifically indicated in only a low percentage of visits, even when antibiotics were prescribed. This may, however, be indicative of recording practice and not clinical presentation.

While the use of a case-control study is cited as a strength of this study, there are several biases and design considerations in the execution of such a study. Such studies are very efficient in terms of time and cost compared to many other designs.[184] Classically, problems with case-control studies can arise because disease and exposure have already taken place and therefore study design is particularly associated with bias from

differential selection of either cases or controls on the basis of their exposure as well as from differential reporting or recording of exposure information between study groups based on disease status.[184] Therefore careful selection of cases and controls is of particular importance for accurate interpretation of study findings. In this study, cases with prevalent disease were selected from the GPMD (the target population) and all cases with demographic data included. This may have introduced prevalence bias,[184] such that patients with active disease, more chronic wounds or with wounds associated with greater morbidity were more likely to have consulted during the study period and triggered the entry of a chronic wound code on to the database. Patients with chronic wounds who were self-treating or were being treated at a referral centres during the study period would not have been identified as cases. Controls however, were not selected through the identification of morbidity and therefore were not subject to prevalence bias in the same way as cases. Therefore prevalence bias could have led to an over-estimation of the consumption of antibiotics by all patients with chronic wounds treated at general practice. To exclude prevalence bias, it would be necessary to study patients following the onset of disease. This would not however be possible using the GPMD (where onset of disease would be difficult to determine) and would entail great cost incurred by following patients prospectively.

It is unlikely that information bias will have affected the study. Data were selected automatically from the database and data entry from the general practice was done automatically for all consultations. Therefore it is unlikely that systematic bias towards identification of more antibiotics for patients with chronic wounds existed.

The selection of controls is of particular importance in case-control studies. The important consideration is whether controls should be representative of the source population from which the cases come, and be similar in all aspects apart from the

disease in question, or whether they should represent the population at whole.[194] In this study, the control patients represent the source population (patients registered at practices participating in the GPMD) rather than the population at whole. However, there is no reason to consider that control patients in this study would have had a higher or lower level of antibiotic consumption than the general population of that age and sex.

2.5.4 Implications for clinicians and for future research

Quantifying the extent to which antibiotics are used for chronic wounds in the primary care setting is an important first step in the investigation of the potential for such wounds to contribute to the prevalence of antibiotic resistance. Antibiotic usage is clearly a fundamental driver of antibiotic resistance, and many recent efforts to control antibiotic resistance have focused on reducing prescribing, particularly in the community.[195] These efforts have frequently focused on the management of respiratory tract infections which account for half of all antibiotic use in the community.[196] However, this study shows that patients with chronic wounds may represent a significant, and often overlooked, population who are being exposed to large quantities of antibiotics. Clinicians should be aware that patients with chronic wounds receive a large number of antibiotics. The necessity and effectiveness of antibiotic prescribing for chronic wounds must now be elicited. Further work is required to clearly define the role antibiotics play in wound healing, the optimal duration of treatment, and the impact these antibiotics may have on the development and prevalence of antibiotic resistance.

Chapter 3 . The prevalence of antibiotic resistant organisms in chronic wounds

3.1 Abstract

The prevalence of antibiotic resistant organisms in chronic wounds in the UK is unknown. This study looked at the prevalence of antibiotic resistance, specifically meticillin-resistant *Staphylococcus aureus* (MRSA), antibiotic-resistant *Pseudomonas aeruginosa* and vancomycin resistant enterococci in patients attending a tertiary care wound healing clinic. The aim was to determine, by identifying the prevalence of antibiotic resistance, whether resistance poses a significant barrier to effective patient care in one specialist wound healing clinic. All patients presenting with chronic wounds at the clinics over a 10-week period were eligible for inclusion. Swabs were taken from patients' wounds and patient and wound data collected. Swabs were processed specifically to investigate the presence of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and enterococci. *S. aureus* isolates were tested for sensitivity to meticillin, using the meticillin 25μg strip method. Disc diffusion methods were used to test susceptibility of *P. aeruginosa* and enterococci isolates. Microbiology swabs and data were collected from 150 patients. The mean (standard deviation) age of patients was 64.5 (15.9) years and 53% were female. Leg and foot ulcers of all aetiologies accounted for 54% and surgical wounds 24% of wounds swabbed. MRSA was present in 10% of patients; 19% of *S. aureus* isolates. Ciprofloxacin resistant *P. aeruginosa* prevalence was 11% of patients; 31% of *P. aeruginosa* isolates. Enterococci were isolated from 9% of patients and all were susceptible to vancomycin and ampicillin. Antibiotic resistant organisms are commonly isolated from chronic wounds. Furthermore, this audit has generated a baseline against which future investigations into the prevalence of resistance at the clinics can be measured.

3.2 Introduction

Antibiotic resistance is of considerable concern to our society. Since the introduction of penicillin in the 1950's bacteria have rapidly evolved mechanisms to survive in the presence of antibiotics. In the UK, today the situation exists where organisms with resistance to multiple antibiotics are ubiquitous in hospitals and bacteria resistant to all but a very limited number of antibiotics cause clinically significant infections.

Chronic wounds cause substantial morbidity; impacting on the quality of life of patients and representing a substantial burden to the healthcare system.[197] The microbiological flora which colonise these wounds is diverse, including skin commensals, *S. aureus*, *P. aeruginosa*, anaerobes and many others. Whilst organisms frequently harmlessly colonise skin, in these wounds they may also cause infection and impede healing.[24]

Antibiotic-resistant organisms are well known to colonise and infect chronic wounds. In one study, half of the *S. aureus* isolates from the skin wounds of patients attending a dermatology clinic were found to be MRSA, and a third of *P. aeruginosa* isolates found to be ciprofloxacin resistant.[139]

Strategies to limit the spread of resistant organisms have been many and diverse. They have ranged from the publication of the SMAC report, through to infection control measures and targeted strategies aimed at behaviour change in relation to the prescription of antibiotics.[196,198,199] MRSA screening of patients that attend at hospital (particularly those at high-risk) is common in many NHS Trusts.

Many of the features of chronic skin wounds and the patients themselves indicate such wounds might be associated with high prevalence of antibiotic resistant organisms. Chronic skin wounds such as leg ulcers and arterial ulcers are more prevalent in the elderly (>65 years old). This aged population are also most likely to use hospitals, be

resident in nursing or residential care facilities and receive antibiotic treatment; all of which are important risk factors for the carriage of antibiotic resistant organisms.[100,105] Moreover, these patients with chronic wounds also receive significantly more antibiotics than patients of the same age and sex attending the same general practice (see Chapter 2).

There are many difficulties in the treatment of infections in chronic skin wounds. Identification of infection is hindered by the frequent absence of clinical symptoms of the acute inflammatory response. Clinicians instead rely on signs and symptoms of infection present in wounds, but these symptoms have been shown to have limited specificity and sensitivity.[37] Laboratory isolation of micro-organisms from a wound swab cannot be used to indicate the presence of infection due to the frequent colonisation of these wounds. However Robson *et al.*[39] suggest that the density of bacteria ($>10^5$ bacteria per gram of tissue) to be an indicator of infection.

Current recommendations state that infection in leg ulcers and other chronic wounds should be treated empirically. For example, Clinical Knowledge Summaries (CKS) recommended flucloxacillin as first-line treatment for venous leg ulcers.[58] Importantly, empirical treatment however requires the likely infecting organisms to be predictable and the likely antibiotic susceptibilities known.

Staphylococcus aureus is the most frequently isolated organism from infected chronic wounds.[24-26] MRSA has an "anti-biogram" that includes antibiotics from the penicillin group, including those that are tolerant to penicillinase. Flucloxacillin is not, therefore, an effective treatment against MRSA, although it is the recommended treatment for staphylococcal skin and soft tissue infections.[62]

In this Chapter the results of an audit undertaken to determine whether antibiotic resistant organisms pose a significant barrier in the effective treatment of chronic wounds, through evaluation of the likely effectiveness of antibiotic treatment protocols and the prevalence of antibiotic-resistant organisms in patients with non-healing skin wounds in a UK specialist clinic are reported. Infections are, for the most part, treated empirically, and therefore little information was available on the prevalence of antibiotic resistance in the specific patient population. Particular concern was present over the level of ciprofloxacin resistance in *Pseudomonas aeruginosa* and meticillin resistant *Staphylococcus aureus* (MRSA). The treatment of resistant-organisms with an ineffective antibiotic is likely to be of no value to the patient, and potentially harmful through delaying effective treatment.

3.3 Methods

3.3.1 Study Population

This work was undertaken as part of an audit at a specialist wound-healing clinic in Cardiff. The wound-healing clinic offers a secondary care service for patients with non-healing wounds. Patients are referred for diagnosis, assessment and treatment of their wound.[200] The majority of patients attend from the local area however some patients with complicated morbidities are tertiary referrals from other parts of Wales and the UK. Twice-weekly outpatient clinics were included in the audit. These were general sessions at which non-healing wounds from a diverse array of conditions were managed (the majority were however leg ulcers or surgical wounds).

The audit was undertaken over a period of 10 weeks from the week beginning 2nd May 2005 to the week beginning 4th July 2005. All patients attending the clinics with a chronic wound were eligible for inclusion in the audit (each patient could be included

only once). The inclusion criterion stated "All chronic wounds e.g. leg ulcers, diabetic foot ulcer, long-term skin wounds."

3.3.2 Ethics

This study was conducted as part of the routine audit process of the Wound Healing Research Unit. Prior to initiating the audit, the proposal was presented to the Wound Healing Audit Group on 22nd March 2005 and discussed by the multi-disciplinary forum that included doctors, nurses and podiatrists. This work was deemed not to require ethical approval by the Audit Group (Appendix 3.1). Under current guidelines, audits of service provision "designed and conducted to provide new knowledge to provide best care" do not require REC approval.[201] The definition of clinical audit stated by National Institute for Clinical Excellence[202] and contributed to by members of the Commission for Health Improvement, Royal College of Nursing and University of Leicester is:

> *"Clinical audit is a quality improvement process that seeks to improve patient care and outcomes through systematic review of care against explicit criteria and the implementation of change...*

The results of the audit provided the first baseline data for the prevalence of resistance in the patients attending the clinic and generated valuable (previously) undetermined information on the antibiotic histories of these patients. Data on antibiotic histories were obtained from both the hospital and general practice. While it would have been possible to have requested this information from the patients' GPs as part of routine care, the Clinical Director considered, as good practice, that consent should be sought from patients to ensure clarity in the purpose of collecting this information. The results were presented to the same group on 13th September 2005 and Unit practice was changed as a result of the audit (Appendix 3.1). The study raised awareness and discussion about the

prevalence and impact of antibiotic resistance in patients being treated at the unit. The expectation had been that MRSA prevalence in this population would be higher than discovered. It was not anticipated however that one-third of *P. aeruginosa* isolates would be resistant to ciprofloxacin. In addition, several specific cases of non-healing patients that had been receiving long-term or recurrent antibiotic treatment were reviewed in light of the antibiotic susceptibilities and management changed. The study therefore clearly informed the audit loop and changes were implemented at the Unit. The definition of audit includes the implementation of change:

> *... changes are implemented at an individual, team, or service level, and further monitoring is used to confirm improvement in healthcare delivery."*[202]

3.3.3 Data Collection

At each clinic included in the audit, a researcher (RH-J) attended the clinic to co-ordinate the collection of microbiology swabs and the patient and wound data. The specialist wound care nurses at the clinics enrolled patients and obtained informed consent. The data from GP's could have been requested as part of the routine management of patients, however it was considered to be good practice by the Clincial Director of Wound Healing to gain consent from patients to ensure clarity of the purpose of the information (Appendix 3.1).

3.3.3.1 Swab collection

Microbiology swabs were taken by nurses using cotton-tipped swabs with Aimes transport medium and charcoal. Due to the needs of the microbiology laboratory the nurses swabbed the whole area of the wound and rotated the swab. If more than one

wound was present the largest wound was swabbed. Swabs were transported to the laboratory and processed on the same day (by RH-J).[17]

3.3.3.2 Patient and wound data collection

Data were collected from four sources, as no individual source was considered sufficiently comprehensive. Unless otherwise specified, data were entered directly from the data source onto an SPSS Version 11 data sheet.

Patient questions

At the time of swab collection, the nurses asked each patient four multiple-choice questions. The nurse completed a paper copy of the questionnaire (Appendix 3.2). Data from the questionnaires were inputted into the SPSS data sheet.

Nursing notes

Each patient attending at the wound clinics has a set of nursing notes in which nurses record patient details, details from the initial patient assessment, and clinical details of each visit. These data are routinely collected on every patient on a standard Departmental form. The researcher searched these notes and extracted the relevant clinical information. This information included:

- Patient demographics: age and sex
- Wound history: number, duration, recurrence, date of first attendance at wound clinic, number of visits to the clinic in previous year, ABPI
- General medical history: related pathologies, medication, nutritional status, smoking status

- Clinical details of the wound: condition of wound bed, edge and surrounding skin, level of exudate, pain frequency and severity, presence of odour, wound size, wound size at previous visit and the presence of undermining
- Treatment details: dressings and other treatments undertaken

Hospital notes

Data were extracted from patients' hospital notes. The search was limited to hospital notes from the University of Wales Hospital and for information regarding antibiotic usage and overnight hospital stays.

General Practitioner's letters

Primary care information was obtained from GPs in relation to risk factors for antibiotic resistance. Patients' GPs were invited to participate by letter and were sent a data collection form (see Appendix 3.3 for an example form) to provide data on antibiotic usage, overnight hospital stays and co-morbidities.

GPs were paid a small sum on return of this information for the time taken to collect the data. Non-responders were sent one follow-up letter, 16 weeks later, in November 2005.

3.3.4 Data analysis

3.3.4.1 Microbiology Analysis

Microbiological analysis of the wound swabs was undertaken at the Cardiff Dental School Microbiology Laboratory (by RH-J). Swabs were processed as soon as possible on the day of collection and plated out onto four different (selective and non-selective) agars. These included blood agar (LabM, Bury, UK; LabM 15; BA) and the following three selective media; Mannitol-Salt agar (Oxoid, Basingstoke, UK; CM0085; MS, for

isolation of *S. aureus*), *Pseudomonas* agar (LabM, 108) supplemented with 200 mg l^{-1} cetrimide and 15 mg l^{-1} nalidixic acid, (PS, for isolation of *P. aeruginosa*) and Bile-Aesculin agar (Oxoid, CM0888) supplemented with 6 μg ml^{-1} vancomycin (BAV, for isolation of vancomycin resistant enterococci). To minimise the numbers of plates used, swabs were inoculated onto an eighth of the plate and then using a sterile loop, samples were streaked for single colonies. All plates were incubated aerobically for 48 h at 37°C.

Mannitol-salt agar is selective for staphylococcal species due to its high salt concentration, with the majority of *S. aureus* isolates able to ferment mannitol and therefore producing distinctive yellow colonies. Following incubation, colonies of staphylococcal appearance (typically large, entire, white colonies on blood agar or large, entire, yellow colonies on mannitol-salt agar) were confirmed as *S. aureus* by Gram-stain and use of a commercially available coagulase kit, the Staphylase Test Kit, (Oxoid, DR0595). Coagulase-positive isolates were tested for susceptibility to meticillin using the meticillin strip-test (Mast Diagnostics, Merseyside, UK). The MRSA control strain used in the strip-test was *S. aureus* NCTC 12493. Coagulase positive, meticillin resistant isolates were then tested for the presence of the *mecA* gene by PCR (see Footnote).

Pseudomonas isolates were selected by their ability to grow on the specialist *Pseudomonas* agar with *P. aeruginosa* isolates exhibiting a characteristic pyocyanin and fluorescein pigment giving them a green and/or metallic appearance on this media. *P. aeruginosa* isolates were confirmed by Gram-stain and subculture on the same media and tested for susceptibility to colistin sulphate 25μg, amikacin 30μg, tobramycin 10μg, ceftazidime 30μg, imipenem 10μg, ciprofloxacin 1μg and piperacillin 75μg discs using a disc diffusion assay on isosensitest agar plates following British Society for

Antimicrobial Chemotherapy (BSAC) guidelines for antimicrobial susceptibility testing. The control strain against which the effectiveness of the discs was tested was *P. aeruginosa* NCTC 10662. Inhibition zones were measured according to standard measures.[203]

Enterococcal isolates were identified by their growth on BAV plates and characteristic intense brown pigmentation on this medium. Lancefield serotyping of streptococcal groups was done using a commercial Streptococcal Grouping Kit (Oxoid, DR0585A). All isolates confirmed as group D streptococci (and therefore established as being enterococci) were tested for susceptibility to ampicillin 10μg and vancomycin 5μg using the disc diffusion assay as described above (BSAC). The clinical isolate V60 was used as a VRE control strain to confirm the findings of the susceptibility testing.

3.3.4.2 Antibiotic history

The range and quantity of antibiotics received by the patients included in the audit was considerable. Systemic antibiotics received from the wound clinic included flucloxacillin, penicillin, co-amoxiclav, cefalexin, minocycline, clarithromycin, erythromycin, trimethoprim and metronidazole, while those received from elsewhere included cefuroxime, doxycycline, gentamicin and vancomycin amongst others. Patients ranged from receiving no antibiotics to receiving eight different antibiotics, while other patients received the same two antibiotics continuously for long periods of time, including up to one year. Therefore following crude assessment of the range and quantity of antibiotics that were received and in consultation with an experienced Consultant in Medical Microbiology (Dr Robin Howe, University of Wales Hospital), the systemic antibiotics were grouped to balance "like with like" and sufficient frequency.

The groups decided upon for the systemic antibiotics were flucloxacillin, cephalosporins and beta-lactamase resistant penicillins, other penicillins, macrolides, ciprofloxacin, clindamycin, metronidazole (systemic) and other systemic antibiotics. While the topical antimicrobial groups were iodine, silver, topical metronidazole and 'other'.

3.3.4.3 Statistical Analysis

A descriptive analysis of the data has been presented. The outcome from the descriptive analysis will be used to build a model of the factors that influence the carriage of antibiotic resistant organisms (Chapter 4). Statistical tests have not been performed on the data at this stage to minimise the number of tests and the finding of spurious significant statistics by chance.

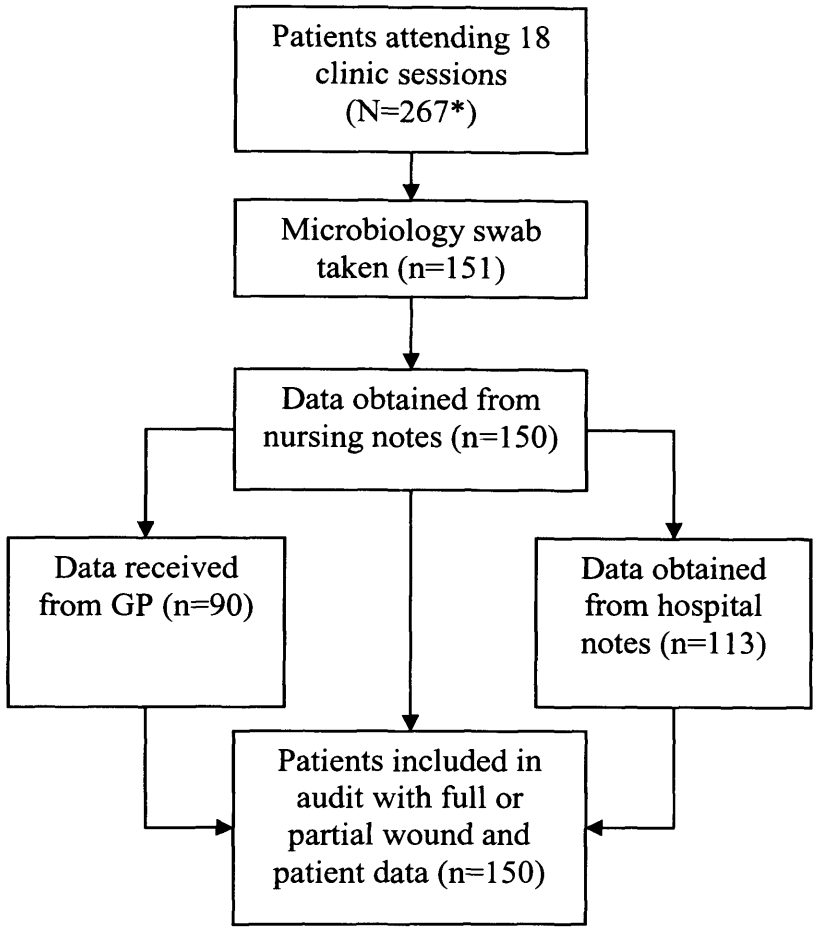
3.3.4.4 Missing data

Variables determined from one data source were classified as missing if the data source was not available for that patient or the data item was missing. For a few variables data were contributed from more than one data source. This was the case for overnight hospital stays and antibiotic usage where data were, for the most part, obtained from GP letters or hospital records. Such variables were defined as missing if data were not available from either the hospital notes or the GP letters (i.e. where data were available from one of these sources only, it was not defined as missing). Hospital and antibiotic usage data were occasionally, but not routinely, recorded in the nursing notes. When available this data were used to supplement the hospital and antibiotic variables. The impact of the available data sources on estimated antibiotic consumption was explored.

3.4 Results

Over the audit period of 10 weeks, 151 patients were included in the audit and had a microbiology swab sample taken from their wound. One patient was excluded from the data summary and analysis due to the quantity of missing data: the nursing notes for this patient were not available and therefore basic data on the patient and the wound were unknown. Data were obtained from GPs and hospital records for 90 patients, from hospital records alone for 38 patients and from neither GP nor hospital records for 12 patients. Figure 3.1 shows the flow of patients included in the study.

Figure 3.1 Flow chart of patients included in the audit



* This is estimated from the recruitment of the first 122 patients included in the audit, from N=216. The same recruitment rate (56.5%) is assumed for the subsequent 29 patients.

3.4.1 Wound classification

Patients included in the audit presented with chronic wounds from a diverse range of aetiologies. Table 3.1 shows the distribution of the wounds, by wound classification and underlying aetiology. Wound classification has been used to give a broad indication of ulcer type (leg ulcer, foot ulcer, surgical wound or miscellaneous) while underlying aetiology indicates the causative pathology for the wound (where this was known). Also shown in Table 3.1 is the distribution amongst the wound classifications of *S. aureus* and *P. aeruginosa*, and the antibiotic resistant isolates. The majority of patients colonised or infected with *P. aeruginosa* (n=47) had leg or foot ulcers (n=42,

82.4%). MRSA was isolated from all wound classifications: five leg ulcer, four foot ulcer, five surgical and one miscellaneous wound patients.

Due to the divergent and often disparate nature of the wounds included in the audit, the summary characteristics of the patients and wounds are presented grouped by wound classification.

3.4.2 Wound Microbiology

S. aureus was present in 52.7% (n=79) of the chronic wounds. Fifteen isolates were confirmed to be MRSA, identified by both meticillin strip-test and the presence of *mec*A gene (10.0% of patients, 19.0% of *S. aureus* isolates). *P. aeruginosa* was isolated from 47 patients (31.3%): 16 isolates were found to be ciprofloxacin resistant (10.7% of patients, 34.0% of *P. aeruginosa* isolates) and 8 were imipenem resistant (5.3% of patients, 17% of *P. aeruginosa* isolates). No isolates were resistant to both ciprofloxacin and imipenem. Figure 3.2 shows the percentage of isolates that were resistant to the antibiotics tested. Only one patient was found to have *P. aeruginosa* sensitive to imipenem and ciprofloxacin but resistant to another antibiotic tested (piperacillin). In total, five *P. aeruginosa* isolates (3.3%) were resistant to more than one antibiotic: two isolates were resistant to ciprofloxacin and piperacillin; one was resistant to ciprofloxacin and ceftazidime; one was resistant to ciprofloxacin, tobramycin and amikacin; and one was resistant to ciprofloxacin, piperacillin, tobramycin and colistin sulphate. Enterococci were isolated from 13 patients (8.7%) and none of these were found to be resistant to ampicillin or vancomycin.

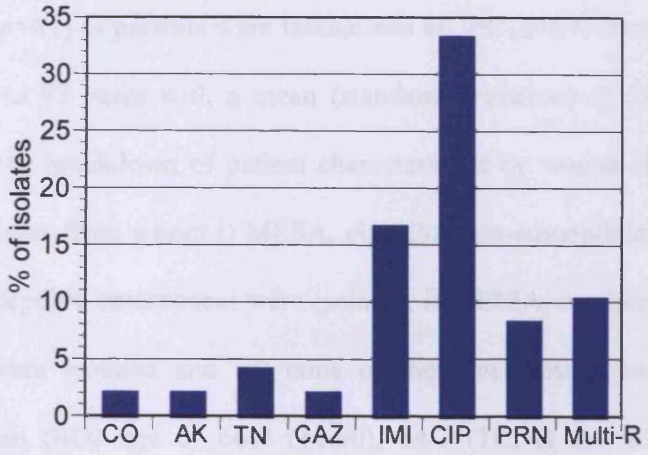
Table 3.1 Classification and aetiology of wounds included in the audit and the occurrence of *S. aureus*, MRSA, *P. aeruginosa* and ciprofloxacin resistant *P. aeruginosa*

Wound classification	Wound aetiology	Number of patients (% of classification) [% of total]	Number (%) of patients organism isolated from			
			S. aureus	MRSA (% of *S. aureus*)	*P. aeruginosa*	Ciprofloxacin resistant *P. aeruginosa* (% of *P. aeruginosa*)
Leg ulcer	Venous	35 (45.5) [23.3]	22 (62.9)	3 (13.6)	11 (31.4)	4 (36.4)
	Arterial	6 (7.8) [4.0]	5 (83.3)	1 (20.0)	1 (16.7)	0 (0)
	Mixed	16 (20.8) [10.7]	11 (68.8)	0 (0)	10 (62.5)	3 (30)
	Vasculitic/Rheumatoid	5 (6.5) [3.3]	2 (40.0)	0 (0)	3 (60.0)	1 (33.3)
	Traumatic	4 (5.2) [2.7]	3 (75.0)	1 (33.3)	0 (0)	0 (0)
	Non-specified	8 (10.4) [5.3]	6 (75.0)	0 (0)	6 (75.0)	2 (33.3)
	Other (burn, eczema, cellulitis)	3 (3.9) [2.0]	0 (0)	0 (0)	2 (66.7)	1 (50)
	Total	77 (100.0) [51.3]	49 (63.6)	5 (10.2)	33 (42.9)	11 (33.3)
Foot ulcer	Arterial	2 (10.0) [1.3]	0 (0)	0 (0)	0 (0)	0 (0)
	Vasculitic/Rheumatoid	3 (15.0) [2.0]	0 (0)	0 (0)	1 (33.3)	1 (100)
	Non-specified	2 (10.0) [1.3]	0 (0)	0 (0)	2 (100)	0 (0)
	Neuropathic	5 (25.0) [3.3]	2 (40.0)	1 (50.0)	3 (60.0)	3 (100)
	Neuro-ischaemic	1 (5.0) [0.7]	0 (0)	0 (0)	0 (0)	0 (0)
	Pressure	4 (20.0) [2.7]	4 (100)	3 (75.0)	2 (50.0)	0 (0)
	Diabetic	2 (10.0) [1.3]	2 (100)	0 (0)	0 (0)	0 (0)
	Calciphyllaxis	1 (5.0) [0.7]	0 (0)	0 (0)	1 (100)	0 (0)
	Total	20 (100.0) [13.3]	8 (40.0)	4 (50.0)	9 (45.0)	4 (44.4)

Table 3.1. continued.

Wound classification	Wound aetiology	Number of patients (% of classification) [% of total]	Number (%) of patients organism isolated from			
			S. aureus	MRSA (% of *S. aureus*)	*P. aeruginosa*	Ciprofloxacin resistant *P. aeruginosa* (% of *P. aeruginosa*)
Surgical wound	Debridement	1 (2.4) [0.7]	0 (0)	0 (0)	0 (0)	0 (0)
	Amputation	1 (2.4) [0.7]	0 (0)	0 (0)	0 (0)	0 (0)
	Pilonidal sinus	3 (7.1) [2.0]	0 (0)	0 (0)	0 (0)	0 (0)
	Hidradenitis	5 (11.9) [3.3]	3 (60.0)	1 (33.3)	0 (0)	0 (0)
	Reconstruction/graft	2 (4.8) [1.3]	2 (100)	1 (50.0)	0 (0)	0 (0)
	Obstetrics/gynaecology	2 (4.8) [1.3]	0 (0)	0 (0)	0 (0)	0 (0)
	Breast	2 (4.8) [1.3]	0 (0)	0 (0)	0 (0)	0 (0)
	Gastrointestinal	12 (28.6) [8.0]	7 (58.3)	2 (28.6)	0 (0)	0 (0)
	Orthopaedic	5 (11.9) [3.3]	1 (20.0)	0 (0)	0 (0)	0 (0)
	Vascular/cardiac	3 (7.1) [2.0]	1 (33.3)	1 (100)	1 (33.3)	0 (0)
	Neurological	1 (2.4) [0.7]	0 (0)	0 (0)	0 (0)	0 (0)
	Abscess	4 (9.5) [2.7]	1 (25.0)	0 (0)	0 (0)	0 (0)
	Renal/Nephrology	1 (2.4) [0.7]	0 (0)	0 (0)	1 (100)	0 (0)
	Total	42 (100.0) [28.0]	15 (35.7)	5 (33.3)	2 (4.8)	0 (0)
Miscellaneous	Abrasive trauma	1 (9.1) [0.7]	1 (100)	0 (0)	0 (0)	0 (0)
	Pressure ulcer	2 (18.2) [1.3]	1 (50.0)	0 (0)	0 (0)	0 (0)
	Penetrating trauma	1 (9.1) [0.7]	1 (100)	0 (0)	1 (100)	0 (0)
	Malignancy	5 (45.5) [3.3]	2 (40.0)	0 (0)	2 (40.0)	1 (50.0)
	Ingrowing toe nail	1 (9.1) [0.7]	1 (100)	1 (100)	0 (0)	0 (0)
	Perineal sinus	1 (9.1) [0.7]	1 (100)	0 (0)	0 (0)	0 (0)
	Total	11 (100.0) [7.3]	7 (63.6)	1 (14.3)	3 (27.3)	1 (33.3)

Figure 3.2 Percentage of *P. aeruginosa* isolates with resistance to the tested antibiotics



CO: Colistin Sulphate, AK: Amikacin; TN: Tobramycin; CAZ: Ceftazidime; IMI: Imipenem; CIP: Ciprofloxacin; PRN: Piperacillin; Multi-R: Multiple resistances

Table 3.2 summarises the number of patients from which *S. aureus*, *P. aeruginosa* and enterococci were identified and the number of patients from which more than one of these organisms were isolated. All three organisms (*S. aureus*, *P. aeruginosa* and enterococci) were isolated from four patients (two leg ulcers and two foot ulcers).

Table 3.2 Identification of *S. aureus*, *P. aeruginosa* and enterococci by wound classification.

Organisms isolated*			Number (%) of patients				
S. aureus	*P. aeruginosa*	Enterococci	Leg ulcer (n=77)	Foot ulcer (n=20)	Surgical wound (n=42)	Miscellaneous wound (n=11)	Total number (%) of patents (n=150)
N	N	N	14 (18.2)	6 (30.0)	24 (57.1)	2 (18.2)	46 (30.7)
Y			29 (37.7)	3 (15.0)	15 (35.7)	5 (45.5)	52 (34.7)
	Y		11 (14.3)	6 (30.0)	2 (4.8)	1 (9.1)	20 (13.3)
		Y	0 (0.0)	0 (0.0)	1 (2.4)	0 (0.0)	1 (0.7)
Y	Y		17 (22.1)	1 (5.0)	0 (0.0)	1 (9.1)	19 (12.7)
Y		Y	1 (1.3)	2 (10.0)	0 (0.0)	1 (9.1)	4 (2.7)
	Y	Y	3 (3.9)	0 (0.0)	0 (0.0)	1 (9.1)	4 (2.7)
Y	Y	Y	2 (2.6)	2 (10.0)	0 (0.0)	0 (0.0)	4 (2.7)

*Y = Yes; N = No

3.4.3 Patient demographics

Overall, 54.0% (n=81) of patients were female and 46.0% (n=69) were male and ranged in age from 24 to 93 years with a mean (standard deviation) of 64.3 (15.96) years. Table 3.3 gives the breakdown of patient characteristics by wound classification. The mean age of patients from whom i) MSSA, ciprofloxacin-susceptible *P. aeruginosa* or vancomycin-susceptible enterococci were isolated, ii) MRSA, or ciprofloxacin-resistant *P. aeruginosa* were isolated and iii) none of these organisms were isolated, were comparable (mean (SD) age of 66.0 (14.60), 64.7 (18.54) and 61.5 (16.22) years respectively). *S. aureus* was isolated from 53.6% of males (n=37) and 56.8% of females (n=46) and these were identified as MRSA in seven males and eight females (18.9% and 17.4% of *S. aureus* isolates respectively). *P. aeruginosa* was isolated from 33.3% of males (n=23) and 29.6% of females (n=24) and found to be ciprofloxacin resistant in seven males and nine females (10.1% and 11.1% of *P. aeruginosa* isolates respectively).

Table 3.3 Patient demographics by wound classification

		Number (%) of patients				Total number (%) of patients (n=150)
		Leg ulcer (n=77)	Foot ulcer (n=20)	Surgical wound (n=42)	Miscellan-eous (n=11)	
Age (years)	Mean (SD)	69.1 (14.0)	63.8 (17.1)	56.2 (15.9)	63.3 (15.9)	64.4 (15.9)
	Range (min, max)	57 (36, 93)	52 (34, 86)	61 (24, 85)	50 (37, 87)	69 (24, 93)
Sex	Female (%)	45 (58.4)	8 (40.0)	24 (57.1)	4 (36.4)	81 (54.0)
	Male (%)	32 (41.6)	12 (60.0)	18 (42.9)	7 (63.6)	69 (46.0)

3.4.4 Wound Measurements

3.4.4.1 Number of wounds

The number of wounds per patient ranged from one to eight, with a mean (SD) of 1.72 (1.09) wounds. Eighty-one patients (54.0%) had a single wound and 62 patients (41.3%) had multiple wounds. Data on the number of wounds was unavailable for seven patients (4.7%). *S. aureus*, *P. aeruginosa* or enterococci were isolated from

74.2% (n=46) of patients with multiple wounds compared with 66.7% (n=54) of patients with single wounds. Furthermore, in 34.8% (n=16) of these patients with multiple wounds and 24.1% (n=13) of these patients with single wounds the organism was found to be either MRSA or ciprofloxacin-resistant *P. aeruginosa.* The number of wounds per patient separated by wound classification is shown in Table 3.4.

Table 3.4 Wound characteristics (including the number of wounds, wound duration and recurrence) by wound classification

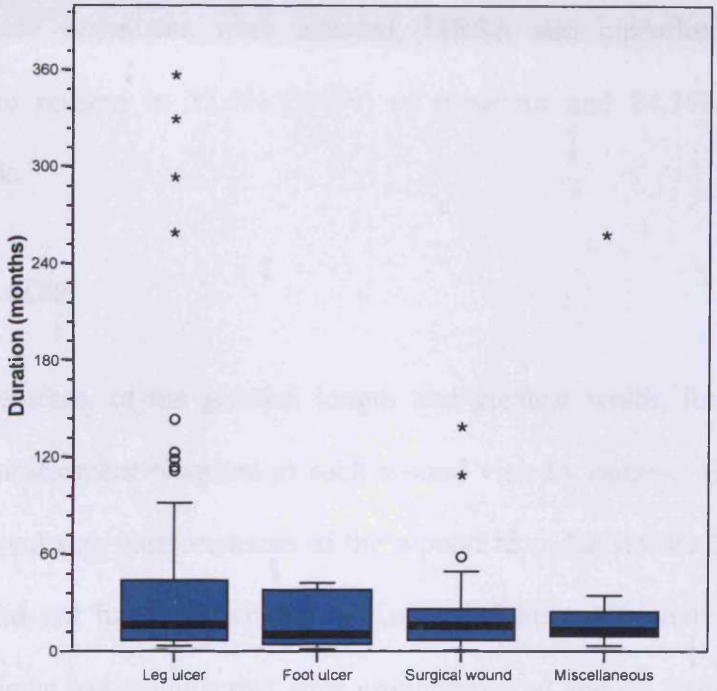
Wound characteristic	Number (%) of patients				Total number (%) of patients (n=150)
	Leg ulcer (n=77)	Foot ulcer (n=20)	Surgical wound (n=42)	Miscellaneous (n=11)	
Number of wounds					
Mean (SD)	1.71 (0.95)	2.22 (1.06)	1.56 (1.25)	1.55 (1.29)	1.72 (1.09)
1	39 (50.6)	4 (20.0)	29 (69.0)	9 (81.8)	81 (54.0)
2	21 (27.3)	9 (45.0)	6 (14.3)	0 (0.0)	36 (24.0)
3	10 (13.0)	3 (15.0)	5 (11.9)	1 (9.1)	19 (12.7)
>=4	3 (3.9)	2 (10.0)	1 (2.4)	1 (9.1)	7 (4.7)
Missing data	4 (5.2)	2 (10.0)	1 (2.4)	0 (0.0)	7 (4.7)
Duration (months)					
Median (IQR)	16.1 (37.5)	10.1 (33.8)	15.5 (21.5)	12.4 (17.1)	15.8 (28.4)
≤ 3months	1 (1.3)	1 (5.0)	5 (11.9)	1 (9.1)	8 (5.3)
> 3 to 6months	15 (19.5)	5 (25.0)	4 (9.5)	1 (9.1)	25 (16.7)
> 6 to 12 months	10 (13.0)	2 (10.0)	8 (19.0)	2 (18.2)	22 (14.7)
> 12 months	42 (54.5)	6 (30.0)	22 (52.4)	7 (63.6)	77 (51.3)
Missing data	9 (11.7)	6 (30.0)	3 (7.1)	0 (0.0)	18 (12.0)
Recurrence					
Yes	35 (45.5)	8 (40.0)	8 (19.0)	5 (45.5)	56 (37.3)
No	33 (42.9)	8 (40.0)	32 (76.2)	6 (54.5)	79 (52.7)
Missing data	9 (11.7)	4 (20.0)	2 (4.8)	0 (0.0)	15 (10.0)

3.4.4.2 Wound Duration

The duration of the wounds swabbed varied greatly, ranging from 15 days to 29 years. The median (IQR) duration for all wounds was 15.8 (28.4) months. Figure 3.3 illustrates the range and extremes in duration of the different wound types, while Table 3.4 presents duration as a categorical variable (less than or equal to 3 months, >3 to 6 months, >6 to 12 months and greater than 12 months). It can be seen that more than 50% of leg ulcers, surgical wounds or miscellaneous wounds had been present for more

than one year. Wound duration could not be determined for 18 patients. Although 6 of these patients were known to have had wounds for more than four years, it was not possible to establish the duration of the wound that was swabbed.

Figure 3.3 Box plot showing range of duration of wounds by wound classification



S. aureus, *P. aeruginosa* or enterococci were isolated from 66.7% (n=22) of the wounds of duration ≤ 6 months and from 68.7% (n=68) of the wounds of duration >6 months. Duration was known for 25 of the 30 wounds with MRSA or ciprofloxacin resistant *P. aeruginosa*. Twenty (80%) of these wounds had duration ≤ 6 months, compared to 48 (73.8%) of the susceptible equivalents.

3.4.4.3 Wound recurrence

Overall, 56 patients (37.3%) were known to have wounds that were recurrent, while 79 patients (52.7%) were thought to be on the first episode of the wound (although this first-episode may have been of extended duration). Table 3.4 shows wound recurrence

by wound classification. As might be expected, the percentage of recurrent wounds is greatest for leg and foot ulcers (45.5% and 40.0% respectively). Information on wound recurrence was not available from the nursing notes for 16 patients (10.6%). The frequency of growth of *S. aureus*, *P. aeruginosa* or enterococci from recurrent and non-recurrent wounds were 76.8% (n=43) and 62.0% (n=49) respectively. In those wounds from which these organisms were isolated, MRSA and ciprofloxacin-resistant *P. aeruginosa* were present in 32.6% (n=14) of recurrent and 24.5% (n=12) of non-recurrent wounds.

3.4.4.4 Wound area

Wound measurements, of the greatest length and greatest width, formed part of the routine wound assessment recorded at each wound visit by nurses. One hundred and eleven patients had size measurements of the wound recorded on the date of the swab. Nine patients did not have the wound size recorded but additional information was given: three patients had wounds that were circumferential and six patients had wounds that consisted of multiple areas. No data or information was recorded for 30 patients. Wound area was calculated by multiplying the maximum length by the maximum width.

The median (IQR) wound areas for leg ulcers, foot ulcers, surgical wounds and miscellaneous wounds were 10.9 (40.6) cm^2, 3.6 (18.8) cm^2, 3.4 (212.5) cm^2 and 1.8 (57.3) cm^2 respectively.

Larger wounds were associated with the growth of *S. aureus*, *P. aeruginosa* or enterococci. Including only those wounds with known measurements, 57.9% (n=44) of those containing any of the above organisms measured >10cm^2, compared with 8.6% (n=3) of those wounds not containing these organisms. There was substantial missing

data on the size of wounds from which MRSA or ciprofloxacin resistant *P. aeruginosa* were isolated (missing data for 8 wounds, known to be circumferential for 2 wounds). In those wounds for which data were present, 70% (n=14) of wounds from which MRSA or ciprofloxacin resistant *P. aeruginosa* were isolated were >10cm^2 compared with 51.7% (n=30) of wounds from which MSSA and ciprofloxacin-susceptible *P. aeruginosa* were isolated.

3.4.5 Wound observations

The condition of the wound itself and the surrounding skin was recorded at each visit in the nursing notes, by the nurses. Three variables were recorded: wound bed, wound edge and surrounding skin using coded descriptors (Appendix 3.4). Data on all three variables were recorded for 127 patients; 15 patients did not have any of these variables recorded, and the remaining 8 patients had partial data recorded. Appendix 3.5 shows the frequency of all descriptors used to describe the wound bed, wound edge and surrounding skin variables, separated by wound classification, while Table 3.5 presents an abridged version of the main factors. The descriptors were not mutually exclusive and any number could be used to describe a wound. Table 3.5 also shows the number of patients classified as having a wound infection. Infection was considered present if cellulitis or evidence of infection was recorded or if the patient had been prescribed a systemic antibiotic for the wound by the wound clinic on the date of the swab.

Overall, 100 (75.8%) wounds had granulation tissue recorded as present: granulating was the most frequently used descriptor of the wound bed in all wound classifications. Over 50% of all wounds had slough on the wound bed, however, this proportion varied greatly by wound classification: 72.5% (n=50) and 52.9% (n=9) of leg and foot ulcers respectively were described as sloughy compared with 16.2% (n=6) of surgical wounds.

Table 3.5 Abridged table of the terms used to describe the bed, edge and surrounding skin of wounds included in the audit

Wound characteristic	Number (%) of patients				Total number of patients (%)
	Leg ulcer	Foot ulcer	Surgical wound	Miscellaneous	
Wound Bed	n=69	n=17	n=37	n=9	n=132
Granulating	56 (81.2)	14 (82.4)	28 (75.7)	2 (22.2)	100 (75.8)
Slough	50 (72.5)	9 (52.9)	6 (16.2)	3 (33.3)	68 (51.5)
Wound Edge	n=68	n=17	n=36	n=10	n=131
Static	47 (69.1)	7 (41.2)	18 (50.0)	9 (90.0)	81 (61.8)
Epithelialising	21 (30.9)	8 (47.1)	15 (41.7)	0 (0.0)	44 (33.6)
Surrounding Skin	n=69	n=17	n=36	n=11	n=133
Erythema	54 (78.3)	11 (64.7)	18 (50.0)	4 (36.4)	87 (65.4)
Dry/flaky	48 (69.6)	9 (52.9)	7 (19.4)	5 (45.5)	69 (51.9)
Eczema	21 (30.4)	3 (17.6)	3 (8.3)	0 (0.0)	27 (20.3)
Oedematous	14 (20.3)	4 (23.5)	2 (5.6)	1 (9.1)	21 (15.8)
Scar	1 (1.4)	0 (0.0)	15 (41.7)	3 (27.3	19 (14.3)
Normal	1 (1.4)	1 (5.9)	7 (19.4)	3 (27.3)	12 (9.0)
Infection					
Wound bed	12 (17.4)	2 (11.8)	5 (13.5)	3 (33.3)	22 (16.7)
Cellulitis	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)
Systemic antibiotic	18 (23.4)	7 (35.0)	15 (35.7)	1 (9.1)	41 (27.3)
Classified as infected	23 (29.9)	8 (40.0)	16 (38.1)	3 (27.3)	50 (33.3)

Only four (3%) wounds had islands of epithelium (two leg ulcers and two foot ulcers): *P. aeruginosa* was isolated from three of these wounds and *S. aureus* was isolated from the fourth. The wound edge was described as epithelialising in 44 wounds (33.6% of wounds with data recorded). Most frequently however the wound edge was static (n=81, 61.8%). Of those wounds that carried *S. aureus* and had wound edge data recorded, 45 (65.2%) had static wound edges and 24 (34.8%) had epithelialising wound edges. Twelve of these patients carried MRSA in their wounds, seven (58.3%) of which had static, and five (41.7%) had epithelialising, wound edges. By contrast, 30 (73.2%) of the 41 wounds that carried *P. aeruginosa* and had wound edge data recorded had static wound edges and 11 (26.8%) had epithelialising edges. Of the 15 patients that carried ciprofloxacin-resistant *P. aeruginosa*, 14 (93.3%) had static wound edges and only 1 (6.7%) had an epithelialising wound edge.

Evidence of infection or cellulitis was recorded as present in 23 wounds (17.7% of those with data). A further 27 patients were prescribed systemic antibiotics on the date of the swab for their wound by the wound healing clinic. Neither *S. aureus* nor *P. aeruginosa* or enterococci appeared to be more frequently isolated from wounds classified as infected. *S. aureus* was isolated from 48.0% (n=24) infected wounds and 55.0% (n=55) non-infected wounds, *P. aeruginosa* was isolated from 32.0% (n=16) infected wounds and 31.0% (n=31) non-infected wounds and enterococci were isolated from 8.0% (n=4) infected and 9.0% (n=9) non-infected wounds. In addition, wounds from which *S. aureus*, *P. aeruginosa* or enterococci were isolated were found to contain MRSA or ciprofloxacin-resistant *P. aeruginosa* in 30.3% (n=10) of infected wounds and 28.2% (n=20) of non-infected wounds.

Pain was recorded both by frequency and severity. Data regarding pain were missing for 17 patients. The majority of patients had intermittent moderate pain (n=62, 82.7% of those with data recorded). The values recorded for pain were dichotomised into those patients that had moderate or severe pain, intermittently or continuously and those who had less frequent or less than moderate pain. A greater proportion of patients with leg ulcers were found to suffer from pain compared to other wound types (72.1% (n=49) patients with leg ulcers compared to 41.2% (n=7), 54.1% (n=20) and 18.2% (n=2) of patients with foot ulcers, surgical wounds and miscellaneous wounds respectively). Overall, *S. aureus, P. aeruginosa* or enterococci were isolated from 65.4% (n=51) of painful wounds and 72.7% (n=40) of less or non-painful wounds. MRSA or ciprofloxacin-resistant *P. aeruginosa* were isolated from 25.5% (n=13) of painful wounds compared with 30.0% (n=12) of less or non-painful wounds.

Focusing only on patients with leg ulcers, 63.5% (n=32) of patients with painful leg ulcers carried *S. aureus* (9.4% (n=3) of which were MRSA) and 36.7% carried *P.*

aeruginosa (33.3% (n=6) of which were ciprofloxacin resistant). While 78.9% (n=15) of patients with non-painful leg ulcers carried *S. aureus* (none of which were MRSA) and 52.6% (n=10) carried *P. aeruginosa* (of which 30.0% (n=3) were ciprofloxacin resistant).

Patients with painful surgical wounds were found to carry *S. aureus* in 40% (n=8) of wounds (5% (n=1) of which were MRSA) but *P. aeruginosa* was not isolated from any painful surgical wounds. *S. aureus* was isolated from 29.4% (n=5) of non-painful surgical wounds (of which 60% (n=3) were MRSA) and 5.9% (n=1) patients carried *P. aeruginosa* (ciprofloxacin susceptible).

Pain was further investigated to identify whether patients with clinically recorded infection had a greater frequency of pain recorded than patients with non-infected wounds. Moderate or severe pain, intermittent or continuous, was recorded as present for 68.9% (n=31) patients with infected wounds and 53.4% (n=47) non-infected wounds.

Odour was recorded as present in the wounds of 17 patients (12.6%), 11 of which had leg ulcers and four had wounds of miscellaneous aetiology. Data regarding the presence of odour were missing for 15 patients. Odour was present in 8 wounds (11.1%) from which *S. aureus* was isolated and 9 wounds (14.3%) without *S. aureus*. In comparison, 10 wounds (24.4%) from which *P. aeruginosa* was isolated were odourous and 7 wounds (7.7%) without *P. aeruginosa* were not odourous.

3.4.6 Patient Characteristics

3.4.6.1 Co-morbidities

Data on co-morbidities that can have a potential impact on the non-healing phenotype of wounds were recorded.

Diabetes

In total 32 patients (21.2%) were identified as having diabetes, and 113 (74.8%) as not having diabetes. The presence of diabetes mellitus was identified using both the nursing notes and the GP letters. In one case there were conflicting data from the nursing notes and GP letter, however, the patient had a neuroischaemic foot ulcer, and therefore has been included as diabetic (as recorded in the nursing notes seven months prior to audit). Twenty-four patients (75.0%) with diabetes carried *S. aureus*, *P. aeruginosa* or enterococci, compared with 74 patients (67.3%) known not to have diabetes. MRSA or ciprofloxacin resistant *P. aeruginosa* were isolated from eight patients with diabetes (33.3%) and from 20 patients (27.0%) known not to be diabetic.

