Typing and epidemiological surveillance show that UK bloodstream *Escherichia coli* with extended-spectrum β-lactamases correspond to human gut strains, but not those from dinner

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Abstract

Background: *Escherichia coli* isolates producing extended-spectrum β–lactamases ('ESBL-*E. coli*') cause >5000 bacteraemias annually in the UK. The contribution of the food chain to this challenge is debated. **Methods:** Selective media were used to seek ESBL-*E. coli* in routinely-submitted human faeces, sewage, farm slurry, and retail foodstuffs in London, East Anglia, Northwest England, Scotland and Wales. Recovered isolates were sequenced and compared with 293 bloodstream and 83 veterinary surveillance ESBL-*E. coli* isolates from the same regions. **Findings:** 10.7% (2157/20243) of human faeces contained ESBL-*E. coli*, rising to 17.0% (678/3995) in London. ESBL-*E. coli* also were frequent in sewage and present in 65.4% (104/159) of retail chicken, but rare in other meats and absent from plant-based foods. Sequence Type (ST) 131 dominated among ESBL-*E. coli* from human blood (188/293, 64.2%), faeces (128/360, 35.6%) and sewage (14/65, 21.5%) with STs 38 and 648 also widespread; CTX-M-15 was the predominant ESBL in these lineages. By contrast, STs 602, 23, 117 - mostly with CTX-M-1 ESBL - dominated among food and veterinary isolates, with only two ST131 organisms recovered. ST10 occurred in both animals and humans: being frequent in surveillance bovines and representing 4.2% (15/360) of human faecal isolates (but only 1% [3/293] from bacteraemias); however both human and animal ST10 isolates were diverse in serotype. **Interpretation:** Most human bacteraemias with ESBL-*E. coli* in the UK involve successful human-associated STs, particularly ST131; non-human reservoirs made little contribution to invasive human disease. **Funding:** NIHR Policy Research.

Introduction

Escherichia coli is a Jekyll and Hyde organism: a few lineages are virulent enteropathogens whereas most are innocuous gut commensals, harmful only if they reach other body sites - notably the urinary tract, where *E. coli* is the commonest pathogen. Most *E. coli* urinary tract infections (UTIs) are uncomplicated cystitis, but some ascend, affecting the kidneys and, at worst, causing overspill bacteraemia. Although such sequelae are rare, *E. coli* is now the commonest bloodstream pathogen in England, with 41060 cases in fiscal 2017/18 - 27.1% more than in 2012/13.[1] Most *E. coli* bacteraemias have a urinary origin [2] and, in the UK *c.* 60% are caused by 'Extraintestinal Pathogenic *E. coli*' (ExPEC) lineages belonging to sequence types (STs) 12, 69, 73, 95 and 131.[3]

Cephalosporin resistance mediated by extended-spectrum β–lactamases (ESBLs] has proliferated in *E. coli* since 2000 [4], now occurring in *c.* 10-12% of UK bloodstream isolates. This proportion suggests around 4900 'ESBL-*E. coli*' bacteraemias annually in England (41060 x 12%), and more across the whole UK. [1], often due to multiresistant ST131 isolates.[3,5], ESBL production and multiresistance increases the risk that empirical treatment will fail, doubling the 17-18% mortality rate typical for *E. coli* bacteraemia.[6-8]

ESBL-*E. coli* also are widespread in sewage, pets, meat and food animals, but the extent of transmission between these milieux and humans is uncertain, with the role of the food chain debated. [9-11] A meta-analysis identified 6 'adequate' studies suggesting food-to-human transmission of ESBL-*E. coli* against 17 finding foodborne transmission was unimportant. [9] We sought to clarify the contribution of foodborne ESBL-*E coli* to human colonisation and infection, using whole genome sequencing (WGS) to compare isolates from multiple sources across the UK.

Materials and methods

Isolates

Consecutive bloodstream ESBL-*E. coli* were obtained during 2013 and 2014 from NHS laboratories in 5 UK Regions, with 5 sites in East Anglia, 2 each in Northwest England, Scotland and Wales and 1 in London. Identification and susceptibility testing were by laboratories' local protocols, with presumptive ESBL-*E. coli* sent to Public Health England (PHE) Colindale to a quota of 80/Region, along with brief, anonymised, patient details.

Isolates from other sources were collected prospectively in the 5 Regions, as detailed below. Isolation involved plating samples onto CHROMagar™ ESBL and CHROMagar™ CTX (CHROMagar, Paris France), prepared according to the manufacturer's directions, and hereafter referred to as 'The two chromogenic media'. For human faecal sampling, which was decentralised, these media were prepared at PHE Colindale and distributed weekly to laboratories; other testing was centralised at PHE Colindale and the Animal and Plant Health Agency.

ESBL-*E. coli* in human faeces

Faecal specimens were as submitted from May 2013 to June 2014 for detection of intestinal pathogens or occult blood screening at Barts Health (London), the Norfolk & Norwich University Hospital (East Anglia), Lancashire Hospitals Trust, Central Manchester University Hospitals (Northwest England), Aneurin Bevan University Health Board (Wales) and NHS Greater Glasgow and Clyde (Scotland). Each laboratory was asked to randomly select and test 15-20 faecal specimens/day to a maximum of 100/week.

