



Contents lists available at ScienceDirect

## Clinical Microbiology and Infection

journal homepage: [www.clinicalmicrobiologyandinfection.com](http://www.clinicalmicrobiologyandinfection.com)

## Original article

## Gut microbiology of UK care home residents: a cross-sectional analysis from a randomised controlled trial

David Gillespie<sup>1,2,\*</sup>, Mandy Wootton<sup>3</sup>, Ruby Ray<sup>2</sup>, Philip C. Calder<sup>4</sup>,  
 Tin Man Mandy Lau<sup>2</sup>, Eleri Owen-Jones<sup>2</sup>, Rachel Lowe<sup>2</sup>, Leanne Davies<sup>3</sup>,  
 Jennifer Richards<sup>3</sup>, Kerenza Hood<sup>2</sup>, Vivian Castro-Herrera<sup>4</sup>, Jane Davies<sup>2</sup>,  
 Nick A. Francis<sup>5</sup>, F.D. Richard Hobbs<sup>1</sup>, Mark Lown<sup>4</sup>, Michael Moore<sup>5</sup>, Victoria Shepherd<sup>2</sup>,  
 Christopher C. Butler<sup>1</sup>

<sup>1</sup> Nuffield Department of Primary Care Health Sciences, University of Oxford, Oxford, United Kingdom<sup>2</sup> Centre for Trials Research, School of Medicine, College of Biomedical & Life Sciences, Cardiff University, Cardiff, United Kingdom<sup>3</sup> Specialist Antimicrobial Chemotherapy Unit, Public Health Wales Microbiology Cardiff, Cardiff, United Kingdom<sup>4</sup> Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, United Kingdom<sup>5</sup> Primary Care, Population Sciences and Medical Education, University of Southampton, Southampton, UK

## ARTICLE INFO

## Article history:

Received 28 March 2023

Received in revised form

28 July 2023

Accepted 1 August 2023

Available online xxx

Editor: K. Andre

## Keywords:

Antimicrobial resistance

Gut microbiology

Long-term care facilities

United Kingdom

## ABSTRACT

**Objective:** To describe the prevalence of potentially clinically relevant gut pathogens and associations with the carriage of resistant organisms in UK care home residents.

**Methods:** Stool samples were collected pre-randomisation from care home residents participating in a randomised placebo-controlled trial. Cultivable clinically relevant bacteria were analysed. Antimicrobial susceptibility testing was performed by agar dilution (amoxicillin, co-amoxiclav, gentamicin, trimethoprim, nitrofurantoin, and ciprofloxacin). We also aimed to detect resistance to third-generation cephalosporins, carbapenems, and vancomycin.

**Results:** Stool samples were available for 159/310 residents participating in the trial (51%) from 23 care homes between 2016 and 2018. In total, 402 bacterial isolates were cultured from 158 stool samples and 29 different species were cultured. The five most common species were *Escherichia coli* (155/158, 98%), *Pseudomonas aeruginosa* (40/158, 25%), *Enterococcus faecalis* (35/158, 22%), *Enterococcus faecium* (30/158, 19%), and *Proteus mirabilis* (25/158, 16%). Enterobacterales isolates were cultured from 157 samples (99%), and resistance to at least one of the tested antimicrobials was found in 119 of these (76%). There were high levels of variation in outcomes by care home.

**Discussion:** We demonstrated that care home residents harbour significant levels of antimicrobial-resistant organisms in their stool. This work emphasises the importance of both enhanced infection control practices and antimicrobial stewardship programmes to support the appropriate use of antimicrobials in this setting. **David Gillespie, Clin Microbiol Infect 2023;■:1**

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## Introduction

Care homes are enclosed environments of mostly frail older adult residents who are more likely to require living assistance, antibiotic treatment and invasive devices such as catheters [1]. Residents are at higher risk from infections, which can partially be

attributed to infection control challenges such as increased risk of infection through diminishing immunity with age, increased proximity in sharing of living space, objects and bathroom facilities, low implementation of infection control procedures and increased antibiotic prescribing [1,2].

