

## Bacterial genotypic and patient risk factors for adverse outcomes in *Escherichia coli* bloodstream infections: a prospective molecular epidemiological study

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**Objectives:** *Escherichia coli* bloodstream infections have shown a sustained increase in England, for reasons that are unknown. Furthermore, the contribution of MDR lineages such as ST131 to overall *E. coli* disease burden and outcome is undetermined.

**Methods:** We genome-sequenced *E. coli* blood isolates from all patients with *E. coli* bacteraemia in north-west London from July 2015 to August 2016 and assigned MLST genotypes, virulence factors and AMR genes to all isolates. Isolate STs were then linked to phenotypic antimicrobial susceptibility, patient demographics and clinical outcome data to explore relationships between the *E. coli* STs, patient factors and outcomes.

**Results:** A total of 551 *E. coli* genomes were analysed. Four STs (ST131, 21.2%; ST73, 14.5%; ST69, 9.3%; and ST95, 8.2%) accounted for over half of cases. *E. coli* genotype ST131-C2 was associated with phenotypic non-susceptibility to quinolones, third-generation cephalosporins, amoxicillin, amoxicillin/clavulanic acid, gentamicin and trimethoprim. Among 300 patients from whom outcome was known, an association between the ST131-C2 lineage and longer length of stay was detected, although multivariable regression modelling did not demonstrate an association between *E. coli* ST and mortality. Several unexpected associations were identified between gentamicin non-susceptibility, ethnicity, sex and adverse outcomes, requiring further research.

**Conclusions:** Although *E. coli* ST was associated with defined antimicrobial non-susceptibility patterns and prolonged length of stay, *E. coli* ST was not associated with increased mortality. ST131 has outcompeted other lineages in north-west London. Where ST131 is prevalent, caution is required when devising empiric regimens for suspected Gram-negative sepsis, in particular the pairing of  $\beta$ -lactam agents with gentamicin.

### Introduction

*Escherichia coli* is, by far, the most common causative organism of bloodstream infection (BSI) and its incidence is increasing in both England and North America.<sup>1–3</sup> Between 2014 and 2018, a 27.2% increase in *E. coli* BSI was recorded in England, Wales and Northern Ireland, rising from 55.2/100 000 to 70.7/100 000

cases.<sup>4</sup> In-hospital mortality of *E. coli* BSI is reported to be 13%–25% in England<sup>5–7</sup> and prolonged length of stay is common.<sup>7–9</sup> Thus, with over 40 000 cases per year,<sup>10</sup> the overall burden of *E. coli* BSIs on individuals and healthcare is considerable. *E. coli* non-susceptibility to several commonly used antimicrobial agents has increased in England<sup>9,11</sup> and this trend is also observed in North America.<sup>3,12</sup>

While many studies have described the factors that may influence outcome from sepsis and *E. coli* BSI,<sup>8,9</sup> remarkably few have examined the impact of bacterial strain background. Extraintestinal pathogenic *E. coli* (ExPEC) form a subgroup of *E. coli* that have sufficient virulence to cause urinary tract infections (UTIs) and BSIs.<sup>13</sup> The most common disease-causing clones of ExPEC in the UK have been reported to be ST131, ST73, ST95, ST69 and ST12.<sup>14</sup> Globally, interest has focused on emergence of the ST131 *E. coli* lineage, in particular the MDR clade ST131-H30-Rx (also known as ST131-C2), which is associated with production of ESBL and resistance to fluoroquinolones.<sup>15–17</sup> Although ST131 to date is not reported to have outcompeted other common clones,<sup>18,19</sup> the emergence of *E. coli* ST131-C2 has reportedly been associated with more severe infections<sup>20</sup> and characterized as highly pathogenic.<sup>17</sup> To provide more granular insight into *E. coli* BSI, we conducted a 1 year prospective study to investigate the burden of infection due to specific *E. coli* genotypes and determine whether *E. coli* genetic background was associated with adverse outcome.

## Materials and methods

### Demographic and clinical data retrieval

Between July 2015 and August 2016, we prospectively collected *E. coli* isolates causing BSIs submitted to the diagnostic laboratory of a large NHS teaching trust in north-west London, serving a population of ~2 000 000 people including adjacent NHS trust hospitals. The following demographic information was collected using an electronic patient administration system (PAS): age, sex, ethnicity, comorbidities and post-infection length of stay (PILOS) (Figure S1, available as [Supplementary data](#) at JAC Online). A modified Elixhauser comorbidity score was calculated for each case.<sup>21</sup> For case definitions and further details, see the [Supplementary methods](#).

### Characterization of isolates and statistical analyses

*E. coli* isolates were processed for WGS. MLST genotypes, virulence factors and AMR genes were determined as described in the [Supplementary methods](#). Antibiotic susceptibility testing results for *E. coli* blood isolates were obtained from the trust's Microbiology Data warehouse ([Supplementary methods](#)). Chi-squared and Fisher's exact tests were used to assess associations between *E. coli* genotype, antibiotic susceptibility results and patient demographic data (sex and age). Multiple logistic regression was used to model the following outcomes: mortality within 7, 30 and 90 days and length of stay, focusing on a core group of 300 patients with outcome data ([Supplementary methods](#)).