Cardiovascular or circulatory pathology

Seventy-four patients (49.0%) were identified as having cardiovascular or circulatory related pathology. *S. aureus*, *P. aeruginosa* or enterococci were isolated from the wounds of 54 patients (73.0%) with and 47 patients (66.2%) without such pathology. These organisms were MRSA or ciprofloxacin-resistant *P. aeruginosa* in 13 patients with and 17 patients without cardiovascular or circulatory pathology.

Connective tissue disease

Connective tissue disease (including diseases such as rheumatoid arthritis, rheumatoid fever, systemic lupus erythematosus) was recorded for 31 patients. A further 13 were identified through the records as having osteoarthritis or non-specified arthritis. One hundred patients had no indication of connective tissue disease in either their nursing notes or GP letters.

Chronic respiratory disease

Chronic respiratory disease was not specifically recorded in the nursing notes, data were only available for those patients with GP data (n=90). Only seven patients (7.8% of those on whom data was available) were known to have chronic respiratory disease.

Immunocompromised patients

Patients were defined as immunocompromised, if they had received immunosuppressant drugs or had an immunocompromising disease. Drugs were recorded in the background data of the nursing notes, and the GP form requested a binary response to chronic systemic immunosuppression. An indication in either of these variables that the patient was immunosuppressed was considered positive for immunosuppression. Drugs included as immunosuppressants from the nursing notes were glucocorticoids (BNF Section 6.3.2), drugs affecting the immune response (BNF Section 8.2, including drugs that are primarily used for transplant patients (BNF Section 8.2.1) and corticosteroids and other immunosuppressants (BNF Section 8.2.2)) and drugs that suppress the rheumatic disease process (BNF Section 10.1.3). In total, 14 patients (9.3%) were considered to be immunosuppressed through medication. No patients were known to have specifically immunocompromising-causing diseases or congenital disorders. Two patients who were immunosuppressed carried *S. aureus*, five patients carried *P. aeruginosa* and one patient carried both organisms. Twenty-six patients were recorded as having a malignancy: five of these had wounds with an underlying malignant aetiology.

Data on patient co-morbidities were missing from both the nursing notes and the GP letters for six patients, although one patient was known to have cardiovascular or circulatory disease.

3.4.6.2 Previous hospitalisation

Hospitalisation was defined as an overnight stay in hospital and was determined for two time-periods: i) previous three months (90 days), and ii) previous year.

Table 3.6 shows the known history of hospitalisation for patients with different wound classifications. A higher percentage of patients with surgical wounds were known to have stayed overnight in hospital in both the previous three months and year compared to patients with leg ulcers.

Overall, 70 patients were not known to have any history of hospitalisation in the previous year. No hospitalisation history could be determined for 12 patients, while three further patients were known to have stayed overnight in hospital in the previous 90 days but hospitalisation history could not be determined for the period up to one year. Data were obtained from the GP forms and hospital notes. For the most part, the nursing notes were not used to populate the hospital variable, unless they specified overnight hospital attendance. Data were considered missing when no GP form had been returned and the hospital notes had not been seen.

Table 3.6 Hospitalisation history of patients by wound classification

Overnight hospital stay in the previous:	Number (%) of patients				Total number (%) of patients (n=150)
	Leg ulcer (n=77)	Foot ulcer (n=20)	Surgical wound (n=42)	Miscellan-eous (n=11)	
90 days	9 (11.7)	5 (25.0)	9 (21.4)	2 (18.2)	25 (16.7)
Year	28 (36.4)	8 (40.0)	28 (66.7)	4 (36.4)	68 (45.3)
Missing	5 (6.5)	2 (10.0)	3 (7.1)	2 (18.2)	12 (8.0)

S. aureus and *P. aeruginosa* were isolated from 52.0% (n=13) and 32.0% (n=13) respectively of patients known to have stayed overnight in hospital in the past three months and from 52.2% (n=59) and 31.0% (n=35) respectively of patients that had not stayed overnight in hospital in the same time period MRSA was identified in 38.5%

(n=5) of patients carrying *S. aureus* with a history of hospitalisation, compared with 11.9% (n=7) of those patients carrying *S. aureus* without previous hospitalisation.

3.4.7 Antimicrobial treatment

3.4.7.1 Previous antibiotic usage

Table 3.7 shows the number of patients that received antibiotics in the previous three months (90 days) and year prior to swabbing the wound. Also given in Table 3.7 are the number of patients that received each antibiotic group and the isolation of *S. aureus* and *P. aeruginosa* and their resistant sub-groups. From the 12 patients with missing hospital notes and GP letters, some data were available from nursing notes on antibiotic usage: 5 patients were known to have received systemic antibiotics and 8 patients to have topical antimicrobials in the previous 90 days. In total, 89 (62.2%) patients received systemic antibiotics and 83 (57.2%) received topical antimicrobials in the previous three months while 124 (87.3%) patients received systemic antibiotics and 109 (75.2%) patients received topical antimicrobials in the year prior to microbial investigation.

Data on antibiotics previously used to treat both wounds and other infections were obtained from the GP forms, hospital notes and, where appropriate, nursing notes. Antibiotics have been excluded if prescribed on the date the swab was taken (as this will not have affected the microbiology data obtained). Antibiotics in the previous three months were defined as those prescribed within 90 days prior to swabbing, or prescribed before this period but known to be of duration extending into the 90-day period.

Table 3.7 Antibiotic use in the previous three months by wound classification and total antibiotic use in the previous year

	Number (%) of patients in the previous 3 months (90 days)				Total number (%) of patients			Number (%) of patients organism isolated from and who received antibiotic in previous 3 months			
	Leg ulcers (n=72)	Foot ulcers (n=18)	Surgical (n=39)	Miscell-aneous (n=9)	Previous 3 months	Previous 12 months	Miss-ing data	*S. aureus*	MRSA (% of *S. aureus*)	*P. aeruginosa*	CIP resistant PA (% of PA)[a]
Systemic antibiotics											
Any antibiotic	47[d] (63.5)	11[e] (57.9)	26[f] (65.0)	5 (55.6)	89 (62.7)	124 (87.3)	[8]	44 (49.4)	10 (22.7)	30 (33.7)	11 (36.7)
Penicillins[b]	8 (11.1)	2 (11.1)	2 (5.1)	0 (0.0)	12 (8.7)	26 (18.8)	[12]	7 (58.3)	0 (0.0)	4 (33.3)	1 (25.0)
Flucloxacillin	23[d] (31.1)	2 (11.1)	10[f] (25.0)	1 (11.1)	36 (25.5)	74 (52.5)	[9]	18 (50.0)	5 (27.8)	11 (30.6)	5 (45.5)
Cephalosporins & β-lactam penicillins[c]	22 (30.6)	4 (22.2)	8 (20.5)	2 (22.2)	36 (26.1)	76 (55.1)	[12]	21 (58.3)	4 (19.0)	14 (38.9)	6 (42.9)
Macrolides	9 (12.5)	3 (16.7)	8 (20.5)	1 (11.1)	21 (15.2)	46 (33.3)	[12]	9 (42.9)	1 (11.1)	7 (33.3)	1 (14.3)
Ciprofloxacin	18[g] (24.7)	6[e] (31.6)	11[f] (27.5)	1 (11.1)	36 (25.5)	58 (41.1)	[9]	10 (27.8)	4 (40.0)	15 (41.7)	9 (60.0)
Clindamycin	2 (2.8)	3[e] (15.8)	5[f] (12.5)	1 (11.1)	11 (7.9)	18 (12.9)	[10]	1 (9.1)	0 (0.0)	3 (27.3)	2 (66.7)
Metronidazole (systemic)	8 (11.1)	1 (5.6)	3 (7.7)	0 (0.0)	12 (8.7)	39 (28.3)	[12]	7 (58.3)	3 (42.9)	4 (33.3)	3 (75.0)
Other	7 (9.7)	4[e] (21.1)	5 (12.8)	3 (33.3)	19 (13.7)	42 (30.2)	[11]	12 (63.2)	7 (58.3)	3 (15.8)	0 (0.0)
Non-specified	2[g] (2.7)	1 (5.6)	0 (0.0)	0 (0.0)	3 (2.2)	21 (15.1)	[11]	2 (66.7)	0 (0.0)	2 (66.7)	1 (50.0)
Topical antimicrobials											
Any topical	39[d] (52.7)	14[h] (70.0)	27[i] (64.3)	3 (33.3)	83 (57.2)	109 (75.2)	[5]	39 (47.0)	12 (30.8)	29 (34.9)	15 (51.7)
Topical silver	24[g] (32.9)	8[e] (42.1)	15[j] (36.6)	2 (22.2)	49 (34.5)	77 (54.2)	[8]	22 (44.9)	8 (36.4)	18 (36.7)	7 (38.9)
Topical iodine	7 (9.7)	10[h] (50.0)	13[f] (32.5)	2 (22.2)	32 (22.7)	53 (37.6)	[9]	15 (46.9)	4 (26.7)	9 (28.1)	5 (55.6)
Topical metronidazole	1 (1.4)	1 (5.6)	2 (5.1)	0 (0.0)	4 (2.9)	10 (7.2)	[12]	1 (25.0)	1 (100)	1 (25.0)	0 (0.0)
Other	14[g] (19.2)	3 (16.7)	5[f] (12.5)	1 (11.1)	23 (16.4)	42 (30.0)	[10]	8 (34.8)	2 (25.0)	11 (47.8)	7 (63.6)
Non specified	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	[12]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

a: CIP Ciprofloxacin, PA *P. aeruginosa*; b: Includes benzyl penicillin, penicillin V, amoxicillin and ampicillin. c: Includes co-amoxiclav, tazacin, cofluampicil; d: n=74 (two identified from nursing notes); e: n=19 (one identified from nursing notes); f: n=40 (one identified from nursing notes); g: n=73 (one identified from nursing notes); h: n=20 (two identified from nursing notes); i: n=42 (three identified from nursing notes); j: n=41 (two identified from nursing notes)

Flucloxacillin, ciprofloxacin and the group of antibiotics classified as cephalosporins and other β-lactamase resistant penicillins (such as co-amoxiclav and co-fluampicil) were the three antibiotic groups most frequently prescribed for these patients.

Thirty-six (25.5%) patients received flucloxacillin in the previous three months and 74 (52.5%) in the previous year. *S. aureus* was isolated from 50% (n=18) of the wounds of patients that had received flucloxacillin in the previous three months (five of which were MRSA). Thirty-six (25.5%) patients received ciprofloxacin in the three months prior to swabbing. *P. aeruginosa* was isolated from the wounds of 15 (41.7%) of these patients and found to be resistant to ciprofloxacin in nine cases (60.0%).

The group of antibiotics included in the classification of cephalosporins and other β-lactamase resistant penicillins were received by 36 (26.1%) patients in the previous three months and 76 (55.1%) patients in the previous year. *S. aureus* was isolated from 58.3% (n=21) of the wounds of patients that had received cephalosporins or other β-lactamase resistant penicillins in the previous three months (four of which were MRSA).

Overall, *S. aureus*, *P. aeruginosa* or enterococci were isolated from 62 (69.7%) of the wounds of patients known to have received antibiotics in the previous three months, compared with 35 (66.0%) wounds of patients that did not receive antibiotics. MRSA or ciprofloxacin resistant *P. aeruginosa* were isolated from 20 patients (22.5%) who had received systemic antibiotics in the previous three months and 6 patients (11.3%) who had not received any systemic antibiotics in this timeframe.

Topical antibiotics were also frequently used in wound care. Overall 57.2% (n=83) of patients had received topical antimicrobials in the previous three months and more than 75% (n=109) of patients received such treatment in the previous year. Overall, topical

silver was used more frequently than iodine. This was due in the most part to the higher use of silver compared to iodine for patients with leg ulcers (32.9% (n=24) and 9.7% (n=7) respectively).

Patients received a mean (SD) of 1.3 (1.33) of the antibiotic groups in the previous three months. The maximum number of different groups received by any patient was five. Approximately 40% of patients, with all types of wound, received multiple (two or more) antibiotic groups in the previous three months. The breakdown of the number of antibiotic groups received by patients with wounds of different aetiologies is given in Table 3.8.

Table 3.8 The number of different antibiotic groups received by patients in the previous 3 months

Number of antibiotic groups[a]	Number (%) of patients				Total number (%) of patients (n=142)
	Leg ulcers (n=74)	Foot ulcer (n=19)	Surgical wounds (n=40)	Miscellaneous (n=9)	
0	27 (36.5)	8 (42.1)	14 (35.0)	4 (44.4)	53 (37.3)
1	18 (24.3)	1 (5.3)	11 (27.5)	2 (22.2)	32 (22.5)
2	14 (18.9)	5 (26.3)	8 (20.0)	2 (22.2)	29 (20.4)
3	9 (12.2)	5 (26.3)	5 (12.5)	1 (11.1	20 (14.1)
4	4 (5.4)	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.8)
5	2 (2.7)	0 (0.0)	2 (5.0)	0 (0.0)	4 (2.8)

a: Antibiotics were grouped in the following categories: flucloxacillin, penicillins, cephalosporins and β-lactamase resistant penicillins, macrolides, ciprofloxacin, clindamycin, metronidazole and other systemic antibiotics.

Table 3.9 shows the relationship between the number of antibiotic groups received by patients and the growth of *S. aureus*, *P. aeruginosa* or enterococci and the resistant isolates. *S. aureus*, *P. aeruginosa* or enterococci were isolated from 70.2% of patients that received two or more antibiotics in the previous three months, compared with 67.1% of patients that received one or less antibiotic group. MRSA or ciprofloxacin resistant *P. aeruginosa* were isolated from 29.8% of patients that received two or more antibiotic groups in the previous three months and from only 10.6% of patients that received one or less antibiotic group.

Table 3.9 Growth of *S. aureus*, *P. aeruginosa* and enterococci and clinically important antibiotic resistances in wounds by the number of antibiotic groups received in the previous three months

Number of antibiotic groups[a]	Number (%) of patients	Number (%) of patients with	
		S. aureus, *P. aeruginosa* or enterococci	MRSA or ciprofloxacin-resistant *P. aeruginosa*
0	53 (37.3)	35 (66.0)	6 (11.3)
1	32 (22.5)	22 (68.8)	3 (9.4)
2	29 (20.4)	23 (79.3)	9 (31.0)
3 or more	28 (19.7)	17 (60.7)	8 (28.6)

a: Antibiotics were grouped in the following categories: flucloxacillin, penicillins, cephalosporins and β-lactamase resistant penicillins, macrolides, ciprofloxacin, clindamycin, metronidazole and other systemic antibiotics.

As previously stated data were collated from several different sources to determine antibiotic usage, including general practitioners, hospital notes and nursing notes where appropriate. Data were not available from all these sources for all patients; indeed complete data were only available for 90 patients. The impact of missing data sources was investigated by comparing the quantity of antibiotics identified for patients with complete data with that of other patients (Table 3.10). It can be seen that for every systemic antibiotic group, patients with complete data had higher antibiotic consumption. This suggests that antibiotic consumption was underestimated in patients for whom general practice data were not available. Overall, 70% of patients with complete data had received systemic antibiotics in the previous 3 months, compared with 46% of patients with only hospital and nursing data. The size of the difference between the two groups differs by antibiotic group, being most pronounced for pencillins and flucloxacillin and smaller but still apparent for macrolides and metronidazole. Cephalosporins and β-lactamase resistant penicillins and ciprofloxacin do not however appear to be underestimated, with differences of less than 2% between the groups. It could be presumed that these antibiotics are more frequently prescribed within the secondary care setting than primary care. It can also be seen from Table 3.10 that 11 antibiotics prescribed for four patients were identified from the nursing notes when both the other data sources were unavailable.

Table 3.10. Identification of antibiotic and topical antimicrobial receipt by the data sources consulted.

	Number (%) of patients in the previous 3 months (90 days) with data collected from nursing notes and		
	GP and hospital data (n=90)	Hospital only (no GP data) (n=48)	No hospital or GP data (n=12)
Systemic antibiotics			
Any antibiotic	63 (70.0)	22 (45.8)	4 (33.3)
Penicillins[a]	12 (13.3)	0 (0.0)	0 (0.0)
Flucloxacillin	28 (31.1)	5 (10.4)	4 (33.3)
Cephalosporins & β-lactam penicillins[b]	24 (26.7)	12 (25.0)	0 (0.0)
Macrolides	16 (17.8)	5 (10.4)	0 (0.0)
Ciprofloxacin	22 (24.4)	11 (22.9)	3 (25.0)
Clindamycin	7 (7.8)	2 (4.2)	2 (16.7)
Metronidazole (systemic)	10 (11.1)	2 (4.2)	0 (0.0)
Other	13 (14.4)	5 (10.4)	1 (8.3)
Non-specified	2 (2.2)	0 (0.0)	1 (8.3)
Topical antimicrobials			
Any topical	49 (54.4)	27 (56.3)	7 (58.3)
Topical silver	29 (32.2)	16 (33.3)	4 (33.3)
Topical iodine	15 (16.7)	14 (29.2)	3 (25.0)
Topical metronidazole	3 (3.3)	1 (2.1)	4 (33.3)
Other	15 (16.7)	6 (12.5)	2 (16.7)
Non specified	0 (0.0)	0 (0.0)	0 (0.0)

a: Includes benzyl penicillin, penicillin V, amoxicillin and ampicillin. b: Includes co-amoxiclav, tazacin, cofluampicil

Data on overall antibiotic consumption were compared to patient-responses on the consumption of antibiotics (Table 3.11). There is greater concordance between the responses and data collected for patients with complete data compared to those without general practice data (85.4% and 68.7% respectively). In total, 10 patients stated that they were prescribed antibiotics but these were not identified through other sources of data. Twenty-one patients stated that they had not had antibiotics in the previous three months, but data from elsewhere indicated that they had received antibiotics in that time frame (although they may not have taken them).

Table 3.11. Comparison between systemic antibiotic consumption identified for patients from GP and/or hospital data sources and that indicated from the patient questionnaire.

Patient questionnaire	Number (%) of patients											
	GP and hospital data (n=89[a])				Hospital data only (no GP data) (n=48)				No hospital or GP data (n=12)			
	No antibiotics		Antibiotics		No antibiotics		Antibiotics		Missing data		Antibiotics	
No antibiotics	20	(22.5)	6	(6.7)	11	(22.9)	15	(31.3)	5	(41.7)	0	(0.0)
Antibiotics	7	(7.7)	56	(62.9)	0	(0.0)	22	(45.8)	3	(25.0)	4	(33.3)

a: data missing from one patient questionnaire

3.4.7.2 Wound dressings

Data regarding the dressing removed from the wound immediately prior to the swab being taken were recorded and have been categorised according to the wound dressing categories in the BNF[62] (Table 3.12). Those dressings that either contained an antimicrobial agent or were applied with one have been placed in subgroups. Data on the dressing removed was missing for one patient (a surgical wound). The most frequently removed dressings were low-adherence dressings or wound contact material (BNF Section A8.1.6). Seventy patients (47.0%) had such a dressing removed; 50% of which contained an antimicrobial (for example inadine and acticoat) or were present together with a separate antimicrobial such as silver sulphadiazine. Hydrocolloid dressings were the second most frequently removed dressings (n=41, 27.5%). These either contained an antimicrobial (for example Aquacel Ag) or were present together with one in 61.0% of cases.

S. aureus, *P. aeruginosa* or enterococci were isolated from 59 wounds from which a non-antimicrobial dressing (or no dressing) had been removed at the clinic visit (71.1%, n=83). This compares with the isolation of these organisms from 27 wounds (75.0%) that had been previously dressed with a silver dressing and 16 wounds (61.5%) that had been dressed with an iodine containing dressing.

MRSA or ciprofloxacin-resistant *P. aeruginosa* were isolated from 23.7% (n=14), 37.0% (n=10) and 37.5% (n=6) of wounds from which a non-antimicrobial dressing (or no dressing), silver containing dressing or iodine containing dressing had been removed respectively.

Table 3.12 Type of dressing removed immediately prior to swabbing the wound

Dressings removed	Number (%) of patients				Total number (%) of patients (n=149)[a]
	Leg ulcers (n=77)	Foot ulcers (n=20)	Surgical (n=41)[a]	Miscellaneous (n=11)	
Low adherence					
- alone	28 (36.4)	0 (0.0)	4 (9.8)	3 (27.3)	35 (23.5)
- with iodine	4 (5.2)	8 (40.0)	8 (19.5)	2 (18.2)	22 (14.8)
- with/and silver	8 (10.4)	0 (0.0)	2 (4.9)	0 (0.0)	10 (6.7)
- and neomycin*	2 (2.6)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.3)
- with chlorhexidine	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Hydrocolloid					
- alone	4 (5.2)	3 (15.0)	7 (17.1)	2 (18.2)	16 (10.7)
- with/and silver	13 (16.9)	2 (10.0)	9 (22.0)	0 (0.0)	24 (16.1)
- and neomycin[b]	0 (0.0)	0 (0.0)	1 (2.4)	0 (0.0)	1 (0.7)
Hydrogel					
- alone	7 (9.1)	2 (10.0)	1 (2.4)	0 (0.0)	10 (6.7)
- with iodine	1 (1.3)	3 (15.0)	0 (0.0)	0 (0.0)	4 (2.7)
Foam					
- alone	2 (2.6)	2 (10.0)	1 (2.4)	0 (0.0)	5 (3.4)
- with silver	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Alginate					
- alone	1 (1.3)	0 (0.0)	3 (7.3)	0 (0.0)	4 (2.7)
- with a silver cream	0 (0.0)	0 (0.0)	1 (2.4)	0 (0.0)	1 (0.7)
Gauze/dry					
- alone	2 (2.6)	0 (0.0)	3 (7.3)	0 (0.0)	5 (3.4)
- with corticosteroids	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Capillary	1 (1.3)	0 (0.0)	0 (0.0)	1 (9.1)	2 (1.3)
Charcoal	0 (0.0)	0 (0.0)	0 (0.0)	1 (9.1)	1 (0.7)
None	1 (1.3)	0 (0.0)	1 (2.4)	2 (18.2)	4 (2.7)

a: Data was missing for one patient with a surgical wound; b: administered as Dermovate NN

3.5 Discussion

3.5.1 Main findings

This audit found approximately 10% of patients to carry MRSA (19% of *S. aureus* isolates) and 10% to carry ciprofloxacin resistant *P. aeruginosa* (34% of *P. aeruginosa* isolates) in their wound. Overall *S. aureus* was present in more than 50% of wounds and *P. aeruginosa* in approximately one-third of wounds. Nine percent of patients carried enterococci in their wounds. Reassuringly, vancomycin–resistant enterococci were not found in the wounds of any patients included in the audit.

Although the numbers of patients included in the audit with each specific wound aetiology was too small to make firm comparisons, there did appear to be a difference in the frequency with which *S. aureus* was isolated from leg ulcers and surgical wounds. Leg ulcers included in this audit frequently grew *S. aureus* (64%) but relatively few were MRSA (10%). In comparison, surgical wounds yielded *S. aureus* less frequently (36%) but a greater proportion was found to be MRSA (33%). This may be due to differential colonisation of leg ulcers and surgical wounds by microbial flora over time. The greater occurrence in MRSA prevalence in patients with surgical wounds may be due to an increased likelihood of previous hospitalisation (a main risk factor for the carriage of MRSA). Interestingly, in this audit only single organisms were isolated from surgical wounds, while in comparison 23 leg ulcers (29.9%) were found to contain at least two of the three organisms investigated.

A total of 150 patients were included in this audit. Seventy-seven patients had leg ulcers, 20 had foot ulcers, 42 surgical wounds and 11 miscellaneous wounds. Leg ulcers were most commonly of venous (n= 35, 45.5%) or mixed (n=16, 20.8%) aetiology, while foot ulcers were most commonly neuropathic (n=5, 25%) or pressure (n=4, 20%) ulcers. Surgical wounds were most frequently due to gastrointestinal

surgery (n=12, 28.6%) and malignancy was the most frequent underlying cause of miscellaneous wounds (n=5, 45.5%).

Fifty-four percent of patients included in the audit were female. The mean age of patients was 64.4 years, although differences in the average age of patients was observed for patients with different wound aetiologies: the mean age of patients with leg ulcers and those with surgical wounds was 69.1 and 56.2 years respectively. Many patients had multiple wounds (>40%) and wound size varied widely both between and within wound classifications. Over 60% of wounds had been present for longer than a year. Although also included were six patients with wounds of less than three months (90 days) duration, five of which were surgical wounds and one was a foot ulcer. Comprehensive, accurate data on the duration of some wounds was difficult to obtain. In part, this was due to the difficulty in distinguishing between recurrent and non-responding wounds. Recurrence of chronic wounds was clearly a problem; 45% of patients with leg ulcers were known to have recurrent wounds.

Few wounds included in the audit were noted as displaying signs of infection (18%). Clinical signs and symptoms are considered to be the optimal way to identify infection, due to the ubiquitous colonisation of chronic wounds by microbes. Due to the expert status of the unit, administration of antibiotic treatment on the day the swab was taken was also considered to be an indicator of infection. There was no reason to suspect that these organisms were more prevalent in the wounds described as infected than the other wounds. In this audit there was no significant relationship between the presence of pain and the recording of signs of infection (as noted by experienced wound specialist nurses), even though previously pain has been implicated as a relatively reliable indicator of infection.[37]

Patients were found to have received a large number, and broad range, of antibiotics in the previous three months. The most frequently used antibiotics were flucloxacillin, ciprofloxacin and the group of antibiotics that included cephalosporins and β-lactamase resistant penicillins. Approximately 20% of patients had received three or more antibiotic groups in the previous three months. The number of different antibiotic groups received by patients is of interest in itself from a wound management perspective, but also has an impact on the likely carriage of antibiotic resistant organisms. Data on antibiotic usage were obtained from a number of sources: GP, hospital and nursing notes. The proportion of patients known to have received antibiotics was compared for patients grouped by the sources from which data were available, in this way it was identified that the total number of antibiotics consumed was likely to be underestimated had GPs not been consulted. This was particularly the case for certain classes of antibiotics, such as flucloxacillin and penicillins and not so important for others such as ciprofloxacin and cephalosporins (including β-lactamase resistant penicillins).

Topical treatments were also regularly used for the patients included in the audit. Prior to taking the swab, a total of 36 patients had received some form of silver topical agent (i.e. dressing) and 26 an iodine product. The inclusion of GP data did not appear so important in determining the proportion of patients who had received topical antimicrobials. This difference is likely due to the use of, and recording of, dressings in the nursing notes.

3.5.2 Relationship to other studies

The frequency with which *S. aureus*, *P. aeruginosa* and enterococci have been isolated from chronic wounds have been previously reported in several studies in the UK.

Moreover, occasionally antibiotic resistance has also been reported, although this is most common for isolates of MRSA.

In a study investigating patients with a range of chronic wounds (n=45), including pressure ulcers, leg ulcers, foot ulcers, abdominal wounds and hand wounds, Bowler and Davis[20] found 33% to be colonised with *S. aureus* and 7% *P. aeruginosa*. Samples included in the study were swabs, pus or fluid samples received at one UK medical microbiology laboratory. The origin of the samples was not stated (i.e. microbial investigation of infection in primary care or specialist wound clinic, or routine wound sampling). The authors do however state that there may have been differences in sampling method, antibiotic treatment and dressings used prior to wound sampling. The prevalence of all these organisms is lower than that identified in this audit.

The bacterial populations of severe non-healing wounds have previously been found to differ according to aetiology (diabetic, venous and arterial).[27] Schmidt *et al.* investigated patients initially presenting at a German hospital surgery department over a four-month period and found 58% of patients with chronic venous insufficiency harboured *S. aureus* and 33% harboured *P. aeruginosa*. The authors also found 50% and 14% of arterial ulcers, 36% and 7% of diabetic ulcers with arterial occlusive disease and 59% and 12% of diabetic ulcers without arterial occlusive disease to contain *S. aureus* and *P. aeruginosa* respectively. Here, in this audit, there also appears to be a difference in the isolation of organisms by wound type, with *S. aureus* and *P. aeruginosa* being less frequently identified in surgical wounds than other wound types.

Previous work investigating micro-organism carriage in leg ulcers includes that by Davies *et al.*[23] who investigated bacterial carriage in venous leg ulcers and recruited patients from the specialist wound clinic at which this audit was conducted. Patients with non-infected venous leg ulcers were recruited to the study that aimed to compare

the microbial findings from surface swabs to that of biopsies. Patients were excluded if they had characteristics or history likely to affect bacterial carriage (e.g diabetes, immunosuppressants, antimicrobial therapy). Sixty-six patients were included in the analysis (6 patients were lost to follow-up). *S. aureus* was isolated from 25.8% of patients in the deep tissue and 34.8% of surface swabs, while *P. aeruginosa* was isolated from 31.8% of deep tissue and 34.8% of surface swabs. Therefore, while the prevalence of *P. aeruginosa* were similar for the two groups (Davies *et al.*[23] and this audit), the prevalence of *S. aureus* is greater in the current audit. This is likely in part due to the different populations involved, although conducted at the same clinic, Davies *et al.*[23] recruited only non-infected venous leg ulcers without previous antibiotic treatment. Here, all patients were eligible for inclusion irrespective of treatment history and infection status.

Other UK studies of leg ulcers include Bowler and Davies's[24] analysis of infected and non-infected leg ulcers. They found *S. aureus* in 53% of non-infected wounds and 43% of infected ulcers. Swabs were used to sample the wounds of patients attending two specialist centres in the UK with both infected and non-infected leg ulcers (infection was determined on clinical criteria). The authors state that isolation of *P. aeruginosa* (and β-haemolytic streptococci) were low in both infected and non-infected leg ulcers, but do not report exact figures.

Comparison of the organisms identified in the surgical wounds included in the audit with national data on the causative organisms of surgical site infections show considerable similarity. Data on the organisms involved in surgical site infections are available for England through the Surgical Site Infection Surveillance Service.[204] This surveillance system collects data from hospitals across England on the number, and likely causative organism, of surgical site infections. Data from October 1997 to

September 2005 indicate that *S. aureus* is the main causative organism for infections in all surgery categories. *S. aureus* accounted for 40% of organisms reported (26% were MRSA and 14% were MSSA) and was the causative organism in 53% of infections. *P. aeruginosa* was isolated from 5%, and Enterococcus species from 8%, of surgical wound infections. The majority of these infections were superficial but data on deep and organ space infections were also incorporated. Initially, this surveillance scheme was voluntary, however, it was later (April 2004) made compulsory for orthopaedic surgery. By 2005, 60,000 operations were included in the surveillance each year. The report does not present figures of the organisms identified by year so it is not possible to determine whether the introduction of a mandatory part of the surveillance system affected the number (and relative proportion) of organisms identified. The authors do state that the number of operations on which data were reported increased by 34% after the introduction of mandatory reporting in orthopaedic surgery. Although the organisms isolated in this audit were not specifically from infected surgical wounds, the percentage of wounds carrying *S. aureus* (36%) and *P. aeruginosa* (5%) were very similar to the national picture of surgical site infections.[204] Enterococci were however slightly less frequently isolated in this audit (2%) than nationally. It is recognised that surgical sites are most likely to become infected with endogenous flora and therefore this comparability is perhaps not surprising.

The prevalence of organisms found in foot ulcers has previously been estimated, using retrospective analyses of wound swabs taken from patients with non-limb or life-threatening infected diabetic foot ulcers attending an outpatient clinic in the UK.[19,140] The prevalence of organisms was investigated over two time period, 1998[19] and 2001,[140] and included 79 and 63 patients respectively. In 1998, 29% (of 104) isolates were *S. aureus*, compared to 48% (of 93) isolates in 2001. Thirty percent of patients had MRSA in 2001, compared with 15.2% in 1998. Four isolates of *Pseudomonas* species

were identified at each time point (<5% of isolates), while there were 6 isolates of Enterococci species in 1998, and none in 2001. The prevalence of *S. aureus* in foot ulcers in this audit (40%) was closer to the later findings by Dang *et al.* *P. aeruginosa* and Enterococci species were more frequently isolated in foot ulcers as part of this audit (45% and 18% respectively) than previously. However, the number of patients with foot ulcers in this audit was small and therefore valid comparisons with other sources are limited, especially regarding Enterococci prevalence. Furthermore, the differences may be due to inclusion criteria: Dang *et al.*[140] and Tentolouris *et al.*[19] included only infected diabetic foot ulcers, whereas all foot ulcers, irrespective of diabetes or infection status, were eligible for inclusion in the audit. Tentolouris *et al.*[19] also suggest that diabetic foot ulcers infected with MRSA heal significantly slower than ulcers infected with MSSA (mean time to healing of 17.8 and 35.4 weeks for MRSA and MSSA infected ulcers respectively).[19] However, this was based on the analysis of 18 ulcers with MSSA and 12 with MRSA, compared using Student's T-test. The main problem with this analysis is that no potentially confounding factors were considered in this relationship, such as duration of ulcer prior to isolation of MRSA.

The prevalence of MRSA by wound classification, can therefore, be seen to be in line with reported estimates for other UK patient populations with wounds. The prevalence of resistance in *P. aeruginosa* has been less frequently reported. In this audit, the finding that one-third of *P. aeruginosa* isolates were resistant to ciprofloxacin was unexpected. Ciprofloxacin and imipenem resistance in the chronic wound patients was considerably higher than that previously determined in clinical samples from the general population. The prevalence of ciprofloxacin resistance amongst clinical samples from non-cystic fibrosis out-patients in 1999 was found by Henwood *et al.*[127] to be 6.3%. Imipenem, cetazidine, amikacin and piperacillin resistance in *P. aeruginosa* isolates

were 4.4%, 2.3%, 3.7% and 4.3%.[127] In this audit, resistance to impenem (16%) and piperacillin (8%) were considerably higher than observed by Henwood *et al.*[127]

The average age of patients included in this audit was 64 years, although patients with surgical wounds were slightly younger than those with other wound types. The older age group found here, is reflected in the literature where wounds of different aetiologies have been found to have increasing prevalence with increasing age. For example, leg ulceration has been found to increase for both sexes with age, up to >8 per 1000 population in those aged 85 years and over.[205] The incidence of pressure ulcers amongst the elderly has been found to significantly increase with increasing age.[191] The incidence of foot ulceration in patients with diabetes has also been seen to increase with advancing age.[206]

In this audit, patients were not more likely to be female, as has been previously described in the literature for patients with chronic wounds, particularly venous leg ulcers. Prevalence studies such as that by Callam *et al.*[6] identified many more women with chronic ulceration of the leg than men (ratio of 1:2.8). However, more recently Margolis *et al.*[11] found (using the UK General Practice Research Database) the incidence of venous leg ulcers per 100 person-years to be overall greater in females than males (1.42 95% CI (1.35, 1.48) and 0.76 95% CI (0.71, 0.83) respectively) but they did not find the difference to be statistically significant at any age group except the 86-91 years, suggesting the overall difference in incidence is reflective of the longer life expectancy of women.[11] In a study of a large wound care database Margolis *et al.*[207] found males with venous leg ulcers were slightly less likely to heal than females (OR 1.10 (95% CI 1.04, 1.16)). It may be that if males are less likely to heal than females, they are more likely to end up being treated in a referral centre and hence the equal representation of males and females in this audit.

As previously highlighted in Chapter 2, systemic antibiotics are frequently given to patients with chronic wounds. The three most frequently prescribed antibiotic groups for patients included in the audit were flucloxacillin, ciprofloxacin and cephalosporins and β-lactamase resistant penicillins. Furthermore, three or more antibiotic groups had been received by 20% of patients in the previous three months, suggesting a wide variety of treatments were being used. In Chapter 2, it was seen that the most commonly used antibiotic for patients with chronic wounds in primary care was flucloxacillin. While this still appears to be frequently used for these patients being treated in tertiary care setting for their wounds, ciprofloxacin is used to a similar level as are antibiotics from the cephalosporin and β–lactamase resistant penicillins.

Topical treatments were regularly used for the patients included in the audit, although the evidence supporting the use of such agents is limited. Iodine has been shown to significantly increase the healing rate and reduce the time to healing compared with patient matched control lesions, although there are numerous flaws with this research as discussed in Chapter 1.[64] A systematic review by O'Meara *et al.*[42] (undertaken before the publication by Fumal *et al.*[64]) found the evidence on the use of silver based products to be conflicting and that there was no evidence to suggest that polynoxylin paste, mupirocin 2% impregnated dressing or povidone iodine 10% were of benefit in the treatment of venous leg ulcers. The review concludes that several topical agents may be helpful but that further research is required to ascertain effectiveness.[42]

Dressings can be used to promote a moist wound environment that can accelerate healing. However, the evidence on which to base dressing choice is limited. This is highlight by a systematic review of dressings and topical agents for surgical wounds healing by secondary intention undertaken by Vermeulen *et al.*[208] This review, which searched five databases and had no limits regarding language or date of publication,

identified 581 potential studies, of which only 14 met the inclusion criteria. These criteria included an outcome measure of objectively measured wound healing or pain (measured either by consumption of painkillers or on a visual analogue scale), as well as an assessment of study quality (randomisation, allocation concealment, sufficient follow-up, intention to treat analysis, blinding, group comparability at baseline and similarity of treatment apart from intervention). There was greatest evidence comparing foam to gauze, where foam was found to be preferable due to pain reduction, patient satisfaction and nursing time. However, while the review itself may have been of a good standard, the evidence available for inclusion in the review meant the authors had to conclude that there was "insufficient evidence to show that the choice of dressing or topical agent affects the healing of surgical wounds by secondary intention". There is an equal lack of evidence for choosing the optimal dressings for other chronic wounds. For example, Hutchinson *et al.*[56] found there to be insufficient evidence to support the effectiveness of any type of dressing or topical agent above another in the treatment of diabetic foot ulcers. Due to this lack of evidence, it is unsurprising that a wide variety of dressing were removed from wounds.

3.5.3 Strengths and Weaknesses

This study was undertaken in a specialist wound healing clinic. The patients treated in these clinics had been referred from primary care, with the exception of surgical patients who could be referred directly from secondary care. This therefore, represents a specific population of patients that are unlikely to be representative of the general population of patients with chronic wounds. This has been indicated by the range of wound-types, long durations of non-healing and general levels of co-morbidity. For example, wound duration has been found to be a significant risk factor for non-healing in VLUs,[209] therefore it is reasonable to expect patients at a referral centre to have

wounds of longer duration than the general population of patients with chronic wounds (the majority of which are treated in primary care). Cornwall *et al.*[192] in a prevalence study of leg ulcers in England found 50% to have been present for longer than a year, compared to more than 60% here.

It is a potential weakness of this work, that the data collected regarding wound and patient characteristics and wound management were retrospectively collected from routinely recorded data. The disadvantages of this method of data collection include potential problems arising from missing data and the lack of means for confirming the accuracy or completeness of records. However, the background and wound characteristic data were recorded by specialist nurses with extensive experience and training in the treatment of chronic wounds, and therefore were likely to be accurate. A further difficulty with the use of routinely recorded data may be that it is not up to date. The nurses' notes included background information completed for every patient at their first visit and wound healing and treatment data recorded for each visit (to enable wound progress to be tracked). For patients who had been treated at the wound clinic for a number of years it is possible that the background data were out of date, for example data indicating co-moribidities. This is, however, unlikely to impact on the main findings of the audit, and would not have affected wound characteristics which were recorded at every visit.

Where appropriate, data were collected from several different sources, including nurses' notes, general practitioners and hospital notes. For antibiotic data it was possible and appropriate to compare data from different sources to give an indication of completeness. It was likely that antibiotic consumption data for patients without GP data were underestimated for certain antibiotics (e.g. flucloxacillin). No differences were observed in the percentage of patients that were prescribed other antibiotic groups

such as cephalosporins and β-lactamase resistant penicillins, and ciprofloxacin. This assumes that there were no systematic differences in the treatment of patient for whom data were and were not available that could potentially introduce bias. The antibiotic data obtained from medical sources were also compared to patients' recalled antibiotic consumption. While for the majority of patients the results (of any antibiotic in the previous three months) were found to concur (79.3%), 31 patients recalled contradicted the other data sources. This was less frequent for patients with data from GP compared to patients just from the nurse and hospital notes (14.6% compared with 31.3%). Patients were only asked about overall antibiotic consumption in the previous three months, and were not asked about specific antibiotics they had received. This may have impacted on the findings, as others have found that the sensitivity of self-reporting of antibiotic use may increase with more detailed questioning (i.e. together with picture cards depicting brand and generic names).[210] Other studies have found that age influences recall accuracy, such that younger patients have more accurate recall.[211]

The microbiological findings in this audit were based on swab samples from the wound. Swabs were taken in accordance with the usual procedure for swab taking at the clinics. In the literature there is no single swabbing method, which has been shown to be of greater accuracy in identifying the organisms involved in infection, and in addition, this audit was interested in identifying organisms colonising the wound as these are the likely pathogens if infection occurs. In terms of identifying wound pathogens, tissue biopsy is considered the gold standard as historically a quota of $>10^5$ bacteria per gram of tissue has been associated with delayed healing. However, Davies *et al.*[23] have shown that biopsies do not have greater predictive power, above that obtained using swabs, in the prediction of healing for non-infected chronic venous leg ulcers. Similarly, the use of swabs for culturing micro-organisms from chronic wounds has been compared with deep tissue specimens taken by surgical debridement and found to

yield identical organisms in 62% of wounds.[212] All organisms yielded by deep tissue methods plus additional organisms identified by the swab were found in 20% of wounds and in only 18% of wounds were organisms identified in the deep tissue that were not identified by swab culture. The accuracy of swabs was found to be greater for wounds which did not extend to the bone (all organisms isolated by deep tissue culture were also found in swabs in 90% of wounds not extending to bone and in 65% of wounds extending to bone).[212] The microbiological work is a strength of this study. Swabs were processed both on selective and non-selective media to identify the organisms of interest and standard isolation procedures were followed. Meticillin resistance was confirmed by isolation of the *mec*A gene.

It is a limitation to the interpretation of the results at this stage that no statistical comparisons were made. Data were instead presented in descriptive format. This makes assessment of the significance of differences between variables difficult; however, due to the number of potentially interesting factors associated with resistance in this population, a statistical association between two variables might be misleading. This is because the impact of other perhaps closely correlated or confounding variables would not be identified. For this reason all statistical investigation of these data is presented in Chapter 4. Furthermore, sample size calculations were not conducted to ensure sufficient patients were included in the audit, but a convenience sample over a ten-week time frame were used. All patients attending the wound healing clinics with chronic wounds were eligible for inclusion in the audit, and an estimated 56.5% were included. There was no reason to consider that this estimate does not represent the true participation rate for the whole study period, despite being estimated from the participation rate of the first 122 (81%) of participants. Reasons for non-participation included healed wounds and nurses forgetting to asked patients. There is no reason to

consider that the population included in the audit do not represent the population of patients that attend this specialist wound healing clinic.

Finally, this work was conducted as an audit to gain specific local intelligence on the extent of antibiotic resistance in this defined population. The main driver in starting this audit was to determine whether patients who were receiving empirical treatment for wound infection were being effectively treated. This was very much a question addressing the optimum treatment practice of the patients attending this wound clinic, and was not intended to be generalisable to all patients with chronic wounds. It has been previously recognised that although routine cultures are rarely indicated in the treatment of individual cases, knowledge of the bacteria growing in ulcers treated at specific centres and the antimicrobial susceptibilities can be useful for predicting the bacteria causing systemic or other infections derived from the ulcers and hence the most appropriate treatment.[22] Empirical treatment of chronic wounds allows for timely treatment of infected wounds, however, like any empirical treatment, this will only be successful if the likely organism and susceptibilities can be predicted. The results of the audit were fed back to clinicians and the changes in practice discussed, such as reduced use of ciprofloxacin. Furthermore, several specific cases of patients who had been receiving long-term or recurrent antibiotics but were still not responding, were reviewed in the light of the audit results and management changed. The audit cycle was completed and the prescription of oral antibiotics has been decresased at the Wound Healing Clinic (Appendix 3.1).

Clinical audit is considered to be the component of clinical governance that offers the greatest potential to assess the quality of care being provided for NHS users. There are many similarities between audit and research, in terms of methods, execution and analysis.[213,214] Here, the use of audit was not intended to bypass the need for ethical

review and, indeed, the project was subject to review by the Wound Healing Audit Group several months prior to starting.

3.5.4 Implications for clinicians and for future research

This audit was designed to identify the prevalence of organisms commonly isolated from chronic wounds and their resistant isolates to ensure effective empirical treatment protocols. This work therefore focussed on MRSA and resistance of *P. aeruginosa* to a number of antibiotics, the most clinically relevant being ciprofloxacin. No previous work reporting antibiotic resistance prevalence in a broad range of chronic wounds has been reported in the UK.

The implications for the clinicians involved in this unit, and the changes in practice that were suggested, included attempts to decrease the use of ciprofloxacin within the unit and review of patients on long-term antibiotics. These ideas and changes may be appropriate for others working in UK specialist wound healing clinics, however the external validity of the audit outputs are likely to be low.

The patients included in this audit have many of the risk factors cited in the literature as being associated with the occurrence of antibiotic resistance. Furthermore, several factors were identified that could potentially be associated with the carriage of MRSA or ciprofloxacin-resistant *P. aeruginosa*, including wound classification, recurrence, size, static wound edge, previous hospitalisation, the type of dressing removed and antibiotic usage. The independent influence of each factor cannot be determined through simple comparison of groups however due to potential confounding and the difficulty in eliciting the true relationship. For example, wound classification and wound size both appeared associated with resistant organisms, however, wound size also differs with wound classification. Establishing the independent association

between such factors and antibiotic resistance requires statistical modelling of data (Chapter 4). Such modelling is of use to the clinicians to identify patients who are at higher risk of infection with an organism resistant to empirical treatment.

Chapter 4 . Risk factors predicting carriage of antibiotic-resistant organisms in chronic wounds

4.1 Abstract

Risk factors previously identified for carriage of antibiotic resistant organisms include previous hospitalisation, antibiotic usage, nursing home residency and co-morbidity. Patients with chronic wounds have a higher prevalence of many of these characteristics compared to the general population. Risk factors for the carriage of antibiotic resistant organisms in chronic wounds in the UK are, however, unknown. Here, those factors associated with carriage of meticillin-resistant *Staphylococcus aureus* (MRSA) and ciprofloxacin resistant *Pseudomonas aeruginosa* in the chronic wounds of patients attending a specialist wound healing clinic were investigated.

The associations between carriage of antibiotic resistant organisms and both known risk factors and wound characteristics were explored. Univariable analysis was used to select significant variables (at three significance levels) for inclusion in multivariable models. Exploratory multivariable models were built using both forward stepwise and backward elimination automated logistic regression procedures. Final models were analysed for fit and predictive ability.

Wound characteristics are of potential importance in the carriage of ciprofloxacin resistant *P. aeruginosa* but not MRSA. MRSA carriage in patients with chronic wounds was associated with previous MRSA and use of 'other' systemic antibiotics and there was evidence of an interaction between these two variables. Wound healing , however, appeared to be protective against the carriage of ciprofloxacin resistant *P. aeruginosa*. Furthermore independent associations were identified between this organism and wound aetiology, ciprofloxacin usage, 'other' systemic antibiotics and 'other' topical antimicrobials. The magnitude of these associations, and the generalisability of the findings to other healthcare settings, requires investigation in further studies.

4.2 Introduction

The carriage of antibiotic resistant organisms has previously been associated with many factors: well known risk factors for antibiotic resistance include hospitalisation, male sex, invasive devices, co-morbidities, and previous antibiotic usage, especially fluoroquinolones, Patients with chronic wounds are frequently exposed to many established risk factors for antibiotic resistance. However the specific risk factors associated with antibiotic resistance in chronic wounds have not previously been investigated. It has been suggested that contamination of wounds by patients themselves, inanimate objects or healthcare staff, long term use of antibiotics, previous hospitalisation and comorbidity may all be risks that could lead to the carriage of antibiotic resistant organisms in chronic wounds.[141]

It is unknown whether any wound specific factors, such as duration or aetiology, are associated with carriage of antibiotic resistant organisms. Wound factors could reasonably be hypothesised to influence the carriage of antibiotic resistant organisms. For example, wound microbial flora is known to differ for wounds of different aetiology,[27] but it is unknown if certain wound aetiologies are more likely to carry antibiotic resistant organisms. Wound duration may be associated with an increased likelihood of carriage of antibiotic organisms, due to increased exposure time for colonisation.

Logistic regression is a statistical method that can be used to explore the association between independent values and a binary outcome variable. Regression analysis has, in the words of Hosmer and Lemeshow, 'become an integral component of any data analysis concerned with describing the relationship between a response variable and one or more exploratory variables'.[215]

The aim of this chapter is to explore the association between antibiotic resistance and wound characteristics and to identify whether wound factors explain the data over and above previously identified and well established risk factors including antibiotic consumption. This study is therefore an exploratory model building exercise to identify the most parsimonious models with the greatest predictive ability to identify cases of antibiotic resistance.

4.3 Methods

4.3.1 Study Population

Anonymised data from an audit of patients attending a specialised out-patient wound healing clinic were utilised. A full description of the study population, data collection and audit outcomes has been given in Chapter 3.