\* Corresponding author. Dr David Gillespie, Centre for Trials Research, School of Medicine, College of Biomedical & Life Science, Cardiff University, Heath Park, Cardiff, CF14 4YS, UK.

E-mail address: [gillespie1@cardiff.ac.uk](mailto:gillespie1@cardiff.ac.uk) (D. Gillespie).

<https://doi.org/10.1016/j.cmi.2023.08.001>

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Care homes have been recognised as an important reservoir for multi-drug resistant organisms [3–6]. Infections are more likely to be caused by antimicrobial-resistant (AMR) bacteria [2,6–8], with urinary tract infections (UTIs) and respiratory tract infections being the most common healthcare-associated infection both in the hospital setting and in long-term care facilities (LTCFs) [9]. A large population study conducted in England demonstrated that residents in long-term care facilities are more than twice as likely as community-dwelling adults of similar age to present with a laboratory-confirmed *Escherichia coli* or *Klebsiella* UTI and four times more likely for the UTI to be caused by resistant bacteria [9].

Recurrent UTI infection has been linked with extended-spectrum (ES) cephalosporin resistance in Enterobacterales, which is a concern both in long-term care facilities and in the acute-care setting [10]. Many of the bacteria resistant to ES cephalosporins are extended spectrum beta-lactamase (ESBL)-producing organisms that are challenging to treat, with ESBL-producing organisms under surveillance by the World Health Organisation (WHO) as a key indicator of AMR evolution [11].

Furthermore, individuals living together have more similar gut bacterial communities than individuals living in other households [7]. Exploring the gut microbiological make-up in residents of care homes may lead to a better understanding of infection and AMR within this setting [8]. The high presence of multi-drug resistant bacteria in the gut, acting as a reservoir for potential future infections, may affect treatment options for this patient group.

Given the interplay between the microbiome and immunity and the additional risk factors associated with care homes and the elderly, interventions aiming to enhance the microbiome constitution may be one strategy to address AMR in this population. As AMR is not currently routinely assessed within this setting in the UK, or even well described, additional research within this area is warranted. In this study, we aimed to categorise and describe the clinically relevant potential pathogens and their associated antibiotic susceptibilities in the gut of UK care home residents, exploring variation between individuals and care homes. The study concentrated on relevant pathogens which are a frequent cause of infection in the elderly and is in contrast to microbiome research, which captures all microbial agents, including non-cultivable species.

## Methods

### Study design, participants, and setting

This was a secondary analysis of microbiological samples taken from participants in a randomised placebo-controlled trial of a daily oral probiotic combination [12–16]. The trial was approved by an NHS research ethics committee (Wales REC3. Ref: 15/WA/0306).

Participants were those living in a residential, nursing or dual-registered care home in England or Wales, aged 65 years or older, and not immunocompromised, or taking on-going regular probiotics who provided a stool sample at study entry (i.e. prior to randomisation). Prior to study participation, written informed consent was obtained from those participants with capacity to do so. For those who lacked capacity to provide consent, a consultee (either a family member or friend) completed a consultee declaration for participation on their behalf.

### Procedures

Key data collected for the study included sex, age, weight, length of time resident in the care home, ethnicity, capacity status, clinical frailty [17], antimicrobial use in the previous

4 weeks, and use of proton pump inhibitors, laxatives, or vitamin D (included as there was interest in the original trial with the correlation between vitamin D and probiotic consumption) in the previous 4 weeks. A stool sample was collected from each participant using a clinically clean bedpan or receiver. Specimens were scooped in order to fill approximately one-third of the container (usually greater than 10 ml) and then placed in “Specisafe” packaging (a rigid plastic container to withstand postal damage) and posted via Royal Mail next day delivery, using compliant mailing envelopes (UN3373), to the Specialist Antimicrobial Chemotherapy Unit, Public Health Wales Microbiology, Cardiff, Wales. For transport, no recommendations were given regarding temperature ranges.