### Ethics statement

The collection of microbial isolates and linkage to routinely obtained healthcare data prior to anonymization was approved by the West London Research Ethics Committee; ethics approval reference number 06/Q0406/20.

## Results

### Description of 1 year *E. coli* BSIs in north-west London

A total of 551 *E. coli* bacteraemia cases, where *E. coli* isolates were available for analysis, were detected between July 2015 and August 2016 (Figure S1). Of these patients, 43.4% were male ( $n=233/537$ ) and 56.6% were female ( $n=304/537$ ). Sixty

percent of *E. coli* bacteraemia cases were  $\geq 65$  years old ( $n=321$ ) (Table S1). Genomic MLST analysis identified 114 different STs among *E. coli* blood isolates, of which 21.2% belonged to ST131 ( $n=117/551$ ), 14.5% to ST73 ( $n=80/551$ ), 9.3% to ST69 ( $n=51/551$ ) and 8.2% to ST95 ( $n=45/551$ ). Therefore, over half of all isolates belonged to ST131, ST73, ST69 or ST95. Although ST131 was the single largest lineage among bacteraemia isolates (with patient demographic data available), within the ST131 lineage there were 60/115 isolates that belonged to the ST131-C2 sublineage (11.2% of total) and 55/115 (10.2% of the total) isolates from 'other' ST131 sublineages (including A, B and C1). Virulence factors and antimicrobial resistance genes segregated with MLST as expected (Tables S5 and S6).

### Association between *E. coli* MLST genotype and antimicrobial susceptibility

Results of routinely conducted phenotypic antibiotic susceptibility testing to five key antibiotic groups (fluoroquinolones, third-generation cephalosporins, carbapenems, gentamicin and piperacillin/tazobactam) were available for 537 isolates, while susceptibility results to amoxicillin, amoxicillin/clavulanic acid and trimethoprim were available for 253 isolates (Figure S1). From the five antibiotic groups tested, 26.6% of isolates ( $n=143/537$ ) were non-susceptible to fluoroquinolones, 19.7% ( $n=106/537$ ) were non-susceptible to third-generation cephalosporins, and 15.1% ( $n=81/537$ ) were non-susceptible to gentamicin (Table S2). Non-susceptibility to amoxicillin (62.8%,  $n=159/253$ ), trimethoprim (43.9%,  $n=111/253$ ) and amoxicillin/clavulanic acid (30.0%,  $n=76/253$ ) was frequently noted. There was a significant association between non-susceptibility to any antibiotic tested and *E. coli* MLST genotype ( $P<0.001$ ), except for amoxicillin, where non-susceptibility was not associated with the MLST genotype ( $P>0.05$ ), and carbapenems, due to low isolate numbers tested (Table S2). As expected, ST131-C2 showed the highest levels of non-susceptibility to all antibiotics tested (Table S2). ST131-C2 accounted for approximately one-third of isolates that were non-susceptible to third-generation cephalosporins and quinolones, and accounted for approximately one-quarter of isolates that were non-susceptible to gentamicin, piperacillin/tazobactam, or amoxicillin/clavulanic acid. Although non-susceptibility to trimethoprim was observed frequently, in 43.9% of all *E. coli* isolates tested (111/253), it was notable that 81.5% of ST131-C2 isolates ( $n=22/27$ ) were non-susceptible to trimethoprim (Table S2).

### Association between *E. coli* MLST genotype and case characteristics

Initial analysis indicated that ST69 and ST95 isolates were more frequently identified in female patients, while ST131 isolates were more likely to affect male patients (Table 1). There was, however, no clear association between specific *E. coli* MLST genotype and age or ethnicity.

Elixhauser comorbidity scores exceeded 5 for 53.0% of the patients (Table 1). We observed that patients infected with ST131, ST73 or ST95 were more likely to have an Elixhauser score of  $>6$ , compared with patients infected with other STs (Table 1). Within the ST131 lineage, patients infected with 'ST131-other'