4.3.2 Data analysis and model building

To explore the influence of wound variables on antibiotic resistance carriage in this population, the following comparisons were explored:

Comparison 1: MRSA compared to no MRSA

Comparison 2: Ciprofloxacin resistant *P. aeruginosa* compared with no ciprofloxacin resistant *P. aeruginosa*

4.3.3 Variables in the model

Models were constructed using a base set of seven variables identified from the literature as associated with carriage of antibiotic resistant organisms. This base set of variables were:

1) Age (*continuous variable*)
2) Male gender
3) Previous hospitalisation
4) Nursing home residency
5) Previous MRSA (for MRSA models)
6) Previous antibiotic usage
7) Co-morbidities

The additional variance that could be explained by wound characteristics, over and above those factors already known to explain some of the variation from the literature, was investigated. The wound characteristics explored were:

8) Wound duration (>1 year)
9) Wound size (>10cm^2)
10) Number of wounds
11) Wound recurrence
12) Wound classification (*4 categories: leg ulcer, foot ulcer, surgical wound or miscellaneous wound*)
13) Evidence of infection
14) Wound healing
15) Number of visits to wound clinic

All variables except wound healing are described in Chapter 3. The variable *wound healing* was constructed from data on the state of the wound bed and edge described in Chapter 3. Wounds were coded as healing when the wound bed was healed, islands of epithelium were present or the wound was described as epithelialising. Where none of these events were stated, wound healing was recorded as absent.

Unless otherwise stated, the variables were binary categorical variables and were coded 0 when absent and 1 when present. Wound classification had four categories coded: 0:leg ulcer, 1:foot ulcer, 2:surgical wound and 3 miscellaneous wound, based on the wound groups as given in Chapter 3 (Table 3.1). The continuous variable, patient age, was transformed to obtain a distribution that did not differ from the normal distribution. The continuous variables, number of antibiotic groups in the previous 90 days, the number of visits to the wound healing clinic and the number of wounds, could not be appropriately transformed and were therefore categorised into binary variables (Appendix 4.1).[216] Antibiotic usage was grouped as described in Chapter 3. Systemic antibiotics were i) penicillins (including amoxcillin and ampicillin), ii) flucloxacillin, iii) cephalosporins, ß-lactamases, iv) macrolides, v) ciprofloxacin vi) clindamycin, vii) metronidazole and viii) other. Topical antimicrobial groups were i) silver, ii) iodine, iii) metronidazole and iv) other.

The impact of missing data on the analysis was minimised using several methods. The *last observation carried forward* method was used to decrease the quantity of missing data for the wound area variable.[217] This involved using the most recently recorded data on wound area and decreased the quantity of missing data from 30 cases to 25 cases. Wounds recorded as circumferential (extending all the way around the leg) or multiple areas were categorised as $\geq 10cm^2$. No appropriate method was available to decrease the quantity of missing data on wound duration. For two variables it was considered appropriate that where there was no indication the variable was present, it could be assumed to be absent. This was only appropriate for wound recurrence and signs of healing because both these variables were determined from data obtained from the nursing notes, which were available for all patients. Values in the variables describing antibiotic use were categorised as missing data when no hospital or GP data were collected, unless use had been specifically recorded in the nursing notes. For cases with

at least one of these data sources, antibiotic use was considered to be zero when it was not identified. Some of the issues surrounding this assumption have been discussed in Chapter 3.

To minimise the number of variables entered into the models and so decrease the risk of over-fitting the models, variables were initially explored using univariable analysis. For categorical variables a Chi-squared statistic was used to investigate the association between each independent variable and the dependent variable. For continuous variables a univariable logistic regression predicting outcome status was fitted to determine the statistical significance of the association between the variable and the outcome.[215,218]

Variables associated with resistance in univariable analysis with a significance level of $P<0.25$ were selected for further analysis in the multivariable logistic regression model. The impact of selecting only those variables with a univariable significance level of $p<0.1$ and $p<0.05$ was also investigated. Variables were excluded if they had a prevalence of <5% or if they had >10% missing data.[218] Where two or more variables were considered similar in the data they represented and had considerable overlap, only one variable was included in the multivariable model.

Automated stepwise logistic regression analysis was undertaken to explore the data and identify which independent variables were likely to be associated with resistance. Initial models were fitted using forward stepwise regression procedures (with removal based on probability of a likelihood ratio statistic). Variables were included in the model if the significance of the score statistic was $p\leq 0.05$ and removed from the model if the probability $p\leq 0.1$, derived from the likelihood-ratio statistic. Further models were fit using backward elimination regression (where variables were excluded based on the likelihood ratio statistic $p\leq 0.05$). Finally, where interaction terms were to be

explored, the models were fitted using only those independent variables significantly associated with the dependent variable in the final stepwise models. In this way, more cases were employed in the analysis, due to the case-wise nature with which SPSS deals with missing data.

Overall fit of the models was assessed using the likelihood ratio chi-squared statistic, testing the null hypothesis that all regression co-efficients are zero, except the constant. The fit of the model to the observed data was tested using Hosmer-Lemeshow's and Pearson Chi-squared goodness-of-fit tests and the strength of association reported using Nagelkerke's R-square. Sensitivity, specificity, positive-predictive and negative predictive values were calculated from the classification tables. Outliers and influential data points were investigated through the residuals and leverage statistic.

All statistical analyses were conducted using SPSS Version 12.0.1.

4.3.4 Sample size

A statistician at Cardiff University calculated the required sample size. Box 4.1 gives details of the calculation, which was based on the number of base and exploratory variables. A minimum sample size of 115 patients was estimated to have 80% power to detect an increase of 0.12 in R^2 associated with the wound variables of interest, over and above the R^2 explained by known risk factors. There were nine variables of interest included in the sample size calculation as at the time of the estimation, the intention was to explore the impact of the wound dressing removed independently, however, these data were later incorporated into the appropriate topical antimicrobial categories, as 50% of the dressings contained a topical antimicrobial or were applied with one.

Box 4.1 Summary of sample size calculation

Power for a test of the null hypothesis

The model will include (A)7 covariates, which will yield an R-squared of .120. It will include (B) 9 variables in the set of interest, which will yield an increment of .120. The model will also include (C) 0 variables entered subsequent to the set of interest, which account for an additional.000 of variance. The total R-squared for the 16 variables in the model is .240.

The power analysis focuses on the increment for the set of interest (B) over and above any prior variables (i.e. 9 variables yielding an increment of 0.12). With the given sample size of 115 and alpha set at .05 the study will have power of 0.80

The test is based on Model 2 error, which means that variables entered into the regression subsequent to the set of interest will serve to reduce the error term in the significance test, and therefore are included in the power analysis.

This effect was selected as the smallest effect that would be important to detect, in the sense that any smaller effect would not be of clinical or substantive significance. It is also assumed that this effect size is reasonable, in the sense that an effect of this magnitude could be anticipated in this field of research.

Notes
Power computations: Non-central F, Model 2 error

Professor P.E. Price, Wound Healing Research Unit, Cardiff University. 2005

4.4 Results

Data were available on 150 patients: 15 patients yielded MRSA and 16 patients were found to have ciprofloxacin-resistant *P. aeruginosa.*

4.4.1 Data transformations

Transformations to normalise the continuous variables were effective for patients' age but not for the number of antibiotic groups taken in the previous 90 days, the number of visits to the wound healing clinic in the previous year or the number of wounds present. Appendix 4.1 shows the data transformations and tests for normality. Patient age was transformed using the square root of the reverse scores (i.e. $\sqrt{((age_{max}+1) - age_i)}$; where age_{max} is the highest value of the variable age and age_i is the age for case i.). The other three variables that could not be appropriately transformed due to the extent of departure from the normal distribution were categorised (Appendix 4.1).

4.4.2 Univariable analysis

The results of the univariable analysis of each independent variable against the dependent variables, MRSA and ciprofloxacin resistant *P. aeruginosa* are shown in Table 4.1. Table 4.1 also shows the prevalence of each variable and the quantity of missing data.

Table 4.1 Association between base and exploratory variables and antibiotic resistant organisms (MRSA and ciprofloxacin-resistant *P. aeruginosa*)

a. Categorical variables

Variable	Number (%) of patients	Prevalence of antibiotic resistance		Missing cases	Univariable analysis MRSA			Univariable analysis ciprofloxacin resistant PA		
		MRSA (%)	CipR PA (%)		Chi-sq value	p-value	Statistic	Chi-sq value	p-value	Statistic
Male	69 (46.0)	7 (10.1)	7 (10.1)	0	0.003	0.956	Pearson	0.037	0.848	Pearson
Overnight stay in hospital in previous 90 days	25 (18.1)	5 (20.0)	2 (8.0)	12	-	0.042	Fishers	-	1.000	Fishers
Nursing home or hospital residency	6 (4.0)	2 (33.3)	0 (0.0)	0	-	0.111	Fishers	-	1.000	Fishers
Previous MRSA	25 (18.0)	8 (32.0)	N/A	11	-	0.000	Fishers	N/A	N/A	N/A
Any systemic antibiotic	89 (62.7)	10 (11.2)	11 (12.4)	8	-	0.211	Fishers	0.814	0.367	Pearson
Number of systemic antibiotic groups (>2 groups)	28 (20.0)	4 (14.3)	4 (14.3)	10		0.258	Fishers	-	0.501	Fishers
Penicillin group	12 (8.7)	0 (0.0)	1 (6.7)	12	-	0.600	Fishers	-	1.000	Fishers
Flucloxacillin group	36 (25.5)	5 (13.9)	5 (13.9)	9	-	0.184	Fishers	-	0.532	Fishers
Cephalosporin group	36 (26.1)	4 (11.1)	6 (16.7)	12	-	0.511	Fishers	-	0.218	Fishers
Macrolide group	21 (15.2)	1 (4.8)	1 (4.8)	12	-	0.692	Fishers	-	0.467	Fishers
Ciprofloxacin group	36 (25.5)	4 (11.1)	9 (25.0)	9	-	0.503	Fishers	-	0.003	Fishers
Clindamycin	11 (7.9)	0 (0.0)	2 (18.2)	10	-	0.598	Fishers	-	0.334	Fishers
Metronidazole	12 (8.7)	3 (25.0)	3 (25.0)	12	-	0.071	Fishers	-	0.125	Fishers
Other systemic antibiotic	19 (13.7)	7 (36.8)	0 (0.0)	11	-	0.000	Fishers	-	0.223	Fishers
Any topical antimicrobial	83 (57.2)	12 (14.5)	15 (18.1)	5	3.541	0.060	Pearson	12.498	0.000	Pearson
Silver	49 (34.5)	8 (16.3)	7 (14.3)	8	-	0.062	Fishers	1.097	0.295	Pearson
Iodine	32 (22.7)	4 (12.5)	5 (15.6)	9	-	0.522	Fishers	-	0.331	Fishers
Metronidazole	4 (2.9)	1 (25.0)	0 (0.0)	12	-	0.308	Fishers	-	1.000	Fishers
Other topical antibiotic	23 (16.4)	2 (8.7)	7 (30.4)	10	-	1.000	Fishers	-	0.004	Fishers
Relevant co-morbidity	103 (71.0)	9 (8.7)	12 (11.7)	5	-	0.370	Fishers	-	1.000	Fishers

a. Categorical variables - continued

Variable	Number (%) of patients	Prevalence of antibiotic resistance		Missing cases	Univariable analysis MRSA			Univariable analysis ciprofloxacin resistant PA		
		MRSA (%)	CipR PA (%)		Chi-sq value	p-value	Statistic	Chi-sq value	p-value	Statistic
Wound duration (> 1 year)	77 (58.3)	9 (11.7)	8 (10.4)	18	0.228	0.633	Pearson	0.377	0.539	Pearson
Wound size (>10cm^2)	58 (46.4)	7 (12.1)	10 (17.2)	25	0.760	0.383	Pearson	7.281	0.007	Pearson
Number of wounds (> 1 wound)	62 (43.4)	7 (11.3)	10 (16.1)	7	0.279	0.597	Pearson	2.688	0.101	Pearson
Wound recurrence	56 (37.3)	6 (10.7)	9 (16.1)	0	0.051	0.822	Pearson	2.739	0.098	Pearson
Wound classification: leg ulcer	77 (51.3)	5 (6.5)	11 (14.3)	0	3.133	0.372	LR	11.971	0.007	LR
foot ulcer	20 (13.3)	4 (20.0)	4 (20.0)							
surgical wound	42 (28.0)	5 (11.9)	0 (0.0)							
miscellaneous	11 (7.3)	1 (9.1)	1 (9.1)							
Evidence of infection	50 (33.3)	4 (8.0)	7 (14.0)	0	0.333	0.564	Pearson	0.875	0.350	Pearson
Wound healing	48 (32.0)	5 (10.4)	2 (4.2)	0	-	1.000	Fishers	3.130	0.077	Pearson
>4 visits to wound healing clinic	66 (46.8)	8 (12.1)	9 (13.6)	9	0.667	0.414	Pearson	0.646	0.421	Pearson

b. Continuous variables

Variable	Missing cases	Univariable analysis MRSA				Univariable analysis Cip R			
		OR	95% CI	p-value	Statistic	OR	95% CI	p-value	Statistic
Age - transformed	0	1.105	(0.776, 1.573)	0.579	Logistic regression	0.777	(0.552, 1.095)	0.149	Logistic regression

PA: *P. aeruginosa*; CipR: ciprofloxacin-resistant

4.4.3 Prediction model for MRSA

4.4.3.1 Model structure

The results of the univariable analyses investigating carriage of MRSA are shown in Table 4.1. Variables eligible for inclusion in the multivariable models investigating MRSA carriage were:

- overnight stay in hospital in the previous 90 days
- previous MRSA
- systemic flucloxacillin in the previous 90 days
- systemic metronidazole in the previous 90 days
- other systemic antibiotics in the previous 90 days
- topical silver in the previous 90 days

Residency in a nursing home or hospital was associated with MRSA in univariable analysis (p=0.111). However only six (4%) patients were resident in such a location and therefore this variable was not investigated further. Any topical antimicrobial in the previous 90 days was also associated with MRSA in univariable analysis, however, topical silver contributed a high proportion of topical antimicrobial usage and subsequently there was considerable overlap between the two variables (116 (77.3%) of data points correlated, X=58.74, p<0.001). The variable regarding any topical antimicrobial was excluded from further analysis and only the more specific variable topical silver investigated further. Similarly, any systemic antibiotic in the previous 90 days was associated with MRSA in univariable analysis (p<0.25), however, any systemic antibiotic was excluded from multivariable analysis due to the high overlap with the specified systemic antibiotic variables included in the model: flucloxacillin, metronidazole or other systemic antibiotics in the previous 90 days. 107 of the 142 (75.4%) data points correlated with 54 (60.7%) of the 89 occurrences of any systemic

antibiotic being contributed by flucloxacillin, metronidazole or other systemic antibiotics.

With significance levels in univariable analysis of $p<0.25$, $p<0.1$ and $p<0.05$ both forward stepwise and backward elimination binary logistic regression methods, without interaction terms, generated models which indicated that carriage of MRSA in chronic wounds was associated with previous MRSA and the use of systemic antibiotics classified as other. This variable 'other systemic antibiotics' included those antibiotics that did not logically fit into the antibiotic categories used for the purposes of this study (Chapter 3). This variable is broken down in Table 4.2.

Table 4.2 Antibiotics and their usage that make up the variable 'Other systemic antibiotics'

Antibiotic	Number of patients	Percentage of patients included in variable*
Trimethoprim	10	55.56
Sodium fusidate	3	16.67
Tetracyclines	2	11.11
Doxycycline	2	11.11
Vancomycin	2	11.11
Gentamicin	1	5.56
Minocycline	1	5.56
Oxytetracycline	1	5.56
Rifampicin	1	5.56
Co-trimoxazole	1	5.56
Total	18	100

* Total percentage >100% as some patients received more than one of the antibiotics

Table 4.3 shows the final model using forward stepwise regression methods (MRSA Model 1), which included 126 MRSA negative patients and 12 MRSA positive patients. A total of 12 patients were excluded due to missing data. Models generated following selection of variables from univariable analysis using significance levels of $p<0.1$ and $p<0.05$ were identical to MRSA Model 1, selected at $p<0.25$ (Table 4.3). Furthermore, models built using backward elimination methods with selection of variables from

univariable analysis at significance levels of $p<0.25$, $p<0.1$ and $p<0.05$ all generated identical models.

The models were refit including only those variables remaining in the final stepwise model. The variables (previous MRSA and 'other' systemic antibiotics) were entered as a block into a logistic regression model. This resulted in 139 patients being included in the analysis, 12 of who carried MRSA (MRSA Model 2, Table 4.4). This model was extended to explore interaction between the two significant variables (MRSA Model 3, Table 4.5). The inclusion of this interaction term, changed the strength of the association between previous MRSA and current MRSA carriage, thus suggesting interaction was present between the variables previous MRSA and other systemic antibiotics.

Table 4.3 MRSA Model 1: final structure of model with independent variables investigated using forward stepwise logistic regression

	B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I. for EXP(B)	
							Lower	Upper
Previous MRSA	1.573	0.713	4.869	1	0.027	4.821	1.192	19.498
Other systemic antibiotics group	2.186	0.716	9.337	1	0.002	8.903	2.190	36.189
Constant	-3.497	0.529	43.770	1	0.000	0.030		

Table 4.4 MRSA Model 2: model with only the variables previous MRSA and other systemic antibiotics entered into the model

	B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I. for EXP(B)	
							Lower	Upper
Previous MRSA	1.634	0.706	5.361	1	0.021	5.123	1.285	20.425
Other systemic antibiotics group	2.118	0.707	8.964	1	0.003	8.316	2.078	33.275
Constant	-3.519	0.532	43.788	1	0.000	0.030		

Table 4.5 MRSA Model 3: model with only the variables previous MRSA and other systemic antibiotics entered into the model, together with their interaction term

	B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I. for EXP(B)	
							Lower	Upper
Previous MRSA	1.655	0.959	2.975	1	0.085	5.231	0.798	34.280
Other systemic antibiotics group	2.140	0.984	4.731	1	0.030	8.500	1.236	58.472
Previous MRSA by Other systemic antibiotics group	-0.045	1.412	0.001	1	0.975	0.956	0.060	15.230
Constant	-3.526	0.586	36.240	1	0.000	0.029		

4.4.3.2 Model fit, predictive value and outliers

i) Classification tables, sensitivity, specificity and predictive values

All models built in a stepwise manor, irrespective of forward or backward methods or the significance level of univariable selection, classified patients according to the Classification Table 4.6 (MRSA Model 1). MRSA Models 2 and 3 classified patients according to the Classification Table 4.7.

Table 4.6 Classification table of observed compared with predicted values for MRSA Model 1 (built using stepwise methods (and including 138 cases))

		Predicted MRSA		Percentage correct
		0	1	
Observed MRSA	0	122	4	96.8
	1	7	5	41.7
Overall Percentage				92.0

The cut value is .500

Table 4.7 Classification table of observed compared with predicted values for MRSA Models 2 and 3 (built including only the variables previous MRSA and other systemic antibiotics (and 139 cases))

		Predicted MRSA		Percentage correct
		0	1	
Observed MRSA	0	123	4	96.9
	1	7	5	41.7
Overall Percentage				92.1

The cut value is .500

The sensitivity, specificity, positive-predictive value (PPV) and negative-predictive value (NPV) associated with the models are shown in Table 4.8.

Table 4.8 Sensitivity, specificity and positive and negative predictive values of the models for predicting MRSA carriage

Model	No. cases	Sensitivity	Specificity	PPV[e]	NPV[f]
None[a]	150	0.0%	0.0%	-	90.0%
1[b]	138	41.7%	96.8%	55.6%	94.6%
2[c] & 3[d]	139	41.7%	96.9%	55.6%	94.6%

a: No model built. All cases presumed to be MRSA negative.
b: MRSA Model 1: Binary Logistic Regression Forward Stepwise Regression ($p<0.25$). These values reflect those obtained in models built with variables selected at significance levels of $p<0.1$ and $p<0.05$, using forward and backward methods.
c: MRSA Model 2: Previous MRSA and other systemic antibiotics only entered (Binary Logistic Regression Enter).
d: MRSA Model 3: Previous MRSA and other systemic antibiotics entered with their interaction term (Binary Logistic Regression Enter).
e: PPV: Positive Predictive Value
f: NPV: Negative Predictive Value

ii) Model fit

In all models, the estimates of Hosmer and Lemeshow Chi-squared statistic from Binary Logistic Regression were non-significant at $p=0.05$ (Table 4.9). Furthermore, re-fitting MRSA Models 2 and 3 using the Multinomial function in SPSS showed Pearson Chi squared goodness-of-fit to be non-significant at $p=0.05$ (Table 4.9). Therefore the models were considered to have adequate fit. Nagelkerke R^2 was 0.311 and 0.306 in MRSA Model 1 and MRSA Models 2 and 3 respectively.

iii) Outliers and leverage points

The three models (MRSA Models 1, 2 and 3) were examined for outliers and leverage measures. Standardised residuals were compared to the predicted probabilities. A good model would not expect to see >5% of the residuals (studentised or standardised) to exceed the absolute value of 1.96 (~2), and no more than 1% to exceed the absolute value of three. All three Models identified the same five (3.6%) outlying cases (standardised and studentised residual > 2 standard deviations) (Table 4.10). All these

cases were MRSA positive but none had received other systemic antibiotics in the previous 90 days, two had (and three had not) previously been colonised with MRSA. In each model, three cases (2.2%) had studentised residuals greater than three.

Two further cases were identified as outliers in Model 2. These cases had studentised residuals greater than two but standardised residuals less than two and neither case was identified as an outlier in Model 1 or Model 3. These two cases were MRSA positive, had not previously been identified as having MRSA but had received antibiotics included in the other systemic antibiotics variable

Table 4.9 MRSA model fit parameters

Model	Model parameter Hosmer-Lemeshow			Nagel-kerke R^2
	Chi-squared	df	p	
MRSA Model 1[a]	0.007	1	0.932	0.311
Binary Logistic Regression Backward elimination ($p<0.25$)	0.441	1	0.507	0.311
Binary Logistic Regression Backward elimination ($p<0.1$ and $p<0.05$)	0.441	1	0.507	0.311
MRSA Model 2[b]	0.001	1	0.999	0.306
MRSA Model 3[c]	0.000	1	1.000	0.306
MRSA Models 2 built using Multinomial Logistic Regression	0.001[d]	1[d]	0.975[d]	0.306
MRSA Models 3 built using Multinomial Logistic Regression	0.000[d]	0[d]	-	0.306

a: MRSA Model 1: Binary Logistic Regression Forward Stepwise Regression ($p<0.25$), 138 cases. These values reflect those obtained in models built with variables selected at significance levels of $p<0.1$ and $p<0.05$.
b: MRSA Model 2: Previous MRSA and other systemic antibiotics only entered (Binary Logistic Regression Enter), 139 cases.
c: MRSA Model 3: Previous MRSA and other systemic antibiotics entered with their interaction term (Binary Logistic Regression Enter), 139 cases.
d: Pearson Chi-squared Goodness of Fit test.

Table 4.10 List of outlying cases (studentised residual greater than 2.000) identified in MRSA Models 1, 2 and 3

Case	MRSA		Model 1[a]				Model 2[b]				Model 3[c]				Variable values	
	Obs	Pred Gp	Pred Val	Unstd Res	Std Res	Studt Res	Pred val	Unstd Res	Std Res	Studt Res	Pred Val	Unstd Res	Std Res	Studt Res	Previous MRSA	Other systemic antibiotics
1021	1	0	0.029	0.971	2.666	5.745	0.029	0.971	2.674	5.809	0.029	0.971	2.679	5.831	No	No
1044	1	0	0.127	0.873	2.079	2.616	0.132	0.868	2.062	2.566	0.133	0.867	2.078	2.550	Yes	No
1057	1	0	0.127	0.873	2.079	2.616	0.132	0.868	2.062	2.566	0.133	0.867	2.078	2.550	Yes	No
1067	1	0	0.029	0.971	2.666	5.745	0.029	0.971	2.674	5.809	0.029	0.971	2.679	5.831	No	No
1106	1	0	0.029	0.971	2.666	5.745	0.029	0.971	2.674	5.809	0.029	0.971	2.679	5.831	No	No
1047	1	0	0.212	0.788	1.830	1.925	0.198	0.802	1.866	2.014	0.200	0.800	1.891	2.000	No	Yes
1150	1	0	0.212	0.788	1.830	1.925	0.198	0.802	1.866	2.014	0.200	0.800	1.891	2.000	No	Yes

a: MRSA Model 1: Binary Logistic Regression Forward Stepwise Regression ($p<0.25$), 138 cases.
b: MRSA Model 2: Previous MRSA and other systemic antibiotics only entered (Binary Logistic Regression Enter), 139 cases.
c: MRSA Model 3: Previous MRSA and other systemic antibiotics entered with their interaction term (Binary Logistic Regression Enter), 139 cases.
Obs: Observed value; Pred Gp: Predicted group; Pred Val: Predicted value, Unstd Res: Unstandardised residuals: Std Res: Standardised residuals; Studt Res: Studentised residuals

Scatterplots of studentised and standardised residuals compared to the predicted outcome of the models were investigated to explore any large residuals, or apparent patterns in the residuals. The difference between studentised and standardised residuals is that studentised residuals include leverage in the equation, so include some part of the influence statistic.[219] Scatterplots of standardised and studentised residuals compared to predicted values are shown in Figure 4.1 and Figure 4.2 for MRSA Models 1 and MRSA Models 2 and 3 respectively. The residuals calculated from the models including the interaction term were very similar to those calculated in the model without the interaction term (and are therefore not reported separately).

Figure 4.1 Scatterplots showing a) standardised and b) studentised residuals plotted against predicted probability for MRSA Model 1

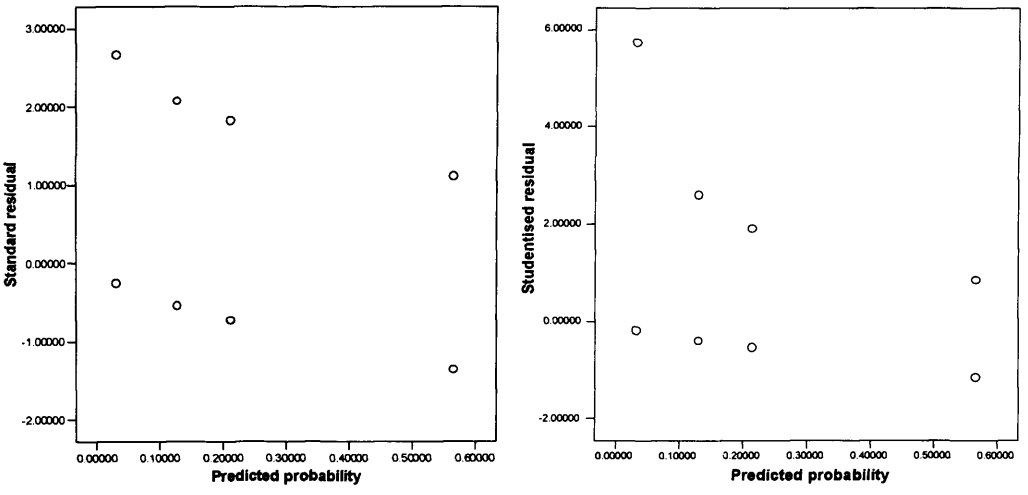
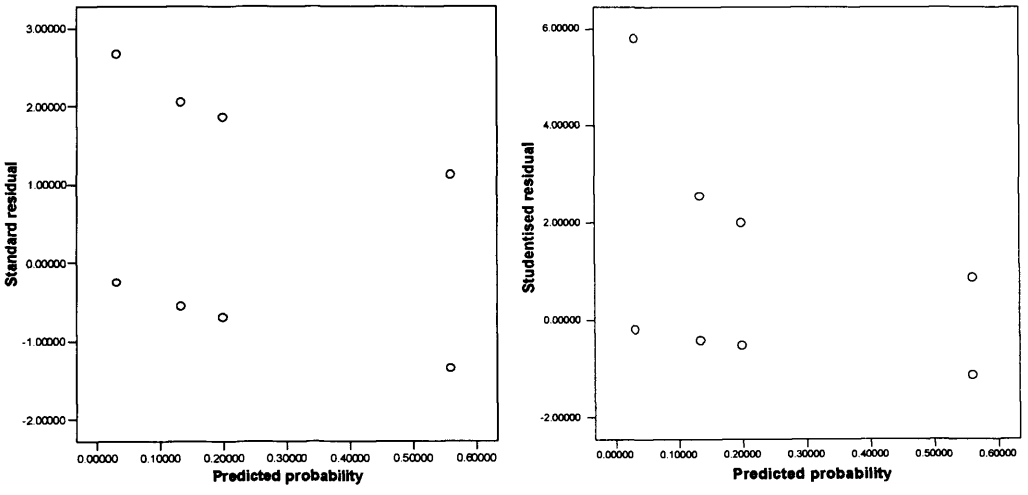


Figure 4.2 Scatterplots showing a) standardised and b) studentised residuals plotted against predicted probability for MRSA Models 2 and 3



It has been suggested that where the leverage statistic is > 2(k+1)/n, cases should be examined in more detail, where k=number of independent variables.[219] When k=2, cases should be examined where leverage >0.04. Thirty-four cases had leverage values >0.04 in all three MRSA Models. Analysis of Cook's distance however, found no values to be greater than one, in any of the three models (indicating cases that might be influential).[219] Cook's distance is a measure of how much all the other residuals would change if each observation were deleted from the analysis.

In conclusion, the different model building techniques all generated predictive models based on previous MRSA status and use of antibiotics classified as other. While the models appear to have reasonable goodness-of-fit through the Hosmer-Lemeshow statistic, examination of the residuals found 2.2% of cases to exceed the absolute value of three in each MRSA Model. This suggests that the models might not have good fit with the data.

4.4.4 Prediction model for ciprofloxacin resistant *P. aeruginosa*

The results from the univariable analysis of the independent variables and their association with ciprofloxacin resistant *P. aeruginosa* are shown in Table 4.1. The following variables were associated with ciprofloxacin resistance in univariable analysis at significance level of p<0.25, and were further explored in multivariable analysis:

- Age
- Cephalosporins in the previous 90 days
- Ciprofloxacin in the previous 90 days
- Metronidazole in the previous 90 days
- Other systemic antibiotics in the previous 90 days
- Other topical antimicrobial in the previous 90 days

- Number of wounds
- Recurrence
- Wound classification
- Wound healing

Two variables (any topical antimicrobials and wound area) were significantly associated with ciprofloxacin resistant *P. aeruginosa* but were not included in further analysis. There was considerable overlap between the variables 'any topical antimicrobial' and 'other topical antimicrobial'. As with the models built to explore MRSA, the more specific variable, other topical antimicrobial, was included in further analysis and any topical antimicrobial excluded. The topical treatments that constituted the variable other topical antimicrobials are shown in Table 4.11.

Table 4.11 Topical antimicrobials and their usage that make up the variable 'Other topical antimicrobials'

Antimicrobial	Number of patients	Percentage of patients included in variable*
Neomycin (Dermovate NN)	17	58.62
Potassium permanganate	10	34.48
Mupirocin/Bactroban	3	10.34
Chloramphenicol	1	3.45
Total	29	100

* Total percentage >100% due to patients receiving >1 antimicrobial

The area of the wound was significantly associated with carriage of ciprofloxacin resistant *P. aeruginosa* in univariable analysis (Pearsons Chi-squared, χ=7.281, p=0.007); however, data were missing for 25 patients (16.7%). This variable was also therefore excluded.

The models generated when all variables significant at a level of $p<0.25$ were explored using forward stepwise and backward elimination multivariable analyses, are shown in Table 4.12. The variables included in the final model were ciprofloxacin, other topical

antimicrobials, wound classification and wound healing. Using backward elimination regression methods, the model included the variables included in the model generated using forward stepwise methods, as well as the variable other systemic antibiotics. Models built using variables significant at $p<0.1$, by both forward stepwise and backward elimination regression methods, included the same variables as the model generated using forward stepwise regression selecting variables with $p<0.25$ in univariable analysis (ciprofloxacin, other topical antimicrobials, wound classification and wound healing). Models built using variables selected by $p<0.05$ in univariable analysis generated final models that included the variables ciprofloxacin, other topical antimicrobials and wound classification. The wound healing variable was not included in the multivariable analysis in this model because it was not sufficiently significant in the univariable analysis.

Table 4.13 shows the model variables and their associated ORs in the final models constructed with variables with a significance level of $p<0.25$, $p<0.1$ and $p<0.05$.

4.4.4.1 Model fit, predictive value and outliers

i) Classification tables, sensitivity, specificity and predictive values

Classification tables are given in Table 4.14, Table 4.15, Table 4.16 and Table 4.17 for the final models built following selection of variables from univariable analysis with significance levels of $p<0.25$ (forward stepwise regression), $p<0.25$ (backward elimination regression), $p<0.1$ and $p<0.05$ respectively. Models built in a stepwise manner, irrespective of forward or backward methods had the same classification tables when $p<0.1$ and $p<0.05$.

The sensitivities, specificities and positive and negative predictive values of four models built using variables with significance level in univariable analysis of $p<0.25$ (forward stepwise regression), $p<0.25$ (backward elimination regression), $p<0.1$ and $p<0.05$ are given in Table 4.18. The model with variables selected at the significance level of $p<0.25$ and built using backward elimination regression had, by most measures, the most accurate predictive ability, with the greatest sensitivity and positive predictive value.

Table 4.12 Final model structure for independent variables association with ciprofloxacin resistant *P. aeruginosa* (built using forward stepwise and backward elimination logistic regression, with variables significant in univariable analysis at p<0.25)

	B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I. for EXP(B)	
							Lower	Upper
Forward Stepwise								
Ciprofloxacin groups	2.163	0.718	9.066	1	0.003	8.693	2.127	35.525
Other topical antimicrobials group	1.937	0.742	6.807	1	0.009	6.938	1.619	29.727
Wound classification								
Leg ulcer			2.116	3	0.549			
Foot ulcer	1.202	0.826	2.116	1	0.146	3.326	0.659	16.791
Surgical wound	-18.983	6063.160	0.000	1	0.998	0.000	0.000	.
Miscellaneous wound	-19.257	12482.964	0.000	1	0.999	0.000	0.000	.
Wound healing	-2.074	0.942	4.845	1	0.028	0.126	0.020	0.797
Constant	-2.687	0.605	19.703	1	0.000	0.068		
Backward Elimination								
Ciprofloxacin group	2.134	0.743	8.240	1	0.004	8.450	1.968	36.284
Other systemic antibiotic	-19.507	8012.179	0.000	1	0.998	0.000	0.000	.
Other topical antibiotic	2.243	0.803	7.807	1	0.005	9.425	1.954	45.471
Wound Classification								
Leg ulcer			3.030	3	0.387			
Foot ulcer	1.559	0.895	3.030	1	0.082	4.752	0.822	27.483
Surgical wound	-18.957	5929.090	0.000	1	0.997	0.000	0.000	.
Miscellaneous	-18.572	11638.227	0.000	1	0.999	0.000	0.000	.
Wound healing	-1.941	0.975	3.962	1	0.047	0.144	0.021	0.971
Constant	-2.685	0.641	17.568	1	0.000	0.068		

Table 4.13 Odds ratios (in the final models) of the variables associated with ciprofloxacin resistant *P. aeruginosa* in chronic wound patients using forward stepwise and backward elimination methods

Variable	Model, P<0.25[a]				Model, P<0.1[a]				Model, P<0.05[a]			
	P-value	OR	95% CI		P-value	OR	95% CI		P-value	OR	95% CI	
			Lower	Upper			Lower	Upper			Lower	Upper
Forward stepwise												
Ciprofloxacin group	0.003	8.693	2.127	35.525	0.003	7.613	1.984	29.209	0.005	5.998	1.718	20.944
Other systemic antibiotic												
Other topical antibiotic	0.009	6.938	1.619	29.727	0.014	5.669	1.417	22.676	0.013	5.269	1.416	19.615
Wound Classification												
Leg ulcer	0.549				0.566				0.814			
Foot ulcer	0.146	3.326	0.659	16.791	0.154	3.046	0.658	14.103	0.330	2.011	0.492	8.215
Surgical wound	0.998	0.000	0.000		0.997	0.000	0.000	.	0.997	0.000	0.000	.
Miscellaneous	0.999	0.000	0.000	.	0.999	0.000	0.000	.	0.999	0.000	0.000	.
Wound healing	0.028	0.126	0.020	0.797	0.047	0.163	0.027	0.972				
Backward elimination												
Ciprofloxacin group	0.004	8.450	1.968	36.284	0.003	7.613	1.984	29.209	0.005	5.998	1.718	20.944
Other systemic antibiotic	0.998	0.000	0.000	0.998								
Other topical antibiotic	0.005	9.425	1.954	45.471	0.014	5.669	1.417	22.676	0.013	5.269	1.416	19.615
Wound Classification												
Leg ulcer	0.387				0.566				0.814			
Foot ulcer	0.082	4.752	0.822	27.483	0.154	3.046	0.658	14.103	0.330	2.011	0.492	8.215
Surgical wound	0.997	0.000	0.000	.	0.997	0.000	0.000	.	0.997	0.000	0.000	.
Miscellaneous	0.999	0.000	0.000	.	0.999	0.000	0.000	.	0.999	0.000	0.000	.
Wound healing	0.047	0.144	0.021	0.971	0.047	0.163	0.027	0.972				

a: Significance level at which variables were selected from univariable analysis

Table 4.14 Classification table of final model built using forward stepwise regression methods to predict ciprofloxacin resistant *P. aeruginosa*, after selecting variables with significance level p<0.25 in univariable analysis

		Predicted ciprofloxacin resistant *P. aeruginosa*		Percentage correct
		0	1	
Observed ciprofloxacin resistant *P. aeruginosa*	0	114	3	97.4
	1	10	5	33.3
Overall Percentage				90.2

The cut value is .500

Table 4.15 Classification table of final model built using backward elimination methods to predict ciprofloxacin resistant *P. aeruginosa*, after selecting variables with significance level p<0.25 in univariable analysis

		Predicted ciprofloxacin resistant *P. aeruginosa*		Percentage correct
		0	1	
Observed ciprofloxacin resistant *P. aeruginosa*	0	115	2	98.3
	1	10	5	33.3
Overall Percentage				90.9

The cut value is .500

Table 4.16 Classification table of model to predict ciprofloxacin resistant *P. aeruginosa*, after selecting variables with significance level p<0.1 in univariable analysis (both forward and backward regression methods)

		Predicted ciprofloxacin resistant *P. aeruginosa*		Percentage correct
		0	1	
Observed ciprofloxacin resistant *P. aeruginosa*	0	121	4	96.8
	1	10	5	33.3
Overall Percentage				90.0

The cut value is .500

Table 4.17 Classification table of model to predict ciprofloxacin resistant *P. aeruginosa*, after selecting variables with significance level p<0.05 in univariable analysis (both forward and backward regression methods)

		Predicted ciprofloxacin resistant *P. aeruginosa*		Percentage correct
		0	1	
Observed ciprofloxacin resistant *P. aeruginosa*	0	122	3	97.6
	1	11	4	26.7
Overall Percentage				90.0

The cut value is .500

Table 4.18 Sensitivity, specificity and positive and negative predictive values of the models for predicting ciprofloxacin resistant *P. aeruginosa* carriage

Model[a]	No. cases	Sensitivity	Specificity	PPV	NPV
P<0.25 F	132	33.3%	97.4%	62.5%	91.9%
P<0.25 B	132	33.3%	98.3%	71.4%	92.0%
P<0.1	140	33.3%	96.8%	55.6%	92.4%
P<0.05	140	26.7%	97.6%	57.1%	91.7%

a: Significance level at which variables selected for inclusion in multivariable model from univariable analysis
b: PPV: Positive Predictive Value
c: NPV: Negative Predictive Value

ii) Model fit

The fit of the models overall is given in Table 4.19. All models adequately fit the data in terms of the Hosmer-Lemeshow statistic. It can be seen however that the overall fit of the models and the variance in the data that they explain decreases as the level of significance required in univariable analysis increased. The model with the greatest fit, that explains the greatest proportion of variance is that built with variables selected at p<0.25, using backward elimination regression methods.

Table 4.19 Model fit parameters for the models of exploring the association between selected variables and ciprofloxacin *P. aeruginosa*.

Model	Model parameter			
	Hosmer-Lemeshow			Nagel-kerke R^2
	Chi-squared	df	p	
Binary Logistic Regression Forward Stepwise (p<0.25)	0.716	6	0.994	0.489
Binary Logistic Regression Forward Stepwise (p<0.1)	1.487	7	0.983	0.453
Binary Logistic Regression Forward Stepwise (p<0.05)	0.260	5	0.998	0.395
Binary Logistic Regression Backward elimination (p<0.25)	2.641	8	0.955	0.539
Binary Logistic Regression Backward elimination (p<0.1)	0.198	7	1.000	0.453
Binary Logistic Regression Backward elimination (p<0.05)	0.260	5	0.998	0.395

iii) Outliers and leverage points

Outliers and influential cases in the model built to explore ciprofloxacin resistant *P. aeruginosa* were examined. The model selected for examination was the one with the greatest predictive values and pseudo R^2: variables selected at p<0.25 in univariable

analysis and determined to be significant in the multivariable analysis using backward elimination regression methods. The model was built using binary logistic regression enter method, including all the appropriate variables.

Outliers

Standardised residuals were compared to the predicted probabilities. Three cases were identified as outliers with both standardised and studentised residuals greater than 2 standard deviations (Table 4.20). Two cases carried ciprofloxacin resistant *P. aeruginosa*, but did not have any of the risk factors association with resistance and one case did not carry ciprofloxacin resistant *P. aeruginosa* but had two risk factors associated with carriage: ciprofloxacin use in the previous 90 days and use of other topical antimicrobials.

One further case was identified as an outlier with studentised residual greater than two (but not standardised residuals). This case carried ciprofloxacin resistant *P. aeruginosa* but had not received ciprofloxacin, other systemic antibiotics or other topical antibiotics in the previous 90 days, the ulcer was not healing and the wound was classified as a foot ulcer. Scatterplots of studentised and standardised residuals compared to predicted values are shown in Figure 4.3.

Figure 4.3 Scatterplots showing a) standardised and b) studentised residuals against predicted probabilities for model predicting ciprofloxacin resistant *P. aeruginosa*

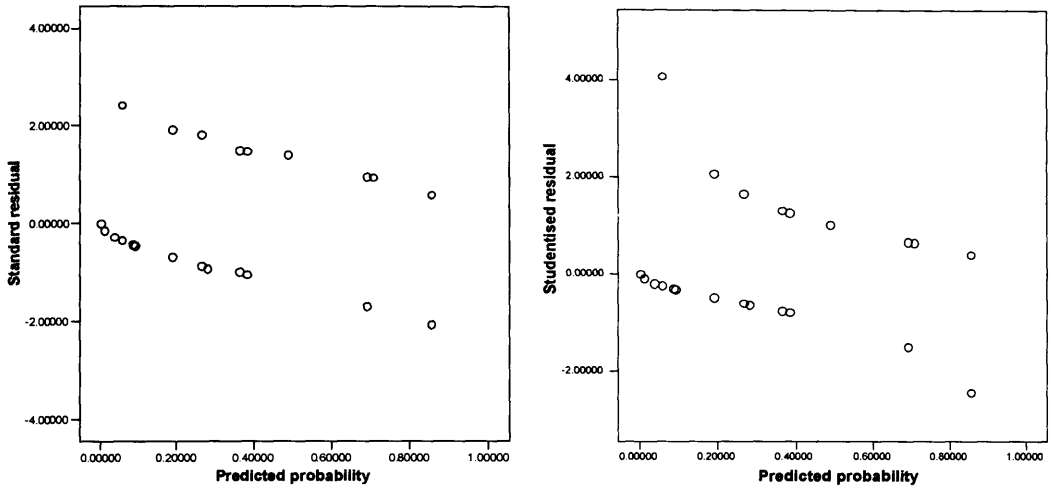


Table 4.20 Outlying cases (studentised residual greater than 2.000) identified in prediction model for ciprofloxacin resistant *P. aeruginosa*

Case	Ciprofloxacin resistance			Residuals			Variable values				
	Observed group	Predicted value	Predicted group	Unstand-ardised	Standard-ised	Student-ised	Ciprofloxacin group	Other systemic antibiotics	Wound classification	Wound Healing	Other topical antibiotics
1006	1	0.057	0	0.943	2.423	4.079	No	No	Leg ulcer	No	No
1063	0	0.856	1	-0.856	-2.066	-2.436	Yes	No	Leg ulcer	No	Yes
1069	1	0.190	0	0.810	1.921	2.064	No	No	Foot ulcer	No	No
1070	1	0.057	0	0.943	2.423	4.079	No	No	Leg ulcer	No	No

Leverage points

Cases should be examined in more detail if the leverage statistic is > 2(k+1)/n. Where, k=5, cases should be examined where leverage >0.08. Forty-one cases had leverage values >0.08. Analysis of Cook's distance (d) found no cases where d>1 (indicating a case that might be influential).[219] Cook's distance is a measure of how much all the other residuals would change if each observation were deleted from the analysis.

In conclusion, ciprofloxacin resistant *P. aeruginosa* in chronic wounds was found to be associated with ciprofloxacin usage, wound classification, other topical antimicrobials and other systemic antibiotics. Four cases had residuals that were greater than desirable in models although a large number of cases appear to have higher than desired leverage, none were identified as unduly influential using Cook's distance. The use of forward stepwise or backward elimination methods led to different models when variables were selected on $p<0.25$ in univariable analysis. The model that included other systemic antibiotics however had greater predictive value.

4.5 Discussion

4.5.1 Main findings

This study found that wound characteristics did not increase the likelihood of carriage of MRSA. Important factors were previous MRSA and use of antibiotics classified, for the purposes of this study, as 'other'. This 'other' antibiotic group included trimethoprim, sodium fusidate and vancomycin amongst others. Modelling indicated that there was a likely interaction between these two significant variables as inclusion of

an interaction term decreased the significance associated with previous MRSA. Interaction occurs between two independent variables when the impact of one variable on the outcome is dependent on the level of the other variable. The potential for interaction was explored due to the link between the variables in clinical terms (these agents can be used as components of MRSA treatment regimens).[220] The inclusion of an interaction term did not improve the predictive ability of the model.

The presence of ciprofloxacin resistant *P. aeruginosa* appeared to be associated with two wound characteristics (wound healing and classification) as well as with previous antibiotic usage. Wound healing appeared to have a protective effect, with wounds showing signs of healing being less likely to carry ciprofloxacin resistant *P. aeruginosa* (OR 0.144, 95% CI 0.021, 0.971). The magnitude of this association should be interpreted with caution due to the exploratory nature of the model building techniques employed. The association with ulcer classification suggested increased carriage in foot ulcers and decreased carriage in surgical wounds and miscellaneous ulcers. It is possible however these wound-related risk factors reflect associations with the carriage of *P. aeruginosa* and not specifically ciprofloxacin resistant strains.

The non-wound factors associated with ciprofloxacin resistant *P. aeruginosa* were related to antibiotic history. These were ciprofloxacin, 'other' systemic antibiotic and 'other' topical antimicrobial usage in the previous 90 days. Ciprofloxacin was the strongest risk factor followed by 'other' topical antimicrobials. The most frequent antimicrobial in the 'other' topical antimicrobial category was neomycin (administered as Dermovate NN). It may be that the association seen between 'other' topical antimicrobials and ciprofloxacin resistant *P. aeruginosa* reflects an underlying skin conditions such as eczema that might favour carriage of pseudomonads rather than having a direct association with ciprofloxacin resistance. The true importance of 'other'

systemic antibiotics on the carriage of ciprofloxacin resistant *P. aeruginosa* was uncertain from the modelling. This uncertainty arose due to the effect the modelling technique had on the inclusion of this variable in the final model structure. The inclusion of 'other' systemic antibiotics did however lead to greater predictive ability of the model, particularly positive predictive value (71.4%).

4.5.2 Relationship to other studies

No study has previously looked at the risk factors for antibiotic resistance in a range of chronic wounds. A few studies have however investigated the risk factors associated with antibiotic resistance in diabetic foot ulcers. Most recently, Kandemir *et al.*[221] investigated the risk factors for multi-drug resistant organisms in diabetic foot ulcers (Texas Wound Classification Grades 1-3) presenting to a tertiary healthcare centre in Turkey over a three-year period from 2002 to 2005. This study included 102 patients with infected DFUs, of whom 36 patients had multi-drug resistant microbes. The most commonly isolated multi-drug resistant organism was MRSA (77.5% of isolates). Both known antibiotic resistant risk factors and wound-related factors were investigated (age, gender, duration of diabetes, glycosylated haemoglobin, neuropathy, wound duration, wound type (neuropathic or ischaemic), history of hospitalisation for the same wound, duration of hospital stay, osteomyelitis, previous antibiotic therapy and antibiotic duration). Multivariable analysis was not undertaken by the authors due to the small number of patients included in the study, however, in univariable analysis hospitalisation duration, history of hospitalisation for the same wound and prior antibiotic therapy were significantly associated with multi-drug resistant infection. This therefore lends support to the findings of this research, that wound-related factors are of limited importance in determining which wounds will carry MRSA.

Hartemann-Heurtier *et al.*[222] also investigated risk factors for multi-drug resistant organisms in DFUs in patients admitted to a specialist diabetic foot unit in France. Microbiology samples were taken from the ulcers (Wagner grade 3-5) of patients consecutively admitted to the unit (and who had at least one overnight stay) during the 3-year period. Although the study aimed to investigate a range of multi-drug resistant organisms, only MRSA and extended spectrum β-lactamase (ESBL) producing enterococci were isolated (from 16% (n=29) and 1.7% (n=3) of wounds respectively).[222] Univariable and multivariable analyses found previous hospitalisation for the same wound and osteomyelitis to be significantly associated with multi-drug resistant organisms. Regular hospital visits and hospitalisation for reasons other than the current wound were not significantly associated with multi-drug resistant organisms. Wound and patient factors such as age, gender, ulcer duration, glycosylated haemoglobin and ulcer type ((neuro)ischaemic or neuropathic) were also not associated with multi-drug resistant organisms. This study, again, emphasises the association between MRSA and hospital related risk factors and the lack of influence of wound-related risk factors, for patients with diabetic foot ulcers.