### Microbiological analysis

Cultivable clinically relevant bacteria only were analysed. A 10 µL loop of stool sample was dissolved in 3 mL sterile saline and vortexed to mix. 50 µL was inoculated onto Columbia Blood Agar, plus a range of the Brilliance chromogenic agars to detect UTI pathogens (PO1110A), isolates harbouring antimicrobial resistance; ES B-Lactamase (PO0530A), Carbapenemase Resistant Enterobacterales (PO1226A) and Vancomycin Resistance Enterococci (PO1175A, Oxoid Ltd, Thermo Fisher, UK) using Spiral plater (WASP, Don Whitley, Bingley, Yorkshire UK). Plates were incubated in air at 35°C ± 1°C for 18–20 hours, then colony counts of all species calculated on each plate.

Cultures were then identified using a Bruker BioTyper Matrix Assisted Laser Desorption Ionisation – Time of Flight mass spectrometer.

Susceptibility testing by agar dilution [18] was performed on the predominant Gram-negative bacteria to a range of antimicrobials: amoxicillin, co-amoxiclav, gentamicin, trimethoprim, nitrofurantoin and ciprofloxacin. Minimum Inhibitory Concentrations were interpreted using the European Committee on Antimicrobial Susceptibility Testing guidelines, version 2021 [19]. Antimicrobial resistance of isolates growing on the ESBL and Carbapenemase Resistant Enterobacterales media were confirmed using ROSCO Phenotyping kits (Rosco Diagnostica) to detect ESBL and ampC β-lactamases plus genotypic confirmation by in-house Real-Time Polymerase Chain Reaction assays for *TEM*, *SHV*, *CTX-M* genes. Minimum Inhibitory Concentrations for potential Vancomycin Resistance Enterococci were confirmed using vancomycin and teicoplanin gradient strips (Etest, BioMerieux, France) and an in-house Real-Time Polymerase Chain Reaction assay for *vanA/vanB* genes.

Stool samples were also inoculated onto Fastidious Anaerobe Agar (FAA, Oxoid Ltd) and incubated in anaerobic conditions for 20–24 hours for *Clostridioides difficile* isolation. Any growth was identified using the Matrix Assisted Laser Desorption Ionisation – Time of Flight.

### Outcomes

Outcomes included the total number of bacterial isolates cultured from stool samples, the frequency of different organisms cultured from stool samples, the number of different organisms per stool sample, the frequency of *C. difficile*, the susceptibility of organisms to antimicrobials, and the prevalence of antimicrobial resistance. Dietary intake was not standardised as part of the trial, and participants continued to follow their usual dietary habits. Care homes are expected to follow regulatory standards which highlight the importance of providing varied and nutritious meals, but meal provision will vary between care homes, and residents may have a less varied diet than community-dwelling individuals.

### Statistical methods

Outcomes are presented descriptively, with frequencies provided with percentages, means with standard deviations, and medians with interquartile ranges as appropriate.

Care home variation was primarily explored descriptively, but the median odds ratio was estimated to assess the risk of having AMR Enterobacterales in a randomly chosen individual if they moved to a care home with a higher risk [20].

### Results

The PRINCESS trial enrolled 310 participants from 23 care homes in England and Wales between December 2016 and May 2018. Out of these, 159 provided stool samples before randomisation (51% of all enrolled participants). Those who provided stool samples had a mean age of 85 years, with one-third being male, and 62% (n = 99) lacked the capacity to consent to the study (Table 1).

One stool sample leaked in transit and was therefore not subject to analysis. In total, 402 clinically relevant bacterial pathogens were identified from 158 stool samples. The median total colony count was  $9 \times 10^6$  CFU/mL (IQR:  $1 \times 10^6$  to  $3.9 \times 10^7$  CFU/mL). Twenty-nine different species were identified (Fig. 1), with the five most common species being *Escherichia coli* (155/158 samples), *Pseudomonas aeruginosa* (40/158 samples), *Enterococcus faecalis* (35/158 samples), *Enterococcus faecium* (30/158 samples), and *Proteus mirabilis* (25/158 samples).