**Table 1.** Summary of patient characteristics and association with specific *E. coli* ST ( $n=300$  patients)

Characteristic	ST131-C2 ( $n=36$ )	ST131-other ( $n=28$ )	ST69 ( $n=31$ )	ST73 ( $n=43$ )	ST95 ( $n=21$ )	Other STs ( $n=141$ )	Total ( $n=300$ )
Sex, $n$ (%)							
Male	20 (55.6)	16 (57.1)	8 (25.8)	20 (46.5)	8 (38.1)	72 (51.0)	144 (48.0)
Female	16 (44.4)	12 (42.9)	23 (74.2)	23 (53.5)	13 (61.9)	69 (48.9)	156 (52.0)
Age (years), $n$ (%)							
<65	11 (30.6)	14 (50.0)	17 (54.8)	19 (44.2)	9 (42.9)	61 (43.3)	131 (43.7)
65–74	12 (33.3)	6 (21.4)	4 (12.9)	7 (16.3)	4 (19.0)	35 (24.8)	68 (22.7)
75–84	6 (16.7)	6 (21.4)	8 (25.8)	8 (18.6)	6 (28.6)	33 (23.4)	67 (22.3)
85+	7 (19.4)	2 (7.1)	2 (6.5)	9 (20.9)	2 (9.5)	12 (8.5)	34 (11.3)
Ethnicity, $n$ (%)							
White	16 (44.4)	10 (35.7)	16 (51.6)	28 (65.1)	10 (47.6)	65 (46.1)	145 (48.3)
Asian	9 (25.0)	5 (17.9)	4 (12.9)	2 (4.7)	2 (9.5)	19 (13.5)	41 (16.7)
Black	4 (11.1)	5 (17.9)	3 (9.7)	2 (4.7)	2 (9.5)	17 (12.1)	33 (11.0)
Any other	6 (16.7)	2 (7.1)	5 (16.1)	4 (9.3)	3 (14.3)	24 (17.0)	44 (14.7)
Not given	1 (2.8)	6 (21.4)	3 (9.7)	7 (16.3)	4 (19.0)	16 (11.3)	37 (12.3)
Elixhauser score, $n$ (%)							
<0	0 (0)	0 (0)	0 (0)	3 (7.0)	0 (0)	7 (5.0)	10 (3.3)
0	11 (30.6)	3 (10.7)	11 (35.5)	8 (18.6)	6 (28.6)	30 (21.3)	69 (23.0)
1–5	8 (22.2)	6 (21.4)	9 (29.0)	8 (18.6)	2 (9.5)	29 (20.6)	62 (20.7)
6–13	9 (25.0)	11 (39.3)	6 (19.4)	18 (41.9)	10 (47.6)	36 (25.5)	90 (30.0)
≥14	8 (22.2)	8 (28.6)	5 (16.1)	6 (14.0)	3 (14.3)	39 (27.7)	69 (23.0)
Onset of infection, $n$ (%)							
Hospital	15 (41.7)	11 (39.3)	7 (22.6)	8 (18.6)	2 (9.5)	32 (22.7)	75 (25.0)
Community	20 (55.6)	17 (60.7)	21 (67.7)	35 (81.4)	19 (90.5)	104 (73.8)	216 (72.0)
NA	1 (2.8)	0 (0)	3 (9.7)	0 (0)	0 (0)	5 (3.5)	9 (3.0)

NA, not available.

isolates were more likely to have higher Elixhauser scores than those infected with ST131-C2 isolates (Table 1).

Based on onset of *E. coli* bacteraemia in relation to time of admission to hospital, three-quarters of patients had community-onset *E. coli* bacteraemia (Table 1). *E. coli* STs were, in general, proportionately distributed among community- and hospital-onset cases, except for ST131 isolates (both ST131-C2 and ST131-other) that were disproportionately associated with hospital-onset cases; approximately 40% of each group, ST131-C2 and ST131-other, were hospital-onset (Table 1). Overall, the ST131 lineage accounted for 34.6% ( $n=26/75$ ) of all hospital-onset cases compared with 17.1% ( $n=37/216$ ) of all community-onset cases. In contrast, the majority (90.5%) of bacteraemia cases caused by ST95 ( $n=19/21$ ) were found to be community-onset (Table 1).

### Association between *E. coli* MLST genotype and patient outcome

Over one-third (33.7%) of study patients with *E. coli* bacteraemia died within 1 year: 7.0% died within 7 days of infection, 11.0% died within 30 days and 15.7% within 90 days (Table 2). There was no association between *E. coli* MLST genotype and death within 7 days or 30 days in the cohort tested; however, analysis may have been affected by low numbers within each *E. coli* genotype group, and mortality being lower than predicted, precluding further analysis at the early timepoints (Table 2). Logistic regression analysis showed increased risk of death within 90 days for

patients infected with *E. coli* genotype 'ST131-other' when compared with all other STs (OR: 2.58; 95% CI: 1.04–6.19). However, once the model was adjusted for patient characteristics, no evidence for any association between *E. coli* genotype and 90 day mortality was found (Table 3). In our study, age and sex did not influence risk of 90 day mortality, although comorbidity, as described by an Elixhauser score of >14, was highly influential. Unexpectedly, we found that *E. coli* non-susceptibility to gentamicin was associated with increased odds of death within 90 days (OR: 3.32; 95% CI: 1.17–9.72) after adjusting for patient characteristics and *E. coli* MLST genotype (Table 3). Mortality at all timepoints was higher in patients infected by isolates that were non-susceptible to gentamicin (7 days, 12.8%; 30 days, 15.4%; 90 days, 25.6%), compared with patients with isolates that were susceptible (7 days, 6.1%; 30 days, 10.3%; 90 days, 14.2%) although numbers at the earlier timepoints were too low for inclusion in comparative analysis. Further analysis also suggested that patients of black ethnicity had increased odds of mortality within 90 days compared with patients of white ethnicity; for those infected with *E. coli* strains that were non-susceptible to gentamicin or piperacillin/tazobactam this was significant (OR: 2.98; 95% CI: 1.03–8.56 and OR: 3.09; 95% CI: 1.02–9.26, respectively; Table 3).