Faeces (*c.* 0.5 g) was mixed with 1 ml 0.85% saline, then 50-µl aliquots were spread on the two chromogenic agars and incubated for 18-24h. Presumptive ESBL-*E. coli* (pink on CHROMagar™ ESBL or blue on CHROMagar™ CTX) were retained.

ESBL-*E. coli* in sewage

Paired inflow and effluent samples (50-1000 ml) were obtained from multiple sewage works belonging to 4 water companies covering Scotland, Northwest England, London and Wales (East Anglia did not participate). Each region provided 4 batches of samples between November 2013 and December 2014, with *c.* 80 samples/region. These were couriered to PHE Colindale at 2-8ºC, stored at 2-10ºC and processed within 24h. Volumes (0.01-10 ml) were filtered through 0.45-µm pore membranes, which were washed with distilled water before transfer to absorbent pads saturated with lauroyl sulphate broth for 4h at 30ºC, then to lauroyl sulphate agar for 14h before enumeration of yellow colonies as presumptive *E. coli*. Lastly, one filter per sample was transferred to each CHROMagar and incubated at 37ºC for 18-24h. Colonies that continued to develop, becoming appropriately coloured for ESBL-*E. coli*, were retained at 4ºC. A simplified method was also followed whereby bacteria were pelleted from *c.* 30 ml sewage, resuspended in 0.5 ml of 'Freezing Broth' and retained at -70 °C. Putative ESBL-*E. coli* were recovered, as red colonies, after plating 100 µl of defrosted material on UTI Brilliance Agar (Oxoid, Basingstoke, UK) containing 10-mg/L cefotaxime.

ESBL-*E. coli* in food

These methods and corresponding results have been published previously. [12] Beef, pork and chicken (n=397 in a 2:1:2 ratio, reflecting market share), also grapes (n=50 samples), strawberries (n=38), raspberries (n=35), blueberries (n=27), celery

(n=50), carrots (n=50), onions or spring onions (n=50), lettuce (n=50), coriander (n=43) and basil (n=7) were bought in each of the 5 Regions.[12] Retailers included leading supermarkets, discount stores, convenience stores and local butchers/greengrocers, in proportion to market share. Beef and chicken were obtained on 5 occasions between August 2013 and February 2014, pork on 4 occasions from October 2013 to February 2014 and vegetables on 15 occasions from January to March 2014. Meat samples were processed by APHA; fruit, vegetables and herbs by PHE, with the two chromogenic agars used to recover presumptive ESBL-*E. coli*.

ESBL-*E. coli* in slurry

Slurry samples (n=97) were collected from representative dairy farms across the 5 Regions in January/February 2014, after milking and before cleaning, sampling 5 floor areas/farm, with 'London' represented by the Home Counties. 1-g samples were incubated overnight at 37°C in 9 ml of Buffered Peptone Water before plating 10-μl amounts on the 2 chromogenic media.

ESBL-*E. coli* from veterinary diagnostic surveillance

These were veterinary diagnostic submissions to APHA or its predecessor laboratories from prospective surveillance across the 5 Regions and from scanning surveillance of food animals. The latter entails laboratory investigations of animal disease, largely post-mortem or through sample submission. Investigation seeks the cause of disease, and *E. coli* may be recovered and characterised. The isolates

155 comprised all ESBL-*E. coli* submitted across the 5 Regions during 2011-13,
156 irrespective of their contribution to disease.

157

158 **Characterisation of presumptive ESBL-*E. coli***

159 Presumptive ESBL-*E. coli*, isolated as above from blood, faeces, sewage, food,
160 animals and slurry were received at PHE and screened for *bla*$_{CTX-M}$, *bla*$_{TEM}$, *bla*$_{SHV}$
161 and *bla*$_{OXA}$ by multiplex PCR.[13] *bla*$_{CTX-M}$-positive isolates were accepted as ESBL-
162 producers, whilst those positive for one of the other β-lactamase genes were
163 subjected to double disc ESBL tests using amoxicillin-clavulanate (20+10 µg) discs
164 *c*. 20 mm apart (centre-to-centre) from cefotaxime (30 µg), ceftazidime (30 µg) and
165 cefepime (30 µg) discs. Expansion of an oxyimino-cephalosporin zone towards the
166 amoxicillin-clavulanate disc implied ESBL production.[14] Isolates positive by these
167 methods were confirmed as *E. coli* by MALDI-ToF (Bruker Maldi-Biotyper, Bremen,
168 Germany); any flagged as *Shigella* were confirmed as *E. coli* based on *o*-nitrophenyl-
169 β-D-galactosidase activity and a 603-bp PCR product for *ipaH.* [15] Definitive
170 confirmation as ESBL-*E. coli* was by WGS (HiSeq 2500, Illumina, San Diego, CA,
171 USA). STs were assigned and β–lactamase genes sought using the in-house
172 'Genefinder' pipeline. [16] ST131 isolates were assigned to clades based on *fimH*
173 sequences [17] serotypes of ST10 isolates (which crossed among host species)
174 were deduced from sequence data. [18]

175

176 **Statistical methods**

Analysis was primarily descriptive, with presentation of proportions as percentages and of continuous variables as mean and standard deviation. Pearson's chi-squared test was used to compare proportions, with R version 3.5.0.