A study by Gadepalli et al. [223] also investigated risk factors for antibiotic resistant organisms in infected diabetic foot ulcers (Wagner grade 3-5). This study, based in one tertiary care centre in India, investigated a range of organisms and multi-drug resistant organisms. *S. aureus* was isolated from 13.7% (n=25) of wounds (56.0% (n=14) were MRSA) and *P. aeruginosa* was isolated from 9.8% (n=18) of wounds (55.5% were ciprofloxacin resistant (n=10)). *S. aureus* was the most frequently isolated species however Gram negative aerobes were the most common group of organisms isolated (51.4%, n=94). Factors found (using multiple logistic regression) to be significantly associated with multi-drug resistant organisms were size of ulcer (less than or equal to 4cm^2 or greater than 4cm^2) and the presence of neuropathy. Significant interaction was

also identified between these two variables. Previous hospitalisation was not investigated in this study due to the lack of available data. It is likely that this model suffers from over-fitting (too few cases for the number of variables) as only 22 patients without multi-drug resistant microbes are included in the study, yet 12 variables are included in the multivariable analysis (no modelling diagnostics are presented). However the study, like the ciprofloxacin resistant *P. aeruginosa* models in this paper, suggests that wound factors such as aetiology and wound size might have an impact on carriage of resistant organisms such as antibiotic resistant Gram-negative aerobes. It is difficult to fully interpret the findings of Gadepelli *et al.* in relation to this research because a number of different organisms, which may have different risk factors, are grouped together and the models are likely to be over-fitted.

Day and Armstrong[141] have noted the lack of literature addressing risk factors associated with antibiotic resistant organisms in chronic wounds previously. In their 1997 study, they did not find any studies that had directly investigated the risk factors associated with MRSA in DFUs in their narrative review of the literature. To my knowledge, there is still only limited published research on this issue and no literature on antibiotic resistance risk factors in non-DFU chronic wounds.

There is a plethora of literature addressing the risk factors for MRSA in other populations of patients, from community residents to intensive care patients. Despite the different populations, and differences in study methods, there are some common risk factors that can be picked out from the literature.

The main risk factor associated with MRSA is hospitalisation. This has been found to be significantly associated with MRSA in many different populations of patients, from those in the community and nursing homes through to those entering hospital.[105,107,109] It was therefore surprising that hospitalisation was not associated with MRSA in this

study. However, this may have been due to the coding used for hospitalisation (binary variable indicating an overnight stay of at least one night). In the study by Jernigan *et al.*[107] investigating patients on admission to hospital in the US, previous hospitalisation was explored using several variables. Case and control patients were found to have the same number of hospitalisations in the previous year, but differed significantly in the binary variable indicating a hospital stay of at least 5 days in the previous year, and the continuous variable indicating the total number of days hospitalised within the previous year. In multivariable analysis, only a hospital stay of at least 5 days was significantly associated with MRSA colonisation.

Many studies do not investigate previous MRSA as a risk factor for current MRSA, and in some studies, patients with known previous MRSA are actively excluded. Tacconelli *et al.*[102] however did investigate previous MRSA in their analysis of risk factors for MRSA bacteraemia diagnosed within 24 hours of admission to one US hospital, over a 5-year period. They found previous MRSA to be the strongest variable associated with MRSA bacteraemia; other significant factors were cellulitis at admission, presence of a central venous catheter and skin ulcer at admission. The authors undertook further modelling in which the variable previous MRSA was specifically excluded on the basis that this information was unlikely to be available to clinicians. In this model, prior hospitalisation in the previous six months was associated with MRSA bacteraemia; other significant variables were presence of a central venous catheter, quinolone therapy in the previous 30 days and diabetes. It may be therefore that the inclusion of previous MRSA in this study was over-riding the influence of previous hospitalisation.

MRSA carriage is clearly a long-term issue and once identified as present, will frequently recur. Even when MRSA colonisation is treated, decolonisation programmes are considered only to be effective in the short-term to decrease bacterial load. The

recently published guidelines on the control and prevention of MRSA, cite previous MRSA as a potential risk factor and consequently a reason for active screening.[198]

Prior antibiotic use is also frequently identified as associated with MRSA carriage or infection. When antibiotics are investigated separately, fluoroquinolones are the group consistently found to be associated with MRSA,[102,110,111,119] although other groups have variously been associated with MRSA, e.g macrolides,[100] and ampicillin.[106] Here, prior ciprofloxacin use was not associated with MRSA carriage in univariable analysis. In this study, it is likely that the group of antibiotics found to be significantly associated with MRSA ('other' systemic antibiotics) reflect previous treatment for MRSA. This is considered likely due to the link in clinical terms and the effect of the inclusion of an interaction term in the statistical modelling.

Risk factors for ciprofloxacin resistant *P. aeruginosa* in wound populations have also not previously been identified. Ciprofloxacin resistance in *P. aeruginosa* has however been addressed in other patient populations and ciprofloxacin use has been associated with isolation of such organisms,[132,136] while broader studies have found fluoroquinolone usage to be associated with fluoroquinolone resistance.[133] Patients with chronic wounds have been shown in Chapter 2 to receive significantly more ciprofloxacin compared to age and sex matched patients from the same general practice.[224]

It is possible that the wound related risk factors (would healing and classification) for ciprofloxacin resistant *P. aeruginosa* reflect risk factors associated with the carriage of *P. aeruginosa* and not specifically ciprofloxacin resistant strains. Schmidt *et al.*[27] investigated bacterial populations in healing and non-healing wounds of differing aetiology. Overall, healing wounds exhibited a significantly different microbial population to non-healing wounds. In non-healing ulcers, pseudomonads were

identified in 12%, 29% and 66% of diabetic-ischaemic, diabetic-neuropathic and venous leg ulcers respectively, while in healing ulcers, pseudomonads were isolated from 0%, 0% and 27% of ulcers respectively.[27]

4.5.3 Strengths and Weaknesses

In this study, model structures were fully explored by using both forward and backward stepwise logistic regression procedures. Variable selection from univariable analysis was based on a liberal significance level of $p<0.25$ and the impact of selecting variables based on significance of $p<0.1$ and $p<0.05$ was also investigated. $P<0.25$ was chosen as the main univariable selection criteria was as it has been used elsewhere and considered an appropriate value.[218] The modelling method (forwards or backwards stepwise regression) affected the final model structure for ciprofloxacin resistant *P. aeruginosa* model. Previously, it has been reported that different model building techniques can result in different final models.[225] An investigation of automated techniques (backward elimination, forward selection and stepwise selection) found over half the prognostic variables were included in less than half the models, when 1000 bootstrap samples were used.[218] This highlights a potential issue with exploratory studies where the finding of interest is the variables included.[218] Due to the size of the database used in this study and the limited number of cases, it was not appropriate to use methods such as data-splitting or bootstrap analysis and so to validate the findings of this study, the analysis should be repeated using an independent sample of patients. It is however a strength of this study that potential models were fully explored using more than one selection method, with different univariable selection criteria.

Variable selection based on univariable analysis and automated algorithms has previously been criticised for generating models in which variable selection is based entirely on mathematical detail and not on biological judgement as to the merits or

otherwise of the included variables.[226] Stepwise and automated selection methods are however recommended for exploratory model building, although variables should not be included without due consideration, for example highly collinear variables may mask the effect of each other.[227] Where two similar variables exist, the most clinically relevant should be chosen for inclusion in multivariable analysis.[226]

Here, all variables investigated in univariable analysis were chosen prior to model building either due to their published association with antibiotic resistance, or as a wound variable of clinical interest to explore. Furthermore, variables were excluded if they had a prevalence of <5%. This figure was higher than used in some previous texts,[218] however it reflects an allowance for the moderate sample size and the small number of cases represented by a cut off of 1%. Furthermore, in the models of both MRSA and ciprofloxacin-resistant *P. aeruginosa*, the variable any topical antimicrobial, although significantly associated with antibiotic resistance in univariable analyses, was excluded from further analyses due to its similarity with another variable (topical silver and 'other' topical antimicrobials respectively). Similarly in the model of ciprofloxacin-resistant *P. aeruginosa* the variable any systemic antibiotic was excluded from further analysis due to the inclusion of more specific antibiotic variables from which a large proportion of the generic variable any systemic antibiotic was constructed.

It is a limitation of this study that the use of routinely recorded and retrospectively collected data did not enable the use of more refined variables and some variables of interest could not be investigated due to the quantity of missing data (namely wound duration and wound area). The manner in which some variables were parameterised, due to the limitations of the data, may have lead to a loss of sensitivity within the models for identifying risk factors. For example the association between previous hospitalisation and MRSA is well established in the literature, but was not identified in

this study. Whilst this may have been due to some peculiarity of the particular population investigated, it is perhaps more likely to be due to the manner in which hospitalisation was included in the model. The hospital variable was a binary variable indicating none or at least one overnight stay in hospital in the previous three months, and although significantly associated with MRSA carriage in the wound in univariable analysis, did not remain so in multivariable analysis. It may be that the association between hospital stay and MRSA in this population of patients would have been better established using a hospital variable which was either continuous for the number of hospitalisations or number of nights in hospital in the previous three months. Alternatively a categorised hospital variable with a higher cut-off point, for example five or more nights in hospital, may have revealed a stronger association between hospitalisation and MRSA in this population.

Similarly, the ability to detect relationships between the dependent and independent variables may have been diminished for the antibiotic variables which were coded as 'had in the previous three months' or 'not had in the previous three months' due to the lack of more detailed data. Ideally, using defined daily doses of each antibiotic group consumed would have been preferable. Furthermore, antibiotic use may have been underestimated for patients on whom data were not available from GP and hospital sources, as discussed in Chapter 3 (patients without data from either of these sources were deemed to have missing data unless antibiotic use had been specifically indicated in the nursing notes). However, antibiotic use was not universally underestimated and those antibiotics that would be anticipated, from the literature, to be associated with antibiotic resistant organisms were not underestimated for patients without general practice data. There was little difference between the percentage of patients who had received ciprofloxacin (24.4% Vs 22.4%), cephalosporins or β-lactamase resistant pencillins (26.7% Vs 25.0%) or 'other' systemic antibiotics (14.4% Vs 10.4%) in the

group for whom general practice data were available and those for which it was not respectively. Greater differences existed for use of penicillin (13.3% Vs 0%), flucloxacillin (31.1% Vs 10.4%) and any antibiotic (70.0% Vs 45.8%). Nonetheless, the potentially missed data from general practice may have decreased the ability of the model to identify significantly relationships between antibiotics and carriage of antibiotic resistant organisms.

It is a potential criticism of this study that some of the risk factors identified could be truly associated with carriage of *S. aureus* or *P. aeruginosa* rather than specifically associated with resistant strains of these organisms. This might particularly be the case with the wound-related factors found to be associated with ciprofloxacin-resistant *P. aeruginosa*. It is plausible that these factors (wound healing and wound category) reflect the likely carriage of *P. aeruginosa* itself and do not distinguish between ciprofloxacin susceptible or resistant strains. This study may have been more informative if a case-case-control design had been used, as advocated and used elsewhere.[110,134,135,228] This would have enabled separation of the risk factors associated with carriage of an organism from those associated with carriage of resistant strains of an organism. This was not considered appropriate in this study however due to the number of cases, number of statistical comparisons already conducted and the problems associated with over analysis and the potential for spurious findings due to Type 1 error.[229]

A further weakness of this study was the small number of cases and therefore the probable over-fitting of the models. Sample size calculations were conducted to determine the number of samples required to detect whether any additional variance could be explained by wound characteristics over and above that of known predictors of antibiotic resistance. With 80% power to detect an addition 0.12 in R^2 associated with wound variables of interest over and above that explained by known risk factors, the

estimated sample size was 115 patients. While this method for estimating sample sizes for logistic regression calculations is well known and recognised as a valid method,[230] the format of the data from the audit and the final number of patients on whom data were available differed from the assumptions made for the sample size estimate. It is probable therefore that the sample size calculation underestimated the number of patients required for modelling. This was due to differences in the manner in which factors of interest, such as antibiotic usage, were described and included in the analyses compared to the manner in which they were expected to be described and included in the analyses for the sample size calculation. In the sample size estimate, antibiotic history was considered as one variable, however 15 antibiotic or antimicrobial variables were actually investigated in univariable analyses, although the majority were not found to be significant (four and five antimicrobial variables were considered in the multivariable analyses of MRSA and ciprofloxacin resistant *P. aeruginosa* respectively). Data were available for 150 patients, which was greater than the estimated sample size of 115.

It is important that there are sufficient cases included in the model both with and without each independent variable.[231] When assessing the adequacy of data already collected, it is generally accepted as a rule of thumb that, to avoid suffering from over-fitting, a model should contain 10 events for each independent variable included in the model. This minimum number has been further supported by recent simulation models.[232] When a model is 'over-fitted' the usual statistical significance tests may be invalid and the confidence intervals difficult to interpret. This assumption of sufficient events per independent variable is considered to be important and if not met, it will have a negative effect on the statistical result.[233] When exploring MRSA carriage, the final model included two independent variables and an interaction term and thereby generated a model with three predictor variables, based on 12 events. The model of

ciprofloxacin resistant *P. aeruginosa* with the greatest fit and predictive value included five independent variables and a dependent variable with 15 events. It is probable that 150 patients, with the level of antibiotic resistance detected, was insufficient for the number of variables investigated and that the models may have been over-fitted.

Large confidence intervals may be a sign of over-fitting in a model.[226] In this study, the 95% confidence intervals of the odds ratios associated with significant variables in both the MRSA and ciprofloxacin resistant *P. aeruginosa* models were not narrow. For example, 95% confidence intervals were 1.97, 36.28 for the variable ciprofloxacin use and 1.95, 45.47 for 'other' topical antimicrobials in the ciprofloxacin resistant *P. aeruginosa* model, and 2.19, 36.19 for the variable 'other' systemic antibiotics, in the MRSA model.

It is particularly likely that the model investigating ciprofloxacin resistant *P. aeruginosa* suffered from over-fitting. The sheer number of variables that appeared, in univariable analysis, to be associated with ciprofloxacin resistance generated several difficulties with model building in this study. The use of higher significance levels to select potentially fewer variables from univariable analysis did not improve model fit or decrease the width of the confidence intervals. Over-fitting was indicated by the lack of stability associated with the models, with the structure changing dependent on the method used for building the model and the significance level at which variables were selected from the univariable analysis. It appears, therefore, that the association between chronic wounds and ciprofloxacin resistant *P. aeruginosa* is complicated and dependent on the interaction of many variables.

Statistical modelling (conducted here using logistic regression) aims to find the best fitting, most parsimonious model that is a feasible explanation in biological terms between the independent and the dependent variables.[215] Prediction models, as used

here (also known as model building or exploratory modelling), have unknown structures at the outset, but explore the model structure to identify that with the best prediction value, while inferential modelling has a known model form at the outset and aims to clarifying the coefficients (the two methods should not be mixed in the same analysis).[234] Prediction models cannot be used to ascertain the magnitude of any association between the independent and dependent variables due to the impact of model structure on the coefficients estimated in the model. The assumptions and limitations of multivariable models are of greater importance when the purpose of modelling is to ascertain the magnitude of an association.[226]

It is a strength of this study that the final models were explored for model fit and logistic regression assumptions: the main components of which are goodness-of-fit, examination of residuals and leverage statistics and conformity to a linear gradient. A fundamental principle of modelling is that the inclusion of additional independent variables in a model will improve the mathematical goodness-of-fit of the model. The inclusion of additional variables can however, as previously discussed, cause problems such as over-fitting of data.[226] Hosmer-Lemeshow goodness-of-fit statistic is a robust measure of fit used in logistic regression, especially for models with continuous variables and studies with small sample sizes.[235] The statistic evaluates goodness-of-fit by creating 10 ordered deciles (by estimated probability i.e. below 0.1, 0.1 to 0.2 etc) of the subjects and comparing, using chi-squared statistic, the number in each group in the observed data and with the number in each group predicted by the model. The desired outcome, therefore, is non-significance, indicating that there is no difference between the observed distribution and that predicted by the model.[236] All models explored in this analysis were non-significant in terms of the Hosmer-Lemeshow statistic and therefore displayed adequate fit. Furthermore the pseudo R^2 statistics suggest that the models explain a modest proportion of variability in the data (approximately 0.2-0.5 for

the different models). However, it has been shown that noise variables can demonstrate good R^2 values if the model is over-fitted.[232]

The standardised and studentised residuals were plotted against the predicted value and outlying cases were identified in the final models for both MRSA and ciprofloxacin resistant *P. aeruginosa*, based on residuals. Residuals are the difference between the observed and the expected values.[231] Models both of MRSA and ciprofloxacin resistant *P. aeruginosa* were found to have higher residuals than desirable. In good models, the residuals centre around zero, with a high proportion (about 95%) lying within ±2, with no pattern to the residuals.[237] Assessment of Cook's distance however found no cases that had great influence on the models. All final models included only binary independent variables and therefore the criterion for nonconformity to linear gradient did not apply.[233]

Finally, these analyses were based on data obtained in an audit of antibiotic resistant organisms in one specialist wound healing centre. The patients attending such a centre were not representative of all patients with chronic wounds or the treatment they receive. The audit was never intended to be generalisable but to address specific local questions. The findings from this analysis should therefore be generalised with great caution.

4.5.4 Implications for clinicians and future research

The study has given an insight into the risk factors associated with antibiotic resistance in two common chronic wound pathogens. This study suggests that no wound-related characteristics are influential in the carriage of MRSA, but that MRSA carriage in chronic wounds is associated with previous MRSA and use of a group of antibiotics that includes trimethoprim, sodium fusidate and vancomycin. The work does suggest that

carriage of ciprofloxacin resistant *P. aeruginosa* is associated with wound characteristics, although it is possible that the model of ciprofloxacin resistant *P. aeruginosa* is over-fitted and may not be a stable model. Wound classification appears to have an association with ciprofloxacin resistant *P. aeruginosa* and wound healing may have a protective effect against such carriage. Furthermore ciprofloxacin use in the previous 90 days, use of the group of topical antimicrobials that includes neomycin (Dermovate NN) and possibly use of the group of antibiotics that includes trimethoprim, sodium fusidate and vancomycin were all associated with carriage of ciprofloxacin resistant *P. aeruginosa.* Knowledge of these factors, particularly in the specialist wound clinic on which the data were based, may help clinicians in their decisions for dealing with wound infections and the use of empirical antibiotic treatment regimens or microbial investigation to determine antibiotic susceptibilities of infecting organisms for patients likely, by these risk factors, to be carrying resistant organisms.

This study should be considered an initial exploration of the relationship between wound characteristics and antibiotic resistance. The model and magnitude of the association with antibiotic resistance should be validated using a further data set.[218] Further research would ideally be conducted to determine whether the factors seen here to be associated with antibiotic resistance in the population of patients attending a specialist wound healing clinic are indeed the same factors that would be identified the general population of patients with wounds. The magnitude of any such association would also be of interest to determine. Further studies that determine whether the wound characteristics found to be significant here for ciprofloxacin resistant *P. aeruginosa* were specifically associated with ciprofloxacin resistance or whether they were truly risk factors for *P. aeruginosa* would also be valuable to interpret the true associations between wound and patient characteristics and carriage of antibiotic resistant organisms.

Chapter 5 . Economic modelling of the treatment of venous leg ulcers

5.1 Abstract

Chronic wounds are expensive to treat, and impose a burden both on patients and healthcare services. Leg ulcers alone have been estimated to cost the NHS £400 million annually. Antibiotic resistance has a financial impact for patients, healthcare providers and society through such factors as longer hospital stays, increased drug costs, infection control measures, and antibiotic resistance surveillance.

This study sought to investigate the impact of antimicrobial resistance on the cost of treatment of venous leg ulcers. A Markov model was built with distinct health states. Infection and antibiotic resistant infection were included in the model as transitional costs that could be incurred by any patient with an open wound. The model structure, probabilities of transitions between health states and costs and benefits were obtained from the literature and earlier work in this Thesis.

The expected cost of one-year of treatment for one patient was £1008.28, which increased by less than £1 when antibiotic resistance to second-line antibiotics was incorporated. The total cost of infection for 1000 patients ranged from £12,147 to £14,590 in Monte Carlo simulations, while the additional cost of antibiotic resistance ranged from £496 to £671. Over a 10-year period (at 3.5% discount rate) the cost of treating one patient was estimated to be £3,917.60, increasing to £3919.09 when antibiotic resistance was included. Variation in the frequency of nurse visits, cost of outpatient appointments and cost of nurses had the greatest impact on one-year treatment costs, while in the 10-year model, the frequency of follow up for patients with healed ulcers had the greatest impact.

5.2 Introduction

The economics of health and healthcare are increasingly important as both costs of and demand for healthcare become ever greater. Economic evaluations can be used to assist with decision-making in the healthcare setting and can take many forms including cost-effectiveness studies, cost-benefit studies, cost-of-illness studies, and modelling studies. Models are frequently used to simulate outcomes in different scenarios. Modelling allows costs to be overtly identified and the factors influencing costs and benefits to be explored, considering both current and future settings.[238] In this way economic modelling has been described as an explicit, quantitative and prescriptive approach to decision making.[238]

Chronic wounds are known to have an impact on morbidity and to be costly to manage. It is estimated that in the UK over £400 million is spent annually on leg ulcer care, with each ulcer costing the NHS £506 to £1240 to treat in 2002 (original figures presented as €814 to €1994; converted to 2002 sterling values using the Bank of England's 2002 annual average spot exchange rate).[150,156] Various aspects of the management of leg ulcers have been the subject of further economic scrutiny, with the aim of identifying the most cost-effective treatment. Such studies include Iglesias *et al.*[151] who compared bandaging regimens (four-layer with short-stretch) and found four-layer bandaging to be both more effective and cheaper, and hence the dominant strategy. While Harding *et al.*[152] investigated the most cost-effective dressing protocol and Morrell *et al.*[153] compared compression bandaging in community clinics with usual care. Markov models have previously been used to investigate, amongst other things, the cost-effectiveness of prevention and treatment of diabetic foot ulcers, and of wound dressing on hard-to-heal VLU.[154,239]

Antibiotic resistance is also known to impact on morbidity and costs. These costs affect patients, healthcare providers and society. Antibiotic resistance is considered to be a negative externality of antibiotic consumption as the true cost of antimicrobial resistance is not however felt directly by the consumers of antibiotics. The costs for patients with antibiotic resistant infections include increased morbidity and the loss of work and family time due to longer hospital stays. The costs to the healthcare provider include increased drug costs, hospitalisation, personnel and investigation costs, while the costs to society include increased surveillance, investment in novel antibiotics, infection control measures and premature deaths.[73,160] Several studies have explored aspects of the costs of antibiotic resistance including investigations of policies to reduce antibiotic use and thereby antibiotic resistance, cost-effectiveness of infection control interventions, the burden of antibiotic resistance to healthcare providers and identification of optimum empirical antibiotic treatment choice according to antibiotic resistance prevalence.[161-166]

Here the impact of antibiotic resistance on the cost of venous leg ulcers will be explored, using a Markov model. Markov models enable explicit examination of costs and benefits, and systematic exploration of uncertainty. This will enable the costs and benefits of VLU treatment to be explored alongside the uncertainty of antibiotic resistance. Furthermore, a Markov model enables this to be explored over time. The main components of such models are health states, transition probabilities and costs and benefits. An outline of key terminology used in Markov models is given in Table 5.1. Health states represent clinically and economically important events in the disease process and must be mutually exclusive.[240] Movement between health states is dependent on transition probabilities. Markov models have a cycle length (the Markov cycle) that should represent a clinically relevant time period and all costs and probabilities must be adjusted to this time period. The costs incurred and benefits

gained in one Markov cycle can be associated with health states or transitions. Health gains and losses are incorporated into the model using utilities, which measure what a person expects to gain from the consumption of a good or a service.[241] Data to populate Markov models can come (and in reality have to come) from a variety of sources, both primary and secondary.[238] All data entered into the model will have varying degrees of uncertainty.[238] Fixed input values are, therefore, used for standard analysis while ranges of values are used in the sensitivity analysis to determine the sensitivity of the outcome to variation in data. The predictions of the model are analysed using cohort analysis and Monte-Carlo microsimulation (see Table 5.1).

Table 5.1 Definition of health economic and Markov modelling terms

	Term	Definition
General health economic terms	Utility	'The satisfaction accruing to a person from the consumption of a good or a service'[242]
	Costs	The value of the opportunity forgone[242]
	Benefits	The sum of the effects of well-being[242]
	Discounting	A technique that allows costs and benefits that will be accrued in the future to be calculated at present value, and are based on a time-preference (for benefit now and cost later)[242]
Markov model building	Markov model	A model process that enables inclusion of risk over time, relative timing of events, repetition of important events and analysis over a period of time using stochastic and deterministic methods[243]
	Health states	Discrete states in which patients in the model must always be[243]
	Transition probabilities	The probability of moving from one health to another during one Markov cycle
	Transitional events	Events that occur during the transition from one health state to another
	Transitional rewards	In cohort analysis the transition rewards are deterministically divided amongst the states to which the transition may lead[244]
	Markov cycle	The length of one cycle which is chosen to represent a clinically meaningful time interval[243]
	Stage	The number of Markov cycle's elapsed in the analysis
	Tracker variables	Variables which are used to count specific events during a Monte Carlo microsimulation
	Half cycle correction	Correction in the model to account for the reality that, on average, patients will move out of the health state half-way through the Markov cycle (and not all at the end)[243]
Markov model analysis	Cohort analysis	Cohort analysis returns the expected value (deterministically calculated using the probabilities of the model)
	Monte Carlo microsimulation trial	A series of individual 'patients' take random walks through the model resulting in a stochastic estimation of the value of the model
	Sensitivity analysis	Investigation of the change in expected value in response to changes in the range of a variable included in the model (always uncertainty to be analysed)
	Tornado diagrams	Sensitivity analyses on a number of variables, presented in graphical format with the variables which cause greatest change in expected value at the top

5.3 Methods

A model was built to investigate the cost associated with infection and antibiotic resistance in the treatment of venous leg ulcers in a hypothetical population of patients in the community. The impact over short and medium-term time periods were investigated and the impact of individual factors on cost and utility established. The model was built using data available from the literature and where appropriate, from data sources used in previous Chapters of this Thesis: Chapter 2, General Practice Morbidity Database (GPMD), and Chapter 3, Antibiotic Resistance Audit.

Many literature searches were conducted to identify the necessary data for creating and populating the models. All literature searches were conducted as described here unless otherwise stated. The databases Medline, EMBASE, all EBM reviews (Cochrane DSR, ACP Journal Club, DARE and CCTR), British Nursing Index and CINAHL (*Cumulative Index to Nursing and Allied Health Literature*) were used. Searches were limited to English language articles published up to December 2006. Publications relating to venous leg ulcers were identified using the expanded MESH terms Varicose Ulcer and Leg ulcer and the non-MESH term 'venous leg ulcer'. Where appropriate searches were refined using terms specific to the data item required. Publications were first screened by their title and the abstracts of potentially relevant articles reviewed. Full publications were obtained when the abstract indicated that the article might contain appropriate data to build and populate the model.

Firstly, the literature was systematically searched (as described above) to identify all potential venous leg ulcer classification systems, on which to base health states. The literature search was refined using the MESH terms severity of illness, classification and wound healing. The classification systems were reviewed and the optimum system

chosen, based in part on the presence of appropriate data available to populate the model.

The options identified from which to define health states were:

1. CEAP classification for venous disease
2. Venous clinical severity score (CVSS)
3. Posnett's VLU states
4. Two disease health states: healed, not healed
5. States reflecting the area and duration of the wound

The clinical classification component of the CEAP system includes Classes 0 to 6. Class 0 is "No visible or palpable signs of venous disease" increasing in severity to the two classifications (5 and 6) which include ulceration: Class 5 "... with healed ulceration" and Class 6 "... with active ulceration".[245] This system only, therefore distinguishes between healed and non-healed ulcers and as such represents the simplest model that could be constructed.

The CVSS (Venous Clinical Severity Score) was developed as an adjunctive scoring system to CEAP. This system includes 10 clinical attributes each graded 0 to 3. With so many possible permutations, this scoring system does not easily lend itself to a Markov model.

Posnett defined health states specifically for venous leg ulcers.[246] These included eight health states depicting unhealed ulcer (four health states indicating increasing severity), healed ulcer, recurrence, amputation and death. These health states were evaluated for patients attending three specialist wound healing clinics and found to be meaningful for classification of patients and Posnett was able to calculate weekly costs associated with venous leg ulcers in these states. However, there was a lack of data regarding transition between these health states and no comparable classifications in the literature.

It has been recognised that dichotomous divisions of wound duration and area at the time of presentation are predictive of healing.[247] Health states defined by wound area and wound duration therefore offer a limited number of discrete states on which data exists regarding transitions. Furthermore categories defined by area and duration offer greater depth to the model than could be achieved with a simple healed or not healed model. The Markov model for this study was built with the health states:

1. Healed ulcer, remained healed for more than one-year
2. Healed ulcer
3. Ulcer less than $10cm^2$, less than 6 months duration
4. Ulcer less than $10cm^2$, more than 6 months duration
5. Ulcer greater than $10cm^2$, less than 6 months duration
6. Ulcer greater than $10cm^2$, more than 6 months duration
7. Death

The value of $10cm^2$ was chosen according to the available data for transition probabilities (see below). For pragmatic reasons, the Markov model cycle length was set at three months. Two healed wound health states were included to enable the increased probability of recurrence in the first year to be incorporated into the model.

Variables were used in to define probabilities and each component of costs and resource use. The costs associated with health states and transition events were built up using variables as part of formulae. Costs and rewards were subject to the half-cycle correction in the initial and final stages. Tunnel states were incorporated to enable transition probabilities to be dependent upon the time spent in a health state. Patients entered the Markov model distributed evenly between the two open ulcer health states of duration less than 6 months. Infection and antibiotic resistant infection were included as transitional events that could be experienced once during each Markov cycle

by patients in an open ulcer health state. Where appropriate, probabilities were converted to fit with the Markov cycle length using the formula $tp_y=1-(1-tp_x)^{1/x}$, where tp_y is the probability over time y, y is the Markov cycle length and tp_x is the probability over time period x.[248]

The data required to populate the model are shown in Table 5.2. Data were chosen from the most applicable literature sources. Priority was given to studies based in the UK population, with preference being given to systematic reviews, large scale randomised controlled trials or cohort studies. Where one good-quality study was available to populate a data point, data were taken directly from the single source. Data points, for which there was no identifiable optimal source, were constructed from a consensus of the available data.

Table 5.2 Data required to populate the Markov model

Health state / transitional event	Probabilities	Resource-use	Utilities
Open wound health states	Healing Hospital visits Death	Dressings Bandages Other treatments Healthcare professional contact Hospital visits	Utilities for each health state
Healed wound health state	Wound recurrence Death	Compression hosiery Healthcare professional contact	Utility
Wound infection	Wound infection Antibiotic resistant wound infection Hospital visit due to infection	Antibiotics (1st, 2nd and 3rd line) Administration costs Microbiology investigation Healthcare professional contact	Loss of utility

The literature searches were conducted as described above and refined using the following expanded MESH terms: severity of illness, classification, wound healing, health resources, cost-utility analysis, infection, wound infection and drug resistance

(microbial and multiple) and the non-MESH term utility. In searches of the British Nursing Index, MESH terms that were not applicable, e.g. infection, were replaced with the same words as non-MESH terms.

Costs of resources were taken from published sources. Staff cost data were obtained from Curtis and Netten, 2006.[249] Cost data for drugs and other treatment commodities were obtained from the British National Formulary (BNF). Where data were not available from either Curtis and Netten[249] or the BNF, other sources from the literature were considered. All costs were included in the model at 2006 UK pound sterling, following adjustment according to the Retail Price Index where necessary.[249]

It is assumed in the model that all infections are due to *S. aureus*, and that treatment failure occurs due to infection by a resistant strain of *S. aureus* rather than an organism innately resistant to the first-line antibiotic treatment.

The model was built and analysed using TreeAge Pro 2006 Suite, TreeAge Software Inc. It was analysed under six conditions:

One-year perspective; no antibiotic resistance
One-year perspective; resistance to first-line antibiotic treatment
One-year perspective; resistance to first and second-line antibiotic treatment
Ten-year perspective; no antibiotic resistance
Ten-year perspective; resistance to first-line antibiotic treatment
Ten-year perspective; resistance to first and second-line antibiotic treatment

In each analysis both cohort and Monte Carlo microsimulation were undertaken.[244] The impact of uncertainty in cost and resource use variables and probabilities on the outcome of the cohort analysis was explored using sensitivity analysis. To explore individual variables, one-way sensitivity analysis was conducted, but to explore the relative impact of several factors, tornado diagrams were constructed. In Monte Carlo

microsimulation, tracker variables were incorporated into the model to count infections, resistant infections, recurrence and time in health states in a simulation of 1000 patients taking random walks through the model. In the 10-year perspective analyses, future costs and rewards were discounted at a rate of 3.5% and the impact of discounting explored from 0% to 6%.

5.4 Results

The results are presented firstly for the model structure and the data used to populate the model, together with the rational. The second part of the results presents the model analysis.

5.4.1 Model Structure

The Markov model is summarised in Figure 5.1. This shows the transitions possible within the model. Generally during each cycle a patient may move to a health state indicated by the arrows in Figure 5.1, remain in the same health state or die. Infection and hospital visits were included as transitional costs that could be experienced once during each Markov cycle by patients in open wound health states. Figure 5.2 shows the Markov node and health states as structured in TreeAge. Figure 5.3 to Figure 5.8 show the branches that emanate from each health state, the possible routes that can be taken and the health states in which they terminate. In Figure 5.3 for example, a patient always taking the top branch would not die, not require a hospital visit, not have a wound infection and would heal within the Markov cycle (3 months) and therefore terminate the cycle in the Healed ulcer (<1 year) health state.

Figure 5.1 Markov health states and possible transitions in the model

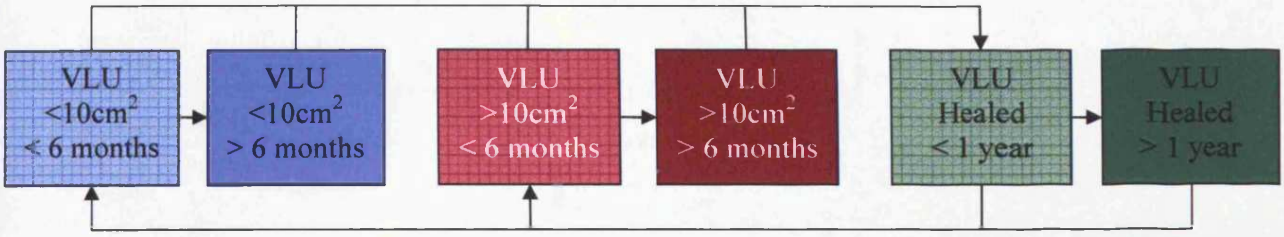


Figure 5.2 Markov node and health states

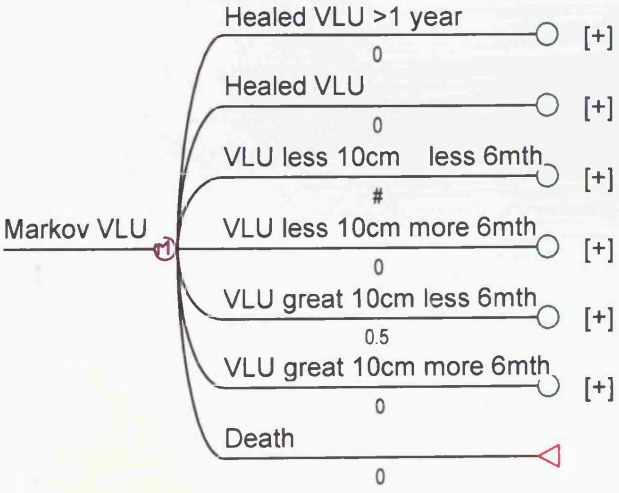


Figure 5.3 Branches from the Markov health state "VLU less than 10cm^2, less than 6 months duration"

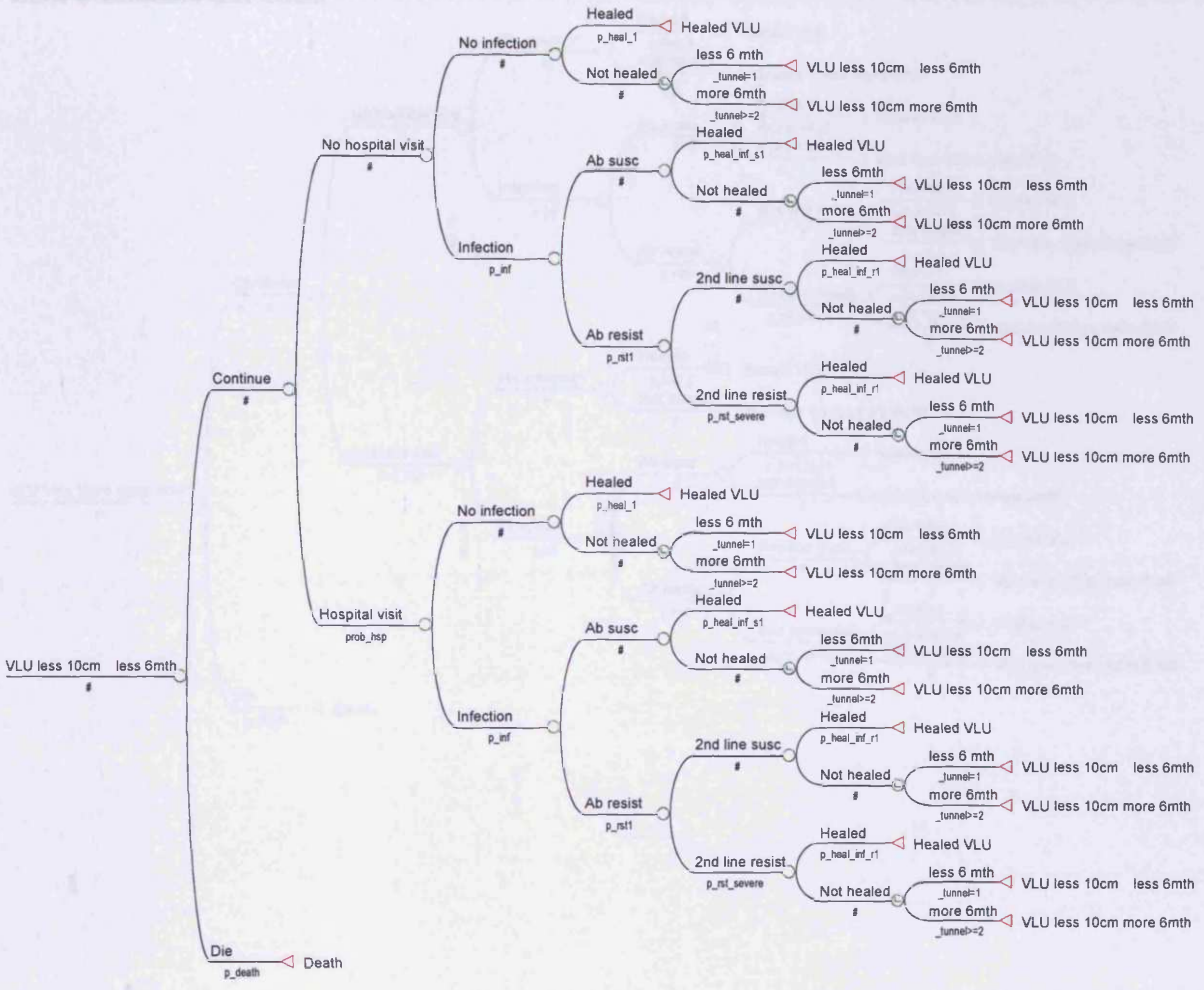


Figure 5.4 Branches from the Markov health state "VLU less than 10cm^2, more than 6 months duration"

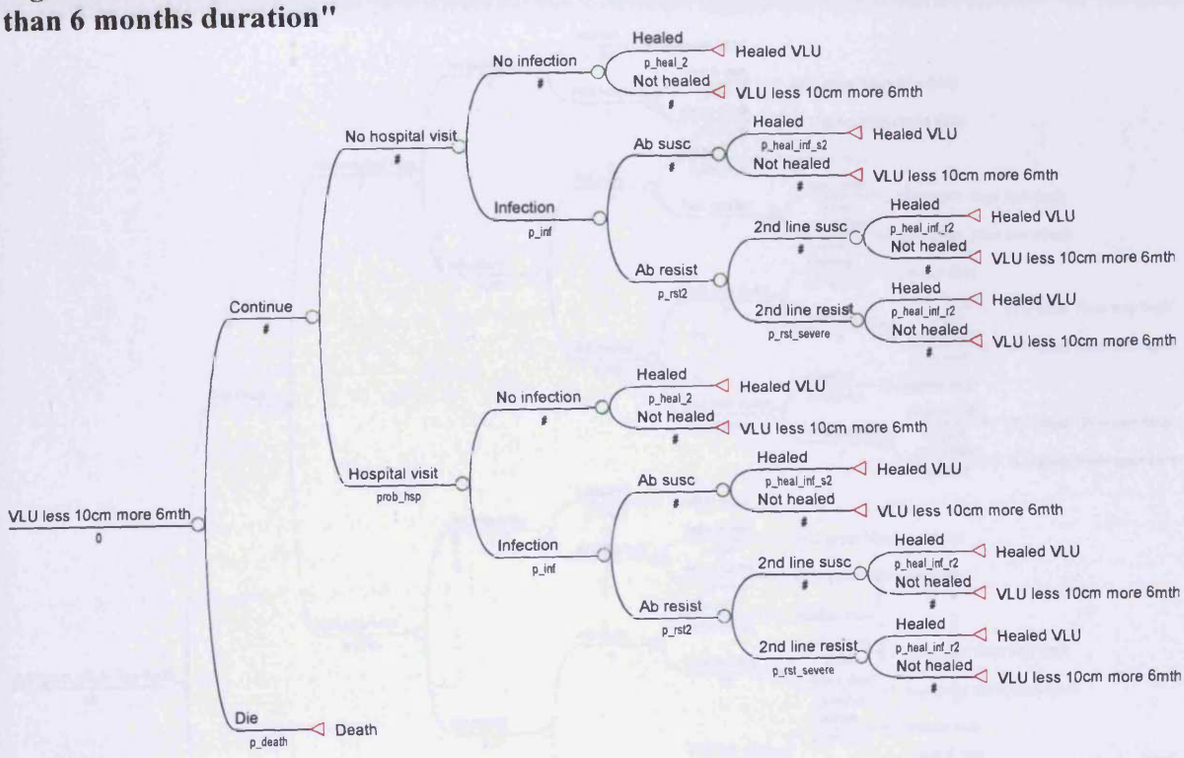


Figure 5.5 Branches from the Markov health state "VLU more than 10cm^2, less than 6 months duration"

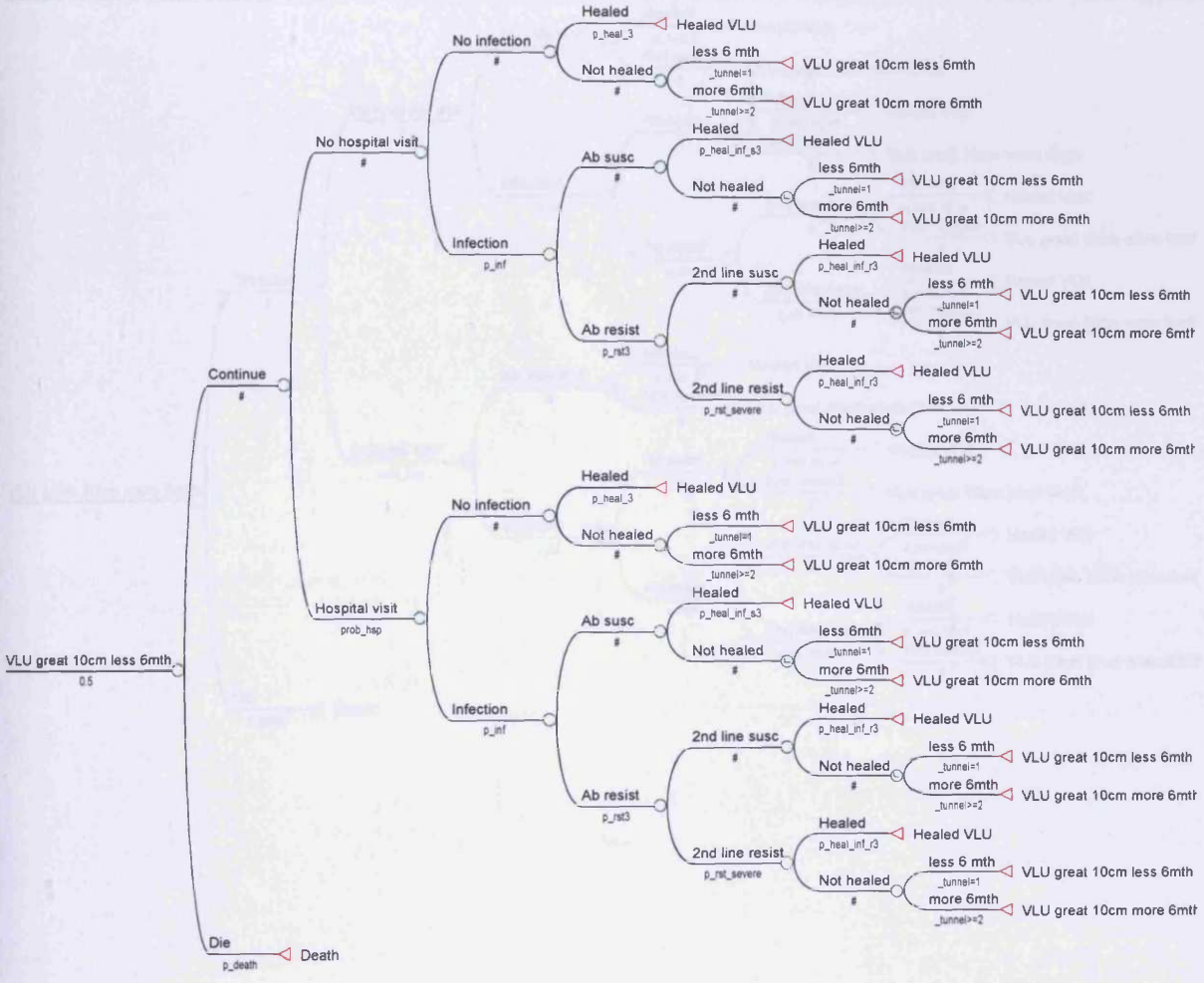


Figure 5.6 Branches from the Markov health state "VLU more than 10cm^2, more than 6 months duration"

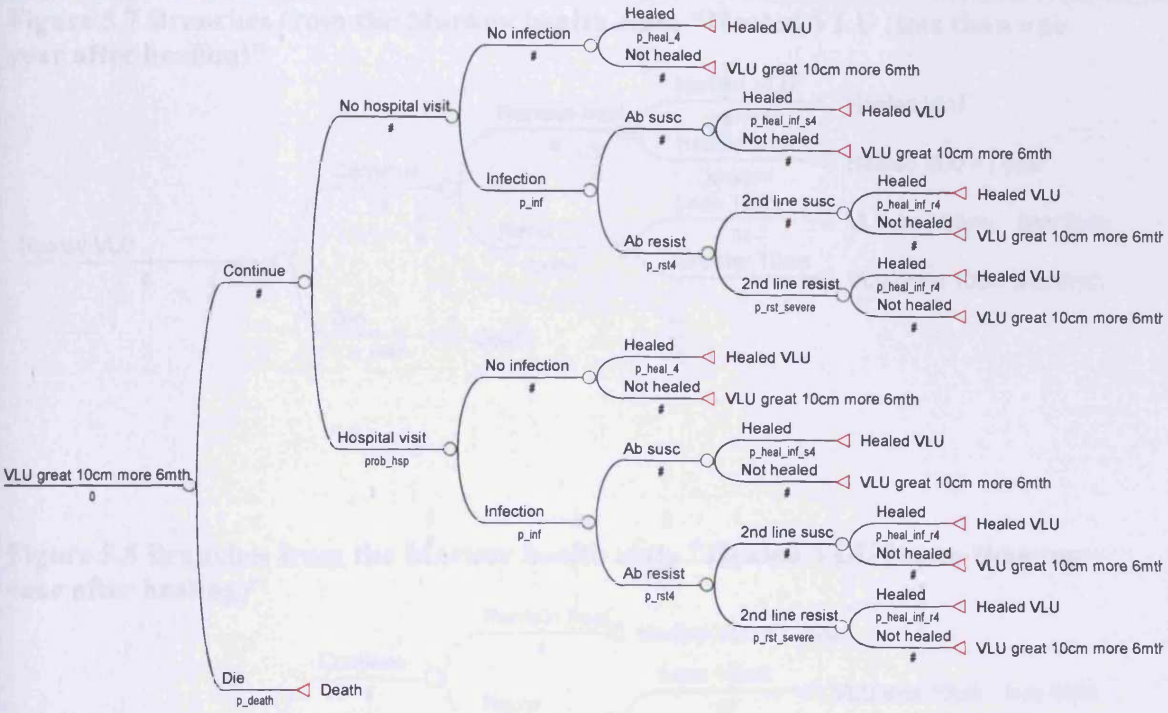


Figure 5.7 Branches from the Markov health state "Healed VLU (less than one year after healing)"

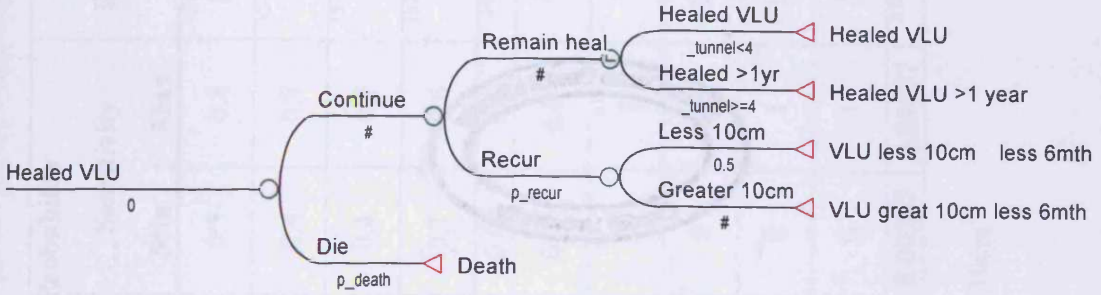
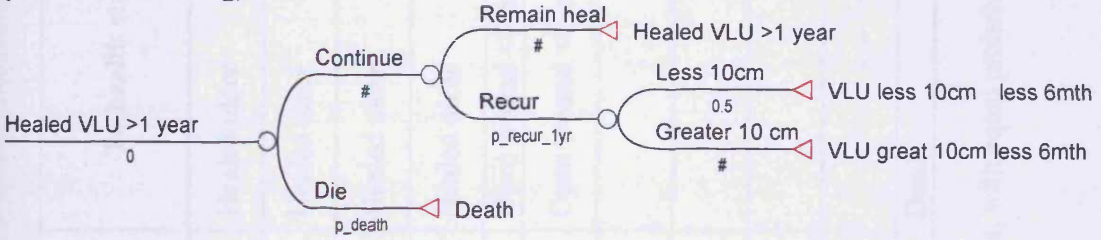


Figure 5.8 Branches from the Markov health state "Healed VLU (more than one year after healing)"



5.4.2 Transition probabilities

No data were available regarding the transition of patients between health states of different ulcer size. In this model, therefore, patients remained in their original size category, being able to move to health states reflecting a healed ulcer, increased wound duration or death. The transition probabilities are summarized in Table 5.3.