Median PILOS was found to be 10 days for *E. coli* bacteraemia patients (Table 2). Overall, patients with bacteraemia caused by ST131 (irrespective of clade) were more likely to stay longer in hospital, with longest PILOS for patients infected with ST131-C2

**Table 2.** Summary of results for testing association between *E. coli* ST and patient outcome ( $n = 300$  patients)

Outcome	ST131-C2 ( $n = 36$ )	ST131-other ( $n = 28$ )	ST69 ( $n = 31$ )	ST73 ( $n = 43$ )	ST95 ( $n = 21$ )	Other STs ( $n = 141$ )	Total ( $n = 300$ )
Mortality, $n$ (%)							
Within 7 days	1 (2.8)	4 (14.3)	1 (3.2)	3 (7.0)	1 (4.8)	11 (7.8)	21 (7.0)
Within 30 days	2 (5.6)	6 (21.4)	2 (6.5)	3 (7.0)	2 (9.5)	18 (12.8)	33 (11.0)
Within 90 days	4 (11.1)	10 (35.7)	3 (9.7)	3 (7.0)	2 (9.5)	25 (17.7)	47 (15.7)
PILOS (days)							
Median (IQR)	18.5 (11.25–27.0)	12.5 (6–28.75)	9 (7–15)	7 (5–13)	11 (6–20)	9 (11.25–27)	10 (6–20)
Min–max	1–75	1–50	1–62	0–90	1–64	0–91	1–62
Patients who stayed $\geq 7$ days, $n$ (%)	32 (88.9)	20 (71.4)	22 (71.0)	25 (58.1)	14 (66.7)	91 (64.5)	204 (68.0)

isolates (median 18.5 days) and ST131-other isolates (median 12.5 days) (Table 2). Unadjusted logistic regression suggested that patients infected with ST131-C2 had six times greater odds of having an extended length of stay (OR: 6.46; 95% CI: 2.02–32.64) and patients infected with ST69 had a 3-fold increase in odds of a long length of stay (OR: 2.85; 95% CI: 1.07–8.52) compared with patients who were infected with ‘other’ ST *E. coli* (Table 4). These differences persisted even when adjusted for patient characteristics. We did not find evidence of a specific association between antibiotic non-susceptibility and length of stay. Female patients had a 3-fold lower risk of an extended length of stay compared with male patients (OR: 0.30; 95% CI: 0.16–0.55) (Table 4). Patients with hospital-onset infection had higher odds of an extended length of stay (OR: 2.69; 95% CI: 1.28–6.08) although these patients may have required treatment for the condition that initiated the original admission to hospital (Table 4). When we examined the smaller subset of patients for whom additional antimicrobial susceptibility data and demographic data were available, we were unable to detect any association between non-susceptibility to trimethoprim, amoxicillin or amoxicillin/clavulanic acid and either PILOS or death within 90 days (Tables S3 and S4).

## Discussion

Our study is one of the largest to comprehensively examine the association between *E. coli* genotype and BSI, examining over 500 consecutive unselected cases of bacteraemia. Over one-fifth of cases could be attributed to isolates of the ST131 lineage, making it the single largest ST, around half of which belonged to the MDR subclone ST131-C2 (also known as ST131-H30-Rx). It has been reported that successful spread of ST131 clades was due to gain of virulence-associated genes, followed by the acquisition of specific antibiotic resistance.<sup>22</sup> Although the ST131-C2 clade is reported to be highly pathogenic<sup>17</sup> we did not find clinical/epidemiological evidence to support this in our patient population when mortality was used as a surrogate for disease severity; the most influential factor in mortality was patient comorbidity. Although a high number of virulence genes were detected for our invasive strains, as expected, they were associated with strain type.

In contrast to mortality, when extended length of hospital stay was considered, certain *E. coli* genotypes, including ST131-C2, were strongly associated with prolonged length of stay, after adjusting for patient characteristics. This observation could potentially be explained by the non-susceptibility of ST131-C2 isolates to available oral antimicrobials that would otherwise be used to expedite patient discharge after initial IV therapy. Interestingly, BSIs due to ST69 were also associated with a prolonged length of stay. Based on the data available, there was no evidence that non-susceptibility to multiple antibiotics alone was associated with extended length of stay, although our ability to detect such a difference may have been limited by study size.