The total number of different species cultured in any one sample ranged from one (14/158 samples, 9% of samples) to six (1/158 samples, 1% of samples), with a median of two different species cultured from a sample (IQR: 2 to 3 species per sample).

Care homes varied in the extent to which they recruited participants provided stool samples (from 0 to 85% of recruited participants) and the mean number of organisms per sample (from 1 to 3.8 organisms per sample). While the most commonly cultured

species was *E. coli* across all care homes, the other most frequently cultured species varied (Table S1).

Four percent of samples, all of which were from participants from different care homes, grew *C. difficile* (toxin positive) on culture (6/158).

Enterobacterales isolates were cultured from 157 samples (99%), and resistance to at least one of the tested antimicrobials was found in 119 of these (76%).

The median odds ratio between care homes for the presence of AMR Enterobacterales was 2.96 (95% credible interval: 2.09 to 4.09), indicating substantial variability between care homes.

Trimethoprim-resistant Enterobacterales were found in 64/157 samples (41%), co-amoxiclav-resistant Enterobacterales in 70/157 (45%), and no samples contained nitrofurantoin-resistant Enterobacterales.

For *E. coli* isolates, 60% (93/154) were resistant to amoxicillin, 42% (65/154) to co-amoxiclav, and 38% (59/154) to trimethoprim. Seven percent (11/154) of *E. coli* isolates were resistant to gentamicin and none were resistant to nitrofurantoin. Susceptibility to all tested antibiotics was found for 30% (46/154) of *E. coli* isolates.

Overall, 39/157 (25%) participants had taken at least one antibiotic during the 4-weeks prior to study entry. While the number of participants taking trimethoprim in the 4-weeks prior to study entry was small (8/157, 5%), seven of these participants subsequently provided a stool sample containing trimethoprim-resistant Enterobacterales.

Three stool samples were confirmed positive for vancomycin resistant *Enterococci*, conferred by a *vanA* gene. Furthermore, 68 (43%) samples were found to contain bacteria that grew on ESBL-selective media, suggestive of resistance to third-generation cephalosporins. Of these, 46% (31/68) were confirmed to harbour an ESBL (and thus in 20%, or 31/158 samples was there ESBL carriage) and the remainder (32 *P. aeruginosa*, two *Citrobacter freundii*, and three *Morganella morganii*) harboured a hyper-expressing native AmpC enzyme conferring third-generation cephalosporin

**Table 1**

Comparison of 310 participants from 23 care homes across England and Wales (2016–2018) according to whether they provided a stool sample at study entry or not\*

Variable	Category	Participants providing a stool sample (N = 159)		Participants not providing a stool sample (N = 151)	
		Frequency	%	Frequency	%
Sex	Female	106	66.7	101	66.9
	Male	53	33.3	50	33.1
Ethnicity	White	158	99.4	146	96.7
	Non-white	1	0.6	5	3.3
Capacity status	Lacks capacity	99	62.3	105	69.5
	Has capacity	60	37.7	46	30.5
Clinical frailty	Very fit to managing well	10	6.3	21	13.9
	Vulnerable to moderately frail	57	35.8	58	38.4
	Severely frail to very severely frail	92	57.9	72	47.7
Prescribed antimicrobials in the last 4 weeks	No	114	71.7	114	75.5
	Yes	45	28.3	37	24.5
Used proton pump inhibitor in last 4 weeks	No	103	64.8	92	60.9
	Yes	56	35.2	59	39.1
Used laxatives in last 4 weeks	No	77	48.4	73	48.3
	Yes	82	51.6	78	51.7
Used vitamin D in last 4 weeks	No	111	69.8	105	69.5
	Yes	48	30.2	46	30.5
Variable	Mean		SD	Mean	SD
Age (y), [n = 309]		85	6.8	85	8.0
Weight (kg) [N = 277]		65	16.5	64	15.3
Variable	Median		IQR	Median	IQR
Length of time resident in care home (months) [N = 307]		17	6–37	13	5–32

\* Data are based on 310 participants unless otherwise stated.