Table 5.3 Definition, values and source for transition probabilities

Variable	Definition	From health state	To health state	Probability			Refs
					Sensitivity		
				Baseline	Min	Max	
p_heal_1	Probability of wound healing	Open wound, <10cm^2, <6 months	Healed ulcer	0.7	0.5	0.8	151,247,250
p_heal_2	Probability of wound healing	Open wound, <10cm^2, >6 months	Healed ulcer	0.5	0.4	0.7	151,247,250
p_heal_3	Probability of wound healing	Open wound, >10cm^2, <6 months	Healed ulcer	0.5	0.4	0.7	151,247,250
p_heal_4	Probability of wound healing	Open wound, >10cm^2, >6 months	Healed ulcer	0.4	0.1	0.6	151,247,250
p_recur	Probability of recurrence	Healed ulcer	Open wound <6 months*	0.05	0.02	0.1	248,251
p_recur_1yr	Probability of recurrence after one year	Healed ulcer	Open wound <6 months*	0.02	0.01	0.05	252
p_hosp	Probability of a hospital visit	All open wound health states	-	0.784	0	1	151
p_inf	Probability of infection	All open wound health states	-	0.12	0	0.3	Chp 2
p_rst	Probability of first-line antibiotic resistance	All open wound health states	-	0.1	0	1	Chp 3
p_rst_severe	Probability of second-line resistance	All open wound health states	-	0.012	0	1	253
p_death	Probability of death	All health states	Dead	0.0126	0.00387	0.04597	254,255

* Patients move to an open wound health state of duration less than 6 months, with equal probability of <10cm^2 or >10cm^2.

5.4.2.2 *Probability of Healing*

The probability of healing and range for sensitivity analysis incorporated into the model, by wound size and duration, is given in Table 5.3. The probability of healing was not affected by any transitional event in the model, such as hospital visit or wound infection. However, the probabilities of healing following infection were included in the model as separate variables (with the same values as the probability of healing for an equivalent wound without infection). This enabled the impact of the probability of healing in infected wounds (both those with antibiotic susceptible infections and those with resistant infections) to be determined separately from the probability of healing in uninfected wounds during the sensitivity analyses.

The probabilities of healing were based on the findings of Iglesias *et al.*[151] who evaluated the cost-effectiveness of compression bandaging in a multi-centred study across the UK and found the probability of an ulcer healing within 12 weeks to be 0.41 for ulcers greater than 10 cm^2 and 0.74 for ulcers less than 10 cm^2. These figures were slightly adjusted to incorporate the findings of Margolis *et al.*[247] and Moffatt *et al.*[250] This adjustment was judgement-based and visual (i.e. not determined using formulae).

Margolis *et al.*[247] published a predictive rule for wound healing following retrospective analysis of a database of patients with VLUs in the US. The study found wounds with area >5 cm^2 and duration >6 months to have a lower probability of healing than wounds with either area <5 cm^2 or duration <6 months. This suggests that there may be an additive effect on delayed wound healing by area and duration (Table 5.4).

Table 5.4 The probability of wound healing by wound size and duration as from Margolis *et al.*[247]

Wound Description	Probability of healing at 24 weeks		Probability of healing in 12 weeks	
	Modelling data	Validation data	Modelling data	Validation data
<5 cm^2 and <6 months	0.93	0.95	0.735	0.776
>5 cm^2 and <6 months; or <5 cm^2 and >6 months	0.65	0.73	0.408	0.480
>5 cm^2 and >6 months	0.13	0.37	0.067	0.206

Moffatt *et al.*,[250] looked at community clinics and their effectiveness for leg ulcer treatment. The study included 550 ulcers, in 475 patients and was based in the UK. Data were presented on the number of patients healed by wound size and duration, which fits with the structure of the model, however data were presented separately according to size and duration and not cross-tabulated (Table 5.5).

Table 5.5 Wound healing and the probability of healing in 12 weeks from study by Moffatt *et al.*, 1992.[250]

VLU category	Number of wounds	Number of wounds healed	Probability of wound healing
All VLU	477	318	0.667
>10 cm^2	128	52	0.406
< 10 cm^2	349	259	0.742
> 6 months	186	101	0.543
1-6 months	178	119	0.669

The values determined by Iglesias *et al.*[151] (which were very similar to those by Moffatt *et al.*[250]) for patients with wounds less than and greater than 10cm^2 were taken as the baseline values for patients with ulcers less than 10cm^2 and less than 6 months duration, and greater than 10cm^2 and greater than 6 months duration respectively. The intermediate values (for wounds less than 10cm^2, greater than 6 months duration or greater than 10cm^2, less than 6 months duration) were set at p=0.5 in line with figures for wounds in the intermediate categories determined by Margolis *et al.*[247] This was an

imperfect science but all values were tested in the sensitivity analysis using a wide range of values.

Many other studies have reported the healing rate of ulcers treated with four-layer (and other) compression bandages, including a systematic review by Cullum *et al.*[256] Cullum *et al.*,[256] found compression treatment to increase the probability of healing compared with no compression and high compression to be more effective than low compression. The percentage of healed ulcers at 12 weeks following treatment with high compression bandages ranged from 34% to 86% in the included UK studies. With the exception of one study, data were not presented stratified by area or duration. One small study involving 29 patients found 75% of patients with ulcers less than $10cm^2$ and 50% with ulcers greater than $10cm^2$ to heal in 12 weeks when treated with four-layer bandaging.[257]

5.4.2.3 Probability of recurrence

Two healed ulcer health states were included in the model to enable the probability of recurrence and use of healthcare services to differ in the first year following healing from later years.

Recurrence rates in the first 12 months following healing were obtained from Gohel *et al.*[248] The probability of recurrence during each Markov cycle was p=0.05 (equivalent to 17% over 12 months). Sensitivity analysis was performed within the range of p=0.02 to p=0.1. The minimum value was equivalent to approximately 8% recurrence rate over 12 months. The maximum value was equivalent to approximately 34% recurrence in one year, as seen in the compression only arm of the study by Barwell *et al.*[251]

Data from Gohel *et al.*,[248] was used as this was a large (1186 patients) study, based in the UK. Furthermore, the findings by Gohel *et al.*[248] are representative of other studies. For example, recently Iglesias *et al.*[151] reported that 13.1% and 25.4% of those treated

with four-layer bandages and short-stretch bandages respectively recurred within 12 months.

The probability of ulcer recurrence for patients who had remained ulcer-free for one year was p=0.02. The range used for sensitivity analysis was p=0.01 to p=0.05. These data were taken from Nelson *et al.*,[252] who reported 35.7% recurrence over 60 months. Recurrence rates for patients followed for more than one year reported in the cohort studies identified ranged from 37 to 38% over 3 years.[258-260]

Recurrence rates from a number of cohort studies and one systematic review were available. In general, recurrence rates reported in cohort studies ranged from 17 to 21% of patients over 12 months,[248,260,261] and therefore the probability of recurrence is highest in the first year: patients followed for one year have a higher annual probability of recurrence than patients followed for 3 to 5 years. Kjaer *et al.*,[261] found 75% of patients that had recurrence in a year post-surgery, did so within 6 months.

Nelson *et al.*,[252] undertook a systematic review to investigate compression for preventing recurrence of venous ulcers. They identified two studies which met their inclusion criteria: Franks *et al.*[262] and Harper *et al.*[263] The conference proceeding by Harper *et al.*[263] has more recently been published as a full article by Nelson *et al.*[264] Franks *et al.*[262] investigated the impact of two brands of Class 2 below knee stockings and found 21% and 34% of patients to have ulcer recurrence in 18 months. Nelson *et al.*[264] found 39% and 32% of patients treated with Class 2 and Class 3 hosiery respectively had ulcer recurrence by 60 months. There was no significant difference in recurrence rates between patients with Class 2 and Class 3 compression hosiery.

5.4.2.4 *Probability of hospital visit*

Hospital visits occurred an average of 0.4 times per month in the study by Iglesias *et al.*[151] Assuming this figure indicates 40% of patients per month visit the hospital, it was transformed to represent the probability of a hospital visit in 3-months and used in the model (p=0.784). For the sensitivity analysis this figure ranged from no hospital visits (p=0) to all patients requiring hospitalisation (p=1).

5.4.2.5 *Probability of infection*

The probability of infection was set to be the same for all wounds in the model, irrespective of size and duration, at p=0.12. The range p=0 to p=0.3 was used for sensitivity analysis. These data were extracted from the GPMD data presented in Chapter 2.

Antibiotic use was recorded in the GPMD and was used as a proxy marker for infection. There were 68 patients with venous leg ulcers (with age and sex data), 28 (41.2%) of who received systemic antibiotics in single diagnosis visits and 48 (70.6%) of who received systemic antibiotics in any visit during one year. The probability of receiving antibiotics using single diagnosis visits was p=0.412 in one year, p=0.124 in 3 months. The probability of receiving systemic antibiotics in any visit was p=0.706 during one year, equating to p=0.264 over a 3 month Markov cycle.

Two systematic reviews were identified that addressed the issue of infection diagnosis and treatment in chronic wounds but did not address the incidence of wound infection.[37,42] No other suitable literature sources were identified to populate this variable.

5.4.2.6 *Probability of resistance to first –line infection*

The probability of resistance to first-line antibiotic treatment was set at 0.1 in relevant models. This figure represents the proportion of patients with chronic wounds, seen in Chapter 3, that are colonised with MRSA. The range of values from no antibiotic resistance to all antibiotic infections was considered in the sensitivity analysis.

5.4.2.7 *Probability of resistance to second-line antibiotic*

The probability of resistance to second-line antibiotic treatment was extrapolated from *S. aureus* bacteraemia susceptibility data.[253] Second-line treatment was assumed to be tetracycline (as recommended in the BNF[62]) BSAC resistance surveillance data for bacteraemia suggest that 98.8% of MRSA isolates are susceptible to tetracycline.[253] Applying the BSAC bacteraemia susceptibility rates to this model results in 1.2% treatment failure when tetracycline is used as second-line treatment. Treatment was assumed not to be tailored according to susceptibility reporting from the laboratory.

5.4.2.8 *Probability of death*

The probability of death for those >65 years in a three month period was p=0.0126 (with the minimum value 0.00387 and maximum value 0.04597 for the sensitivity analysis).

Population estimates were used to calculate the death rate for patients with VLUs in this model as Nelzén *et al.*,[265] found 5-year survival for patients with VLUs, not to differ from age and sex matched patients without leg ulcers. Age-specific death rates and population sizes obtained from the Office for National Statistics, for the year 2005,[254,255] were used to calculate the probability of death for the population over 65 years old and separately by age-band and sex. The sensitivity range is from the age and sex categories

with the lowest to the highest probability of death (females aged 65-74 years and males aged 85 years and over respectively).

5.4.3 Costs and Benefits

5.4.3.1 *Utilities of health states*

The utilities gained and lost during the model cycle are summarised in Table 5.6.

Table 5.6 Definition, values and source for utility variables

Variable	Description	Utility			Ref.
			Sensitivity analysis		
		Baseline	Min	Max	
u_unheal	Utility associated with one cycle in an open ulcer health state	0.175	0.1425	0.2	151,266,267
u_heal	Utility associated with one cycle in a healed ulcer health state	0.2	0.175	0.25	151,267
u_infection	Utility loss due to antibiotic susceptible wound infection	0	0	-0.1	-
u_resist	Utility loss due to antibiotic resistant wound infection	0	0	-0.125	-

The utility associated with unhealed VLUs was set at 0.7 for one year (0.175 for 12 weeks), as found by Iglesias *et al.*[151] For the sensitivity analysis, this value was varied from 0.57 (0.1425 for 12 weeks) as found by Walters *et al.*[266] up to 0.8 (0.2 for 12 weeks) as found by Jull *et al.*[267] Patients with healed ulcers had a utility of 0.8 (0.2 for 12 weeks),[267] ranging from 0.7 to perfect health for sensitivity analysis (0.175 to 0.25 respectively for 12 weeks).

Only three studies were identified in the literature that reported utilities for patients with VLUs. Jull *et al.*[267] investigated leg ulceration and perceived health in a New Zealand population. Utility was calculated from preference-based assessment and found to be

0.8 for cases (defined as "any break in the skin on the lower leg (below the knee) or on the foot, which had been present for more than 6 weeks") and 0.89 for controls matched by 10-year age-band after adjustment for age and sex. Unadjusted figures were 0.68 (SD 0.14) for cases and 0.79 (SD 0.13) for controls.

Walters *et al.*,[266] used several methods for evaluating quality of life for leg ulcer patients, and found pain to have a large influence. Using the EuroQol DSI score that ranges from –0.2 to 1.0, they found a mean of 0.57 (SD 0.18) in patients with VLUs.

Iglesias *et al.*[151] also assessed quality of life of patients with VLUs in the UK as part of their RCT of compression bandages. They used the EuroQol EQ-5D generic measure of health status, where utilities can range from –0.57 to 1, with negative values representing health states worse than death. The mean (SD) utility for patients was 0.7 (0.3) following the first quarter of treatment.

No data were available regarding the loss of any utility associated with infection or antibiotic resistant infection. These values were therefore set as zero for the model analyses but explored using sensitivity analysis. The loss of utility associated with antibiotic susceptible infection was explored from 0 to -0.1 utilities and the loss associated with first-line antibiotic resistant infection explored from 0 to –0.125 utilities. In the model structure second-line antibiotic resistant infections experienced a repeat loss of the utility associated with first-line antibiotic resistant infection.

5.4.3.2 Costs in health states

Costs associated with one cycle in each health state were calculated from the variables and formulae given in Table 5.7.

Table 5.7 Formulae for calculating the cost associated with health states

Health State	TreeAge cost formula
Open wound	Discount((c_nurse * freq_nrs) + (c_dr * freq_dr) + (c_bandage_unh * freq_nrs) + (c_dressing_unh * freq_nrs) ; dis_rate ; (_stage/4))
Healed wound	Discount((c_hosiery_hld * freq_followup + c_nurse * freq_followup); dis_rate ; (_stage/4))
Death	0

Definitions of the variables included in the cost calculations are given in Table 5.8. Also included in Table 5.8 is the basic cost of each item, the range for sensitivity analysis and data source.

Table 5.8 Definition, values and source for health state cost variables

Variable	Description	Cost/frequency			Refs.
		Baseline	Sensitivity analysis Min	Max	
c_nurse	Cost of a nurse consultation	£25.34	£16.87	£44.64	151,249
freq_nrs	Frequency of nursing visit over 3 months	12	6	36	151
c_dr	Cost of a doctor consultation (10 minutes at surgery)	£25	-	-	249
freq_dr	Frequency of doctor visit over 3 months	0.6	0	12	151
c_bandage_unh	Cost of bandages for an unhealed ulcer (one application)	£8.62	£6.16	£11.09	62,151,250
c_dressing_unh	Cost of dressings for an ulcer	£1.56	£0.33	£4.00	62
c_hosiery_hld	Cost of support hosiery for healed ulcers (average Class 2 and 3 standard below-knee hosiery)	£10.00	£9.37	£12.36	62
freq_followup	Frequency of nurse visits for patients with healed ulcers	3	0.5	6	-

Health care professional costs

The cost per visit included in the model was £25.34. This assumed visits to be evenly divided between the clinic and home setting. For sensitivity analysis, the minimum cost was £16.87 and the maximum £44.64. The minimum cost was that of a 22-minute consultation in the clinic setting, and the maximum value that of a 40-minute consultation in the home setting.

It was assumed in this model that consultations were evenly split between two settings: patients home and clinic. Estimates for the cost of nursing care in different settings were calculated using duration and frequency data from Iglesias *et al.*[151] and professional's time costs from Curtis and Netten.[249] Iglesias *et al.*[151] calculated that on average VLU consultations took place four-times a month. They estimated, following a small trial, that a consultation lasts 22 minutes in the clinic setting and 30 minutes in the home setting. These figures formed the basis of the cost per visit in the model. (Iglesias *et al.*[151] however used an average visit time of 40 minutes in the home setting.) In the clinic setting, the cost per hour of patient contact with a district nurse is £46 and in the home setting, the cost per hour of contact is £65, plus £1.30 travel costs for each visit.[249]

Patients were assumed to have weekly consultations with a nurse (Iglesias *et al.*[151]). The sensitivity analysis investigated the impact on costs associated with visit frequency from fortnightly to three-times a week.

The frequency with which patients consult a doctor was taken from Iglesias *et al.*[151] (mean of 0.2 per month, i.e. 0.6 per three months). For the sensitivity analysis this ranged from no visits (n=0) through to weekly visits (n=12). The cost of a 10-minute

consultation with a doctor was taken from Curtis and Netten (£25), and not subjected to sensitivity analysis.[249]

Patients with healed ulcers were assumed to have monthly follow up consultations with a nurse. For the sensitivity analysis this ranged from 6-monthly to fortnightly follow up consultations.

Dressing, bandage and hosiery costs

A summary of the costs for dressings, bandages and hosiery are given in Table 5.8. These costs of dressings, bandages and hosiery are reported for one application. In the model the total cost during the three month cycle was calculated by multiplying the cost per application by the frequency of nurse visits as part of the cost formulae (Table 5.7). It is therefore assumed that all dressing, bandage and hosiery costs will be incurred on each contact with a nurse.

There is limited evidence regarding the relative effectiveness of different wound dressings, although low-adherent dressings have been shown to be as effective as hydrocolloid dressings beneath compression bandages in the treatment of leg ulcers.[268] To estimate the costs associated with dressings, used underneath the bandage system, data from the wound healing clinic audit reported in Chapter 3 was used. As part of the audit, data were collected on the dressings removed from the wound and were available for 35 patients with VLUs. The most commonly removed dressing for these patients was NA (28.6%, n=10) a low adherent dressing, and the second most frequently removed dressing was Aquacel Ag (14.3%, n=5), a silver impregnated hydrocolloid dressing. Overall silver dressings were removed from 11 patients (31.4%). The wounds that did not have NA or Aquacel Ag, had a broad range of products including intrasite conformable, mepitel and inadine. The cost of dressings was calculated as a

weighted average between NA ultra and Aquacel Ag at a 2:1 ratio. Four-layer bandage systems include a knitted viscose primary dressing, of which NA is one type. NA costs 33p for 9.5cm by 9.5cm and 63p for 9.5cm by 19 cm, however, in this analysis it is assumed that this cost is included in the cost of four-layer bandages. Aquacel Ag has a range of prices from £1.68 for 5cm by 5cm up to £18.70 for 20cm by 30cm. For the purposes of this study, it has been assumed that 10cm by 10cm is used at a cost of £4.00. The cost for one dressing application included in this analysis is therefore, £1.33. For sensitivity analysis, £0.00 was the minimum value (representing all knitted viscose primary dressings included in four-layer bandage systems) and £4.00 was the maximum value (all Aquacel Ag).

It was assumed that all patients in the model were treated with four-layer compression bandages. Several different four-layer bandage systems are available.[62] Iglesias *et al.*[151] listed three standard kits in their information for nurses conducting the trial; Profore, System 4 and Original (consisting of Velband/Softban, Crepe 10cm, Elset and Coban, referenced to Moffatt *et al.*,[250]). Costs for these three kits, in 2006 prices, were obtained from the BNF. Assuming an ankle circumference of 18-25cm, Profore, Smith and Nephew, costs £8.92, System-4, Med-lock, costs £8.29 and the components of Original sum to £8.65. The average cost of these three systems was taken as the basic cost of four-layer bandaging in this model (£8.62). The minimum and maximum values for sensitivity analysis were the cheapest and most expensive four-layer bandaging kits listed in the BNF: £6.16 (Ultra-Four, Robinson) and £11.09 (Profore, Smith and Nephew, for ankle circumference above 30cm).[62]

There is evidence that recurrence rates in patients wearing high compression hosiery are lower than those wearing medium compression hosiery.[252] However, compliance may not be as great in patients wearing high compression. Both Class 2 and Class 3

compression hosiery are used for ulcer prevention and therefore the average cost of below-knee, standard Class 2 and Class 3 hosiery was included as the basic cost in the model (£10.00).[62] This cost was varied from a minimum of £9.37 (below-knee standard Class 2 compression hosiery) to £12.36 (thigh length standard Class 3 compression hosiery) for the sensitivity analysis.

5.4.3.3 Costs incurred at transitional events

The formulae for calculation of costs associated with transitional events are shown in Table 5.9. The values assigned to the variables involved are summarised in Table 5.10.

Table 5.9 Formulae for calculating the cost associated with transitional events

Transitional event	TreeAge cost formula
Hospital visit	Discount(c_hosp ; (_stage/4))
Infection	Discount(c_swab + c_abx1 + c_nurse; (_stage/4))
Infection resistant to first-line treatment	Discount(c_abx2 + c_nurse; dis_rate ; (_stage/4))
Infection resistant to second-line treatment	Discount((c_vanc + c_vc_inpt) * c_vc_days; (_stage/4))

Table 5.10 Definition, values and source for transitional events cost variables

Transitional variable	Description	Cost			Refs
		Baseline	Sensitivity analysis Min	Max	
c_hosp	Cost of a hospital visit (cost of general outpatient attendance for an adult)	£113	£93	£1085	249
c_swab	Laboratory sample	£34.93	£16.02	£46.78	269
c_nurse	Cost of nurse visit	£25.34	£16.87	£44.64	249
c_abx1	First-line antibiotics: flucloxacillin 7 days	£7.59	-	-	58,62
c_abx2	Second-line antibiotics: tetracycline 7 days	£3.84	£3.84	£7.68	62
c_vanc	Cost of vancomycin including its preparation, administration and monitoring	£27.51			62,270 ,271
c_vc_inpt	Cost of one inpatient bed-day	£217	£197	£249	249
c_vc_days	Number of inpatient days for vancomycin treatment	5	0	10	272

The cost of a hospital visit (c_hosp) was assumed to be the cost of a general adult outpatient visit as reported by Curtis and Netten (£113).[249] In the sensitivity analysis this ranged from the lowest national cost associated with one outpatient visit (£93) to the cost of a five-day inpatient stay for the elderly (5 days at £217).

Three layers of potential infection costs were included in the model: infection, infection resistant to first-line antibiotic treatment and infection resistant to second-line antibiotic treatment. The first costs incurred due to infection included the cost of microbial investigation of the wound, one nurse visit and first-line empirical antibiotic treatment. Where first-line treatment failed due to antimicrobial resistance, additional costs incurred were the cost of second-line antibiotics and a further nurse visit. Finally, for patients that did not respond to second-line treatment, the costs associated with a period of hospitalisation and intravenous antibiotics were incurred. These costs were cumulative, such that patients who had infections resistant to first-line antibiotic treatment incurred the costs of both susceptible infection and infection resistant to first-line antibiotic treatment. Similarly, patients with infection resistant to second-line antibiotic treatment incurred the costs of susceptible infection, infections resistant to first-line antibiotic treatment and infections resistant to second-line antibiotic treatment.

First-line treatment, as recommended by Clinical Knowledge Services (CKS) (previously called Prodigy),[58] was 500mg oral flucloxacillin, four times a day for 7 days; total cost £7.59.[62] CKS advises that the leg ulcer is reviewed in light of the microbiology report 3 days after starting treatment. Although for the most part in the UK, a consultation with a doctor is required to obtain an antibiotic prescription, this is not included as a cost in the model. A consultation with a nurse is however included. It is assumed that a patient would see only one healthcare professional when presenting with infection. There is little difference in the cost of a consultation with a doctor or

nurse in this model due to differing length of consultation (£25.34 for an average length consultation with a nurse and £25 for a 10-minute consultation with a GP).

The cost of a wound swab taken for microbiology analysis for culture and antibiotic susceptibilities was taken from a study by Brezmes *et al.*,[269] conducted in a Spanish hospital as no figures were identified regarding the cost of processing a wound swab in the UK. Brezmes *et al.*,[269] found the cost associated with swab sample culture to be €32.32 in 1995-1996. This figure ranged from an average cost of €14.82 for a negative swab to €43.29 for a positive swab. These figures were converted to pound sterling at the annual average exchange rate for 1996[156], and inflated to 2006 prices using the retail price index.[249] The equivalent sterling cost in 2006 was calculated to be £34.93, for sensitivity analysis a minimum value of £16.02 (negative swab) and maximum value of £46.78 (positive swab) were used.

As stated previously, second-line treatment in the model was assumed to be tetracycline. Tetracycline or clindamycin are recommended as suitable second-line antibiotics, given appropriate susceptibility for MRSA.[62] However, tetracycline had both a lower cost and higher susceptibility rate in BSAC bacteraemia data. The basic cost of tetracycline was £3.84 and ranged from £3.84 to £7.68 (the price a 7 day course of 500mg qds) for sensitivity analysis.

Those patients that were unsuccessfully treated by second-line antibiotics were assumed to have a severe skin and soft tissue infection that required hospitalisation and intravenous antibiotics. The BNF states that either vancomycin or linezolid can be used for severe skin and soft-tissue infection.[62] Vancomycin costs include intravenous injection, plasma-concentration monitoring and drug cost. The recommended dose for the elderly (>65 years) is 500mg twice daily (2x £8.05) or 1g once daily (£16.11).[62] The cost for vancomycin preparation, administration and monitoring costs were taken

from Davey *et al.*,[270] and inflated to current prices (£8.96 per day in Davey *et al.*, at 1993 prices; £11.40 in 2006[271]). The total drug and administration cost per day for vancomycin treatment was therefore £27.51 (the *c_vanc* variable). The national average cost for one hospital bed-day for the elderly (the *c_vc_inpt* variable) was £217 (lowest national quartile £197, highest national quartile £249 (figures used for sensitivity analysis)).[249] A value of 5 bed-days was used as this has been estimated as the mean length of inpatient stay for patients with skin and soft tissue infections (with a minimum of zero days and maximum of 10 days for sensitivity analysis; the *c_vc_days* variable).[272]

5.4.4 Model analysis

Three different scenarios of antibiotic resistance were analysed over two time periods (one year and 10 years). For each analysis the expected value (cost and utility) derived from the cohort analysis are presented together with the sensitivity analysis and followed by the results of a micro-simulation trial.

5.4.4.1 One-year perspective: No antibiotic resistance

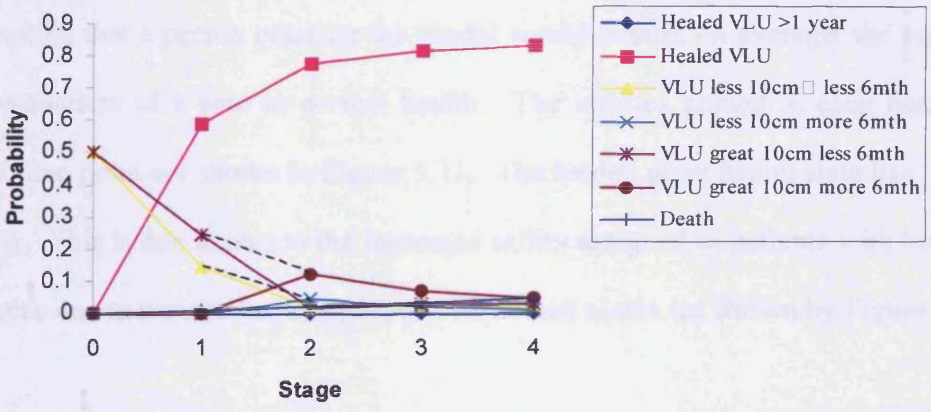
Cohort analysis

The model was initially run for one year with zero probability of antibiotic resistant infection. The expected value of a one-year cycle was £1008.28 and the average utility was 0.75. The cost for one quality adjusted life-year (QALY) was therefore £1348.34.

Figure 5.9 shows the probability of being in each health state at each stage. Patients start in one of the two open wound categories with wound duration less than 6 months. It can be sent that the probability of being in either of these states decreases as the probability of being in the healed ulcer state increases. At 6 months (Stage 2) patients move from the 'less than 6 month' categories to the 'more than 6 month' categories

(represented by the dotted lines on Figure 5.9). Patients who started with an ulcer greater than 10 cm^2 can be seen to move out of the ulcer state (to the healed wound state) more slowly than patients with an ulcer less than $10cm^2$, reflecting the lower probability of healing for wounds of larger size. There remains a small probability and cost associated with wounds of duration less than 6 months after Stage 3 due to recurrence. After one year, 83.7% of patients had a healed ulcer, 11.4% had an open wound and 4.9% had died.

Figure 5.9 Probability of being in each health state at each stage in the model

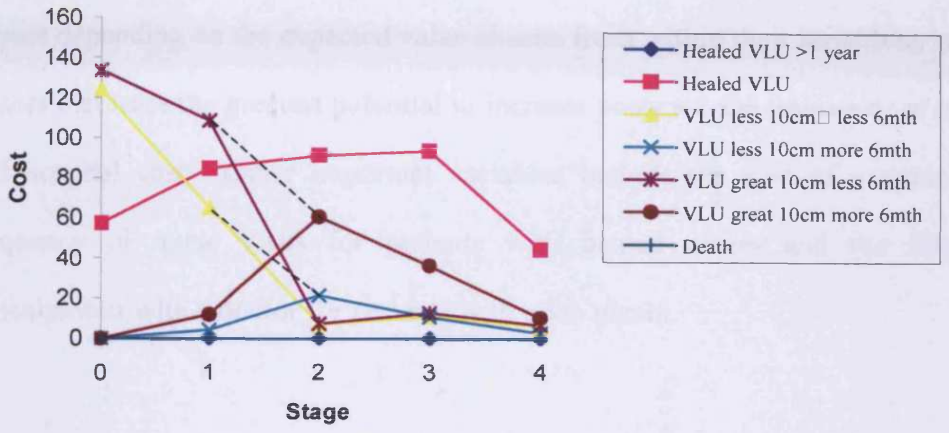


*

Dotted lines represent transition between two health states that only differ due to the duration of active ulceration (i.e. from a wound health state of $<10cm^2$, <6 months to $<10cm^2$, > 6 months).

The cost associated with each health state over one year is shown in Figure 5.10. It can be seen that in the first quarter, the treatment of wounds greater than $10cm^2$ and less than 6 months duration has the greatest cost. However, after 6 months, as more ulcers heal, the greatest cost is associated with the treatment of healed ulcers.

Figure 5.10 The cost of each health state at each stage in the model



*

Dotted lines represent transition between two health states that only differ due to the duration of active ulceration (i.e. from a wound health state of $<10cm^2$, <6 months to $<10cm^2$, > 6 months).

As previously stated, the mean utility value associated with one year was 0.75; indicating that a person entering the model would obtain, on average, the equivalent of three-quarters of a year in perfect health. The utilities gained in each health state at each time point are shown in Figure 5.11. The healed ulcer health state has the greatest utility. This is due in part to the increased utility assigned to patients with healed ulcers but also due to the number of patients with healed ulcers (as shown by Figure 5.9).

Figure 5.11 Utilities in each health state throughout the model

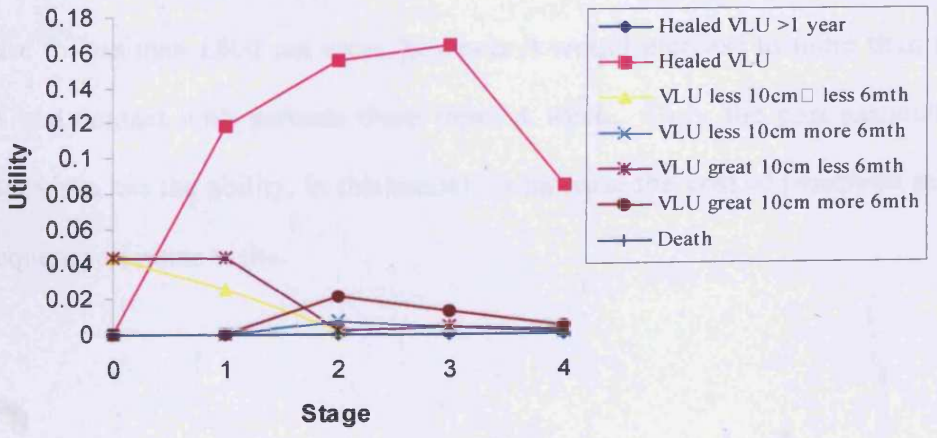
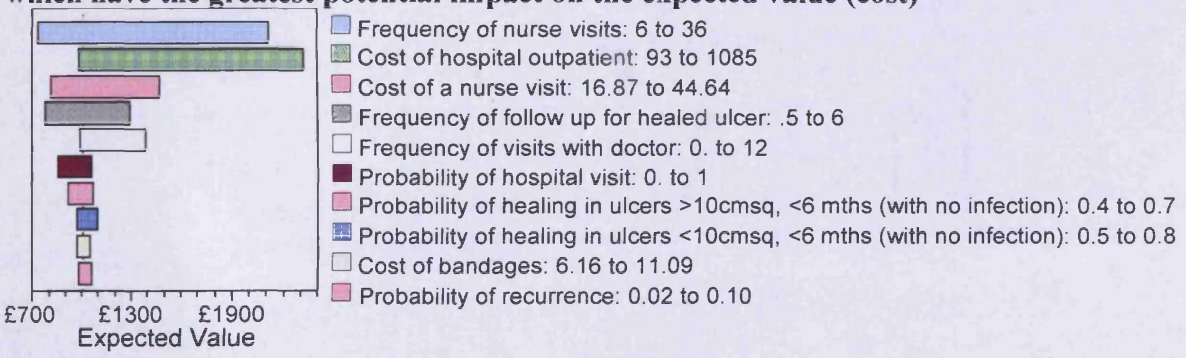


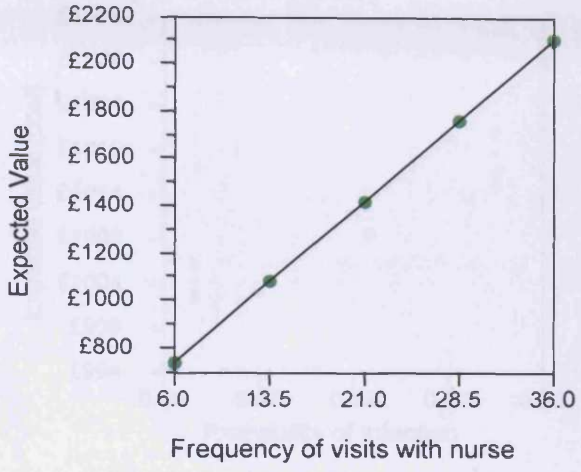
Figure 5.12 demonstrates the potential effect of the ten variables that have the greatest impact depending on the expected value chosen from within their sensitivity range. The factors that have the greatest potential to increase costs are the frequency of nurse visits and hospital cost. Other important variables include the cost of a nurse visit, the frequency of nurse visits for patients with healed ulcers and the frequency of consultations with a doctor for patients with open ulcers.

Figure 5.12 Tornado diagram showing the effect of variation in the ten variables which have the greatest potential impact on the expected value (cost)



Variation in the frequency of nursing visits from once a fortnight to three times a week can be seen from Figure 5.12 to have the largest impact on the expected value. Figure 5.13 shows that if all patients were seen fortnightly by nurses, the expected value would decrease to less than £800 per ulcer, however it would increase to more than £2000 if nurses had contact with patients three times a week. Only the cost associated with hospital visits has the ability, in this model, to increase the cost of treatment more than the frequency of nurse visits.

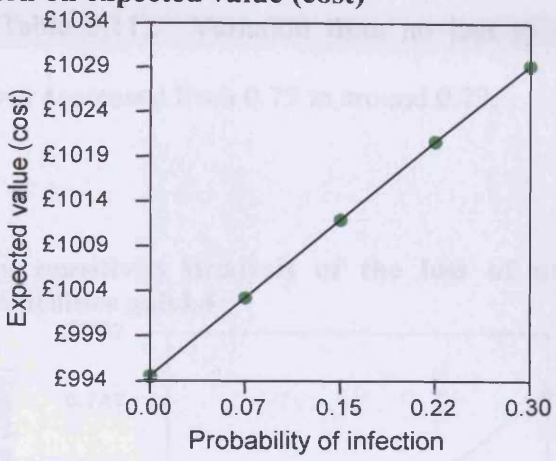
Figure 5.13 One-way sensitivity analysis of the frequency of nurse visits for patients with open ulcers



It can also be seen from Figure 5.12 that if all nursing contacts took place in the clinic setting (at a cost of £16.87), the expected cost associated with each wound would decrease to about £800, while if all contacts took place in the patient's home and took an average of 40 minutes (at a cost of £44.64), the cost would increase to approximately £1450.

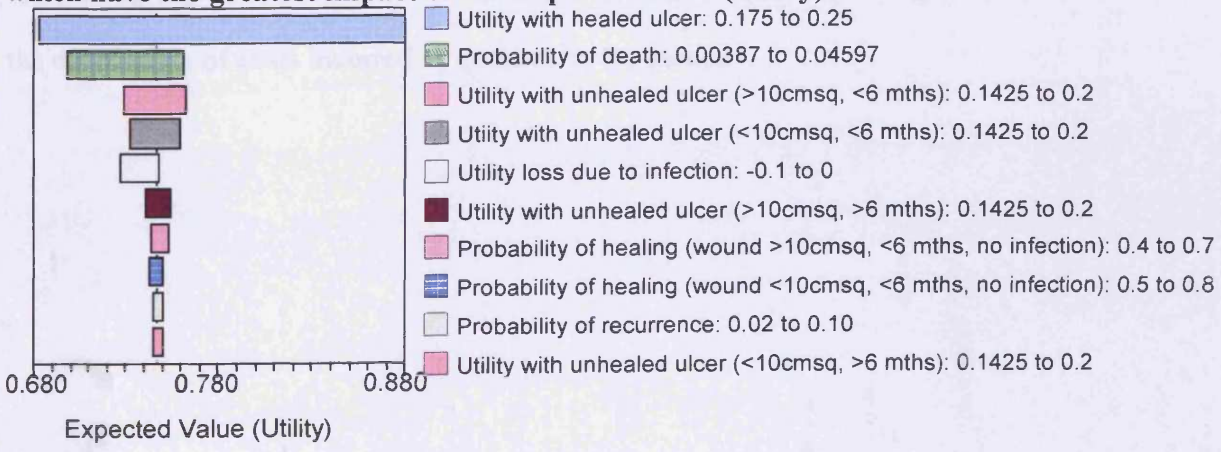
The influence of the probability of infection is explored in Figure 5.14. Variation in the probability of infection is not as influential on expected cost as variation in the frequency of nurse or doctor visits, probability of hospital visit or costs associated with nurse visits.

Figure 5.14 One-way sensitivity analysis showing the impact of variation in the probability of infection on expected value (cost)



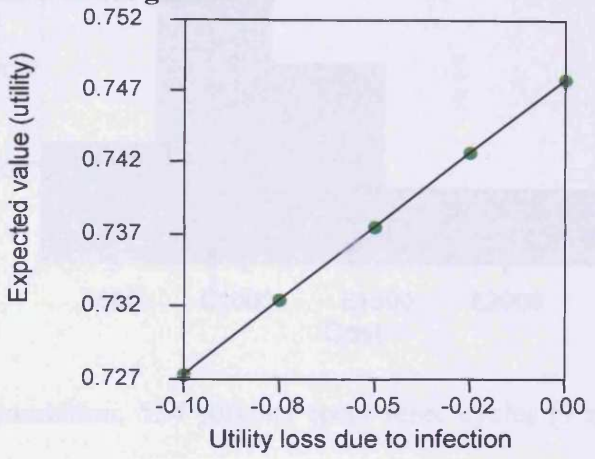
The ten variables with the greatest impact of divergence, within the sensitivity range, on expected utility are shown in Figure 5.15. It can be seen that change in the utility associated with healed ulcers has the greatest impact on expected utility, with changes in the estimated probability of death being the second most influential variable at reducing expected utility. If the utility associated with a healed ulcer decreased to 0.175 (0.7 per annum) then the expected utility would decrease to 0.685, however if the utility associated with a healed ulcer increased to 0.25 (1 per annum; perfect health) then the expected utility associated with a year in the model would be 0.880.

Figure 5.15 Tornado diagram showing the effect of variation in the ten variables which have the greatest impact on the expected value (utility)



The impact of any loss of utility due to infection was investigated using one-way sensitivity analysis (Table 5.11). Variation from no loss to –0.1 utility, found the expected utilities gained decreased from 0.75 to around 0.73.

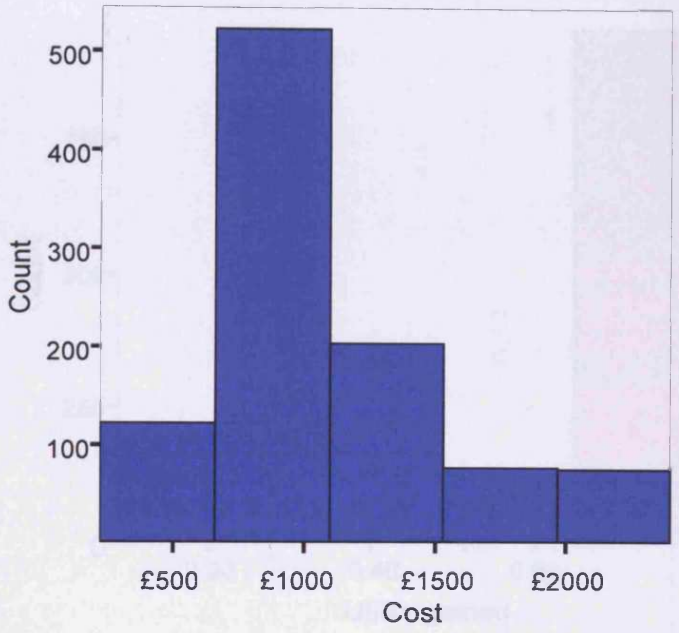
Table 5.11 One-way sensitivity analysis of the loss of utility associated with infection on expected utilities gained



Micro-simulation analysis

The mean cost (standard deviation) of treatment of a microsimulation of 1000 patients taking 'random walks' through the model was found to be £997 (£456), when all infections were susceptible to first-line antibiotic treatment. The minimum cost incurred by any patient was £219 and the maximum cost was £2409. Figure 5.16 shows the distribution of costs incurred by patients in the model.

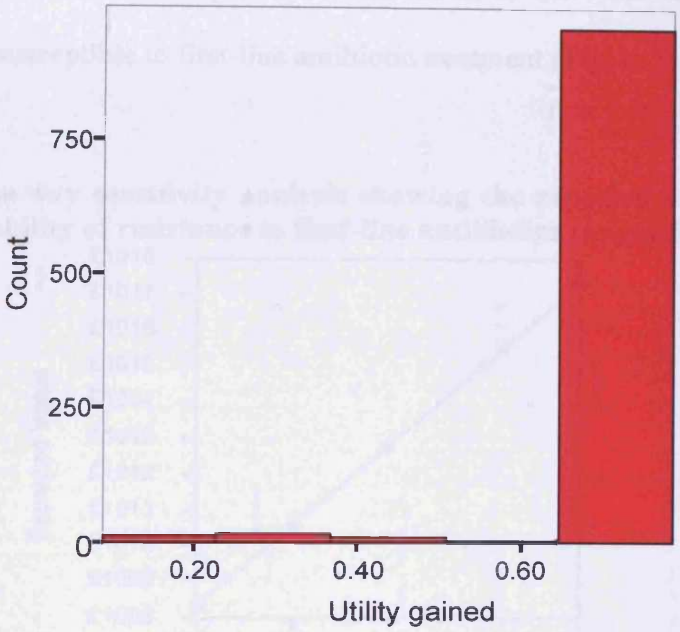
Figure 5.16 Distribution of costs incurred by patients in Monte Carlo microsimulation analysis, when all infections are susceptible to first-line antibiotic treatment



During this micro-simulation, 534 patients spent three cycles (9 months), 251 patients spent two cycles (6 months) and 102 spent one cycle (3 months) in the healed ulcer state. Only 113 patients did not heal for any period of time. Recurrence occurred once in 102 patients and no patients experienced two episodes of recurrence during the year. In total, 161 patients experienced infection: 144 a single infection, 16 patients two infection episodes and one patient had three episodes of infection. The cost incurred by each infection episode was £67.86 and therefore the total cost of infection during the year was £12,146.94.

The range of utilities gained in the microsimulation is shown in Figure 5.17. Thirty-nine patients gained less than 0.5 utilities during the year. The minimum utilities gained was 0.09 and the maximum was 0.79. The mean (SD) utility gained was 0.747 (0.107).

Figure 5.17 Distribution of utilities gained by patients in Monte Carlo microsimulation analysis, when all infections are susceptible to first-line antibiotic treatment



5.4.4.2 One-year perspective; resistance to first-line antibiotics

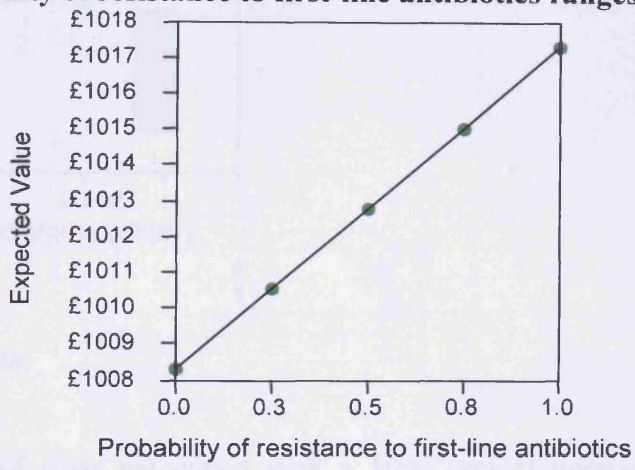
The cost of resistance to first-line antibiotic treatment was explored over one year using a base probability of resistance of 0.1, and the range p=0 to p=1 for sensitivity analysis.

Cohort analysis

The expected value in this model was £1008.88: an increase of 60p compared with the model when all infections were susceptible to first-line antibiotic treatment. This cost is a direct consequence of incurring the cost of second-line antibiotic treatment. The model assumes patients respond to second-line antibiotic treatment. Furthermore, there is no impact on the probability of healing in the model and only one extra consultation with a healthcare professional. When all infections are resistant to first-line antibiotics, the expected value increased by less than £10 (Figure 5.18).

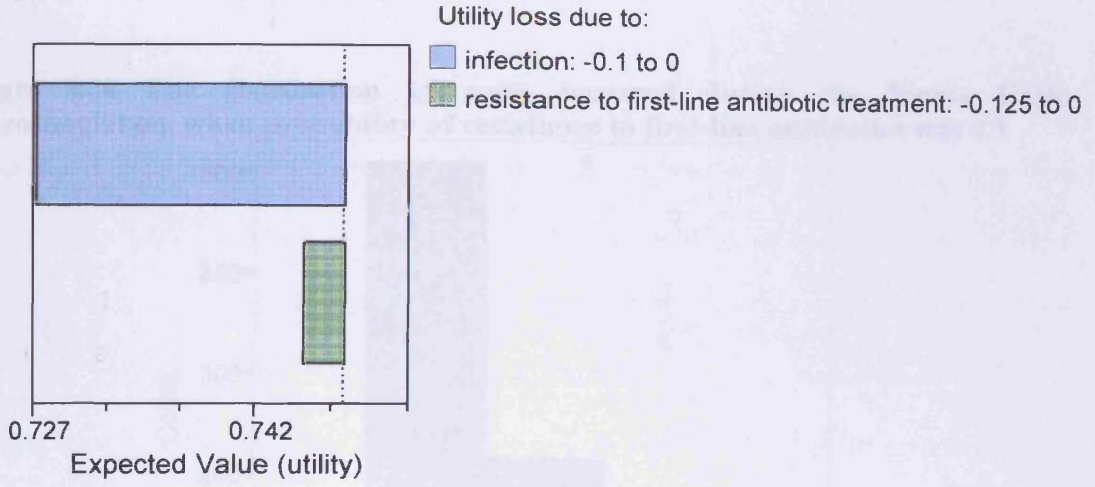
The factors in which variation had the greatest influence on expected cost in this model were identical to those factors previously identified as the most influential when all infections were susceptible to first-line antibiotic treatment (Figure 5.12).