A number of other studies have examined the factors that influence outcome following *E. coli* bacteraemia. Most have identified the importance of comorbidity and age on mortality. Some have identified factors that are amenable to intervention such as timing of effective antibiotics.<sup>23,24</sup> Only two have examined the role of bacterial genotype, but did not identify an association with adverse outcome such as mortality.<sup>20,25</sup> Strain genotype can be associated with mortality in invasive bacterial infections caused by other species, and is seen to reflect a salient role for the bacterium in pathogenesis.<sup>26</sup> The lack of identifiable link between mortality and any one genotype may reflect that *E. coli* is largely an opportunistic pathogen, and emergent lineages may simply represent strain types that are well adapted to colonize the gut or cause disease in the elderly and those with comorbidity. *E. coli* is the leading cause of BSI in developed countries, and will account for the majority of cases that are designated as sepsis; factors that influence sepsis outcome will therefore be dominated by the largely host-related factors that impact *E. coli* outcome.<sup>2,8,9</sup>

Genotyping of the *E. coli* isolates in this study also provided insight into the MLST genotype-specific associations with markers of antimicrobial resistance. Two recent studies have highlighted an unexplained increase in quinolone non-susceptibility in *E. coli* bacteraemia isolates from London between 2011 and 2015<sup>9</sup> and from the USA between 2009 and 2016,<sup>3</sup> while a recent large-scale study from the USA has emphasized a doubling (from 5.46% to 12.97%) of *E. coli* isolates resistant to cephalosporins.<sup>3</sup> The expansion of ST131 may provide an explanation for increases observed in London: ST131-C2 is known to be associated with

**Table 3.** Multiple regression modelling of association between *E. coli* genotype, patient characteristics, antibiotic resistance and mortality risk within 90 days

Factor	ST group— unadjusted	Model 1: ST group adjusted for patient characteristics	Model 2: Model 1 + fluoroquinolone non-susceptibility		Model 3: Model 1 + gentamicin non-susceptibility		Model 4: Model 1 + third-generation cephalosporin non-susceptibility		Model 5: Model 1 + piperacillin/tazobactam non-susceptibility	
			OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
<i>E. coli</i> ST (other STs = reference group)										
ST131-C2	0.58 (0.16-1.63)	0.68 (0.18-2.21)	0.38 (0.09-1.43)	0.43 (0.10-1.52)	0.37 (0.08-1.54)	1.06 (0.25-3.89)				
ST131-other	2.58 (1.04-6.19)*	2.70 (0.97-7.50)	2.18 (0.75-6.22)	2.42 (0.86-6.78)	2.22 (0.74-6.51)	2.55 (0.87-7.39)				
ST69	0.50 (0.11-1.55)	0.68 (0.11-2.81)	0.89 (0.15-3.74)	0.76 (0.13-3.24)	0.68 (0.12-2.84)	0.69 (0.11-2.84)				
ST73	0.35 (0.08-1.06)	0.55 (0.13-1.83)	0.65 (0.15-2.25)	0.68 (0.15-2.34)	0.56 (0.13-1.89)	0.70 (0.16-2.42)				
ST95	0.49 (0.07-1.83)	0.80 (0.14-3.20)	1.02 (0.18-4.26)	0.90 (0.15-3.70)	0.74 (0.13-3.00)	0.82 (0.14-3.30)				
Patient's age group, years (<65 years = reference group)										
65-74	0.87 (0.28-2.59)	0.87 (0.28-2.59)	0.81 (0.25-2.46)	0.84 (0.26-2.60)	0.91 (0.28-2.78)	0.74 (0.22-2.30)				
75-84	1.10 (0.43-2.78)	1.10 (0.43-2.78)	1.17 (0.45-3.02)	1.18 (0.45-3.05)	1.24 (0.48-3.21)	1.19 (0.45-3.12)				
>85	1.85 (0.52-6.22)	1.85 (0.52-6.22)	1.95 (0.54-6.63)	2.01 (0.56-6.88)	1.90 (0.51-6.71)	1.73 (0.47-5.90)				
Patient's sex (male = reference group)										
Female	0.68 (0.31-1.49)	0.68 (0.31-1.49)	0.75 (0.33-1.69)	0.71 (0.32-1.58)	0.71 (0.32-1.59)	0.73 (0.32-1.65)				
Patient's ethnicity (white = reference group)										
Asian	1.19 (0.38-3.51)	1.19 (0.38-3.51)	0.92 (0.28-2.82)	1.28 (0.40-3.90)	1.12 (0.32-3.54)	1.04 (0.31-3.25)				
Black	2.60 (0.90-7.44)	2.60 (0.90-7.44)	2.27 (0.76-6.58)	2.98 (1.03-8.56)*	2.91 (0.97-8.58)	3.09 (1.02-9.26)*				
Other	1.32 (0.37-4.45)	1.32 (0.37-4.45)	1.12 (0.30-3.87)	1.35 (0.35-4.82)	1.33 (0.36-4.54)	1.37 (0.38-4.58)				
Not stated	1.70 (0.49-5.36)	1.70 (0.49-5.36)	1.77 (0.50-5.70)	2.04 (0.58-6.59)	1.55 (0.45-4.88)	1.68 (0.43-5.79)				
Elixhauser index (<0 = reference group)										
0-5	1.58 (0.28-10.09)	1.58 (0.28-10.09)	1.61 (0.29-10.40)	1.11 (0.18-7.50)	1.46 (0.26-9.22)	1.88 (0.34-11.91)				
6-13	3.66 (0.99-19.79)	3.66 (0.99-19.79)	3.81 (1.01-20.99)	3.35 (0.89-18.21)	3.90 (1.05-21.10)*	3.51 (0.93-19.05)				
≥14	18.09 (5.24-96.31)***	18.09 (5.24-96.31)***	19.91 (5.58-109.77)***	18.16 (5.21-97.23)***	16.48 (4.81-87.18)***	16.57 (4.74-88.42)***				
Onset of infection (community-onset = reference group)										
Hospital	1.01 (0.44-2.21)	1.01 (0.44-2.21)	0.90 (0.39-2.02)	0.94 (0.41-2.05)	0.87 (0.36-2.00)	1.13 (0.47-2.59)				
Antibiotic susceptibility (reference)										
Antibiotic non-susceptibility			2.46 (0.97-6.37)	3.32 (1.17-9.72)*	2.15 (0.67-6.74)	0.64 (0.10-3.07)				