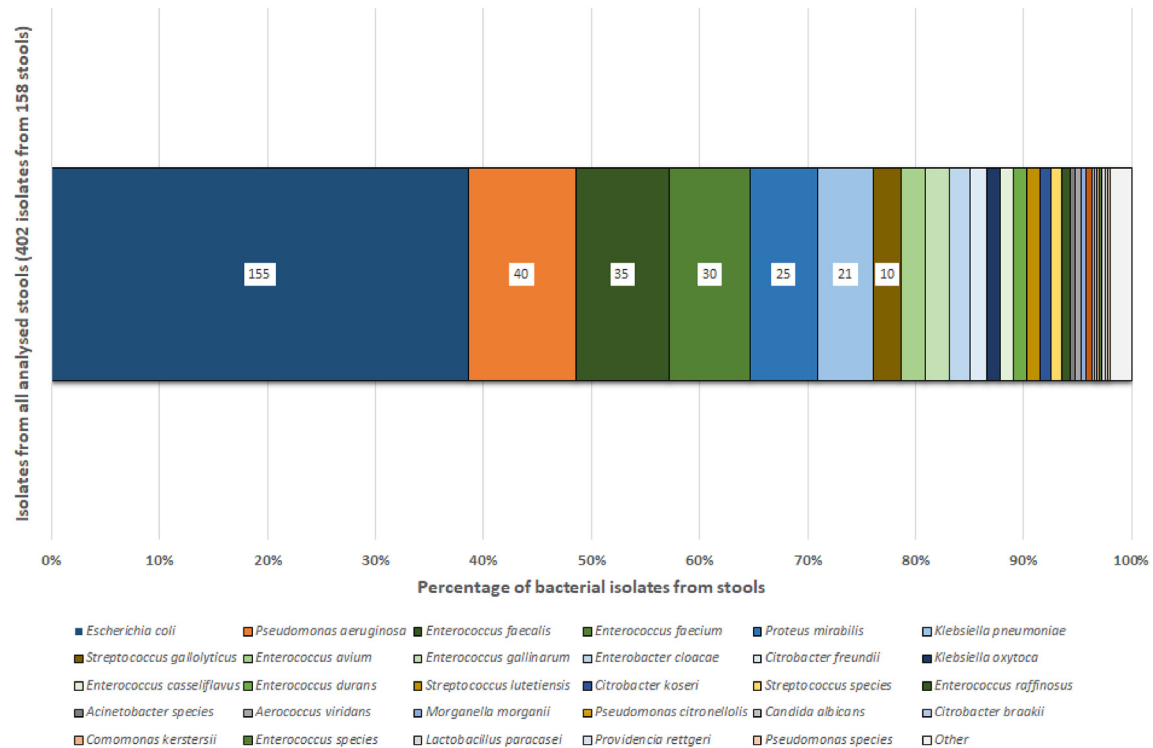


Fig. 1. Microbial species cultured from stool samples of 158 care home residents from 23 care homes across England and Wales (2016–2018)\*.

\*Ordered by most frequently occurring species. Data labels were provided for species where the number of isolates was  $\geq 10$ . Colour coded by species type (blue = Enterobacteriales; orange = *pseudomonas* species; green = *Enterococci* species; yellow = *streptococcus* species; grey = other species).

resistance. All ESBL-positive bacteria were identified as *E. coli* and harboured CTX-M group 1 (27/31) or CTX-M group 9 (4/31) ESBL genes. Seven samples contained isolates that grew on the carbapenem-resistance plate, seven were *P. aeruginosa*, one *A. johnsonii* and one *E. coli*. However, no transferable carbapenemase resistance determinant was confirmed.