Figure 5.18 One-way sensitivity analysis showing the expected value of treatment when the probability of resistance to first-line antibiotics ranges from p=0 to p=1



Utility was not directly affected by first-line antibiotic resistance, and therefore the ten most influential variables associated with the utility were identical to those found in the analysis when all infections were antibiotic susceptible (Figure 5.15). The impact on the expected utility of a loss of utility associated with infection and further loss of utility associated with antibiotic resistant episodes of infection was explored in sensitivity analysis (Figure 5.19). Loss of utility associated with antibiotic susceptible infection had a greater influence than utility lost due to antibiotic resistant infection: a loss of 0.1 utility for an infection caused the expected value to decrease to 0.727, while a loss of an additional 0.125 for any antibiotic resistant infection caused the expected value to decrease to 0.745.

Figure 5.19 Tornado diagram showing the impact of utility loss associated with infection and resistance to first-line antibiotic treatment on expected value (of utility)



Micro-Simulation

The mean (SD) of costs associated with a 1000 patient microsimulation when the probability of resistant infection was 0.1 was £1019 (£466). The minimum cost for any patient in the model was £219 and the maximum cost was £2342. The distribution of costs incurred by patients travelling through the model is shown in Figure 5.20. The mean (SD) utility gained by patients in the model was 0.747 (0.100): the minimum utility was 0.09 and the maximum gained was 0.79. The distribution of utility gained during the trial shown in Figure 5.21.

Five hundred and sixteen patients spent three cycles in the healed ulcer state, 263 patients spent two cycles and 105 spent one cycle in the healed state. One hundred and sixteen patients did not heal for any period of time. Recurrence occurred once in 105 patients and twice in one patient during the year. One hundred and ninety-one patients experienced infection, of whom 17 (8.9%) had two infections. Of these 208 episodes of infection, 23 (11.1%) were resistant to first-line treatment (21 patients had one, and one patient had two, infections resistant to first-line treatment). The cost of infection in this

microsimulation was therefore £14,114.88 and the additional cost of resistance to first-line treatment (£29.18 per infection) was £671.14.

Figure 5.20 The distribution of costs incurred during the Monte Carlo microsimulation, when probability of resistance to first-line antibiotics was 0.1

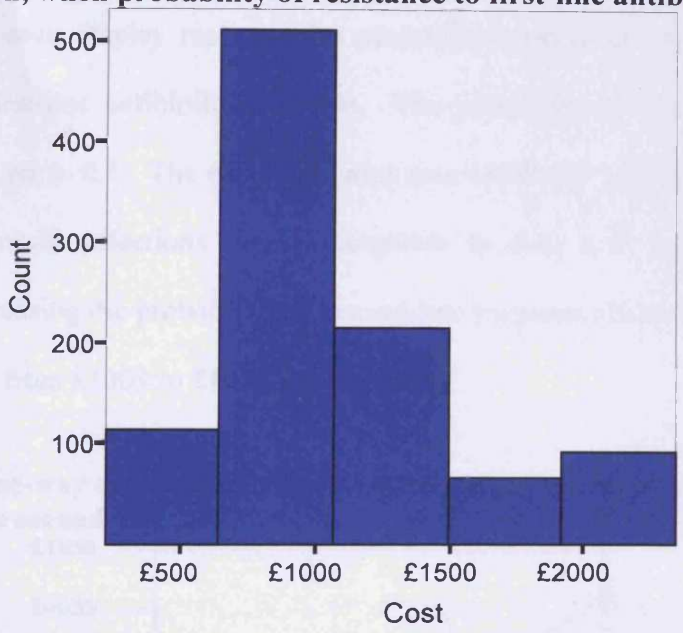
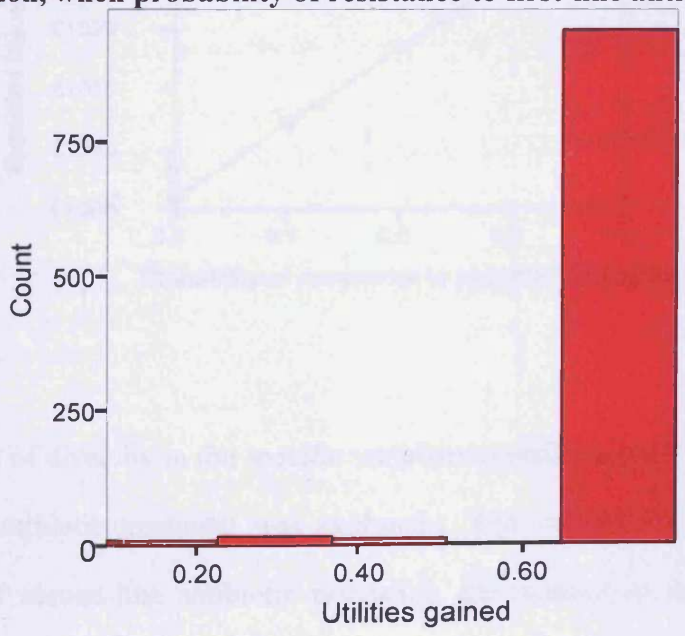


Figure 5.21 The distribution of utilities gained during the Monte Carlo microsimulation, when probability of resistance to first-line antibiotics was 0.1

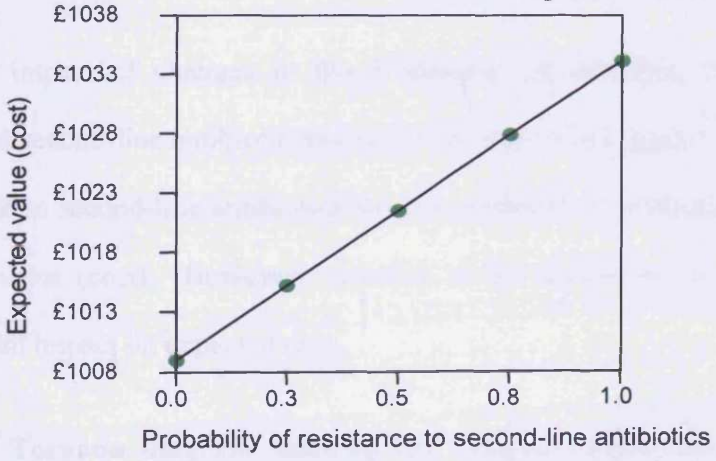


5.4.4.3 One year perspective; resistance to first and second-line antibiotics

Cohort analysis

Resistance to second-line antibiotics was explored using the base value of p=0.012. Patients could only display resistance to second-line antibiotic treatment if they had resistance to first-line antibiotic treatment. The probability of resistance to first-line antibiotics was set to 0.1. The expected value was £1009.18: 90p more than the cost of treatment when all infections were susceptible to first and second-line antibiotic treatment. Increasing the probability of second-line resistance from 0 to 1, increased the expected value from £1009 to £1033 (Figure 5.22).

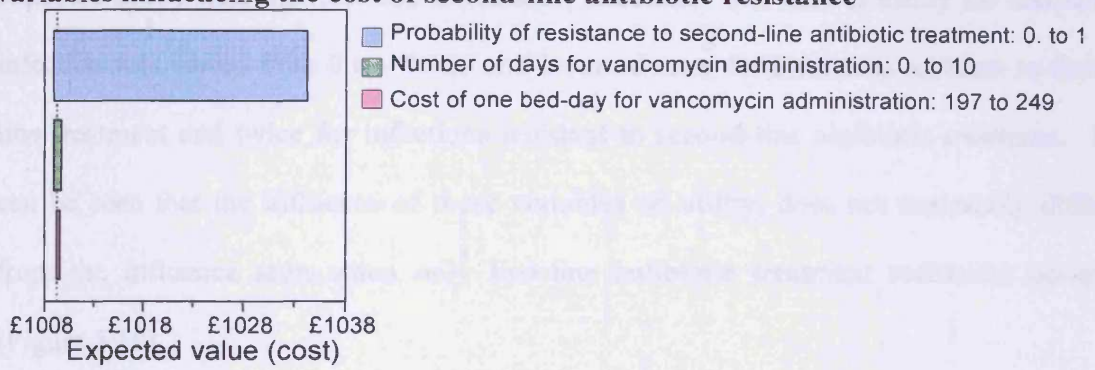
Figure 5.22 One-way sensitivity analysis investigating the impact of the probability of resistance to second-line antibiotic treatment on expected value



The influence of diversity in the specific variables contributing to the additional cost of second-line antibiotic treatment was explored. The variables investigated were the probability of second-line antibiotic resistance, the number of days for vancomycin treatment and the cost of an inpatient stay while vancomycin was administered (Figure 5.23). The probability of resistance to second-line treatment was the most influential on expected value (cost). However, this was of less importance to the overall cost than the

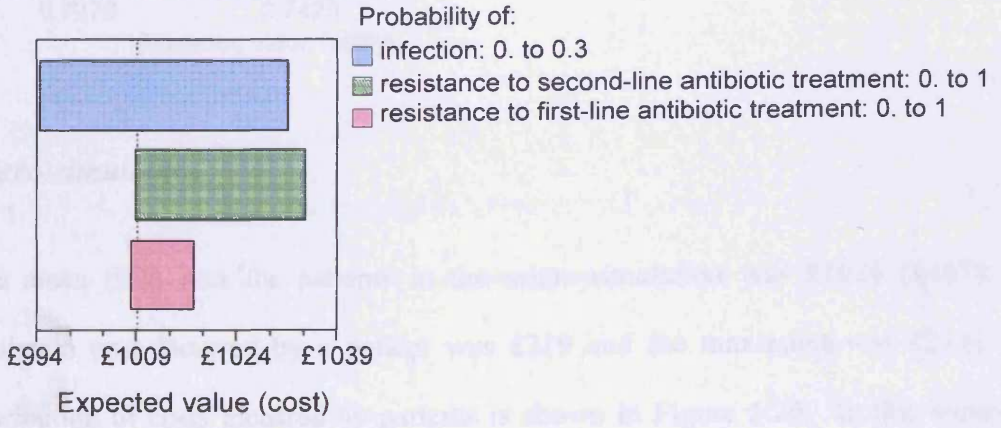
probability of infection, cost of dressings and probability of healing in ulcers greater than 10cm^2 and greater than 6 months duration. The ten variables that had the greatest affect on the expected value were the same variables seen to have the greatest impact when all infections were susceptible to first-line antibiotic treatment (Figure 5.12).

Figure 5.23 Tornado diagram showing the relative influence on expected cost of variables influencing the cost of second-line antibiotic resistance



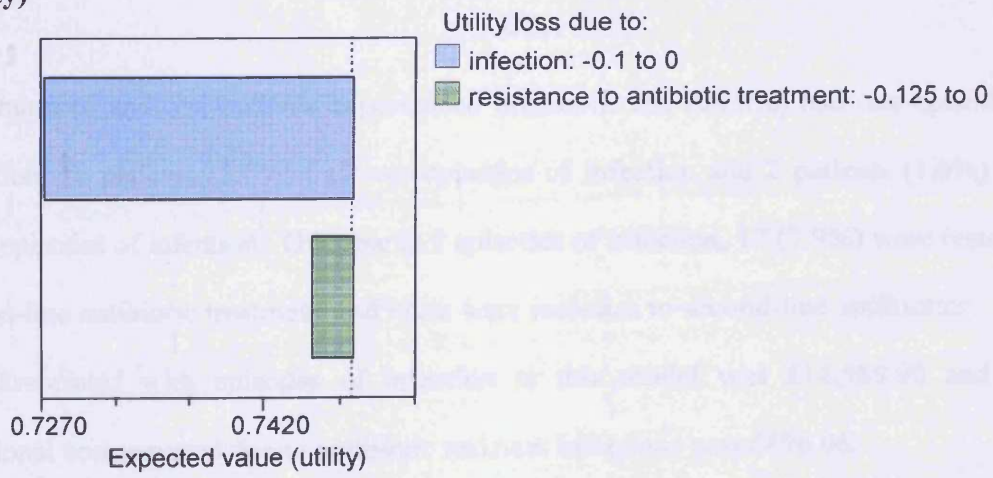
The relative impact of changes in the probability of infection, first-line antibiotic resistance and second-line antibiotic resistance are shown in Figure 5.24. It can be seen that resistance to second-line antibiotics has the potential to cause the greatest increase in expected value (cost). However, variation in the probability of infection has the greatest overall impact on expected cost.

Figure 5.24 Tornado diagram showing the relative impact of variation in the probability of infection, resistance to first-line antibiotic treatment and resistance to second-line antibiotic treatment



The impact of changes in the utility associated with antibiotic resistance was explored. Resistance to second-line antibiotic treatment was associated with a further loss of utility of the same magnitude (and represented by the same variable) as that already lost due to first-line antibiotic treatment resistance. Figure 5.25 shows that change in the utility lost due to infection (from 0 to –0.1 per Markov cycle) is of greater influence on expected utility than that lost due to resistant infection. The loss of utility for resistant infection was varied from 0 to –0.125 and incurred once for infections resistant to first-line treatment and twice for infections resistant to second-line antibiotic treatment. It can be seen that the influence of these variables on utility, does not noticeably differ from the influence seen when only first-line antibiotic treatment resistance occurs (Figure 5.19).

Figure 5.25 Tornado diagram showing the relative influence of changes in the utility loss associated with infection and antibiotic resistance on expected value (utility)

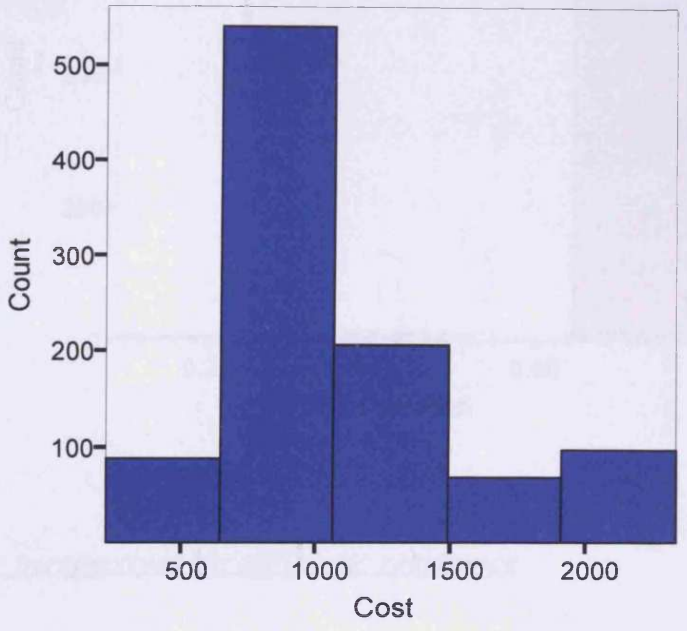


Micro-simulation

The mean (SD) cost for patients in the micro-simulation was £1029 (£467). The minimum cost incurred by a patient was £219 and the maximum was £2342. The distribution of costs incurred by patients is shown in Figure 5.26. In this simulation, 116 patients had ulcers that did not heal, while 108 experienced healed ulcers for one

cycle (3 months), 255 had healed ulcers for two cycles (6 months) and 521 had healed ulcers for three cycles (9 months).

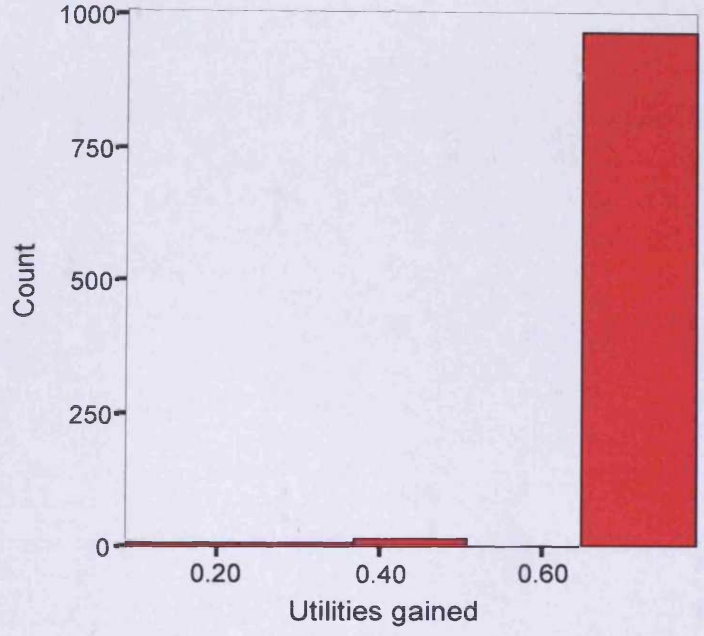
Figure 5.26 The distribution of costs incurred by patients during the Monte Carlo microsimulation where the probability of resistance to second-line antibiotic treatment was 0.012



Two-hundred and one patients experienced infection: 189 (94.0%) had one episode of infection, 10 patients (5.0%) had two episodes of infection and 2 patients (1.0%) had three episodes of infection. Of these 215 episodes of infection, 17 (7.9%) were resistant to first-line antibiotic treatment and none were resistant to second-line antibiotics. The cost associated with episodes of infection in this model was £14,589.90 and the additional cost incurred due to antibiotic resistant infections was £496.06.

The mean (SD) utility experienced by patients in the simulation trial was 0.752 (0.087). The minimum utility gained was 0.09 and the maximum was 0.79. The distribution of the utility gained by patients in the microsimulation trial is shown in Figure 5.27.

Figure 5.27 The distribution of utility gained by patients during the Monte Carlo microsimulation where the probability of resistance to second-line antibiotic treatment was 0.012



5.4.4.4 Ten-year perspective: No antibiotic resistance

Cohort analysis

The expected value for each patient in the 10-year cohort was £3917.60, with 3.5% discounting of future costs and all infections susceptible to first-line antibiotic treatment. The expected value when future costs were not discounted was £4471.10 and £3596.49 when 6% discount rate was applied (Figure 5.28).

The expected utility gained over the 10-year period was 5.327 when future benefits were discounted at a rate of 3.5%. When future benefits were not discounted the expected utility was 6.211 and when a rate of 6% applied it was 4.816. The cost per QALY was therefore £719.87, £735.42 and £746.78 when discount rates of 0%, 3.5% and 6% were applied respectively.

Figure 5.28 One-way sensitivity analysis showing the impact of discounting future costs on expected value (costs)

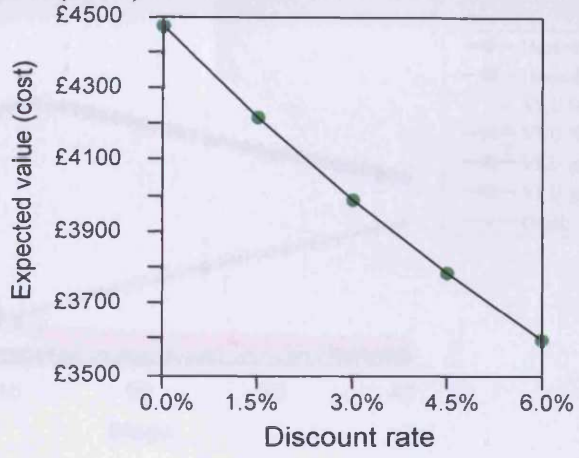
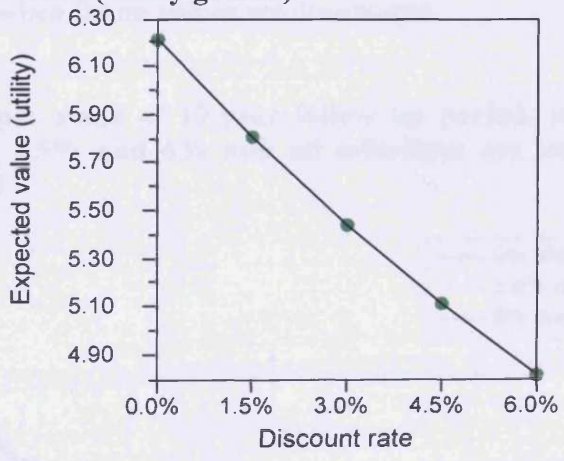
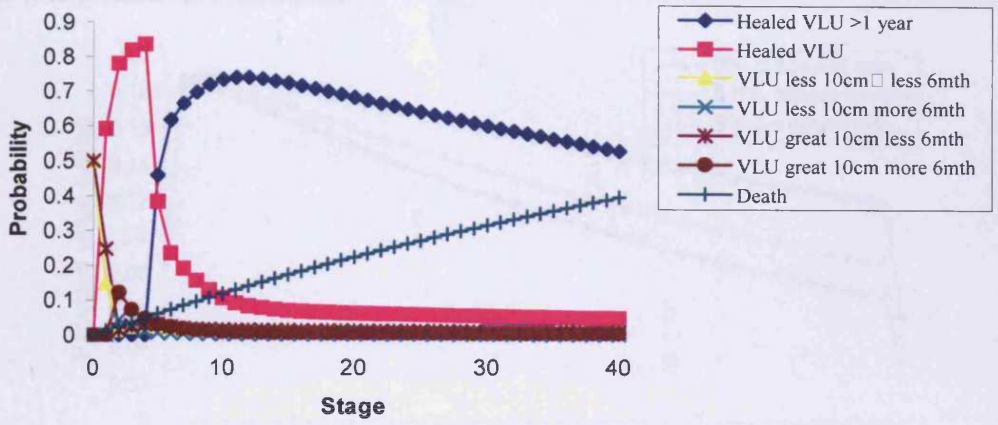


Figure 5.29 One-way sensitivity analysis showing the impact of discounting future benefits on expected value (utility gained)



The probability of being in each health state in the 10-year period is shown in Figure 5.30. Around 85% of patients have a healed ulcer between 15 and 30 months (stages 5 to 10). The proportion of patients with healed ulcers then slowly declines as the proportion of patients in the model who have died increases.

Figure 5.30 The probability of patients being in each health state during the 10-year follow-up period



The costs incurred and utilities gained at each stage of the model are shown in Figure 5.31 and Figure 5.32. It can be seen that both costs and utilities decrease over time and do so at a faster rate when future values are discounted.

Figure 5.31 Costs per stage of 10-year follow up period, when future costs are discounted by 0%, 3.5% and 6% and all infections are susceptible to first-line antibiotic treatment

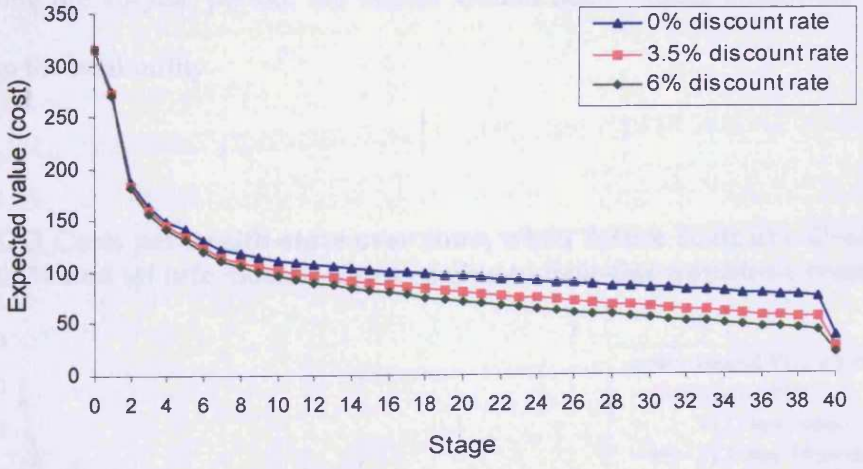
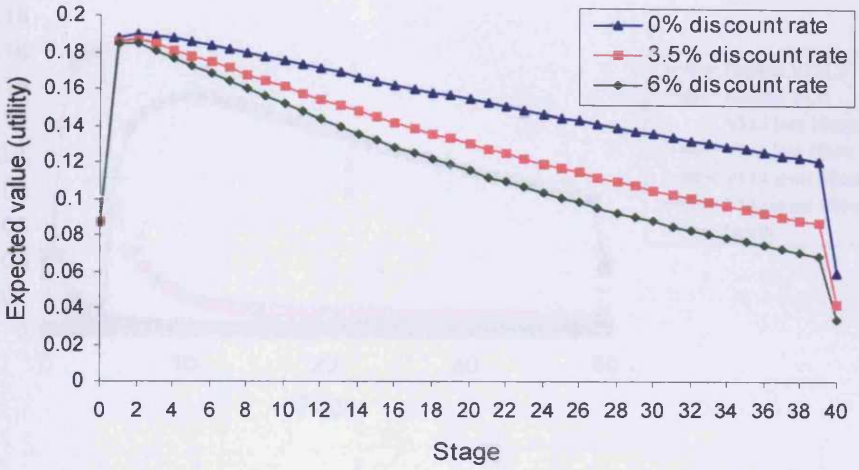


Figure 5.32 Utilities gained per stage of 10-year follow up period, when future benefits are discounted by 0%, 3.5% and 6% and all infections are susceptible to first-line antibiotic treatment



The costs per health state over the model period are shown in Figure 5.33, when future costs are discounted by 3.5% and all infections are susceptible to first-line antibiotic treatment. It can be seen that the main cost is associated with healed ulcers subsequent to the very initial stages of the model. Figure 5.34 shows the gain in utilities by health state during the 10-year period: the healed wound health states contribute the greatest amount to the total utility.

Figure 5.33 Costs per health-state over time, when future costs are discounted at a rate of 3.5% and all infections are susceptible to first-line antibiotic treatment

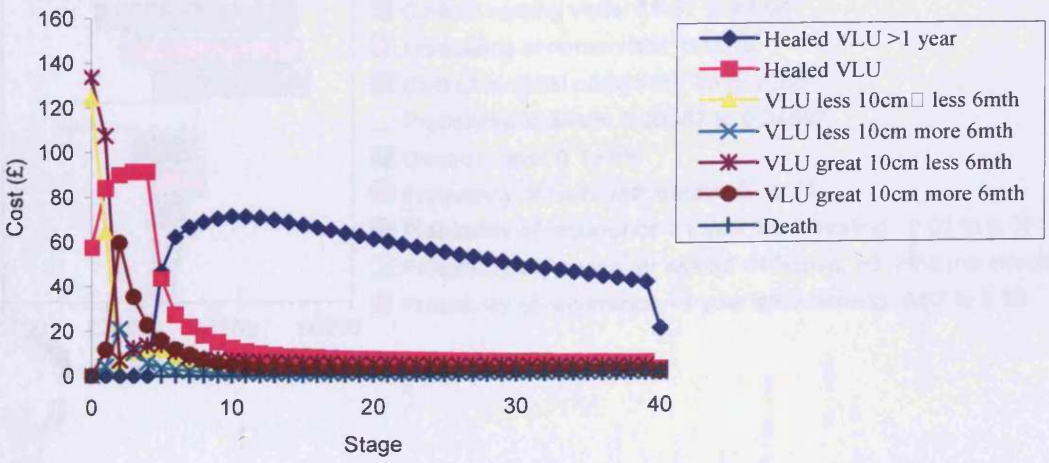
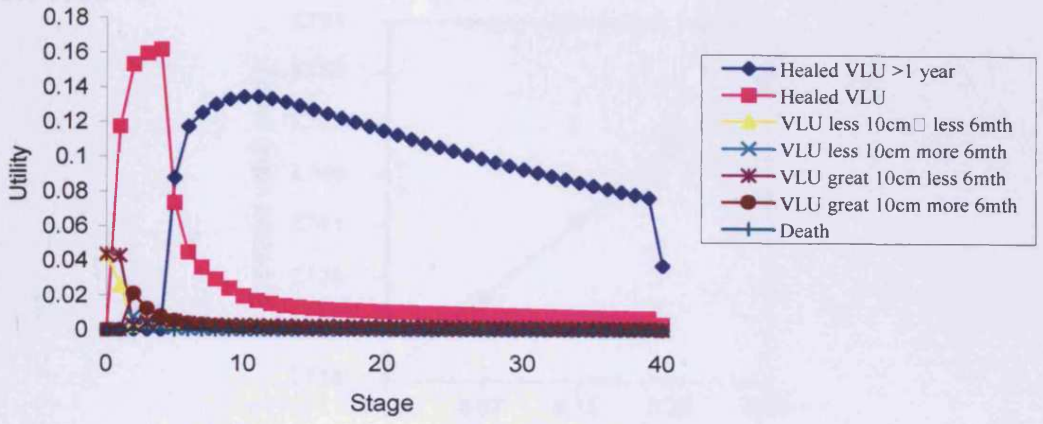


Figure 5.34 The utilities gained in each health state during the model, when future benefits are discounted at a rate of 3.5% and all infections are susceptible to first-line antibiotic treatment



The variables, in which diversity has the greatest impact on expected value, when future costs are discounted by 3.5%, are shown in Figure 5.35. The main factor is the frequency of follow up visits for healed ulcers. Fortnightly visits with a nurse could increase the expected value to more than £6300 while six-monthly visits could decrease it to around £1800. The impact of variation in the probability of infection is shown in Figure 5.36. The expected cost is decreased by approximately £30 when the probability of infection decreases from p=0.3 to p=0.

Figure 5.35 Tornado diagram showing the ten variables in which variation has the greatest impact on the expected value (cost), with future costs discounted at 3.5% and all infections susceptible to first-line antibiotic treatment

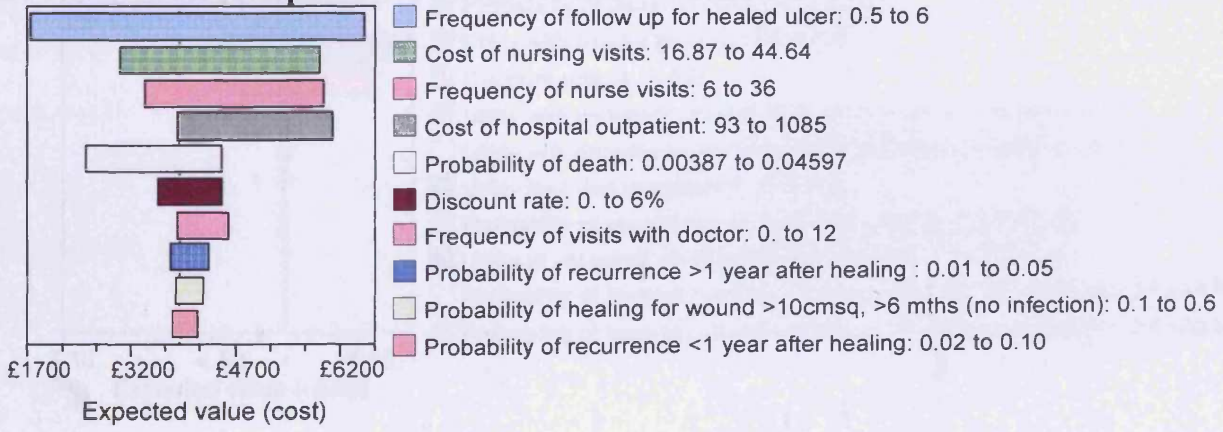
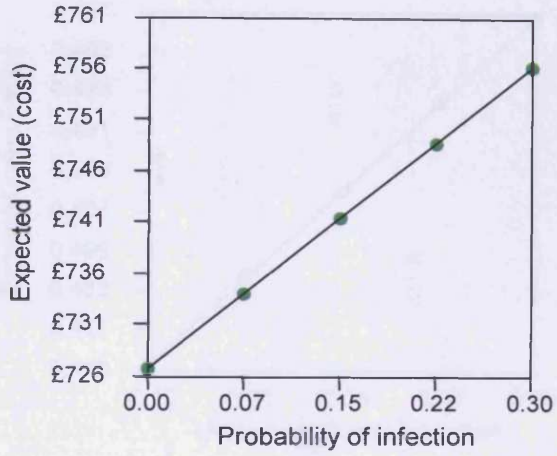


Figure 5.36 One-way sensitivity analysis showing the impact on expected cost, in the 10-year model, of variation in the probability of infection when all infections are susceptible to first-line antibiotic treatment and future costs are discounted at a rate of 3.5%



Divergence in three variables was seen to have considerable impact on expected utility: probability of death, utility associated with a healed ulcer and the discount rate applied (Figure 5.37). There was very little impact on expected utility by variation in any of the other variables in the model. Loss of utility associated with infection was associated with a slight decrease in the expected utility: from 0.497 to around 0.479 (Figure 5.38).

Figure 5.37 Tornado diagram showing the 10 variables in which variation has the greatest impact on the expected utility, when future benefits are discounted at a rate of 3.5%

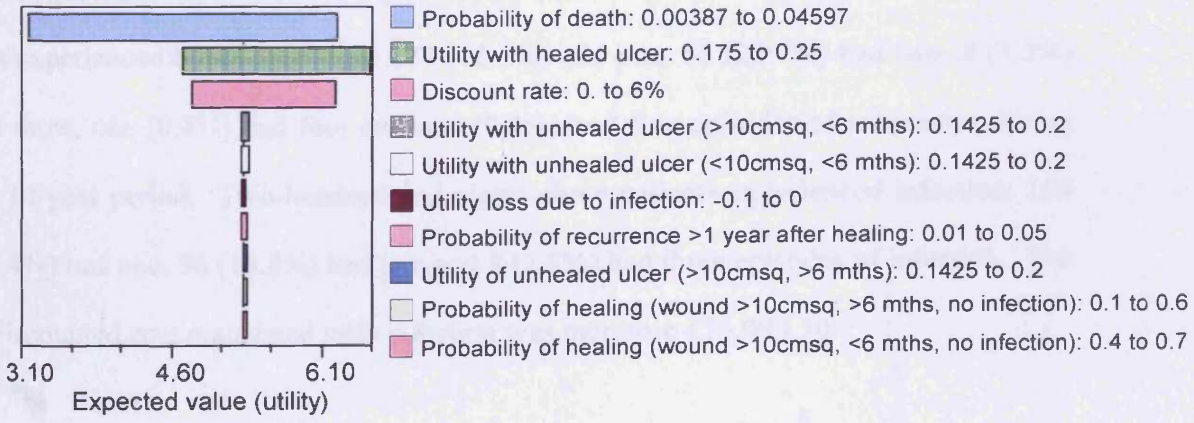
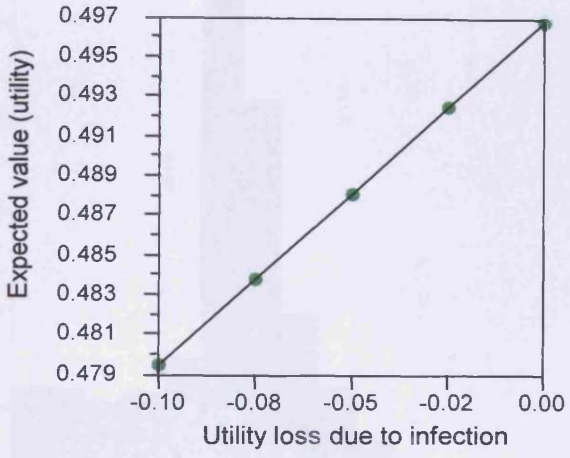


Figure 5.38 One-way sensitivity analysis of the impact of loss of utility associated with infection during the 10-year model period, when future benefits are discounted at a rate of 3.5% and all infections are susceptible to first-line antibiotic treatment



Micro-simulation

The mean (SD) expected value after running a microsimulation of 1000 patients through the model for 10 years was £3897 (£1616). The minimum value was £219 and the maximum value was £10,298. The range of costs incurred during the microsimulation is shown in Figure 5.39 and the range of expected utility gained is shown in Figure 5.40. The mean (SD) utility gained from the model was 5.25 (2.14). All patients spent at least one Markov cycle in a healed ulcer health state. The mean (SD) number of stages spent in a healed ulcer state was 29.0 (12.64): equivalent to 7 years 3 months. Recurrence was experienced by 242 patients: 177 (73.1%) had one, 55 (22.7%) had two, 8 (3.3%) had three, one (0.4%) had four and one (0.4%) had five episodes of recurrence during the 10-year period. Two-hundred and eighty-three patients experienced infection: 219 (77.4%) had one, 56 (19.8%) had two and 8 (2.8%) had three episodes of infection. The undiscounted cost associated with infection was therefore £24,090.30.

Figure 5.39 Distribution of costs incurred by patients during the Monte Carlo microsimulation with 10-year follow-up, when all infections are susceptible to first-line antibiotic treatment and future costs are discounted at a rate of 3.5%

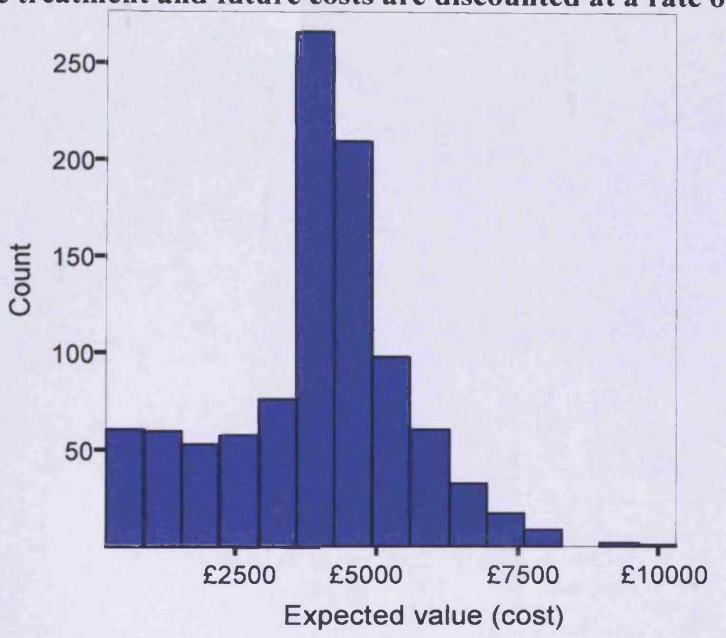
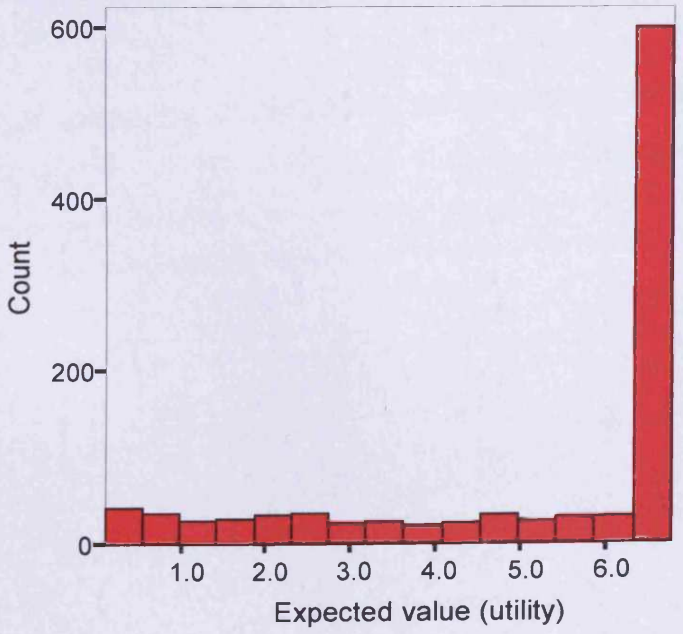


Figure 5.40 Range of expected values (utilities) gained during microsimulation when all infections are susceptible to first-line antibiotic treatment and future benefits are discounted at a rate of 3.5%



5.4.4.5 *Ten-year perspective; resistance to first-line antibiotics*

Cohort analysis

Resistance to first-line antibiotic treatment had an extremely small impact on the expected cost of an ulcer after 10 years. The expected value was £3918.59 when future costs were discounted by 3.5%: an increase of 99p above the expected value when all infections were susceptible to first-line antibiotic treatment. The expected value when future costs were discounted by 0% and 6% was £4472.16 and £3597.45 respectively. The impact of discounting future values, both costs and utility, are very similar to that shown in Figure 5.28 and Figure 5.29. The expected utility was 5.327, including a 3.5% discount rate (equal to the utility gained when all infections were susceptible to first-line antibiotic treatment).

The 10 variables in which variation most greatly affected the expected value in the model were the same when the probability of resistance to first-line antibiotic treatment was 0.1 as when all infections were susceptible to first-line treatment. The difference in expected cost when the probability of resistance to first-line antibiotic treatment was varied from 0 to 1 was approximately £10, as shown in Figure 5.41. As with the one-year model, the probability of resistance to first-line antibiotic treatment is of less influence on cost that the probability of infection (Figure 5.42). The impact of changes in the utility associated with infection and resistant infection are shown in Figure 5.43.

Figure 5.41 One-way sensitivity analysis showing the impact of variation in the probability of resistance to first-line antibiotic treatment from 0 to 1, when future costs are discounted at a rate of 3.5%

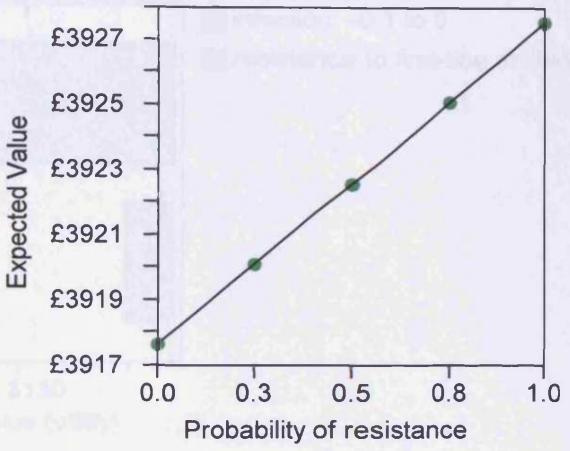


Figure 5.42 Tornado diagram showing the relative impact of changes in the probability of infection and first-line antibiotic treatment resistant infection, when future costs are discounted at a rate of 3.5%

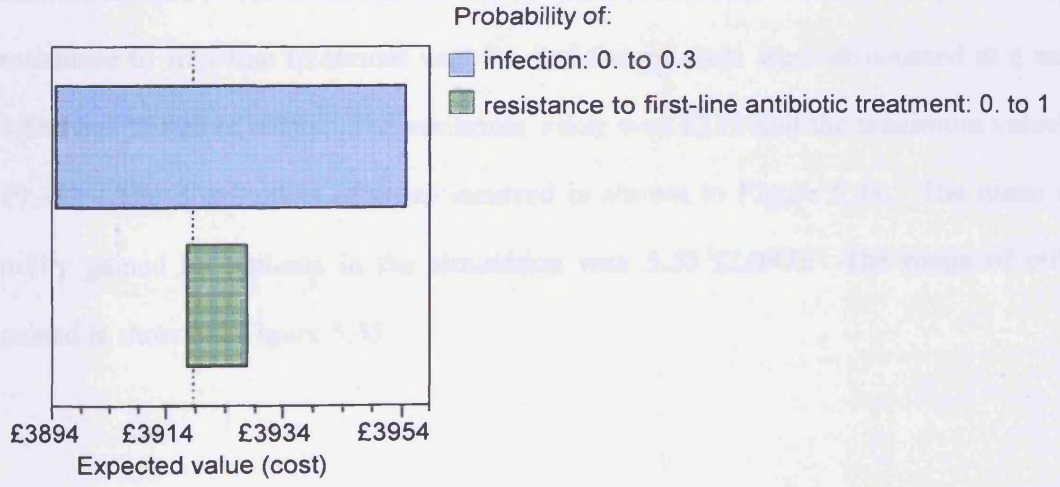
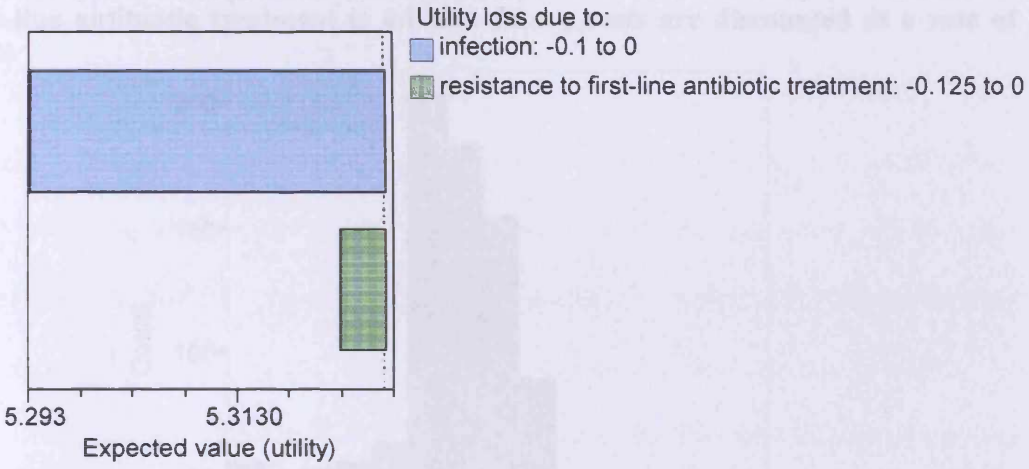


Figure 5.43 Utility loss due to infection and antibiotic resistant infection, when future costs are discounted at a rate of 3.5%



Monte Carlo simulation

The mean (SD) value in Monte Carlo microsimulation when the probability of resistance to first-line treatment was 0.1 and future costs were discounted at a rate of 3.5% was £3890 (£1618). The minimum value was £219 and the maximum value was £9,352. The distribution of costs incurred is shown in Figure 5.44. The mean (SD) utility gained by patients in the simulation was 5.33 (2.097). The range of utilities gained is shown in Figure 5.45.

Figure 5.44 Distribution of costs incurred by patients during the Monte-Carlo microsimulation trial, with 10-year follow-up, when the probability of resistance to first-line antibiotic treatment is 0.1 and future costs are discounted at a rate of 3.5%

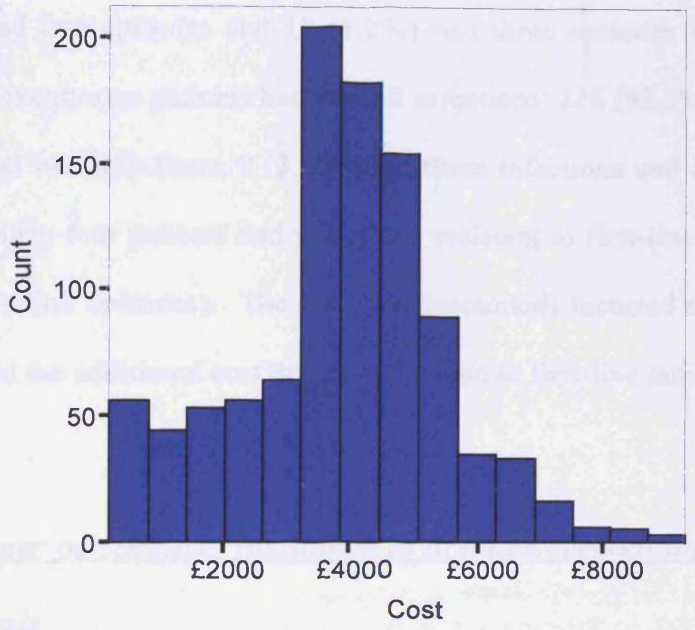
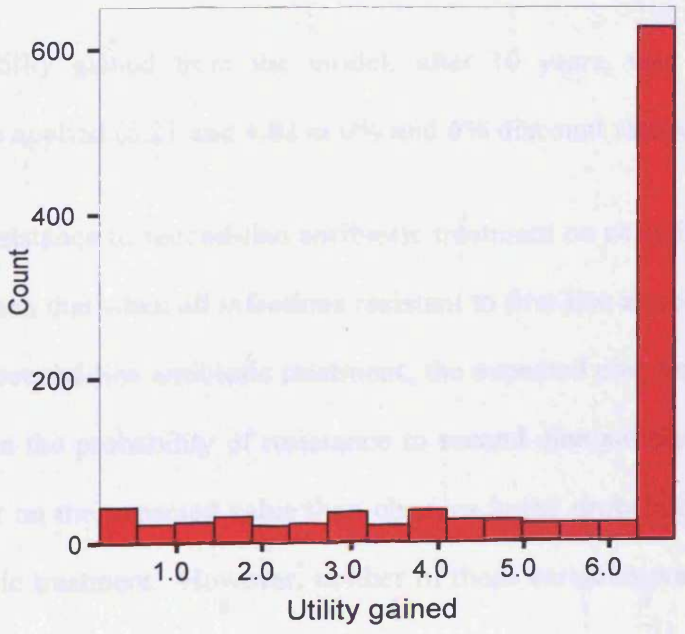


Figure 5.45 Distribution of utilities gained by patients during the Monte Carlo microsimulation trial, with 10-year follow up, when the probability of resistance to first-line antibiotic treatment is 0.1 and future benefits are discounted at a rate of 3.5%



The mean (SD) number of stages spent in a healed ulcer state was 29.6 (12.32). Two-hundred and thirty-one patients had recurrent ulceration: 183 (79.2%) had one episode, 36 (15.6%) had two episodes and 12 (5.2%) had three episodes of recurrence. Two hundred and seventy-one patients had wound infections: 216 (93.5%) had one infection, 45 (19.5%) had two infections, 9 (3.9%) had three infections and one (0.4%) had four infections. Thirty-four patients had infections resistant to first-line antibiotic treatment (10.1% of infection episodes). The cost (undiscounted) incurred due to infection was £22,868.82 and the additional cost due to resistance to first-line antibiotic treatment was £992.12.

5.4.4.6 Ten-year perspective; resistance to first and second- line antibiotics

Cohort analysis

The expected value when resistance to second-line antibiotic treatment occurred with a probability of 0.012 was £3919.09 when future costs were discounted at a rate of 3.5% (£4472.69 and £3597.92 when discount rates of 0% and 6% were applied respectively).

The expected utility gained from the model, after 10 years, was 5.33 when 3.5% discount rate was applied (6.21 and 4.82 at 0% and 6% discount rate respectively).