Outcome data were available for 300 *E. coli* bacteraemia cases. ORs (95% CIs) are presented; *P* values were calculated using chi-squared test; \**P* < 0.05; \*\*\**P* < 0.001.

**Table 4.** Multiple regression modelling of association between *E. coli* genotype, patient characteristics, antibiotic resistance and length of stay ( $\geq 7$  days)

Factor	ST group— unadjusted	Model 1: ST group adjusted for patient characteristics	Model 2: Model 1 + fluoroquinolone non-susceptibility	Model 3: Model 1 + aminoglycoside non-susceptibility	Model 4: Model 1 + third-generation cephalosporin non-susceptibility	Model 5: Model 1 + piperacillin/tazobactam non-susceptibility
<i>E. coli</i> genotype (other STs = reference group)						
ST131-C2	6.46 (2.02–32.64)***	6.06 (1.75–32.15)**	4.81 (1.23–27.10)*	5.80 (1.63–31.13)**	8.93 (1.85–89.15)**	4.54 (1.25–24.94)*
ST131-other	1.20 (0.51–3.01)	0.81 (0.32–2.17)	0.71 (0.27–1.95)	0.77 (0.30–2.11)	0.79 (0.30–2.17)	0.84 (0.32–2.39)
ST69	1.72 (0.70–4.76)	2.85 (1.07–8.52)*	3.06 (1.14–9.20)*	2.97 (1.10–8.95)*	3.13 (1.17–9.37)*	2.82 (1.04–8.59)*
ST73	0.69 (0.34–1.39)	0.70 (0.33–1.53)	0.79 (0.36–1.77)	0.84 (0.38–1.86)	0.79 (0.35–1.77)	0.82 (0.35–1.96)
ST95	0.96 (0.38–2.61)	1.29 (0.46–3.85)	1.38 (0.49–4.18)	1.31 (0.46–3.96)	1.40 (0.50–4.22)	1.15 (0.40–3.54)
Patient's age group (<65 years = reference group)						
65–74		1.43 (0.69–3.04)	1.46 (0.70–3.12)	1.41 (0.68–3.00)	1.40 (0.66–3.02)	1.48 (0.70–3.23)
75–84		1.61 (0.74–3.57)	1.67 (0.77–3.71)	1.75 (0.80–4.00)	1.58 (0.72–3.55)	1.87 (0.82–4.46)
>85		1.64 (0.65–4.38)	1.87 (0.72–5.16)	1.79 (0.69–4.95)	1.53 (0.58–4.29)	1.84 (0.68–5.33)
Patient's sex (male = reference group)						
Female		0.30 (0.16–0.55)***	0.33 (0.18–0.62)***	0.29 (0.15–0.54)***	0.29 (0.15–0.53)***	0.30 (0.15–0.57)***
Patient's ethnicity (white = reference group)						
Asian		1.26 (0.52–3.26)	1.15 (0.47–3.02)	1.56 (0.62–4.24)	1.14 (0.46–3.01)	1.53 (0.58–4.36)
Black		1.20 (0.45–3.46)	1.16 (0.43–3.35)	1.24 (0.46–3.61)	1.03 (0.37–3.05)	0.93 (0.33–2.80)
Other		0.78 (0.34–1.83)	0.77 (0.34–1.81)	0.78 (0.34–1.84)	0.68 (0.28–1.64)	0.81 (0.34–1.98)
Not stated		0.78 (0.33–1.87)	0.75 (0.32–1.80)	0.79 (0.34–1.90)	0.79 (0.34–1.90)	0.74 (0.30–1.85)
Elixhauser index (<0 = reference group)						
0–5		2.15 (0.98–4.79)	2.10 (0.96–4.68)	2.09 (0.95–4.70)	1.93 (0.87–4.37)	2.08 (0.93–4.79)
6–13		2.71 (1.28–5.87)**	2.70 (1.27–5.91)**	2.91 (1.36–6.41)**	2.43 (1.11–5.44)*	3.36 (1.52–7.66)**
$\geq 14$		2.02 (0.87–4.80)	2.06 (0.89–4.89)	1.91 (0.82–4.56)	1.85 (0.79–4.46)	2.27 (0.94–5.67)
Onset of infection (community onset = reference group)						
Hospital onset		2.69 (1.28–6.08)**	2.51 (1.18–5.71)*	2.62 (1.24–5.92)*	2.55 (1.17–5.95)*	2.55 (1.15–6.10)*
Antibiotic susceptibility = reference group						
Antibiotic non-susceptibility			1.42 (0.65–3.20)	1.14 (0.41–3.44)	1.12 (0.41–3.18)	0.53 (0.13–2.31)