Role of funder

This paper reports independent research commissioned and funded by the NIHR Policy Research Programme (Defining Reservoirs of ESBL-Producing *E. coli* and the Threat Posed to Personal, Animal and Public Health in the UK). The views expressed are those of the author(s) and not necessarily those of the NHS, NIHR, Department of Health and Social Care, 'Arms-Length Bodies' or other government departments. The sponsor had no role in study design, data collection, analysis or interpretation, nor in writing this report. The corresponding author had full access to all study data and had final responsibility for the decision to submit for publication.

Results

Bloodstream isolates: reference group

ESBL production was confirmed in 293/327 (89.6%) bloodstream isolates received as ESBL-*E. coli* (Table 1). Case Record Forms were available for 244/293 (83.2%), with a lower availability for London isolates (32.7%; Pearson's chi-squared test $p < 0.001$). There was a small excess of men; the mean age was 70 years, though younger in London (58.9 years; Kruskal-Wallis rank sum test $p = 0.002$); 61.4% of the 285 cases with data were community presentations, or hospitalised <48h. Data on the origin of bacteraemia were available for *c.* 50% of patients, with genitourinary (60.0%) and gastrointestinal/hepatobiliary sources (18.3%) predominating; few

patients were identified as post-surgical (9.1% of 209 with data), but post-discharge re-presentations may be under-recorded.

Faecal ESBL-*E. coli*

20,243 faecal samples were screened, comprising 3995-4112 per region (Table 2). 2107 (10.4%) gave colonies of the appropriate colour for *E. coli* on CHROMagar™ ESBL, 1302 (6.4%) on CHROMagar™ CTX, and 1252 (6.2%) on both. If appropriately-coloured growth on either medium – as seen for 2157 (10.7%) specimens – was taken as positive, regional ESBL prevalence ranged from 8.5-9.8%, except for London, 17.0% ($p < 0.001$) (Table 2). 400 of the presumptive ESBL-*E. coli* (80/region) were forwarded to PHE, and WGS found ESBL genes in 360 of these (90%). The 40 isolates lacking ESBL genes split between cephalosporin-susceptible *E. coli* (n=20), *E. coli* with other resistance mechanisms (n=18) and non-*E. coli* (n=2). Accordingly ESBL prevalence may be up to 10% lower than suggested in Table 2; though some detection failures likely reflect plasmid loss, reducing this correction factor.

Data were available for 355/360 carriers (Table 2). Their age distribution was bimodal, peaking at <5 and 75-79 years. Males comprised 50-63.6%, according to Region, and in-patients 29.9% (London) to 65.2% (Scotland). Overseas travel was reported for 99 patients (27%), with South and Southeast Asia the leading destinations (n=33); recent travellers accounted for 41.6% and 57.5% of the London and Wales patients but <20% elsewhere. Significant gastrointestinal pathogens were identified by local laboratories in only 11% of patients whilst recent exposure to antimicrobials was established for 32 (9%); 11 had received piperacillin/tazobactam.

225

226 **ESBL-*E. coli* from sewage**

227 163 inflow and 162 effluent samples were submitted. Failure of the selective media
228 adequately to suppress developing colonies of ESBL-negative *E. coli* on the transfer
229 membranes precluded accurate calculation of ESBL prevalence; nevertheless, a
230 panel of 65 sewage ESBL-*E. coli* was assembled, 41 from Wales, 18 from London
231 and three each from Scotland and Northwest England.

232

233 **ESBL-*E. coli* from food, bovine slurry and animals**

234 Results of screening foodstuffs have been published separately.[12] ESBL-*E. coli*
235 were recovered from 65.4% (104/159) of chicken samples, with positivity rates from
236 40.6% (13/32 Scotland) to 80.6% (25/31 Northwest England ($p < 0.0001$).
237 Contamination may arise from the original bird or be acquired during slaughter and
238 processing. Even with enrichment, only 1.9% (3/159) of beef and 2.5% (2/79) of pork
239 samples yielded ESBL-*E. coli*, based on growth on either chromogenic medium. No
240 ESBL-*E. coli* were recovered from 400 fruit and vegetable samples, many of
241 international origin.

242 19 to 20 dairy-farm slurry samples were tested per region, with an ESBL-*E.*
243 *coli* positivity rate, based on growth on either medium, of 27.8% (27/97). Regional
244 rates were from 15% (Scotland, 3/20) to 40.0% (Northwest England, 8/20).

245 These prospective collections were supplemented with 83 ESBL-*E. coli* from
246 the APHA's scanning surveillance of food animals. These were from the same
247 Regions as the other series, with 'London' again including the Home Counties; 51
248 isolates were from cattle, 29 from chickens with singletons from other species.