The impact of resistance to second-line antibiotic treatment on costs is shown in Figure 5.46. It can be seen that when all infections resistant to first-line antibiotic treatment are also resistant to second-line antibiotic treatment, the expected cost increased by around £40. Variation in the probability of resistance to second–line antibiotic resistance was of greater impact on the expected value than changes in the probability of resistance to first-line antibiotic treatment. However, neither of these variables was as significant as the probability of infection (Figure 5.47).

Figure 5.46 One-way sensitivity analysis showing the impact of change in the probability of resistance to second-line antibiotic treatment, when future costs are discounted at a rate of 3.5%

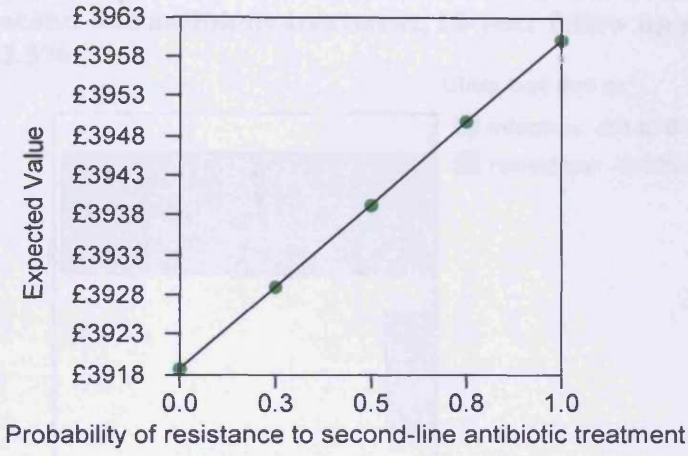


Figure 5.47 Tornado diagram showing the relative impact of variation in the probability of infection, resistance to first-line antibiotic treatment and resistance to second-line antibiotic treatment for a 10-year cohort, when future costs are discounted at 3.5%

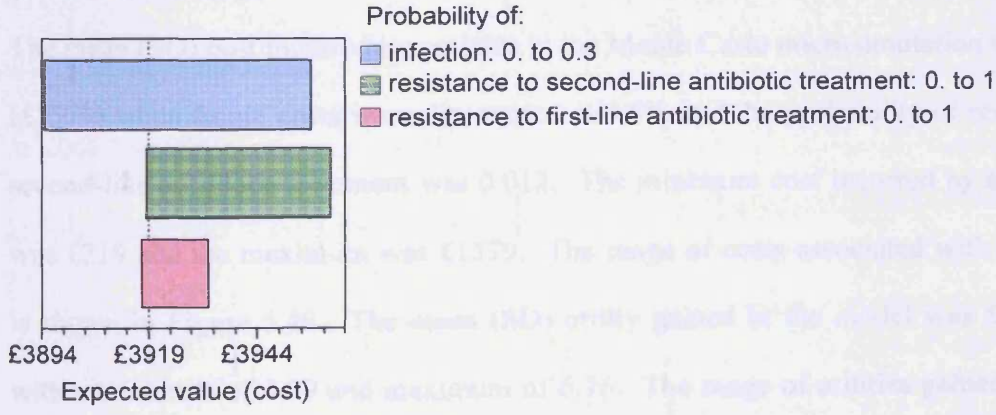
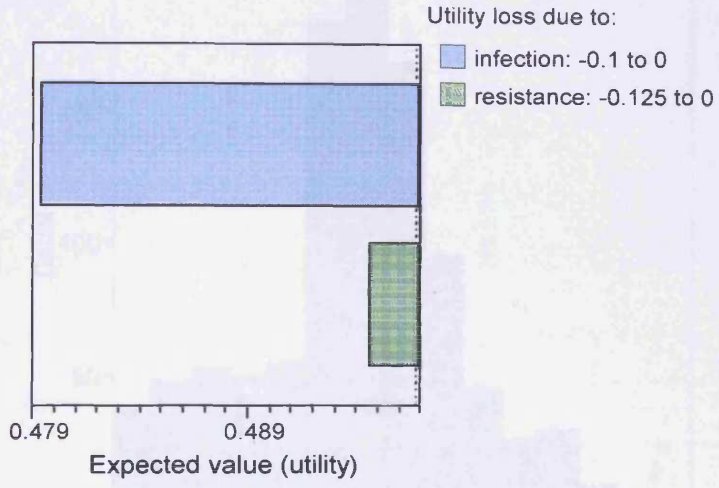


Figure 5.48 Tornado diagram showing the relative impact of loss of utility associated with infection and antibiotic resistant infection, in a model with 0.1 probability of resistance to first-line antibiotic treatment, 0.012 probability of resistance to second-line antibiotic treatment, 10-year follow up and future benefits discounted at 3.5%



Microsimulation trial

The mean (SD) cost incurred by patients in the Monte Carlo microsimulation was £4042 (£1579) when future costs were discounted at 3.5% and the probability of resistance to second-line antibiotic treatment was 0.012. The minimum cost incurred by any patient was £219 and the maximum was £1579. The range of costs associated with the model is shown in Figure 5.49. The mean (SD) utility gained in the model was 5.44 (1.99) with a minimum of 0.09 and maximum of 6.76. The range of utilities gained is shown in Figure 5.50.

Figure 5.49 Distribution of costs incurred by patients in the Monte Carlo microsimulation trial followed for 10 years, when the probability of resistance to second-line antibiotic treatment is 0.012, and future costs are discounted at a rate of 3.5%

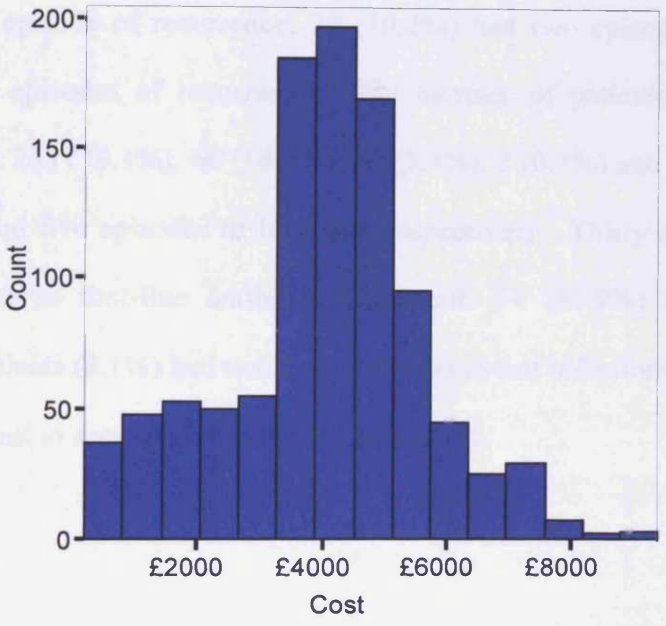
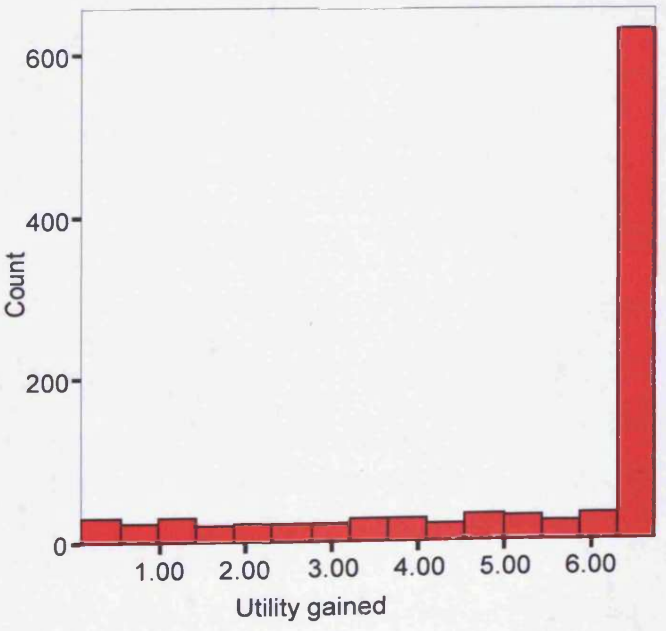


Figure 5.50 Distribution of utilities gained by 1000 patients in the Monte Carlo microsimulation followed for 10 years, when the probability of resistance to second-line antibiotic treatment is 0.012, and future benefits are discounted at a rate of 3.5%



The mean (SD) number of stages spent in a healed ulcer state was 30.0 (11.73) (equivalent to seven years and six months). Recurrence occurred for 248 patients: 211 (71.8%) had one episode of recurrence, 30 (10.2%) had two episodes and 7 patients (2.4%) had three episodes of recurrence. The number of patients that experienced infection was 294: 233 (79.3%), 48 (16.3%), 10 (3.4%), 2 (0.7%) and 1 (0.3%) had one, two, three, four and five episodes of infection respectively. Thirty-seven patients had infections resistant to first-line antibiotic treatment: 34 (91.9%) had one resistant infection and 3 patients (8.1%) had two episodes of resistant infections. No patients had an infection resistant to second-line antibiotic treatment.

5.5 Discussion

5.5.1 Main findings

This study has estimated the cost of treating a venous leg ulcer for one-year following incidence to be £1008.28. The cost per QALY was determined to be £1348.34, with an average 0.75 utility gained during one year of treatment in the absence of antibiotic resistance. Failure to respond to first or second-line antibiotic treatment was associated with a very small increase in the overall cost of treatment: 0.09% to £1009.18 when 10% of infections were resistant to first-line antibiotic treatment and 1.2% of these were resistant to second-line antibiotic treatment. In Monte Carlo microsimulation, the mean (SD) cost of treating each leg ulcer when there was no antibiotic resistance was £997 (£456) and £1029 (£467) with resistance to first and second-line antibiotic treatments. Costs ranged from £219 to £2409 for patients in the Monte Carlo microsimulation when there was no antibiotic resistance and from £219 to £2342 when there was resistance to first and second line antibiotics. The cost of treating leg ulcers in the light of infection and antibiotic resistant infection has not previously been estimated in the UK.

In the longer term, over a 10-year period, when future values were discounted at a rate of 3.5%, the cost of treating an ulcer was estimated to be £3,917.60, increasing to £3,919.09 when resistance to first and second-line antibiotic treatment were included. The Monte Carlo microsimulation when all infections were susceptible to antibiotic treatment found treatment costs to range from £219 to £10,298.

In the one-year Monte Carlo microsimulation trials, the number of patients who had infections ranged from 161 to 201, and patients had up to three episodes of infection. The overall cost of antibiotic susceptible infections ranged from £12,147 to £14,590 and the additional cost of antibiotic resistance from £496 to £671. Individually each episode of infection incurred a cost of £67.86 and each infection resistant to first-line antibiotic

treatment an additional £29.18. The further cost incurred by infections resistant to second-line treatment was £1222.55. In this model, however the probability of an infection resistant to second-line treatment was low, and no patients in the simulation of 1000 patients had such an infection.

During the first year of treatment, the variables in which divergence within the specified sensitivity range had the greatest impact on the expected cost of treatment, were the cost of hospital visits and frequency of visits with a nurse during the first year of treatment. When the costs were investigated over a 10-year period the frequency of follow-up had the greatest impact on costs, although the cost and frequency of nurse visits for patients with open ulcers also remained important. The probability of recurrence was of greater consequence to the expected cost when the population was modelled for 10-years rather than one-year. These main cost drivers remained the same irrespective of first-line or second-line antibiotic resistance.

The variables that had the greatest impact on expected utility gained in the one-year model were the utility gained by patients with healed ulcers, probability of death and utility gained by patients with unhealed ulcers greater than $10cm^2$ and duration greater than 6 months. In the 10-year model, where the average expected utility gained was 5.33 and future benefits were discounted at a rate of 3.5%, the costs per QALY was £735.42. Only probability of death, utility of a healed ulcer and the discount rate applied had any discernable affect on the total expected utility gained. The loss of utility due to infection was of greater impact than the additional loss due to antibiotic resistant infection on expected utility. This is due to the higher likelihood of, and therefore number of patients with, antibiotic susceptible infections compared to resistant infections.

In summary, this study has shown the cost per QALY of treating a leg ulcer for one year after incident to be £1348.48, and that viewed over a longer time period (10 years) this decreases to £735.42 per QALY (when the costs included are those from the physician's perspective). Infection and antibiotic resistant infection have little impact on the overall costs while there are still effective alternative treatments available. The additional cost incurred due to infection with antibiotic resistant organisms was minimal. The cost associated with MRSA has previously been suggested to vary according to setting, being highest in intensive care units and other high-risk locations. It may be that isolation from leg ulcers is similar to that of superficial sites and in long-stay patients in the community and has little impact on costs.[163]

5.5.2 Relationship to other studies

The cost to the National Health Service (NHS) of treating a venous leg ulcer for one year was estimated to range from £506 to £1240 per ulcer in 2002 by Tennvall and Hjelmgren.[150] These costs equate to £568 to £1393 per ulcer when they are increased in line with the retail price index to 2006 sterling prices.[271] The costs estimated in this model, were therefore similar to those determined by Tennvall and Hjelmgrem.[150]

Tennvall and Hjelmgren used stochastic (Monte Carlo) models to determine the costs of treating venous leg ulcers in both the UK and Sweden. They stratified their analysis for ulcers by the same size and duration categories used here (defined by less than or greater than $10cm^2$ and less than or greater than 6 months duration). The majority of data used to populate the models were obtained from a patient-specific database of 252 patients with venous leg ulcers treated between 1993 and 1997 in a specialist leg ulcer clinic in Sweden. Further data were obtained from a UK expert panel (consisting of five experienced nurses and one surgeon) and data on the frequency of recurrence were

extracted from a published paper (although no search strategy or criteria were given as to how this study was identified and chosen).

The model structure described by Tennvall and Hjelmgrem differed from that used here and was more rigid. Patients were categorised by ulcer size and duration and followed separately for one year. Three wound states were defined for each category: initial ulcer state, an ulcer-free state and a second (recurrent) ulcer state. Patients could only move through the health states in this order and therefore there was a maximum of one recurrence per person. Recurrent ulcers healed at an average rate (i.e. considered to be of average size and average duration). In this study, there was fluidity between these wound categories, such that patients who had not healed after six months moved to the health state for ulcers of duration more than 6 months, and all patients started in the wound health states with duration less than six months. Furthermore, the application of a healing probability to recurrent ulcers was dealt with in a slightly different manner here compared to Tennvall and Hjelmgrem. Here, all recurrent ulcers were deemed to be less than 6 months duration at recurrence and to have an equal probability of being greater than or less than $10cm^2$, while Tennvall and Hjlemgrem's model gave recurrent wounds an average size and duration (and thereby probability of healing). In neither model, therefore, did the probability of healing (based on size and duration) of the first ulcer have any impact on the probability of healing of any recurrent ulcer.

This study and that of Tennvall and Hjelmgrem found comparable results with respect to the most influential factors on overall treatment costs for venous leg ulcers. In this study, these factors were found to be cost of hospital visits and the frequency of nursing visits when considering the cost of treatment over one year. While Tennvall and Hjelmgrem found labour to account for most of the costs in both Sweden and the UK.

Tennvall and Hjelmgrem did not consider wound infection in their model and the materials used did not include antibiotics or the microbial investigation of wounds. Furthermore, the main source of data was a Swedish-based clinic database. The advantage of using this population was that patients were being routinely treated and were followed over a long period of time, however, these patients were attending a specialist venous leg ulcer clinic and therefore may not represent the general population of patients with venous ulcers and furthermore, the Swedish data were also used to populate probabilities in the UK model. This is considered acceptable when there are no other sources, and the authors did consult a UK-based expert panel and used UK-based cost estimates. The study did not consider the quality of life associated with venous leg ulcers. The main disadvantage of the study by Tennvall and Hjelmgrem however is that they do not present any sensitivity analyses (as they only include Monte Carlo simulation results). This makes it difficult to interpret how uncertainties in the model may affect the outcome.

Harding *et al.*[152] identified time to healing, along with nurse-time, to be key influences in the cost-effectiveness analysis comparing gauze with modern dressings over a 12-week period from the health services perspective. This research, which was sponsored by Convatec Ltd, assessed the cost-effectiveness of a hydrocolloid dressing, compared to gauze and skin replacement therapy, for the treatment of venous leg ulcers in the UK and found the hydrocolloid dressing to be the most cost-effective. The study derived protocols of care for venous leg ulcers based upon the identified literature and expert opinion. Literature was used to define healing rates (between 4 and 12 weeks) and wear-time for dressings and searches were conducted using Medline (not indicated to be systematic). The healing rates from the literature were pooled for inclusion in the model. Expert opinion was mainly relied upon to inform use of physicians' time, use of debridement, antibiotics in the case of wound infection and compression bandaging.

Infection rates were determined from published literature and expert opinion, but were not reported. Infected wounds were assumed to be treated with amoxicillin 500mg three-times a day for 10 days. This was based on expert opinion (consisting of members from across Europe) and does not appear to reflect the most common antibiotic treatment for chronic wounds identified in Chapter 2, using General Practice Morbidity Database.

The modelling method used in the study by Harding *et al.*[152] and its structure were not reported which makes it difficult to compare with other studies. No sensitivity analyses were conducted so the impact of uncertainty in the data could not be determined. Furthermore no patient benefits were included (such as QALYs).

Guest and Ruiz[273] have previously identified the cost of hospitalisation and domiciliary nurse visits to be the key cost drivers in the treatment of both abscesses and surgical wounds over an eight-week period in the UK. Similarly, these key drivers were identified here in the management of venous leg ulcers over a much longer time period. Guest and Ruiz constructed a model, using TreeAge, to investigate the cost implication of using carboxymethylcellulose dressing (Aquacel) compared with gauze in the management of surgical wounds healing by secondary intention (sponsored by ConvaTec Ltd, the manufacturers of Aquacel). The cost of dressing was found to account for 5% or less of the total management costs, although the number of daily changes required for dressings did have an impact on the costs due to the nurse-time required. They also found, through one-way sensitivity analysis that changes in the probability of healing had little impact on the overall costs. The probability of healing was determined by pooling the primary estimates from eight studies identified through a systematic review of the literature (and sensitivity analysis was carried out using 95% confidence intervals on these estimates). Data from which the probability of healing

could be estimated were only identified for wounds treated with gauze. Healing rates for Aquacel were assumed (based on the authors' opinion) to be the same as those reported for gauze. All wounds were assumed to heal within 8 weeks. Resource use data were based on expert opinion. While the study by Guest and Ruiz adds to the knowledge on cost drivers for wound-healing caution can be expected in the interpretation of a model funded by the manufacturer of the dressing under investigation. However, in this model on the treatment of venous leg ulcers, the cost of dressings and probability of healing were also found to be minimal, compared to other factors.

No studies were identified which looked at the economic costs associated with antibiotic resistant organisms in chronic wounds. Few studies have investigated the additional costs incurred due to MRSA infection for other morbidities. Those studies that have been published have focused on severe infections such as septicaemia, bacteraemia and pneumonia, taken a hospital perspective and do not include measures of quality of life. Studies that have investigated the costs associated with resistant organisms in skin and soft tissue infections include Engemann *et al.*,[274] Shah *et al.*[168] Vinken *et al.*[165] and to a lesser extent Rubin *et al.*[166]

Engemann *et al.*[274] compared costs for resistant and susceptible surgical site infections and found the mean cost attributable to meticillin resistance was $13,901 per surgical site infection (2000 US Dollar value). Here, the additional cost of an infection resistant to second-line treatment was £1,223. Although not directly comparable, the attributable cost identified by Engemann *et al.* is considerably higher than that included in this model. The differences are likely to arise due to differences in healthcare costs between the two countries as well as the severity of the infections being investigated.

Engemann *et al.*[274] collected data prospectively (and analysed) for 121 patients with MRSA surgical site infection, 165 with MSSA surgical site infection and 193 control

patients (no infection, but similar surgery in same year) from two hospitals in Durham, North Carolina, US. Only deep or organ-space infections were included (superficial infections were specifically excluded). In addition to identifying an increased likelihood of death in those infected with MRSA compared with MSSA (OR 3.6, 95% CI 1.7 to 7.4), the authors found the median hospital cost was approximately $40,000 more for patients with MRSA than MSSA infection. After adjusting for duration of surgery, hospital, length of hospitalisation before infection, length of ICU stay before infection, renal disease and diabetes mellitus, MRSA was associated with 1.19 fold increase in median hospital costs (p=0.03).

The importance of hospital costs on the overall cost of MRSA infections has been shown by Shah *et al.*[168] They explored the direct medical costs incurred during vancomycin use for skin and soft tissue infections (and three other infections) caused by MRSA. Again, this study was US based, and took the hospital perspective. The authors constructed a model that was populated using data from the literature (Medline searches for citations 1995 to 2003) and Medicare costs. They found drug acquisition costs to be a minimal part of the costs associated with vancomycin (mean (SD) of $770 ($536)). Most of the costs arose due to hospital costs (mean $23,659 ($3,849)). The authors explicitly state however that they have not included the costs of vancomycin resistance and therefore that they are underestimating the true costs to society and the hospital, but that these costs have not yet been adequately addressed.

Rubin *et al.*[166] did attempt to establish the additional cost incurred due to MRSA infections compared to MSSA infections using hospital discharge data from the New York State Department of Health. The additional costs incurred due to MRSA for bacteraemia, pneumonia, endocarditis, surgical site infections, osteomyelitis and septic arthritis were investigated. Although this study was structured around hospital

discharge data, all data informing the additional costs that would be incurred for resistant infections compared to susceptible infections, and furthermore, the prevalence of antibiotic resistance, were determined from an expert panel. An additional cost of $2,500 was estimated to be incurred for each MRSA infection compared with MSSA. However, due to the methods used to gain this figure, and the US setting, it is not particularly appropriate for comparison with the findings of this study.

Presentation of these studies highlights that there are few studies available with which to compare the antibiotic resistance outcomes of this study. Most commonly published economic studies of MRSA infections compare the cost-effectiveness of different treatment regimens and do not explicitly explore the additional cost incurred due to antibiotic resistance. For example Vinken *et al.*[165] used a decision-analysis model to investigate the most cost-effective empirical treatment for cellulitis (flucloxacillin switching to vancomycin in the presence of resistant organisms; vancomycin switching to flucloxacillin in the presence of sensitive organisms; or linezolid treatment from the outset). The cost of treatment, using flucloxacillin and vancomycin increased from £2320 to £3659 when resistance increased from 0% to 25% (assuming flucloxacillin to be completely ineffective for resistant infections and 80% of infections to be of unknown susceptibility).

5.5.3 Strengths and weaknesses

The economic perspective taken in this study, as with many models, is that of the healthcare provider, taking only direct costs into consideration, such as dressings, healthcare professional's time and so on. The wider costs to society or individual patients were not included. For patients themselves the costs can be complex, involving both tangible costs (e.g. loss of earnings, travel costs) and intangible costs (e.g. quality of life, pain).

The view of antibiotic resistance in this cost analysis, was very much that of the physician, as defined by McGowan *et al.*[159] McGowan *et al.*[159] state that the physician's perspective focuses on the individual, investigates the outcome of health and looks at the short-term approach to treatment. The economic problem is, at its basic level, ineffective treatment regimens, caused by antibiotic resistance. In this model, the costs of administering tetracycline and if this fails, the costs associated with vancomycin treatment, were effectively the only costs of antibiotic resistance considered. From this perspective, the economic impact of antibiotic resistance depends on the availability of effective alternatives. The costs incurred due to antimicrobial resistance are however many and diverse. These may include the development of new antibiotics and other drugs, surveillance of resistant organisms, implementation of infection control measures, laboratory techniques to detect antibiotic resistance, educational programmes for healthcare professionals and the public, efforts to optimise administration of antimicrobial agents and to influence drug choice. None of these costs were included in the model and therefore the true cost of antimicrobial resistance to the population has not been considered.

Here, a Markov model has been used to investigate the costs and benefits associated with venous leg ulcers and specifically with infection and antibiotic resistance. The output of any model is wholly dependent on its structure and the data used to populate it. The results from the model were as expected and comparable with other published data, which suggests predictive and face validity.[152,273,275] The validity of the modelling process itself however cannot be established as no other models have thus far independently addressed the same question.[275] However, the overall cost of treating venous leg ulcers for one year was comparable with that determined by Tennvall and Hjelmgrem.[150]

Good modelling practices were followed in the construction of the model. These included the use of the half-cycle correction to prevent over or under estimates of costs,[240] discounting future values, analyses using both cohorts and Monte Carlo simulation and systematic investigation of uncertainty using sensitivity analysis.[240,243] Future costs and benefits were discounted in the ten-year analyses at the standard rate of 3.5%, with the range 0% to 6% explored in sensitivity analysis. Discounting future costs by 6% resulted in a reduction in the expected costs (at current value) of just under £1000, compared with no discounting. Similarly, the impact of discounting of the expected gain in utilities was a reduction of approximately 1.4 utilities. Furthermore, the model was explored over two time periods (one year and ten years) to obtain the short and medium-long term perspective. These time periods were however chosen purposely chosen but not evidence-based.

The health states used as the basis of the model structure were clinically meaningful and mutually exclusive. Unfortunately, there does not exist for venous leg ulcers a clinical scoring system that could be translated into appropriate health states. Such classification systems do exist for other chronic wounds, such as the Wagner or the University of Texas systems for classifying diabetic foot ulcers, and the pressure ulcer grades PU1 to PU4, and these have been used as the basis for model structure.[154] CEAP, CVSS and Posnett's categories all had potential advantages and disadvantages compared with ulcers defined by duration and size in this model. The CEAP classification represents a progressive graduation of clinical severity, however, it is a relatively static scale whereby improvements in clinical presentation do not result in a change of category due to the continuing presence of underlying disease.[276] The system developed to improve short-term responsiveness of CEAP, the CVSS, consisted of 10 clinical attributes each graded from 0 to 3 (0=absent, 3=severe).[276] This would have meant 30 health states for which only extremely limited data were available regarding

transitions and was therefore not considered appropriate. Posnett's health states were specifically designed for economic analysis and incorporated grades of ulcers and infection. However, data were not available for transitions between these states. Ultimately data were required to determine transitions, costs and benefits and these were most commonly available from the literature for wounds defined by duration and size.

Even though the model was structure around the commonly reported wound descriptors of size and area, not all required data for the model were available to determine either transitions between health states or the costs and utility associated with each health state. For the costs and utility these deficiencies were addressed using sensitivity analyses, however for the model structure assumptions were made which could not be tested. For example, there were no data on the rate at which patients might move between health states with wounds less than $10cm^2$ to health states with wounds greater than $10cm^2$. A simple model structure was therefore adopted whereby patients could not move between health states of different wound sizes, which clearly does not reflect the true clinical picture. Furthermore, on occurrence of an ulcer (either at the start of the model or at recurrence) patients were assumed to have equal probability of a wound less than or greater than $10cm^2$.

Lack of movement between health states indicating different wound sizes may have lead to an underestimate of the costs and utility loss associated with ulcers of less than $10cm^2$ (as they were assumed never to become more than $10cm^2$) but may also have over estimated the costs associated with wounds of greater than $10cm^2$ as these were assumed never to decrease in size below $10cm^2$). Furthermore, the paucity of some data with which to populate the model meant that patients did not have different outcomes according to their possible co-morbidities or ulcer history. The addition of these factors

would not have added to the model as the data were not available to accurately described transitions, benefits and costs. Although these assumptions are not ideal, they are a necessary part of model building where there are data deficiencies and are accepted in the field provided they are clearly stated.

Healing rates for ulcers are frequently reported in the literature at three months follow up. Therefore, this clinically relevant time-period was chosen as the Markov cycle length. It has previously been recognised that the availability of data is frequently the deciding factor in the selection of the Markov cycle length.[243]

There is no generally accepted or tested method to collect data with which to populate analytical models.[277] A large range of data are required, including treatment effects, baseline event rates, utilities, resource use and unit cost. It is rare for one source to be able to provide all that is necessary.[277] There is a generally accepted hierarchy of data sources with databases and epidemiological data in the literature taking preference over expert opinion. In this model, all data were obtained from the literature or databases.

Antibiotic prescriptions were used as a proxy marker for infections in this study. This is likely to have over-represented the incidence of true infection in venous leg ulcers, however it represents the actual costs incurred due to perceived infection. Defining infection in chronic wounds is a highly debated topic. As previously discussed in Chapter 1, the presence of microbes themselves is unreliable as an indicator of infection as all chronic wounds are colonised. However, the clinical signs and symptoms commonly used generally have low predictive value[36] (and Nelson HTA[37]). The literature review did not identify any studies reporting the frequency of infection in venous leg ulcers.

Wound infection was directly reported in the General Practice Morbidity Database for patients with chronic wounds attending general practice (Chapter 2). However, this was not considered to reflect the true frequency of infection but to be subject to recording bias. Wound infection was only specified in 2.1% of visits by patients with chronic wounds, however patients received antibiotics in 7.0%. In single-diagnosis visits, the proportion of wounds for which wound infection was stated, was again only a fraction of the number of visits on which antibiotics were prescribed (wound infection was recorded as present in 15.7% of single diagnosis visits which resulted in an antibiotic prescription). Therefore, data relating to venous leg ulcer single-diagnosis visits from the General Practice Morbidity Database (Chapter 2) were used to give a conservative estimate of the probability of receiving systemic antibiotics during the three-month period. Sensitivity analyses were conducted with the lowest value of no antibiotics, and the highest value similar to that of all antibiotic consumption by patients with venous leg ulcers (which includes antibiotics prescribed for other conditions such as respiratory and urinary tract infections). Flucloxacillin was the most frequently prescribed antibiotic, both in single diagnosis visits (38%) and all visits (25%) by patients with venous leg ulcers. Furthermore, it is clear from Chapter 2, that the majority of wound patients receive antibiotics for 5-7 days, which supports the choice of a 7-day course of flucloxacillin as the first-line of treatment in the model.

First-line antibiotic treatment resistance was considered to be treated with a tetracycline as indicated in the BNF.[62] The cost of this was estimated to be £3.84. However, it may be that much more expensive antibiotics would be used, for example clindamycin at £23.03 for an oral course of 24 tablets. Use of clindamycin in this model would have increased the cost of treating first-line antibiotic resistant infections by approximately £460 (in the Monte Carlo simulation following patients for one year, with resistance only to first line antibiotics).

There are two aspects to the inclusion of costs in economic models. Firstly, there is the question of which resources and what quantities are used, and secondly there is the question of price. Sculpher *et al.*,[278] in their review of economic evaluation studies in healthcare recommend that resource use data are collected separately from cost data. In this study, most cost data were collected from two respected UK based sources, the British National Formulary[62] and the Unit Costs of Health and Social Care publication by Curtis and Netten.[249] The Department of Health publish tariffs for NHS services, listing the prices that will be paid for a variety of health care services, which could have been used an alternative to Curtis and Netten.[279]

Where cost data were not available from the BNF or Curtis and Netten, data were obtained from elsewhere in the literature. The cost of processing a microbiology swab was taken from Brezmes *et al.*[269] in Spain and subjected to the appropriate exchange rate and inflated according to the retail price index. A wide range was used in the sensitivity analysis due to the indirect nature by which the cost had been obtained and therefore the lack of confidence in the value. The cost of processing a microbiology swab, however, had little impact on the expected value.

Data derived from different countries can be used, although this is generally considered more appropriate for clinical outcome data than costs.[238] There are however instances where country-specific data may be important, such as antibiotic resistance which can vary greatly between countries.[280] In this study, data were obtained from the same country and healthcare setting when possible. Where this were not available however sources from further afield were used. This was the method employed by Ortegon *et al.*,[154] in their study investigating the cost-effectiveness of the prevention and treatment of diabetic foot ulcers based in Sweden. The authors stated that they used Swedish data where these were available, then data from other European countries with similar

demographics and health care systems, such as the UK, and finally data from elsewhere, for example the US. In this study, the factors that had the greatest impact on cost (the cost of nursing and frequency of visits) were taken from UK sources.

The probability of treatment failure to first-line antibiotic treatment was taken from the prevalence of MRSA identified in chronic wounds found in Chapter 3. It is however possible for *S. aureus* to be resistant to flucloxacillin without being MRSA and rarely *vice versa*, and patients treated at the tertiary referral clinic may not represent those treated in the community. The probability of failure to second-line treatment was taken from the proportion of MRSA bacteraemia that are resistant to tetracycline. The population of patients with and the strains causing *S. aureus* bacteraemia may not reflect the population of patients with venous leg ulcers or the organisms that cause their wound infections.

Due to the uncertainty surrounding the true prevalence of resistance to first and second-line antibiotic treatment in this population, the probabilities were varied from 0 to 1 for investigation in the sensitivity analysis. A similar method was used by Fleurence *et al.*,[155] who investigated the cost-effectiveness of treatment and prevention of pressure ulcers with pressure-relieving devices in UK hospitals. They found a lack of data on which to build the model, and were required to extrapolate data and explore uncertainty using large intervals in the sensitivity analysis.

Furthermore, the model assumed all infections to be due to *S. aureus*. *S. aureus* is the most frequently isolated pathogen from infected VLUs,[27] and the reason why recommended empirical treatment is flucloxacillin.[58] However, it is not the only pathogen and non-response will not, therefore, solely be due to antibiotic resistance. Pseudomonads and anaerobes, amongst others, cause wound infections and do not

respond to flucloxacillin. This is not however addressed in the model where all infections are assumed to be *S. aureus*.

In this study, confidence can be placed in the parameters that have the largest impact on the costs in the model: cost of nursing and frequency of nursing visits. Data regarding the cost of nursing was extracted from the Curtis and Netten[249] and the frequency of nursing visits taken from a HTA randomised controlled trial (RCT).[151] Both of these sources relate to the UK population, in the community setting.

Nurses' time was one of the most influential factors on the expected value of the analysis. In this model, the time for one visit was taken from Iglesias *et al.*[151] who conducted a small investigation into the time taken for VLU consultations with nurses. The use of this objective and measured value is a strength of this study as many previous studies have relied on expert opinion to provide data on the time taken to perform tasks.[150,152] The base value used in this model was an average of costs associated with clinic and home visits. The duration of nurse-consultations from Iglesias *et al.*,[151] and used in the model, was similar to that reported in other studies.[152] It is possible that nurses other than district nurses, such as practice nurses or nurse specialists would treat patients with chronic wounds. Costs in the model were based on district nurses which were representative of a range of nursing groups. Furthermore, district nurses still manage the majority of the treatment of venous leg ulcers in the community.

The HTA RCT by Iglesias *et al.*,[151] investigating different bandaging regimens, contributed a large quantity of data to this model including transition probabilities and resource-use. RCTs are frequently considered to lack external validity.[238] However, the study by Iglesias *et al.*,[151] was multi-centred (based in several locations across the UK) and moreover left many of the treatment decisions to individual clinicians. For

example, clinicians were free to use a four-layer bandaging system of their choice to treat patients randomised to receive such bandages.

Movement within the model was dependent on transition probabilities obtained from the literature. Transition probabilities for healing were based on figures from Iglesias *et al.*,[151] with slight visual adjustment to try to incorporate the different effect of duration and size on delayed wound healing.[247] The randomised controlled study by Iglesias *et al.*,[151] compared two systems of compression bandaging; here the probability of healing was taken from the proportion of patients whose ulcers healed in 12 weeks in the four-layer compression bandages arm of the study. Healing rates did not however have great influence on the overall cost of ulcers when explored through sensitivity analysis. Improving healing rates of ulcers greater than $10cm^2$ and duration greater than 6 months from a probability of healing of 0.1 to 0.6 (i.e. from 10% to 60% of ulcers healing in three months) changed the expected cost by around £500 over 10 years, and the expected utility <0.05.

The probability of healing following infection did not differ in the model from the probabilities of healing without infection. In reality however, infection will likely delay healing. The probability of healing in each health state following infection were however incorporated as separate variables but were not found to be of great significance to the overall cost or utility of healing. However, the probability of healing for patients with infected ulcers was included in the model as a separate variable to allow the impact of the probability of healing in infected ulcers to be determined separately from that of uninfected ulcers (the impact was found to be negligible).

The model structure separates the probability of recurrence in the first year following healing from the probability of recurrence after remaining ulcer free for one year. This reflects the findings of several studies that recurrence more frequently occurs in the first

year following healing. It is possible that the probability of recurrence more than one year after healing has however been slightly over-estimated in the 10-year model. This is because the probability was calculated from a study that followed patients with healed ulcers for five years, without removing from the calculation those that recurred within the first year (this was not possible from the data reported). The impact of surgery on recurrence (or costs) has also not been included in the model, although Barwell *et al.*,[251] found 35% of patients had recurrence in the first 12 months in the compression only arm of a surgical intervention RCT, compared with 15% of patients given surgery and compression.

Costs to the healthcare provider which have not been incorporated into this model, include such items as wound cleansers/irrigation, debridement, surrounding skin treatment, topical products, analgesics and surgery costs. This is due to the lack of quality data regarding the frequency of use of these items. Some of these items, such as wound cleansers (which may be tap water) would be likely to be of little importance to the overall cost (as found by Harding *et al.*[152]). However, surgical intervention could be expected to have considerable impact on cost, wound healing and possibly quality of life. These costs were not explicitly included because neither an estimate of the average surgical costs for VLU treatment, nor the frequency of this occurrence for patients treated in the community could be established. General hospital costs were included, based on the findings by Iglesias *et al.*,[151] with the maximum cost investigated in sensitivity analysis representing 5-days inpatient care.

Many studies have investigated the health related quality of life of patients with venous leg ulcers, few of these however have used tools or reported the results in such a way that they can be used in economic analyses. Data regarding the quality for life for patients with leg ulcers were obtained from Iglesias *et al.*,[151] with sensitivity analysis

based on data from Walters *et al.*[266] and Jull *et al.*[267] (Jull *et al.*[267] was based on a New Zealand population, however evidence suggest that country-specific preference values are not necessary.[281]) No data were available to differentiate quality of life according to ulcer size or duration. Therefore the assumption was made that quality of life was the same for all open ulcers, but better for patients with healed ulcers.

Quality of life in the study by Iglesias *et al.*,[151] was measured using the EQ-5D generic measure of quality of life. There is concern that these measures do not accurately reflect utility. Utility is not necessarily equal to the rating of health. Utility makes use of the idea of area under the curve to assess preferences. Therefore the product of utility and length of time must reflect the true preferences of a person. For example 20 years at 0.5 utility must be as preferable to 10 years in perfect health.[282] Preference can be measured using several methods including rating scales, standard gamble, time-trade off and willingness to pay amongst others. However only standard gamble is considered to truly measure utility as it reflects decision making with uncertainty, although time-trade off methods are considered acceptable too.[282] No studies measuring utilities associated with leg ulceration using standard gamble or time trade off were identified in the literature searches and therefore Iglesias *et al.*,'s measurements with EQ-5D were used.[151]

There is conflicting evidence regarding the overall improvement in quality of life of patients treated for venous leg ulcer. Iglesias *et al.*,[151] found no overall improvement in utility despite a large percentage of patients' ulcers healing over time. This may however reflect information drop out. The number of people contributing to the quality of life measurements decreased over time. Information drop-out is more frequently associated with drop out due to decreased quality of life.[283] However, it may be that patients with healed ulcers were less concerned with completing the regular

questionnaire compared to patients with active ulceration (results are not presented separately by ulcer status). Alternatively, it may reflect a deterioration or plateau in general health, rather than ulcer-specific, health-related quality of life.[153] Or it may reflect the lack of sensitivity in the EQ-5D to detect such changes. A study by Walters *et al.*,[266] looking at health related quality of life for patients with venous leg ulcers using three different measurement tools, found only one (the McGill Short Form Pain Questionnaire) to identify a difference in the quality of life reported by those patients whose ulcer had healed compared with those in which it had not, during a three month follow up period. The EuroQol DSI score (which uses the same question base as the EQ-5D) did not identify any difference. Therefore the lack of difference identified by Iglesias *et al.*[151] may reflect the insensitivity of the measurement tool they used.

In contrast to Iglesias *et al.*,[151] Franks *et al.*,[284] demonstrated a significant improvement in quality of life measured using the Nottingham Health Profile during a randomised trial of two bandaging systems (4-layer bandaging and single layered adhesive compression bandage). At the start of the trial, patients with leg ulcers had significantly higher perceived pain, and poorer mobility compared to age, sex and social class norms. After 24 weeks of treatment, there was significant improvement in pain, sleep, mobility, emotion and energy, with the largest changes being in pain and sleep. Healed patients had significantly improved quality of life compared to patients with open ulcers.[284]

Loss of utility associated with infection and antibiotic resistance was not included in the main model, due to a lack of data from which to estimate the magnitude of loss. However, sensitivity analysis was used to explore the impact of any loss which was found to be minimal.

5.5.4 Implications for clinicians and for future research

This study and others[285] suggest that one of the most effective ways to reduce the cost to the healthcare provider during the first year of treatment, would be to treat all patients in the clinic setting and to minimise, where possible, the frequency of visits. In the mid to long-term, decreasing the frequency of follow-up visits could potentially reduce the cost to the health-care provider of leg ulcer treatment, however any association between follow-up visits and ulcer recurrence would need to be investigated.

In summary, this study has found the treatment costs of venous leg ulcers to be substantial, but that antibiotic treatment and antibiotic resistance do not greatly influence their magnitude. This lends support to the argument proposed by Coast *et al.*[160], that the true costs of antibiotic resistance are unlikely to be felt directly by the consumer of antibiotics and that MRSA in superficial sites may be of little financial impact. This study has found this to be the case, while there are cheap, effective alternatives for resistant organisms. The costs of antibiotic resistance would be considerably greater if alternative drugs were not available. The main cost drivers in the treatment of venous leg ulcers are the frequency of nurse visits, cost of hospital visits, costs of a nurse visit and frequency of visits for healed ulcers.

Some of the key parameters in this study were from good quality, UK-based data sources. Nonetheless, the source document for many data points of the model was a randomised controlled trial.[151] Further work could be conducted to determine with greater accuracy the cost of those values that had the greatest influence on the cost incurred, such as the frequency of nurse visits and cost of hospitalisation of patients with venous leg ulcers. The model could be refined, if data become available, to incorporate infections caused by organisms other than *S. aureus* and the impact and cost of resistance in these organisms, such as *P. aeruginosa*.

Markov models have been used to determine the impact on cost of new technologies. Work of this nature has already been conducted by many wound-dressing manufacturers to show that although the cost of modern dressings may be more expensive per item, they produce cost-savings elsewhere due to their design i.e. less frequent dressing changes and therefore fewer labour costs (i.e. nurse time). Similar studies to determine the value of novel antibiotics for treatment of venous leg ulcer infection would also be of importance. Studies could also be conducted to determine at what prevalence of antibiotic resistance does another antibiotic regimen become preferable to empirical treatment with flucloxacillin.

Finally, microbes and antibiotic resistance are ever-evolving: new organisms with increased pathogenicity may evolve (for example Panton Valentine Leukocidin (PVL) producing *S. aureus* strains) as may new antibiotic resistant strains of organisms. It will be important to monitor such changes and to assess the impact such organisms may have on both the health and costs of treating venous leg ulcers.

Chapter 6 . General Discussion

This Thesis has focused on the interaction between two common problems: chronic wounds and antibiotic resistance. Both of these present their own challenges and burden to patients and healthcare services but the relationship between them is still very much unknown. The main findings of the study are presented below together, where appropriate, the relationship to other research and the strengths and weaknesses of the research. Finally, the implications of the Thesis are discussed.

6.1 Literature review summary

Microbes are consistently associated with chronic wounds, can cause serious infections in such wounds and contribute to non-healing. The exact mechanisms by which microbes contribute to non-healing are yet to be fully elicited.

Evidence regarding the optimum treatments for infection that leads in faster wound healing does not exist.[286] A systematic review of the literature has showed recent research to focus on showing non-inferiority of new antibiotics compared to established treatments for serious skin infections, rather than demonstrate improved wound healing. The lack of evidence does not indicate that antibiotics will not be effective against clinically infection. Unfortunately however, there is also little evidence to support the accuracy of clinical indicators of infection.[36,37] Consequently, there is a substantial reliance on expert clinical opinion regarding which wounds to treat with antibiotics and which antibiotic to choose. Guidelines frequently recommend flucloxacillin as first-line empirical treatment because *S. aureus* is considered to be the most common infecting pathogen.[58-60]

There is no evidence to suggest how frequently chronic wounds in the UK were being treated with antibiotics and which antibiotics were being used, despite anecdotal suggestion that they are frequently and unnecessarily employed.

The literature on antibiotic resistance and its association with chronic wounds was also reviewed. Antibiotic resistance is clearly a growing problem, both in the UK and elsewhere. MRSA is, perhaps, the most renowned antibiotic resistant phenotype, strains of which have spread across the world. Antibiotic resistance can evolve rapidly and one of the next concerns is whether strains shall develop that are resistant to both meticillin and vancomycin. Antibiotic resistance in other organisms is also an important cause of morbidity, including ciprofloxacin and other fluoroquinolone resistance in *P. aeruginosa* as well as vancomycin-resistant enterococci.

Antibiotic resistant organisms are frequently isolated from chronic wounds, with many studies focussing on diabetic foot ulcer colonisation or infections, due to serious sequalae of ineffective antibacterial treatment for such infections. The likely prevalence of antibiotic resistance in a specialist wound healing clinic in the UK could not be predicted from the available evidence, yet this knowledge is vital to effective and prompt treatment of wound infections.

Risk factors associated with MRSA and antibiotic resistant *P. aeruginosa* were reviewed. Common risk factors for MRSA were found to be hospitalisation, nursing home residency, other healthcare intervention such as indwelling device and ICU stay, antibiotic use (particularly ciprofloxacin) and to a lesser extent co-morbidities. Ciprofloxacin resistant *P. aeruginosa* was most commonly associated with previous fluoroquinolone use. Chronic wounds were on occasion investigated, and found to be associated, or confounders in a relationship, with MRSA. The impact of chronic wounds on antibiotic resistant *P. aeruginosa* infections has not been investigated. Furthermore, no studies were identified that focussed on the population of patients with chronic wounds and investigated risk factors for antibiotic resistance in this specific population (with the exception of diabetic foot ulcers).

Finally, the review focussed on the economic implications of chronic wounds. Several studies were identified that had investigated different aspects of the treatment of chronic wounds, ranging from overall treatment costs, to cost-effectiveness studies of wound dressings. No studies were identified that explored the impact of antibiotic resistance on the treatment costs of chronic wounds.

The review of the literature was undertaken to explore current research in the field. For the most part a narrative review was conducted. Narrative reviews have their limitations, and cannot be easily repeated, but are appropriate when a broad topic is reviewed rather than a specific question. It was not appropriate to report details of the literature searches, which were undertaken ad-hoc as part of the narrative review. The search strategies employed frequently varied according to the subject under consideration and changed over time. A systematic review was conducted to address one specific question (evidence for antibiotic treatment and choice of treatment in chronic wounds). For the systematic review, a comprehensive search strategy was used and explicitly reported.

6.2 Aims

In the light of the gaps identified in the literature, the aims for this Thesis were composed:

i. To describe and quantify antibiotic prescribing for chronic wounds in the primary care setting and compare this with antibiotic usage to patients without chronic wounds

ii. To determine whether antibiotic resistance posses a significant barrier to effective patient care in one specialist wound healing clinic

iii. To investigate which factors are associated with the carriage of resistant organisms in chronic wounds

iv. To model and explore the economic aspects of chronic wound care, particularly in relation to changing levels of antibiotic use and antibiotic resistance.

6.3 Original research

The range of questions to be addressed here dictated that a broad range of research skills and methodologies were utilised. The studies that were undertaken, their main findings, relationship to other research and strengths and weaknesses are discussed in the following section.

i. To describe and quantify antibiotic prescribing for chronic wounds in the primary care setting and compare this with antibiotic usage to patients without chronic wounds

The General Practice Morbidity Database for Wales (GPMD) was used to explore antibiotic consumption by patients with chronic wounds in primary care and to compare their quantity and type of consumption with that of the age, sex and general practice matched population without chronic wounds. Patients with chronic wounds were found to be prescribed significantly higher quantities of antibiotics than matched patients. In the GPMD, 68% of patients with chronic wounds received antibiotics from their general practitioner in the previous year. In only those visits in which wound treatment or diagnoses codes were recorded, patients with chronic wounds received greater quantities of flucloxacillin, co-amoxiclav and metronidazole than non-wound patients received in all visits. In all visits, patients with chronic wounds also received significantly more ciprofloxacin, erythromycin, trimethoprim, cefaclor and cefalexin.

The increase in antibiotic consumption was not due to other factors such as diabetes and the number of visits.

These findings were in line with other published work in the area, specifically Tammelin *et al.*[61] in Sweden who found 60% of patients with chronic wounds to have received antibiotics in the previous 6 months, and Leistevuo *et al.*[174] who exposed chronic skin wounds as one of the most common reasons for antibiotic prescription in the elderly in Finland. The high level of flucloxacillin use in the *single-diagnosis* visits suggest that primary care treatment is in line with UK recommendations for empirical treatment of venous leg ulcers and other chronic wounds.[58-60]

One of the main strengths of this study was that it made use of the GPMD; a large primary care database. It was known that this database does not capture all prescribing data in some practices and therefore only those practices considered by Health Solutions Wales to have accurate prescribing data were included in the study. A further weakness of the database was that it is not geographically representative for all Wales and the geographical representativeness of the sub-set of practices included in the analysis was not assessed.