Outcome data were available for 300 *E. coli* bacteraemia cases. ORs (95% CIs) are presented. *P* values were calculated using the chi-squared test. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001.

non-susceptibility to quinolones, third-generation cephalosporins and gentamicin<sup>27</sup> and this was confirmed in our study, as was non-susceptibility to amoxicillin (85%) and amoxicillin/clavulanic acid (67%). Wider susceptibility testing against antimicrobials that are used in the community, for UTIs rather than BSIs, further demonstrated that a striking proportion (>80%) of ST131-C2 isolates were trimethoprim resistant. Trimethoprim was used in management of uncomplicated UTI until 2017 so may have contributed to emergence of ST131 in those with frequent urosepsis and the predominance of ST131-C2 in bacteraemia cases.

Multivariable regression analysis unexpectedly highlighted an association between gentamicin resistance and increased odds of 90 day mortality (OR: 3.32; 95% CI: 1.17–9.72). Mortality at earlier timepoints also appeared greater in this group of patients, although the low numbers precluded statistical evaluation (mortality at 7 days: gentamicin susceptible 6.1% versus non-susceptible 12.8%; at 30 days: gentamicin susceptible 10.3% versus non-susceptible 15.4%). Empiric single-agent aminoglycoside therapy has previously been proposed as treatment for UTIs, although there is no evidence to support this in the setting of sepsis.<sup>28</sup> Emergence of ESBL-producing Gram-negative bacteria may have encouraged use of aminoglycosides as a carbapenem-sparing strategy;<sup>29</sup> there is no evidence that aminoglycosides confer overall harm, when ESBL Enterobacteriaceae are susceptible.<sup>30</sup> Nonetheless, our frequent finding of gentamicin non-susceptibility in ST131 isolates (34%) points to a risk of reliance on gentamicin as treatment in populations where ST131 is present. In our study, 27% of isolates resistant to amoxicillin/clavulanic acid, and 47% of isolates resistant to cephalosporins, were also resistant to gentamicin. Our observation of a 3-fold increased risk of death in such cases underlines the importance of local surveillance of resistance patterns and adjustment of protocols accordingly. Though we did not examine amikacin susceptibility in this study, alternative dual-agent regimens that include amikacin may be more effective.<sup>29</sup>

Surprisingly, the multivariable models also identified black ethnicity as a predictor of 90 day mortality; this could not be explained by patient comorbidities or specific *E. coli* lineages. It is feasible that ethnicity may play a role in late recognition of bacteraemia, with consequent impact on management and therefore sepsis outcomes, although this might be expected to impact mortality at the earliest timepoints. Ethnicity is a complex trait that has frequently been associated with increased sepsis mortality in studies undertaken in North America.<sup>31</sup> In our study, black ethnicity was associated with a mortality of 6% at 7 days, 18.2% at 30 days and 33.3% at 90 days, in contrast to white ethnicity where mortality was 6.9%, 8.3% and 11.7% at the same timepoints, respectively, albeit that numbers were too low at earlier timepoints to analyse. We do not know if similar data exist for other infections, or other types of hospital admission in the UK; however, the recent COVID-19 pandemic has highlighted inequalities in healthcare outcomes.<sup>32</sup> Socioeconomic status and travel history were not collected in our study, and we cannot rule out the possibility that specific groups may be over-represented among patients from long-term care facilities or specific healthcare settings, for example dialysis or haematology. Our findings mandate more detailed study of the impact that ethnicity and other social factors might play in *E. coli* bacteraemia outcome within the UK.

The analysis also revealed that female patients were three times less likely to have a prolonged length of stay compared with male patients, even when adjusted for confounding factors, resonating with findings in an earlier national retrospective cohort study.<sup>7</sup> Conversely, length of stay was markedly prolonged in those patients with hospital-onset *E. coli* BSIs, as found in a previous retrospective study carried out in our NHS trust.<sup>9</sup> The models applied in the current work accounted for hospital-onset and comorbidities, but not the original reason for hospital admission, which may be the main driver for prolonged length of stay. There may also be important associations between reason for admission, onset of infection and gender. Larger samples and more detailed information would allow these more complex associations and possible interactions to be examined.

There are limitations to our study. The study took place over 1 year and was based in an urban and socially diverse area in London, hence our findings might not be relevant to other settings. The study was underpowered to detect significant differences in mortality between *E. coli* genotype at early timepoints, as the observed mortality at 30 days was much lower than predicted,<sup>6</sup> nonetheless, it was clear that ST131-C2 alone was not hypervirulent. Other factors that could potentially have influenced the mortality include the appropriateness of antibiotic treatment prescribed, which we were not able to include in our analysis but is underlined by the finding of an association of gentamicin resistance with mortality. The treatment for urosepsis was, however, governed by a standard antibiotic-prescribing policy during the time of the study, which included revision of empiric antimicrobial therapy in response to culture and susceptibility testing. Ethnicity is self-reported, with missing data in many cases, and we had no information on the social status of the patients to adjust for in the analysis of association between *E. coli* genotype, severity of the infection and ethnicity. Information on other risk factors such as previous antibiotic exposure and transfer from other facilities was not available to include in our analyses. Finally, although our study included over 500 cases of BSI and isolates, full outcome data were only available for 300 patients, limited to the local NHS trust, while antimicrobial susceptibility data were limited to the tests reported by the local diagnostic laboratory.