249

ESBL-*E. coli* STs in relation to specimen source

Table 3 lists the top 10 ESBL-*E. coli* STs for each specimen type, so long as these had ≥3 representatives. ST131 greatly predominated in human bacteraemias, comprising 188/293 (64.2%) isolates. It was also the most prevalent ST, though less overwhelmingly so, in faeces (128/360 isolates, 35.6%) and sewage 14/65 (21.5%). Regional proportions of ST131 among bloodstream isolates were: London 36/55 (64.5%); East Anglia 40/66 (60.6%); Northwest England 29/61 (47.5%); Scotland 28/37 (75.5%) and Wales 55/74 (74.3%); corresponding proportions among faecal isolates were London 16/77 (20.8%), East Anglia 16/67 (23.9%), Northwest England 27/75 (36.0%), Scotland 37/68 (54.4%) and Wales 32/72 (44.4%). Regional variation in the ST131 proportion was significant for both blood (p 0.01) and faeces (p <0.0001).

Other frequent bloodstream STs, in descending rank order, were 38, 648, 405, 73, 69, 636, 95, 1193 and 10. Several of these were also prominent in other human-related sources: thus, STs 38, 405, 636 and 648 were among the top 10 types among faecal isolates, with ST38 in second rank and ST648 fourth; ST38 was the second ranked ST from sewage, followed by ST10. By contrast, the top-ranked STs from meat and animals were 602, 23, 117 (or its single locus variant, ST6284) and ST10. There was species specificity within the animal isolates, with STs 23 and 602 dominating for chickens and chicken meat, whereas STs 10, 117 and 6284 dominated in cattle and their slurry (Table 3).

The top-ranked human types were rare in meat, animals and slurry. Just two ST131 isolates were recovered from animal-related sources: one from chicken meat and another from a surveillance chicken; both belonged to ST131 clade B whereas over 95% of bloodstream, faecal and sewage ST131 isolates belonged to clades C1

and (mostly) C2. STs 38, 648, 405, 73, 636, 95 and 1193 were not seen in animal-associated sources, and ST69 was seen in just one isolate from chicken meat and one from a cow. Only ST10, which accounted for 15/360 (4.2%) human faecal isolates and 3/293 (1.0%) from blood was widely seen in bovines and their slurry, though not in meat (Table 3). This human/animal overlap for ST10 was more apparent than real, however: the total of 38 ST10 isolates were deduced to belong to 26 different combinations of O (somatic) and H (flagellar) serotype, with the 3 human bloodstream isolates and 12/15 (87%) of human faecal isolates belonging to serotypes not seen from animal sources.

The predominant animal-related STs were infrequent in humans. ST602 - the top ST from meat (specifically chicken) - was not seen from human bacteraemias and had only 2 representatives from human faeces. Among all 293 human bacteraemia isolates just 5 (1.6%) belonged to top-ranked types from any animal-related source, specifically the 3 ST10 isolates and single representatives of STs 23 and 117.

ESBLs in relation to ST

CTX-M-15 enzyme predominated in human bloodstream, faecal, and sewage isolates (Table 4). This substantially reflected its association with ST131, but it remained the most prevalent ESBL in other major STs from these sources except ST38, where CTX-M-14 narrowly predominated. A sizeable minority (14.2%, 24/188) of ST131 isolates had CTX-M-27, not CTX-M-15.

By contrast, CTX-M-1 was considerably the most frequent ESBL among meat (chicken) isolates, whereas CTX-M-15 was not seen and most other ESBLs were

SHV or TEM types. CTX-M-1 also predominated (20/29 cases) in surveillance chickens whilst CTX-M-14 dominated in cattle, with 30 examples *versus* 12 CTX-M-15, 3 CTX-M-27 and 7 isolates with other CTX-M types. Major hosts of CTX-M-1 enzyme in chickens and their meat were STs 23 and 602, whereas ST10 and ST117/ST6284 were the frequent hosts of CTX-M-14 among bovines. Despite its frequency in *E. coli* from chickens and their meat, CTX-M-1 enzyme was seen in only 10/293 human bloodstream isolates, 21/360 from faeces and 7/65 from sewage. It mostly occurred in minor human STs, with only one or 2 representatives apiece. The solitary exception (again) was ST10, where CTX-M-1 was present in 3/15 human faecal isolates. The ST23/CTX-M-1 and ST602/CTX-M-1 combinations, widespread in chickens and their meat, were only seen in single human faecal isolates and never in blood. CTX-M-14 - the most frequent ESBL from the bovine isolates - was widely seen in major human blood and faecal isolates, including ST131 and ST38, but the ST10/CTX-M-14 combination, frequent in cattle, had only single representatives from human faeces and blood, whilst ST117/ST6284 CTX-M-14 was not seen. There was a single bloodstream ST117 isolate with CTX-M-1 enzyme, matching a combination seen in 10 isolates from chickens or their meat.