A further strength of the study was the use of a case-control design. All cases within the database were identified using appropriate diagnostic and treatment Read Codes (the status of cases was not externally verified). Each case was matched with four control patients to increase the power of the study; due to the database nature of the study no extra costs were incurred. Controls were carefully selected to represent the source population without disease. This was achieved by matching cases and controls by age-band, sex and general practice. It could be perceived as a criticism of this study that patients were not matched on any co-morbidity, such as diabetes, however the impact of diabetes and the frequency of visits (which could reflect overall morbidity) were

investigated in the analysis. Antibiotic data for one year were compared between the cases and the controls (for the cases, antibiotic use both before and after the Read Code indicating wound status was included).

ii. To determine whether antibiotic resistance posses a significant barrier to effective patient care in one specialist wound healing clinic

The prevalence of MRSA, antibiotic-resistant *P. aeruginosa* and vancomycin-resistant enterococci in chronic wounds being treated at a tertiary referral centre were investigated. *S. aureus* was present in 53% of chronic wounds, 19% of which were MRSA. *P. aeruginosa* was isolated from 31% of patients, 34% of which were ciprofloxacin resistant and 17% of which were imipenem resistant. Enterococci were isolated from 9% of patients and all were found to be ampicillin and vancomycin susceptible. No previous study has reported the prevalence of MRSA, resistance to *P. aeruginosa* and vancomycin resistant enterococci in a range of chronic wounds at a specialist wound healing clinic in the UK.

Sixty-three percent of patients attending the wound-healing clinic had received systemic antibiotics in the previous 3 months and 87% had received systemic antibiotics in the previous year. Echoing the findings of the GPMD, however, flucloxacillin was the frequently used antibiotic: 25.5% of patients had received flucloxacillin in the previous three months, and 52.5% in the previous year. Ciprofloxacin was also extremely commonly used: 25.5% and 41.1% of patients received ciprofloxacin in the previous three and 12 months. Also frequently used were cephalosporins and β-lactamase-resistant penicillins (including co-amoxiclav, tazacin and co-fluampicil), which were received by 26.2% and 55.1% of patients in the previous three and 12 months respectively. It is probable that the use of flucloxacillin, but not ciprofloxacin, was

those patients for whom data were not available from general practice. Topical silver and iodine products were also frequently used.

As might be expected from a tertiary referral centre, the prevalence of MRSA was higher than that found in the community, but slightly lower than previously reported in diabetic foot ulcers (in the UK and elsewhere).[19,28,105,222] Ciprofloxacin-resistance in *P. aeruginosa* however was higher than previously reported in a retrospective study of leg ulcer swab submissions (outside the UK), and higher than that reported for clinical specimens from non-cystic fibrosis outpatients in the UK[22,127].

This study was conducted as part of an audit of care in one out-patient specialist wound healing clinic. The study was not therefore intended to be generalisable, but to focus on the needs of, and benefits for, patients attending the clinic. The results of the audit were fed back to clinicians and changes in practice occurred. Many types of chronic wounds were represented in the audit. Data that were used for the study were routinely recorded and retrospectively collected, which could have potentially lead to missing, out-of-date or inaccurate data. However, the potential for such problems was considered minimal, especially in the nursing notes, because specialist nurses, for the purpose of tracking patient progress, recorded data.

It was strength of this study that data were obtained from several distinct sources, including nursing notes, general practitioners, hospital notes and the patients themselves. A potential weakness is that complete data were only available for approximately 60% of patients. However, differences in the frequency of antibiotic consumption, by antibiotic group, were compared for patients with and without general practice data. Overall antibiotic use was also compared with patient self-reported antibiotic consumption. It was thereby determined that the usage of flucloxacillin particularly, had likely been underreported.

The microbiology investigation of the wounds was a strength of this study and used both selective and non-selective media and meticillin resistance was confirmed by the presence of the *mec*A gene.

iii. To investigate which factors are associated with the carriage of resistant organisms in chronic wounds

The influence of known risk factors, such as antibiotic consumption, and the potentially influence of wound characteristics were explored. Risk factors for carriage of MRSA were found to be previous MRSA and 'other' systemic antibiotics (of which more than 50% were trimethoprim). Likely interaction between these two terms was identified, and it is possible that 'other' systemic antibiotics, such as trimethoprim or sodium fusidate, were acting as proxy markers for previous treatment associated with MRSA. No wound characteristics were found to be associated with carriage of MRSA.

The investigation of risk factors associated with ciprofloxacin-resistant *P. aeruginosa* suggested that both antibiotic history (specifically ciprofloxacin usage, other topical antimicrobials and other systemic antibiotics) and wound factors (wound classification and healing status) might influence carriage. In the case of wound healing, this may be a protective effect of healing against ciprofloxacin-resistant *P. aeruginosa* (or ciprofloxacin sensitive *P. aeruginosa*) colonisation, or it may reflect that wounds colonised with ciprofloxacin-resistant (or sensitive) *P. aeruginosa* are less likely to heal. The risk factors identified here should, however, be regarded as exploratory and only suggestive of the risk factors that should be fully explored in future studies. This is due to the large number of potentially interesting variables that were identified through univariable analysis, compared to the number of cases (less than the accepted standard of 10 events for each risk factor) and the lack of stability in the models which suggests over-fitting.[231]

No studies have previously investigated the risk factors associated with antibiotic resistance in a range of chronic wounds. Studies have however investigated MRSA in diabetic foot ulcers, and support the finding of this study that wound characteristics are of little influence on the carriage of MRSA.[221,222] Previous MRSA is a recognised risk factor for existing MRSA carriage and has been incorporated into current MRSA screening guidelines.[198]

Although not identified in this study, prior hospitalisation and other healthcare associations are found in the literature to be the main risk factors for MRSA.[105,109] It may be that the manner in which prior hospitalisation was considered (as a binary variable indicating an overnight stay of at least one night) was not sufficiently sensitive to elicit a relationship which may be dependent on the duration of stay. Alternatively, it may be that when previous MRSA is recorded in the model, prior hospitalisation is no longer an independent effect. This theory, would need further investigation, but is supported by work from Tacconelli *et al.*[102] who found the significance of prior hospitalisation to differ depending on the inclusion or exclusion of previous MRSA: only when previous MRSA was excluded from the model was prior hospitalisation a significant risk factor.

No prior studies were identified that investigated the risk factors associated with antibiotic resistance in *P. aeruginosa* in patients with chronic wounds. Ciprofloxacin usage has been previously identified as a risk factor for ciprofloxcin-resistant *P. aeruginosa*.[132,136]

In this study, risk factors were explored using logistic regression models. It is a strength of the study that models were thoroughly explored both for structure and with model diagnostics. Furthermore good modelling practices were followed in the construction of the models. The models were found to have relatively good fit with the data, but due to

the small study size (and low number of cases) it is likely that they were over-fitted (too many variables were included for the number of cases).

Finally, criticism could be made of the choice of comparator group in the model. Patients with resistant organisms were compared to all other patients and not to patients with carriage of susceptible organisms. This later comparison would have enabled the difference to be elicited between variables that were risk factors for colonisation and those that were risk factors for colonisation with a resistant organism. This would have aided interpretation of the ciprofloxacin resistant *P. aeruginosa* model in particular. In this model, wound healing was found to be protective against ciprofloxacin resistant *P. aeruginosa*, however, it may have been that wound healing was protective against *P. aeruginosa* colonisation irrespective of its ciprofloxacin resistance status. Previously, a higher prevalence of *P. aeruginosa* in non-healing, compared to healing, wounds has been identified by Schmidt *et al.*[27]

The risk factors identified in these exploratory models should be confirmed in further studies. This would also enable the generalisability of data from one specialist wound healing clinic to be gauged.

iv. To model and explore the economic aspects of chronic wound care, particularly in relation to changing levels of antibiotic use and antibiotic resistance.

The final part of this study aimed to model, in economic terms, the impact of antibiotic resistance in chronic wounds, from the perspective of the healthcare provider. Specifically, the impact of MRSA on the treatment of venous leg ulcers was modelled. Study findings from the GPMD and the prevalence and risk factors identified for patients attending the tertiary wound-healing clinic as well as published literature were used to form the basis of, and then to populate, the Markov model. The cost of

treatment for venous leg ulcers was found to be a considerable burden, reaching more than £1000 per patient for the year following presentation of the ulcer to healthcare services. The cost per QALY was £1348.34, with an average 0.75 utility gained during one year of treatment, in the absence of antibiotic resistance.

In this study, the main cost factors for the first year of treatment for venous leg ulcers were the frequency of nursing visits and the cost of hospital outpatient appointments. The probability of infection, which neither improved nor lessened the probability of healing, did not considerably affect cost: costs increased by approximately £20 in response to a change in the probability of infection from 0.12 to 0.30, when all infections were susceptible to first-line treatment. Antibiotic resistance, in the manner in which it was incorporated into the model had very little impact on the cost of treatment. This was due to the overall low probability of resistance to first-line antibiotic treatment and an even lower probability of resistance to second-line treatment. The cost incurred due to infection with first-line antibiotic resistant organisms was not severe and resulted in only one extra course of relatively cheap antibiotics and one additional visit with a healthcare professional. The costs incurred did increase considerably for patients that failed to respond to second-line therapy as it was assumed at this point that patients had a severe skin infection that required intravenous antibiotics and a period of hospitalisation. However, the risk of getting to this point was small and indeed, none of the 1000 patients taking a random walk through the model in the Monte Carlo microsimulation, when there was a chance of such an infection, took this route.

When a cohort of patients were followed for a period of ten years, the total cost became less dependent on factors relating to the cost of treatment for active ulcers and more dependent on the costs incurred during periods of healed ulcers, for example the frequency of follow-up visits. The cost was estimated at £3918, when future costs were

discounted at a rate of 3.5%, and again there was a negligible increase when the probability of infection resistant to first or second-line antibiotics increased. The cost per QALY in the 10-year model, when future costs were discounted at 3.5% was £735.42.

Other studies that have investigated the cost of treatment for leg ulcers have also found them to be a significant expensive. Tennvall and Hjelmgrem[150] suggest that the annual cost to the NHS of treating one venous leg ulcer is £568 to £1393 per ulcer in 2006 sterling prices (when they are adjusted by year and currency). Tennvall and Hjelmgrem[150] did not however consider the costs associated with infection in their model. Furthermore they did not present any sensitivity analyses. Harding *et al.*,[152] in a company sponsored cost-effectiveness study of wound dressings, did include costs for antibiotic treatment and stated that infection rates were determined from the literature, but these were not reported. They did not report the model structure or sensitivity analyses, making it difficult to assess the model. The main cost drivers, in studies that have reported sensitivity analyses, were found to be similar to those identified in this study (costs related to healthcare professional's time).[273]

There were few available studies with which to compare the costs associated with antibiotic resistance determined in this model. However, these results concur with the finding by Cooper *et al.*[163] that the cost and impact associated with MRSA is likely to vary with setting, being greatest in settings such as intensive care units and possibly negligible when isolated from superficial sites.

A strength of the economic model was its explicit exploring of the costs associated with wound care and sensitivity analyses to identify the main cost drivers (both actual cost values and probabilities). Furthermore good modelling techniques were used including a half-cycle correction, discounting of future values, cohort and Monte Carlo analyses

and (multivariable) sensitivity analyses. Systematic literature searches were conducted to identify data sources and much of the data used to populate the model were obtained from a large, multi-centred, UK-based randomised controlled trial of compression bandaging. Where possible all data were obtained from the UK, and use was made of the data previously presented in this Thesis.

The model structure used clinically meaningful health states that stratified wounds by duration and size to enable more realistic prediction of healing and resource use. A potential criticism of this study is that wound infection and antibiotic resistance did not impact on the probability of healing in the model, and therefore the costs associated with infection may have been underestimated. The probabilities of wound healing after infection in the different wound categories were included as separate variables to the non-infected wound healing. Through the use of tornado diagrams, the impact changing the probability of wound healing for ulcers following infection (within the sensitivity range) was seen to be negligible on overall costs.

The models constructed did not however considered the full costs of antibiotic resistance and their relationship with venous leg ulcers. The costs to society of antibiotic resistance can be large and varied and include such expenses as the cost of surveillance, drug discovery, infection control measures and so on.[159] These costs could not realistically have been included in the Markov model, the aim of which was to investigate the impact of antibiotic resistance in one treatment area. The perspective taken, therefore, was one that could be supported by the available literature, and has been previously described as that of the physician, whereby the main cost is ineffective treatment, and the impact depends on the availability of other treatment options.[159]

6.4 Implications

Antibiotic usage is clearly a common part of the treatment of chronic wounds in primary care and in a specialist wound healing clinic. Antibiotic use is known to be a risk factor in the development and spread of antibiotic resistance, and therefore inappropriate use should be limited. Studies that investigate the extent of inappropriate antibiotic use for chronic wounds, and suggest alternative treatment options, should be encouraged.

The high level of ciprofloxacin consumption by patients with chronic wounds is of concern as exploration of risk factors for antibiotic resistance in a specialist wound healing clinic, suggested that previous ciprofloxacin usage is associated with increased likelihood of ciprofloxacin resistant *P. aeruginosa*. Those risk factor associated with clinically relevant antibiotic resistance, such as MRSA and ciprofloxacin resistant *P. aeruginosa* should be confirmed.

Clinicians should be aware of the high prevalence of antibiotic resistance in chronic wounds and be vigilant to wounds that are not responding as anticipated to antibiotic treatment. It may be that greater use of microbiology investigations for the purposes of identifying antibiotic susceptibilities, not determining infection, is valid.

Further work needs to be undertaken to establish the impact of antibiotic resistance on the morbidity associated with chronic wounds. Such data could then be incorporated in to the economic model presented here to further define the costs associated with antibiotic resistance in chronic wounds. The basic cost of treating venous leg ulcers was not found to be significantly increased in response to antibiotic resistance, however, should antibiotic resistant organisms increase the frequency of nursing visits, or delay the healing process, then this situation is likely to change.

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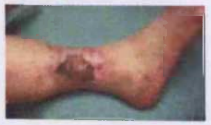
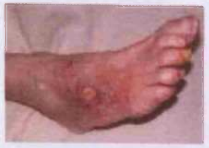
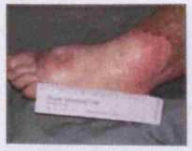
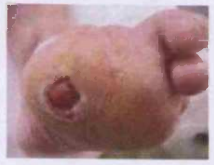
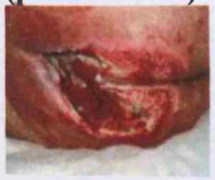
Appendices

Appendix 1.1. Outline of the different aetiologies and pathogenesis of chronic wounds

Wound Aetiology	Clinical description	Underlying physiology	Contributory factors	Main treatment	Population at risk	Prevalence
Venous leg ulcers 	**Location:** gaiter area of leg **Clinical appearance:** • Signs of venous abnormality • No signs of arterial disease in the lower limb[287]	Venous hypertension[287]	Venous disease, obesity, immobility[287]	Compression therapy[288]	Elderly (>65 years)[177]	37-81% of all leg ulcers[177]
Arterial leg ulcers 	**Location:** anterior shin, toe joints, malleoli, under heel[288] **Clinical appearance:** • No odema[289] • Punched out appearance (clear demarcation of borders from surrounding tissue)[289] • Pale and dry[289] • Surrounding skin shiny, cool to touch, pale with elevation, diminished or absent pulses[289] • ABPI below 0.80[289]	Arterial insufficiency leading to ischemia in tissues and eventually necrosis[289]	Main causes of arterial insufficiency: atherosclerosis and acute ischaemia (commonly caused by an embolism).[289]	Correction of arterial abnormality, e.g. angioplasty[288] Exercise program to treat peripheral arterial disease[290]	Risk factors for peripheral arterial disease: smoking, diabetes, age >40 years, hypertension, hyperlipidema, hyperhomo-cystenema[290]	20% of all leg ulcers[289]

Wound Aetiology	Clinical description	Underlying physiology	Contributory factors	Main treatment	Population at risk	Prevalence
Mixed leg ulcers 	**Location:** below the knee **Clinical appearance:** • Mixed arterial and venous factors resulting in chronic wound formation	An ulcer with more than one underlying factor, frequently venous disease and arterial insufficiency[54]	Includes venous disease, obesity immobility, atherosclerosis and acute isachemia	Balance between treatment for venous and arterial disease[54]	Elderly, with risk factors for peripheral arterial disease. Frequently ulcers appear as venous ulcers and then as the patient ages, arterial insufficiency leads to further delayed wound healing[54]	8-26% of all chronic leg ulcers[177]
Diabetic foot ulcers 	**Location:** toe joints, inner side of first metatarsal head, malleoli, under heel, under metatarsal head[288] **Clinical appearance:** Neuropathic feet - warm with pulses and ulcers develop on tips of toes and plantar surfaces of metatarsal heads. Neuroischaemic - may not be warm or have pulses, and ulceration often on margins of foot, tips of toes and heels.[291]	Underlying neuropathy resulting in: repeated unrecognised trauma, structural abnormalities and changes to local regulation of inflammation[33]	Diabetes. Many patients also have arterial disease (ischaemic foot ulcers).[288]	Control of glucose levels, relief of pressure (e.g. total contact casting), surgical debridement, control of infection, arterial reconstruction, if necessary.[292]	Diabetic patients with peripheral neuropathy and excessive plantar pressure.[293]	1.7% of patients with diabetes; >2% of community based diabetic patients develop foot ulcers each year.[294]

Wound Aetiology	Clinical description	Underlying physiology	Contributory factors	Main treatment	Population at risk	Prevalence
Decubitis ulcers (pressure sore) 	**Location:** heel, malleoli, sacral and trochanter.[292] **Clinical appearance:** Four stages: • Nonblancable erythema • Partial thickness loss of skin layers • Full thickness loss, exposing subcutaneous fat • Exposed muscle or bone (deep ulcer or necrosis)[292]	Compression of tissue between bony prominence and external surface,[292] causing the blood supply to be inadequate[295]	External mechanical forces (pressure, friction and shear).[296] Additional risk factors include incontinence, bad nutritional state, increased body temperature, diabetes, peripheral arterial disease and age.[292]	Surgical removal of necrotic tissue, pressure relief, dressings.[292] Prevention: diminish pressure, frequent position changes, special mattresses/cushions.[292]	Patients unable to change position.	0.3-0.7% of >65 years in primary care[191] 6.8-14.6% in home care setting[292] 5.1-15.6% in general hospital[292] 25-41% in geriatric nursing homes[292]
Non-healing surgical wounds	**Location:** site around surgical intervention **Clinical appearance:** • Wounds resulting from surgical intervention with delayed healing or wound breakdown	Surgical trauma	Morbidity resulting in surgery, e.g. malignancy, amputation[295]	Healing by primary or secondary intention.	Healing by secondary intention more likely when surgery involves infected, necrotic or contaminated tissue[208]	23% of adult patients visited at home by nurses (in Michigan, US).[297]

Appendix 3.1. Letter from the Clinical Director of Wound Healing

Wound Healing Research Unit
Yr Uned Ymchwil Gwella Clwyfau

CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD

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Mynydd Bychan
Caerdydd CF14 4UJ

19 December 2007

Rebecca Howell-Jones
Department of Oral Surgery
Dental School
Cardiff University

Dear Rebecca

Thank you for your recent enquiry seeking clarification of matters relating to the audit that you undertook with us in the Directorate of Wound Healing as part of your PhD work.

I am writing to confirm that work was in-part generated by myself asking the question "Do we prescribe excessive amounts of antibiotics which would increase the risk of patients attending my clinics developing resistant organisms and how much impact does general practitioner prescribing have on the pattern of bacteria present and bacterial resistance in patients who attend my clinics?".

I can confirm that the ideas were discussed and the final plan was agreed in a departmental audit meeting in Spring 2005 and the results of the study were presented to us in autumn 2005.

The access to records that are held by my clinic was not an issue as this was an agreed departmental audit. The obtaining of information regarding the same patients from their general practitioner to determine whether additional courses of antibiotics had been prescribed for those patients were, in my opinion, an extension and a legitimate question to ensure validity of the audit findings. While it would have been possible to have requested this information from the patient's GP as part of their routine care I made the decision as Clinical Director to ask patients for consent to ensure there was clarity of purpose in requesting this information.

We did complete the audit cycle as a result of the information generated by the project and I have reduced further my prescription of oral antibiotics for patients seen in my clinics.

I trust this clarifies the position. If you or anyone else wishes to discuss this matter with me further I would be delighted to do so.

Yours sincerely

Keith Harding
Clinical Director of Wound Healing Cardiff & Vale NHS Trust
Head, Department of Wound Healing
Professor of Rehabilitation (Wound Healing)

Appendix 3.2. Patient questionnaire

Wound Healing Research Unit

CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD
coleg meddygaeth cymru
wales college of medicine

Wound Healing Audit - Questions for patients

To help us find out more about antibiotic resistance and chronic wounds would you please answer the four short questions below:

1. Since the beginning of [*3 months previous*] 2005, have you been prescribed or taken any antibiotics to treat your wound?

Yes ☐ No ☐ Don't know ☐

2. Since the beginning of [*3 months previous*] 2005, have you been prescribed or taken antibiotics to treat any illness apart from your wound?

Yes ☐ No ☐ Don't know ☐

3. Which one of the following options best describes where you live at the moment?

Own home

Residential care

Nursing home

Other .....

Don't know

4. Have you stayed overnight in a hospital since the beginning of June 2005?

Yes ☐ No ☐ Don't know ☐

You do not have to answer these questions unless you want to.

Appendix 3.3. GP letter

[Headed paper]

Private and Confidential

Date 2005

Dear,

Subject: Antibiotic resistance audit in patients with wounds

We are currently undertaking an audit, of the patients we treat at our wound healing clinics in Cardiff, with which we would be very grateful for your support.

As part of the audit, we are investigating our prescription of antibiotics and the levels of antibiotic resistant organisms in patients' wounds. We are also interested in looking at those factors which predispose patients to having such organisms when they attend at our clinics.

To be able to place our prescribing for wound infection in the context of antibiotics that patients may have received for other conditions, we require information about patients' previous exposure to commonly known risk factors. Therefore, we would be most grateful if you would assist us with our enquiry by completing the attached single-sided form for the following patient(s):

Name Date of birth:, Patient's postcode:

We understand that it takes time to find this information and to complete the forms and, we are able to provide payment of £3 per patient, on return of the information.

Thank you in advance for your co-operation with this interesting and relevant investigation. If you have any queries, feel free to contact Rebecca Howell-Jones on (029) 2074 4252.

Yours sincerely

Prof. Keith G. Harding

Clinical Director of Wound Healing
Head of University Department of Surgery
Professor of Rehabilitation Medicine (Wound Healing)

PRIVATE AND CONFIDENTIAL

Patient Name:; Date of Birth:

1. Please list below all antibiotic courses the patient has been prescribed since (if it is more convenient to attach a print-out of the relevant information, this will be perfectly acceptable):

	ANTIBIOTIC (Generic name)	**Dose**	**Daily frequency** (eg. bds, tds)	**Duration** (days)	**Date prescribed**	**Prescribing condition** (Wound or Other)
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

2. Please indicate below any co-morbidities suffered by the patient:

Co-morbidity	**Please tick if present**
Diabetes mellitus	
CHD – Coronary Heart Disease	
Malignancy – (excluding skin malignancy)	
Long term systemic immunosupression	
Chronic respiratory disease	
Others: Please specify	

3. Please indicate below any time the patient has spent in hospital since 11 May 2004:

	Name of hospital	**Length of stay**		
		Date of admission	**Date of discharge**	**Number of nights**
1				
2				
3				
4				

Appendix 3.4. Frequency of all descriptors used for the wound bed, edge and surrounding skin

Condition	Leg ulcer (%)	Foot ulcer (%)	Surgical (%)	Miscell-aneous (%)	Total (%)
Wound Bed	(n=69)	(n=17)	(n=37)	(n=9)	(n=132)
Bleeding	2 (2.9)	0 (0.0)	3 (8.1)	1 (11.1)	6 (4.5)
Granulating	56 (81.2)	14 (82.4)	28 (75.7)	2 (22.2)	100 (75.8)
Slough	50 (72.5)	9 (52.9)	6 (16.2)	3 (33.3)	68 (51.5)
Necrosis	4 (5.8)	1 (5.9)	0 (0.0)	1 (11.1)	6 (4.5)
Evidence of Infection	12 (17.4)	2 (11.8)	5 (13.5)	3 (33.3)	22 (16.7)
Overgranulating	3 (4.3)	0 (0.0)	4 (10.8)	0 (0.0)	7 (5.3)
Indolent	0 (0.0)	1 (5.9)	0 (0.0)	0 (0.0)	1 (0.8)
Scab	0 (0.0)	0 (0.0)	0 (0.0)	2 (22.2)	2 (1.5)
Healed	1 (1.4)	0 (0.0)	0 (0.0)	1 (11.1)	2 (1.5)
Islands of epithelium	2 (2.9)	2 (11.8)	0 (0.0)	0 (0.0)	4 (3.0)
Easy to bleed	0 (0.0)	0 (0.0)	0 (0.0)	1 (11.1)	1 (0.8)
Not able to see	0 (0.0)	0 (0.0)	5 (13.5)	1 (11.1)	6 (4.5)
Plastic button	0 (0.0)	0 (0.0)	1 (2.7)	0 (0.0)	1 (0.8)
Calcium/calcification	2 (2.9)	1 (5.9)	0 (0.0)	0 (0.0)	3 (2.3)
Fibrin	0 (0.0)	0 (0.0)	1 (2.7)	0 (0.0)	1 (0.8)
Bone	1 (1.4)	1 (5.9)	1 (2.7)	0 (0.0)	3 (2.3)
Wound Edge	(n=68)	(n=17)	(n=36)	(n=10)	(n=131)
Epithelialising	21 (30.9)	8 (47.1)	15 (41.7)	0 (0.0)	44 (33.6)
Callus	0 (0.0)	3 (17.6)	0 (0.0)	1 (10.0)	4 (3.1)
Rolled edge	0 (0.0)	1 (5.9)	1 (2.8)	0 (0.0)	2 (1.5)
Static	47 (69.1)	7 (41.2)	18 (50.0)	9 (90.0)	81 (61.8)
Surrounding Skin	(n=69)	(n=17)	(n=36)	(n=11)	(n=133)
Normal	1 (1.4)	1 (5.9)	7 (19.4)	3 (27.3)	12 (9.0)
Macerated	7 (10.1)	5 (29.4)	2 (5.6)	0 (0.0)	14 (10.5)
Erythema	54 (78.3)	11 (64.7)	18 (50.0)	4 (36.4)	87 (65.4)
Excoriated	3 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	3 (2.3)
Oedematous	14 (20.3)	4 (23.5)	2 (5.6)	1 (9.1)	21 (15.8)
Cellulitis	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)
Lipodermasclerosis	8 (11.6)	0 (0.0)	0 (0.0)	0 (0.0)	8 (6.0)
Fragile	2 (2.9)	3 (17.6)	1 (2.8)	1 (9.1)	7 (5.3)
Dry/flaky	48 (69.6)	9 (52.9)	7 (19.4)	5 (45.5)	69 (51.9)
Eczema	21 (30.4)	3 (17.6)	3 (8.3)	0 (0.0)	27 (20.3)
Haemosiderin	2 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.5)
Scar	1 (1.4)	0 (0.0)	15 (41.7)	3 (27.3	19 (14.3)
Keloid scar	0 (0.0)	0 (0.0)	1 (2.8)	0 (0.0)	1 (0.8)
Bridge scar	0 (0.0	0 (0.0)	1 (2.8)	0 (0.0)	1 (0.8)

Appendix 4.1. Exploration of variables and transformations to normalise the data.

Transformations were conducted on the following variables to try to normalise the data:

- Number of wounds
- Age of patients
- Number of antibiotic groups in the previous 90 days
- Number of visits to the wound healing clinic.

The distribution of each variable was examined prior to transformation to assist with the selection of appropriate methods. For example, the impact of logistic transformations was explored when data were positively skewed (i.e. towards the left-hand side of the graph).[274] Other transformation used included the square root and inverse. For negatively skewed data the scores were reversed prior to transformation.[204] Normality was examined using Q-Q plots and the Kolmogorov-Smirnov statistic.

Table A4.0.1 to Table A4.0.4 show the distribution of these variables and their transformations.

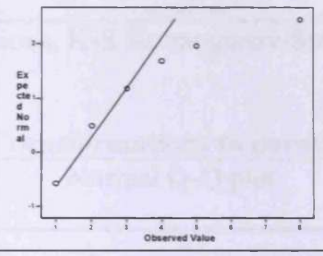
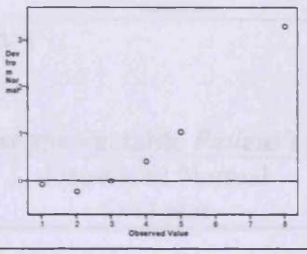
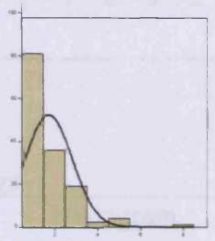
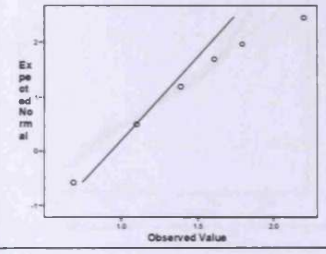
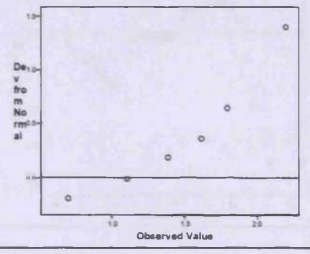
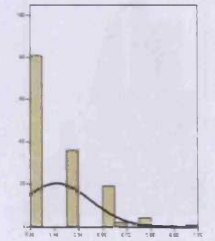
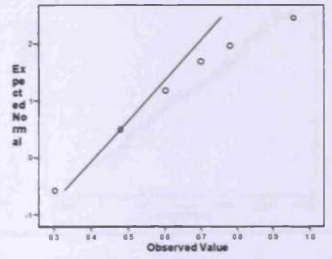
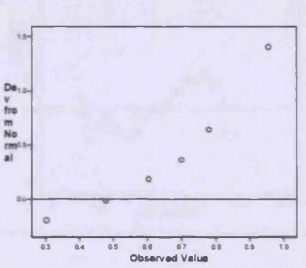
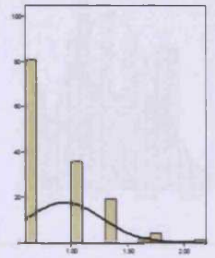
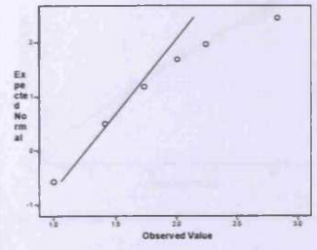
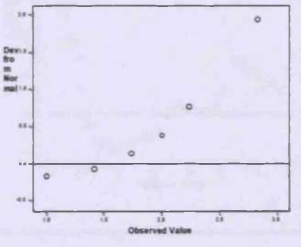
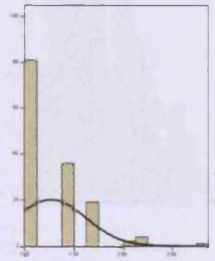
Transformations of the variable *Number of visits to wound healing clinic* were also investigated while excluding the outlying data point (32 visits to wound healing clinics in the previous year). The transformations were conducted in the same way as shown in Table A4.0.4 and all were found to differ significantly from the normal distribution using the Kolmogorov-Smirnov statistic.

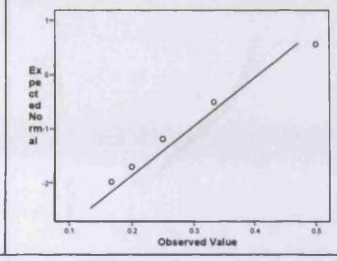
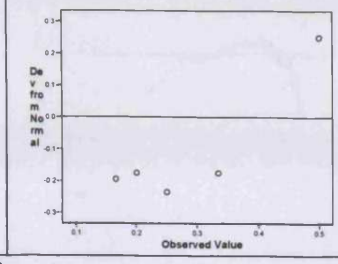
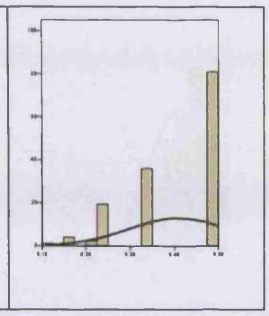
Transformation was only successful at normalising data on the age of patients. The remaining three variables could not be successfully normalised and were therefore categorised. Histograms of these three variables, and details of their distribution (Table A4.0.5), were explored prior to devising appropriate categories:

- Number of wounds: 1 wound, >1 wound
- Number of antibiotic groups: ≤ 2 antibiotic groups, >2 antibiotic groups

- Number of visits to wound healing clinic: ≤ 4 visits, >4 visits.

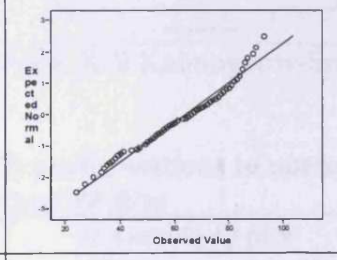
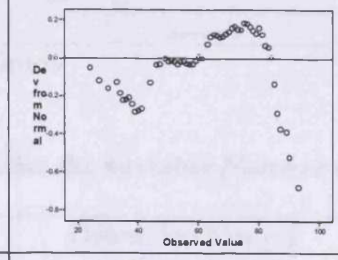
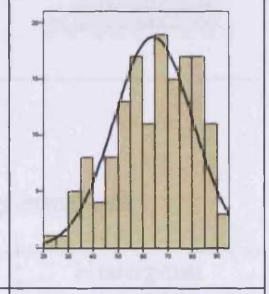
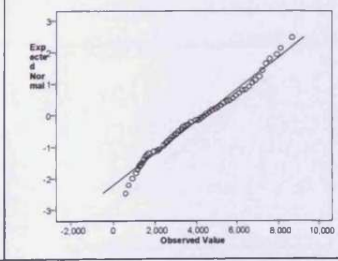
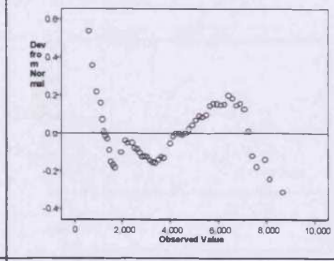
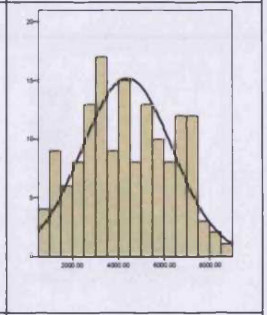
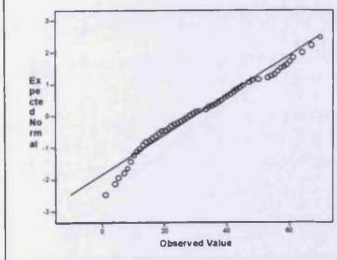
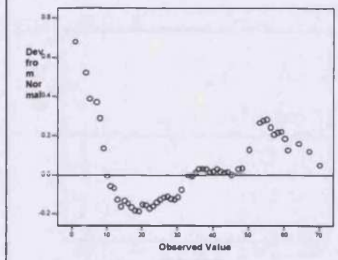
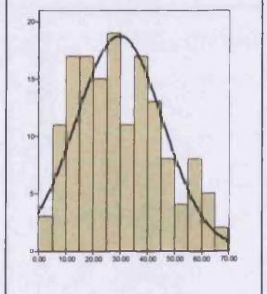
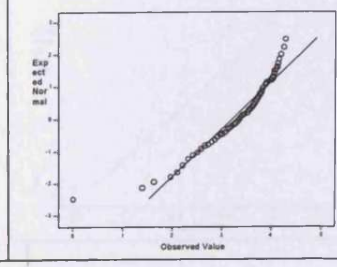
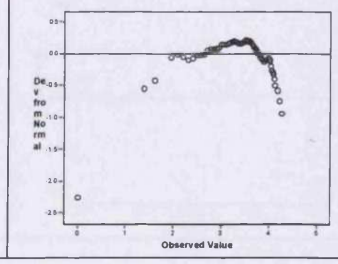
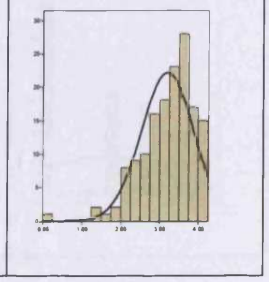
Table A4.0.1 Transformations to normalise the variable *Number of wounds*

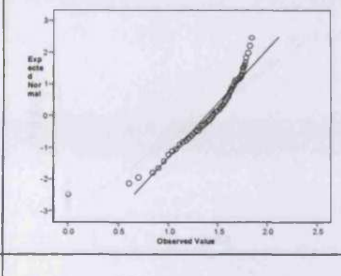
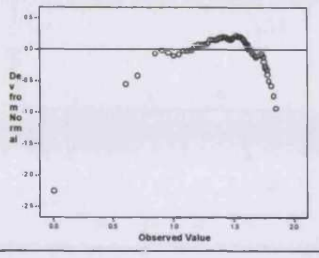
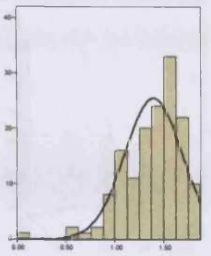
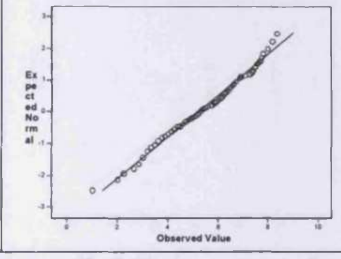
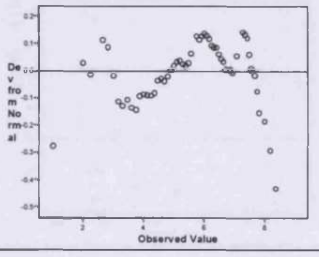
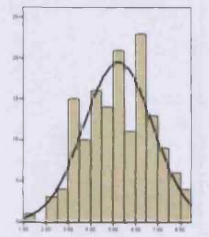
	Normal Q-Q plot	Detrended Normal Q-Q plot	Histogram
T: None K-S: 0.312 (p=0.000)			
T: Natural logarithm (+1) K-S: 0.344 (p=0.000)			
T: Log^{10} (+1) K-S: 0.344 (p=0.000)			
T: Square root K-S: 0.337 (p=0.000)			

T: Inverse K-S: 0.360 (p=0.000)			

T: Transformations, K-S Kolmogorov-Smirnov

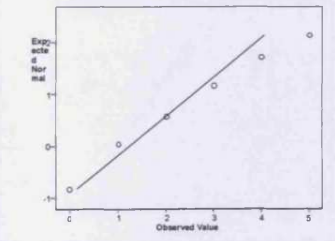
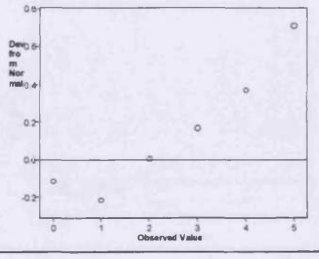
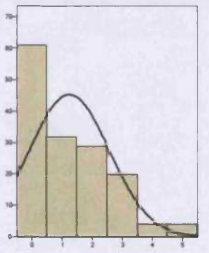
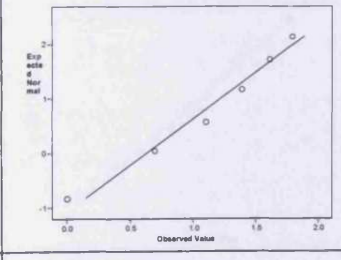
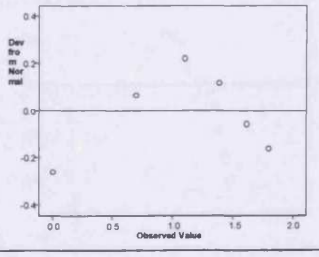
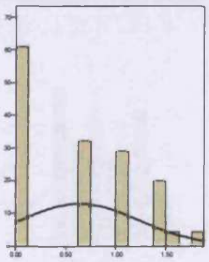
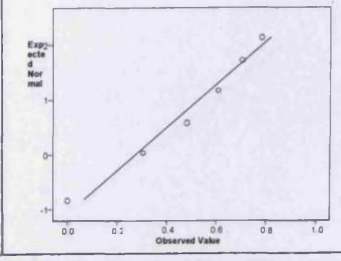
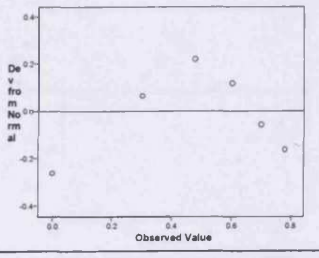
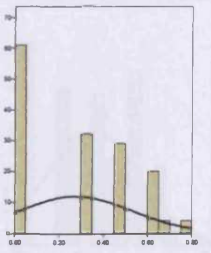
Table A4.0.2 Transformations to normalise the variable *Patient's Age*

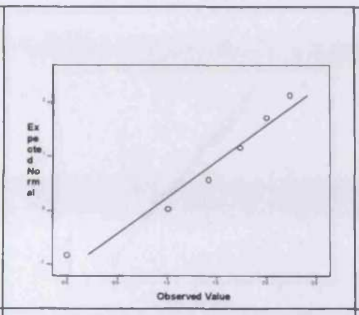
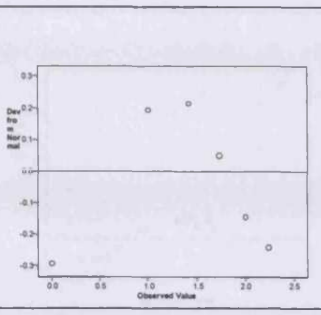
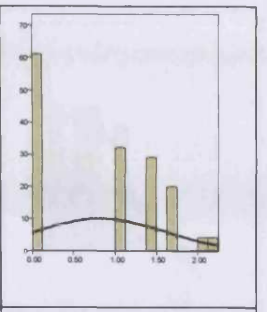
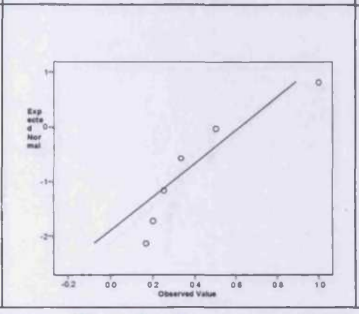
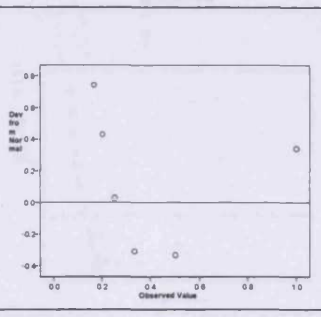
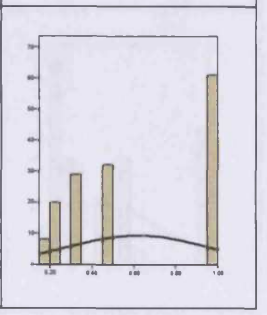
	Normal Q-Q plot	Detrended Normal Q-Q plot	Histogram
T: None K-S: 0.74 (p=0.042)			
T: Squared K-S:0.067 (p=0.099)			
T: Reversed (i.e. all values subtracted from max value + 1) K-S: 0.74 (0.042)			
T: Natural logarithm of reversed data K-S: 0.099 (p=0.001)			

T: Log^{10} of reversed data K-S: 0.099 (p=0.001)			
T: Square root of reversed data K-S: 0.065 (p=0.2)			

T: Transformations, K-S Kolmogorov-Smirnov

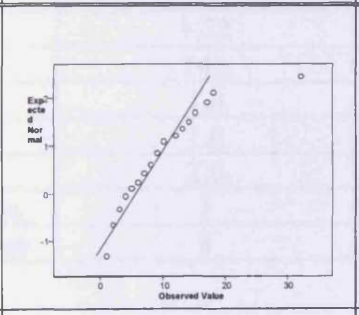
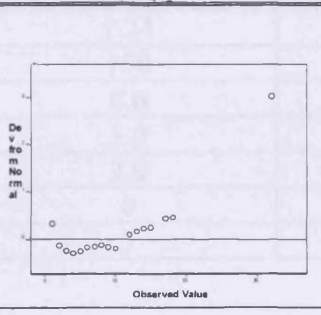
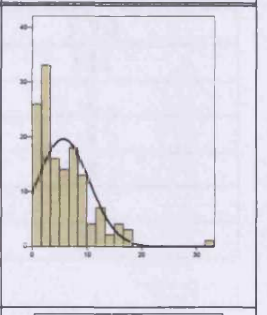
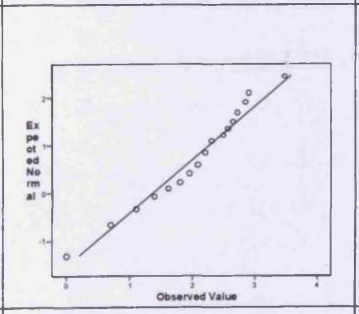
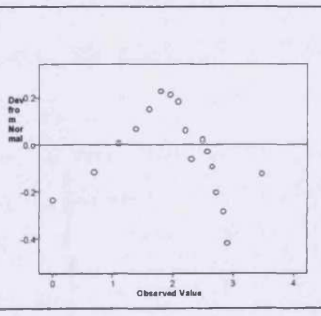
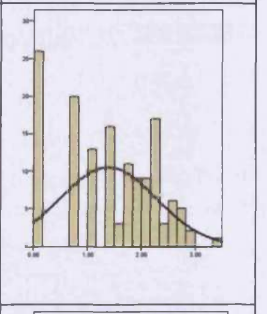
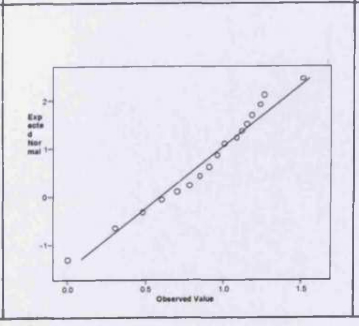
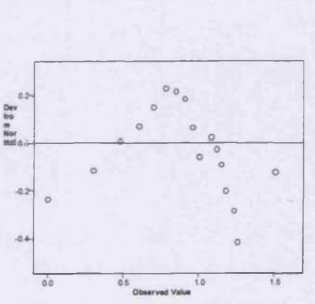
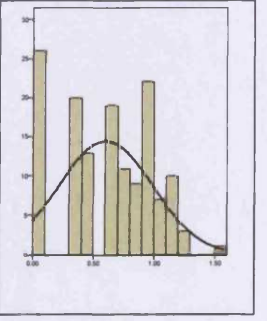
Table A4.0.3 Transformations to normalise the variable *Number of antibiotic groups in previous 90 days*

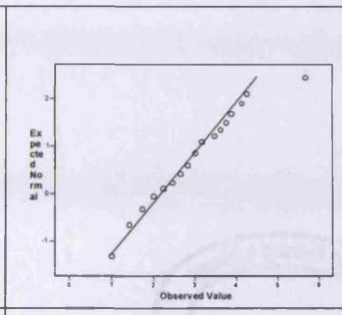
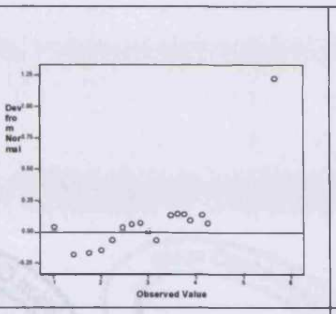
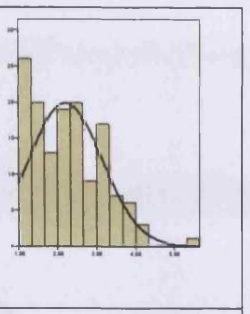
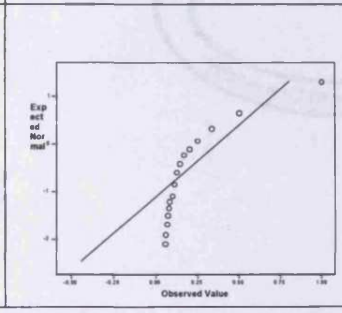
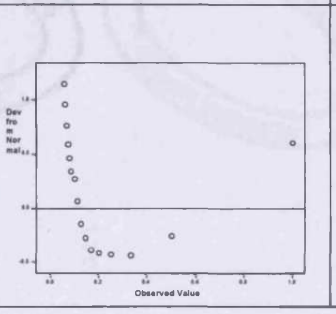
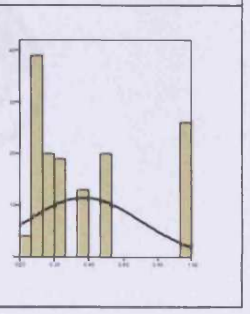
	Normal Q-Q plot	Detrended Normal Q-Q plot	Histogram
T: None K-S:0.232 (p=0.000)			
T: Natural logarithm (+1) K-S: 0.267 (p=0.000)			
T: Log^{10} (+1) K-S: 0.267 (p=0.000)			

T: Square root K-S: 0.275 (p=0.000)			
T: Inverse K-S: 0.283 (p=0.000)			

T: Transformations, K-S Kolmogorov-Smirnov

Table A4.0.4 Transformations to normalise the variable *Number of visits to wound healing clinic*

	Normal Q-Q plot	Detrended Normal Q-Q plot	Histogram
T: None K-S: 0.170 (p=0.000)			
T: Natural logarithm K-S: 0.124 (p=0.000)			
T: Log^{10} K-S: 0.124 (p=0.000)			

T: Square root K-S: 0.123 (p=0.000)			
T: Inverse K-S: 0.224 (p=0.000)			

T: Transformations, K-S Kolmogorov-Smirnov.

Table A4.0.5 Descriptive statistics for the variables *Number of wounds*, *Number of antibiotic groups* and *Number of visits to wound healing clinic*

	Number of wounds	Number of antibiotic groups	Number of visits to wound healing clinic
Mean	1.72	1.24	5.70
Number	143	150	141
25th percentile	1.0	0.0	2
50th percentile	1.0	1.0	4
75th percentile	2.0	2.0	8
Minimum value	1	0	1
Maximum value	8	5	32