## Discussion

This study of cultivable gut bacteria found that care home residents across the UK have a varied diversity of clinically relevant bacterial species. Stool samples from care home residents contained between one and six clinically relevant pathogens, some with high levels of resistance to antimicrobials used to treat common infections. The most common species found in the stools are also the most prevalent cause of blood stream infections [21]. A significant level of antimicrobial resistance was found, with the resistance rates similar to those nationally reported found in blood stream infection *E. coli* [21]. In this study, third-generation cephalosporin resistance in Enterobacteriales was significantly higher than for those isolates causing bloodstream infections and UTI in UK population-based studies [21,22].

Unrecognised and untreated UTIs are often a causative factor in the development of systemic infections, and in the elderly, delayed prescriptions for UTIs have been associated with increased septicæmia [23]. *E. coli*, *Enterococcus faecalis*, *K. pneumoniae* and *Proteus mirabilis* are the most common pathogens associated with UTIs and were the most prevalent found in this study [24]. When considering antimicrobial agents commonly used to treat UTIs, resistance rates were similar for those found in both community and in-patient Welsh isolates for trimethoprim and ciprofloxacin [22,25]. Co-amoxiclav resistance rates in the *E. coli* from stools were higher than for Welsh UTI isolates. For the first line UTI therapy

nitrofurantoin, the resistance rate in our study was zero compared with 3.1% and 3% found in the Welsh UTI community and in-patient isolates. These differences may reflect the settings in which these studies were carried out, the age ranges considered in the Welsh surveillance work, and the longer time frame over which the surveillance work was carried out. For nitrofurantoin, resistance rates are naturally low amongst urinary isolates in general, but the lack of resistance in our data set could be influenced by the low levels of nitrofurantoin available in the gut.

We found substantial variation among care homes regarding the presence of AMR Enterobacteriales. This implies that in some care homes, the level of AMR Enterobacteriales is high, consistent with previous work suggesting that the enclosed environments of care homes may act as a reservoir for the transmission of AMR infections [4,26].

The study has several limitations. Stool samples were provided by just over half of included residents. There may be factors which may indicate that these residents are a selective group. Identified barriers to the collection of stool samples relate to dignity and practical issues [27]. Future research aiming to study the gut microbiology of care home residents should consider the representativeness of those sampled and could consider different sampling strategies or oversampling in some circumstances.

This study has demonstrated that care home residents harbour significant levels of culturable AMR organisms in their gut. Given their living environment, increased frailty, and infection frequency (in particular UTIs), this work emphasises the importance of both enhanced infection control practices to limit the transmission of infections and antimicrobial stewardship programmes, aligned to guidelines around local resistance patterns, to support the appropriate use of antimicrobials in this setting. Further work is needed to understand transmission patterns within care homes, as well as the microbiome health and diversity in this population, as these

may be important areas to target for interventions aiming to reduce AMR transmission.

### Author contributions

DG drafted the paper along with RR and MW. The statistical analyses were conducted by DG. Early drafts were reviewed by PC, TMML, and CCB. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work. DG and CCB have directly accessed and verified the underlying data reported in the manuscript.

### Transparency declaration

All authors declare that they have no conflict of interests. This project was funded by the EME programme, which is funded by the MRC and NIHR, with contributions from the Chief Scientist Office—Scotland, Health and Care Research Wales, and the Health and Social Care Research and Development Division (part of the Public Health Agency) Northern Ireland (grant 13/95/10, Probiotics to Reduce Infections in Care Home Residents [PRINCESS]). The funding and sponsoring organizations had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication. In addition, they had no right to veto publication or to control the decision regarding which journal the paper was submitted.

### Acknowledgements

We would like to thank all those who participated in the trial, and their consultees (if applicable), without whom this trial would not have been possible. We would also like to acknowledge the contribution of Mr Alun Toghil and Mr James Downs as the public and patient involvement representatives in the Trial Management Group. Two UK Clinical Research Collaboration-registered clinical trials units were involved in the study. The Centre for Trials Research receives funding from Health and Care Research Wales and Cancer Research UK, Cardiff University and the University of Oxford Primary Care Clinical Trials Unit.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cmi.2023.08.001>.

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