Discussion

We compared ESBL-*E. coli* from human bacteraemias with those from human faeces, sewage, food, slurry and animals across 5 UK regions. Bloodstream isolates followed expected patterns: largely from older patients with community-associated infection of genitourinary or gastrointestinal origin.[2] Faecal ESBL-*E. coli* were often linked to foreign travel, particularly to South or Southeast Asia or prior antibiotics, in

keeping with the literature.[19,20] Greater contamination of chicken than other meats also concurs with previous findings (see also [12]).

Typing and ESBL results (Tables 3 and 4; summarised in figure 1), indicated commonality between human bloodstream ESBL-*E. coli* and those from faeces and sewage, with STs 131 (especially), 38 and 648, prominent in all, largely with CTX-M-15 enzyme. Likewise, there was commonality between the lineages from surveillance chickens and chicken meat, with STs 23 and 602 dominating, often with CTX-M-1 ESBL, and between cattle and their slurry, where ST10 (with CTX-M-14 or -15) dominated. On the other hand, there was little crossover between types from humans, chickens, and bovines, with only (serotype diverse) ST10 among the top-10-ranked types from humans, animals and meat; ST117 was widely seen from both bovines and chickens. Other foodstuffs besides chicken showed little contamination.

Our findings do not support the assertion that the ESBL-*E. coli* causing invasive human infections are disseminating via the food chain. Rather, they support the view that host-adapted ESBL-*E. coli* lineages are circulating, with limited inter-species transmission. This conclusion agrees with the majority of studies included in recent meta-analysis.[9] ST131, which dominated among human-related isolates, is well-known and often multiresistant.[5,21] Although it occasionally occurs in food animals, (as in 2 instances here) the animal ST131 clades are generally different,[22] as here. At the upper edge of the reported prevalence range, Johnson *et al.*[23] in the US found 5/25 ESBL-*E. coli* from chickens or chicken meat belonged to ST131. By contrast, we - and a previous investigation covering the UK, Germany and the Netherlands [24] found only occasional ST131 isolates from food and animals. This rarity is supported by a major review,[5] cataloguing many individual detections of ST131 from food or food animals, but no dissemination.

Other common types from bacteraemia – ST38 and ST648 (each accounting for *c.* 5% of cases versus 64.2% for ST131) – were absent from food or animals. The literature carries reports of ST38 (with CMY-2, rather than ESBLs) from poultry, humans and wildlife [25]; ST648 too is largely reported from humans, though carriage was seen in horses and dogs.[26] Among the major meat and animal types, ST23 was reported from an outbreak in a French hospital,[27] with various further one-off reports but, as here, is largely a poultry type,[28] as is ST117,[21] which has spread in Nordic broiler production.[29] ST602, although frequent here, is less reported previously. ST10, as the sole lineage to appear in the 'top 10' of both human bloodstream and food-animal or meat-associated groups has been repeatedly noted by others in both animals and humans; nonetheless the serotype diversity seen here argues against simple direct flows of ST10 along the food-chain. The present results are in keeping with those of a comparison of ESBL-*E. coli* from human bacteraemias and livestock in the East of England – one of the regions also surveyed here – which also found that these isolate groups and their resistance determinants are largely distinct.[30]

Rather than the food chain, the likeliest frequent route of transmission for human-adapted ESBL-*E. coli* is human to human oro-faecal. This would account not only for the strain and enzyme distributions summarised in Figure 1 but also the regional variation in gut carriage of ESBL-*E. coli* (Table 2) with higher rates in London, where sampling was solely from the Royal London Hospital, which predominantly serves poor, crowded areas and populations with frequent travel to and from south Asia. A study in the UK West Midlands similarly observed that human gut carriage of ESBL-*E. coli* was more prevalent in inner city conurbations (i.e. around Birmingham) than in rural Shropshire.[31] We cannot exclude that some

small minority of human infections may have a direct origin from food, nor that local clusters may occur. Canadian authors [32,33] stress local finding of near-identical ST131 and ST117 *E. coli* (ESBL-producing or not) from both retail chicken meat and human infections; nevertheless these putative 'crossovers' accounted for only a tiny minority of all the human and animal *E. coli* they collected. Further, we cannot exclude the possibility that some future multi-resistant *E. coli* lineage from one or more food animal species will also prove adept at colonising and infecting humans. And, one further caveat remains: we do not know when, where, or how often *bla*$_{\text{CTX-M}}$ genes escaped from *Kluyvera* spp. (where they are endogenous and chromosomal) to mobile DNA, nor the chain of transmission to human-adapted *E. coli* lineages. However, it seems logical that the hazard of such gene escape will multiply with the range of animal species - and intestinal microbiotas - exposed to selective antibiotics.[34]

The present findings suggest that efforts to stop the rise of ESBL-*E. coli* in invasive infections should concentrate upon (i) disrupting oro-faecal transmission by good post-toilet hygiene, e.g. in care homes; (ii) on prevention of UTIs by good hydration and catheter care, and on (iii) prompt effective treatment of preceding UTIs. Vaccines may provide a future answer, with promising early results for cystitis in younger women.[35] Efforts to counter the spread of ESBL-*E. coli* in food production seem unlikely to impact greatly on the tally of invasive human infections but remain important in ensuring that veterinary infections remain tractable.

Panel

Research in context. *E. coli* producing acquired extended-spectrum β–lactamases ('ESBL-*E. coli*') are the largest group of multi-resistant pathogens from bacteraemias

in the UK, presenting major challenges. *E. coli* is also the major aerobic component of the human and animal gut biota and a frequent contaminant of meat and the environment. Extensive literature reviews in 2011-2 were summarised in a joint 2012 report of ESBL-*E. coli* by UK Government Advisory Committees. [11] This, and subsequent publications, recorded considerable uncertainly on the contribution of food-borne and environmental ESBL-*E. coli* to human colonisation and invasive infection. Thus, for example, early Dutch studies suggested some match between ESBL-*E. coli* from humans and poultry farming whereas a larger subsequent study covering the UK, Netherlands and Germany did not support such a linkage. [24] A meta-analysis[9] identified 6 'adequate' studies suggesting food-to-human transmission of ESBL-*E. coli* versus 17 that argued against this view. These uncertainties led initiation of a competitive NIHR Policy Research Programme and, among various activities, this programme funded the present comparison of ESBL-*E. coli* from human and animal sources. Reviews of the recent literature, to support the present paper, were undertaken by searching PubMed with combinations of the terms *Escherichia coli,* ESBL, CTX-M, meat, poultry, bacteraemia, faeces and UTI.

Added value of this study. We showed, comprehensively, that the ESBL-*E. coli* strains from bacteraemias in the UK match those prevalent as human gut colonists and in sewage. However they are largely distinct – in respect of strain and ESBL types – from those in food animals and retail food.

Implications of all available evidence. In 2016 the UK Government indicated its aim to achieve a 50% reduction in serious Gram-negative infections by 2020. A reduction in the numbers of infections due to ESBL-*E. coli* is especially desirable, given their incidence (>5000 cases p.a.) and the treatment challenges. The present data shows that actions on the food chain, however desirable for animal husbandry,

are unlikely to contribute to reducing human infection. Better potential control points are (i) prevention of transmission by good post-toilet hygiene e.g. in care homes and (ii) prevention of severe infection by good patient care and rapid effective treatment of initial uncomplicated UTIs, which precipitate most of the bacteraemias. Vaccines may be a future answer.

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Author contributions

MJD contributed to the design of the study and led on the central laboratory processing and sequencing of isolates from all sources; KLH contributed to the design of the study and acted as overall project manager; DWW led the design, analysis and co-ordination of the faecal screening programme and managed local aspects of the project in London; MT contributed to the design of the study, managed the project in Wales and led analysis of the sewage data; NE managed all non-meat food sampling and sewage analyses; LR and CT contributed to the design of the study, managed the meat and slurry work, and sourced the veterinary surveillance isolates: PC designed and undertook all statistical analyses and managed the project in Northwest England; CW contributed to the design of the study and managed all aspects of the study in Scotland; MD and MJE conducted the bioinformatic analyses of whole genome sequencing data; NW authored the original funding application and led on overall project design and co-ordination; DML co-ordinated the project in East Anglia and led the writing and revising of this paper; ALL AUTHORS commented on the draft manuscript and contributed to the final version.

Transparency declaration

DML: Advisory Boards or ad-hoc consultancy: Accelerate, Allecra, Antabio, BioVersys, Centauri, Entasis, Integra-Holdings, Meiji, Melinta, Mutabilis, Nordic, ParaPharm, Pfizer, QPEX, Roche, Shionogi, Taxis, T.A.Z., Tetraphase, VenatoRx, Wockhardt, and Zambon. Paid lectures – Accelerate, Astellas, bioMerieux, Beckman Coulter, Cepheid, Correvio, Merck, Pfizer, and Nordic. Relevant shareholdings or options – Dechra, GSK, Merck, Perkin Elmer, Pfizer, T.A.Z, amounting to <10% of

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601 Table 1. **Sources of ESBL-*E. coli* isolates from bloodstream infections**

	E Anglia	London	Northwest	Scotland	Wales	All
Total no. cases	66	55	61	37	74	293
No with data	55	18	61	37	72	243
Basic demographics						
Age (mean (SD))	71.5 (24.0)	58.9 (20.4)	65.7 (20.2)	73.3 (17.0)	74.3 (13.9)	70.0 (18.7)
Male %	45.5	61.1	44.3	56.8	55.6	51.2
Source of isolate (%)						
Community/Outpatient [a]	2 (8.0)	10 (55.6)	6 (10.9)	0 (0.0)	2 (4.2)	20 (10.9)
Inpatient (> 48h)	5 (20.0)	4 (22.2)	21 (38.2)	12 (32.4)	29 (60.4)	71 (38.8)
Inpatient (≤ 48h)	18 (72.0)	4 (22.2)	28 (50.9)	25 (67.6)	17 (35.4)	93 (50.3)
Specialty (%)						
A & E	13 (23.6)	6 (33.3)	12 (22.6)	20 (54.1)	16 (22.2)	67 (32.1)
Intensive care	0 (0.0)	3 (16.7)	3 (5.7)	2 (5.4)	2 (2.8)	10 (4.8)
Medical	29 (52.7)	8 (44.4)	24 (45.3)	9 (24.3)	25 (34.7)	95 (45.5)
Paediatrics	2 (3.6)	0 (0.0)	3 (5.7)	0 (0.0)	0 (0.0)	5 (2.4)
Surgical	11 (20.0)	0 (0.0)	4 (7.5)	2 (5.4)	2 (2.8)	19 (9.1)
Other	0 (0.0)	1 (5.6)	7 (13.2)	4 (10.8)	1 (1.4)	13 (6.2)
Origin (%)						
Gastrointestinal/biliary	7 (17.5)	5 (33.3)	3 (11.1)	6 (19.4)	1 (14.3)	22 (18.3)
Genitourinary tract	27 (67.5)	6 (40.0)	14 (51.9)	23 (74.2)	2 (28.6)	72 (60.0)
Line related	0 (0.0)	1 (6.7)	2 (7.4)	1 (3.2)	2 (28.6)	6 (5.0)
Respiratory	4 (10.0)	1 (6.7)	1 (3.7)	1 (3.2)	2 (28.6)	9 (7.5)
Skin/soft tissue	0 (0.0)	0 (0.0)	2 (7.4)	0 (0.0)	0 (0.0)	2 (1.7)
Surgical site infection	0 (0.0)	2 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.7)
Other	2 (5.0)	0 (0.0)	5 (18.5)	0 (0.0)	0 (0.0)	7 (5.8)

602

603 [a] This underestimates community onset infection, as evidenced by the much larger
604 proportion of patients categorised as 'Accident and Emergency'. Patients presenting
605 at Accident and Emergency with suspected bacteraemia and sepsis are likely to be
606 admitted, with their isolates recorded as 'inpatient <48h'.
607 Figures in the table are shown as percentages of available data. Overall
608 completeness of each variable (for regions East Anglia / London / Northwest /

609 Scotland / Wales / All) was: age 38/33/97/100/97/72%; sex 83/33/100/100/97/83%;
610 source of isolate 38/33/90/100/65/62%; and origin 61/27/44/84/9/41%.
611

612 **Table 2.** Faecal carriage of ESBL-*E. coli* in relation to patient demographics

Overall isolation rates		East Anglia	London	Northwest	Scotland	Wales	Overall
CHROMagar™ ESBL		309/4107 7.5%	678/3995 17.0%	366/4019 9.1%	393/4010 9.8%	361/4112 8.8%	2107/20243 10.4%
CHROMagar™ CTX		169/4107 4.1%	363/3995 9.1%	258/4019 6.4%	282/4010 7.0%	230/4112 5.6%	1302/20243 6.4%
Either medium		349/4107 8.5%	678/3995 17.0%	366/4019 9.1%	393/4010 9.8%	371/4112 9.0%	2157/20243 10.6%
No. isolates reviewed in detail and subjected to sequencing		64	77	75	66	73	355
Mean age, years; (SD)		56.9 (26.1)	33.4 (25.7)	48.3 (28.5)	60.3 (24.5)	64.2 (22.9)	52.1 (27.8)
Sex (%)	Female	32 (50.0)	41 (53.2)	38 (50.7)	42 (63.6)	42 (57.5)	195 (54.9)
	Male	32 (50.0)	36 (46.8)	36 (48.0)	24 (36.4)	31 (42.5)	159 (44.8)
	Missing data	0 (0.0)	0 (0.0)	1 (1.3)	0 (0.0)	0 (0.0)	1 (0.3)
Overseas travel (%)	Yes	6 (9.4)	32 (41.6)	13 (17.3)	6 (9.1)	42 (57.5)	99 (27.8)
	No	58 (90.6)	45 (58.4)	62 (82.7)	60 (90.9)	31 (42.5)	256 (72.1)
Source of isolate (%)	Community	44 (68.8)	54 (70.1)	42 (56.0)	23 (34.8)	36 (49.3)	199 (56.1)
	Inpatient (>48h)	9 (14.1)	20 (26.0)	20 (26.7)	32 (48.5)	26 (35.6)	107 (30.1)
	Inpatient (≤48h)	9 (14.1)	3 (3.9)	7 (9.3)	11 (16.7)	11 (15.1)	41 (11.5)
	Missing data	2 (3.1)	0 (0.0)	6 (8.0)	0 (0.0)	0 (0.0)	8 (2.3)

Recent antibiotics	Yes	5 (7.8)	20 (26.0)	12 (16.0)	1 (1.5)	15 (20.5)	72 (20.3)
	No	13 (20.3)	43 (55.8)	0 (0.0)	2 (3.0)	14 (19.2)	53 (14.9)
	Missing data	46 (71.9)	14 (18.2)	63 (84.0)	63 (95.5)	44 (60.3)	230 (64.8)

613 **Table 3.** Major STs among ESBL- *E. coli* found, by sample type

Rank	**Bacteraemia**		**Faeces**		**Sewage**		**Meat**		**Slurry**		**Animals**	
	ST	No. repre-sentatives	ST	No. repre-sentatives	ST	No. repre-sentatives	ST	No. repre-sentatives	ST	No. repre-sentatives	ST	No repre-sentatives
1	131	188	131	128	131	14	602	21	10	6	23	16[a]
2	38	17	38	29	38	6	23	8	641	3	117	11[b]
3	648	16	10	15	10	3	117	8			10	11[c]
4	405	9	648	11			155	6			6284[d]	6[e]
5	73	6	69	10			57	4			602	4[f]
6	69	4	405	10			371	4			88	4[g]
7	636	4	410	10			3776	4				
8	95	3	636	7			6285	4				
9	1193	3	162	6			665	3				
10	10	3	443	6			2040	3				
No included in above major types		253		232		35		65		9		52
Total isolates, all STs		293		360		65		111[h]		24		83[i]

614
615 The top 10 STs are listed, except where a group has fewer than 3 representatives
616
617 [a] 14/16 from chickens

[b] 9/11 from cattle
[c] 11/11 from cattle
[d] Single locus variant of ST117: if these were grouped collectively, they would be the top ST from livestock
[e] 6/6 from cattle
[f] 4/4 from chicken
[g] 2/4 from chicken; 2/4 from cattle
[h] 106 chicken; 3 beef; 2 pork
[i] 51 cattle; 29 chicken, 3 other.

627 **Table 4**. ESBL types among major STs of *E. coli* from different sources

	CTX-M ESBLs										TEM: known or possible ESBLs			
	-15	-27	-14	-1	-24	-2	-3	-9	Other	SHV	-52	-117	-191	Other
Bacteraemia														
131	159	24	5	-	-	-	2	-	-	-	-	-	4	-
38	8	-	8	-	-	-	-	-	-	-	-	-	-	-
648	16	-	-	-	-	-	-	-	-	-	-	-	1	-
405	8	-	1	-	-	-	-	-	-	-	-	-	-	-
73	4	-	-	1	-	-	-	1	-	-	-	-	-	-
All	229	27	20	10	1	2	2	1	-	4	0	1	8	0
Faeces														
131	98	18	7	1	-	-	-	-	4[a]	-	-	1	-	2
38	11	1	15	1	-	-	-	-	-	1	-	-	-	0
10	8	-	1	3	-	-	-	-	1	-	-	1	-	1
648	10	-	1	-	-	-	-	-	-	-	-	-	-	-
69	6	-	2	-	-	-	-	-	1	-	-	-	-	-
All	256	24	38	21	0	0	0	0	20	11	1	6	2	5
Sewage														
131	13	1	-	-	-	-	-	-	-	-	-	1	-	3
38	2	-	5	-	-	-	-	-	-	-	-	-	-	5
73	1	-	-	-	-	-	-	-	-	-	-	1	-	-
648	2	-	-	-	-	-	-	-	-	-	-	-	-	1
10												2		1
All	21	1	5	0	0	0	0	0	3	3	0	6	0	14
Meat														
602	-	-	-	21	-	-	-	-	-	-	-	-	-	-

23	-	-	-	8	-	-	-	-	-	-	-	1	-	4
117	-	-	-	8	-	-	-	-	-	-	-	-	-	-
155	-	-	-	6	-	-	-	-	-	-	-	-	-	-
57	-	-	-	1	-	-	-	-	-	3	-	-	-	-
All	0	0	0	82	0	2	0	0	4	13	8	3	2	4
Slurry														
10	1	-	2	1	-	-	-	-	-	-	-	1	-	0
641	-	-	1	2	-	-	-	-	-	-	-	-	-	1
All	4	1	4	6	0	0	0	0	4[b]	0	0	2	0	1
Animals														
23	1	-	1	12	-	-	-	-	-	-	-	-	-	9
117	1	-	3	2	-	-	3	0	2[c]	-	-	1	1	-
10	3	-	7	-	-	1	-	-	-	-	-	1	-	-
6284	-	-	6	-	-	-	-	-	-	-	-	-	-	-
602	-	-	0	4	-	-	-	-	-	-	-	-	-	-
All	13	0	31	32	0	1	3	1	2	0	0	2	2	9
Chicken				29										9
Cattle	12	0	30	3	0	1	3	1	2	0	0	2	2	0

[a] Includes one isolate with Asn173Ser variant of CTX-M-27
[b] Includes one isolate with novel Ser205Arg variant of CTX-M-1
[c] All with CTX-M-214

NB, some totals exceed numbers of isolates belonging to the ST as some isolates had >1 ESBL. The top 5 STs are included, except where they had <3 representatives

Sunburst diagram (MS Excel) showing different E. coli strains and ESBL types predominating in specimen types from one-health compartments that relate to humans and animals. The inner circle presents the sources of ESBL-*E. coli,* segments are scaled according to the numbers of isolates found, single representatives of an ST are aggregated into the category 'singletons'. The middle circle represents the numbers of isolates from each ST in relation to each ESBL and the outer circle represents the number of isolates with an ESBL-type.