Systematic linkage of bacterial genome data with patient-level demographic and clinical outcome data has provided a unique insight into the burden of *E. coli* BSI. The data highlight key areas for future research in a larger cohort, while underlining the value of genetic surveillance of strains when developing antibiotic prescribing algorithms.

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## Author contributions

E.J., M.E., N.W. and S.S. contributed to the study design. K.H., O.B., E.J., S.S. and C.E.C. performed verification of methods and data analysis. E.J. performed bioinformatics analysis. O.B. and K.H. did statistical analysis. O.B., K.H., M.M., F.A.R., M.P., J.P. and E.J. contributed to data collection and preparation of samples for WGS. O.B. and S.S. wrote the ethics application and got the approval. E.J., K.H. and S.S. took the lead in writing the manuscript. All authors provided critical feedback of the results and review of the manuscript.

## Supplementary data

[Supplementary methods](#) and references, Figure [S1](#) and Tables [S1](#) to [S7](#) are available as [Supplementary data](#) at JAC Online.

## References

- Wilson J, Elgohari S, Livermore DM et al. Trends among pathogens reported as causing bacteraemia in England, 2004–2008. *Clin Microbiol Infect* 2011; **17**: 451–8.
- Bonten M, Johnson JR, Van Den Biggelaar AHJ et al. Epidemiology of *Escherichia coli* bacteremia: a systematic literature review. *Clin Infect Dis* 2021; **72**: 1211–9.
- Begier E, Rosenthal NA, Gurtman A et al. Epidemiology of invasive *Escherichia coli* infection and antibiotic resistance status among patients treated in US hospitals: 2009–2016. *Clin Infect Dis* 2021; **73**: 565–74.
- Public Health England. Laboratory surveillance of *E. coli* bacteraemia in England, Wales and Northern Ireland 2018. Health Protection Report 2019; **13**. [https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment\\_data/file/844788/hpr3719\\_ecoli18.pdf](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/844788/hpr3719_ecoli18.pdf).
- Vihta K-D, Stoesser N, Llewelyn MJ et al. Trends over time in *Escherichia coli* bloodstream infections, urinary tract infections, and antibiotic susceptibilities in Oxfordshire, UK, 1998–2016: a study of electronic health records. *Lancet Infect Dis* 2018; **18**: 1138–49.
- Bhattacharya A, Nsonwu O, Johnson AP et al. Estimating the incidence and 30-day all-cause mortality rate of *Escherichia coli* bacteraemia in England by 2020/21. *J Hosp Infect* 2018; **98**: 228–31.
- Naylor NR, Pouwels KB, Hope R et al. The health and cost burden of antibiotic resistant and susceptible *Escherichia coli* bacteraemia in the English hospital setting: a national retrospective cohort study. *PLoS One* 2019; **14**: e0221944.
- Lillie PJ, Johnson G, Ivan M et al. *Escherichia coli* bloodstream infection outcomes and preventability: a six-month prospective observational study. *J Hosp Infect* 2019; **103**: 128–33.
- Blandy O, Honeyford K, Gharbi M et al. Factors that impact on the burden of *Escherichia coli* bacteraemia: multivariable regression analysis of 2011–2015 data from West London. *J Hosp Infect* 2019; **101**: 120–8.
- Wilson J. Applying Pareto analysis to reducing *Escherichia coli* bloodstream infections. *J Infect Prev* 2018; **19**: 208–10.
- Otter JA, Galletly TJ, Davies F et al. Planning to halve Gram-negative bloodstream infection: getting to grips with healthcare-associated *Escherichia coli* bloodstream infection sources. *J Hosp Infect* 2019; **101**: 129–33.
- Kaye KS, Gupta V, Mulgirigama A et al. Antimicrobial resistance trends in urine *Escherichia coli* isolates from adult and adolescent females in the United States from 2011 to 2019: rising ESBL strains and impact on patient management. *Clin Infect Dis* 2021; **73**: 1992–9.
- Köhler CD, Dobrindt U. What defines extraintestinal pathogenic *Escherichia coli*? *Int J Med Microbiol* 2011; **301**: 642–7.
- Day MJ, Doumith M, Abernethy J et al. Population structure of *Escherichia coli* causing bacteraemia in the UK and Ireland between 2001 and 2010. *J Antimicrob Chemother* 2016; **71**: 2139–42.
- Banerjee R, Johnson JR. A new clone sweeps clean: the enigmatic emergence of *Escherichia coli* sequence type 131. *Antimicrob Agents Chemother* 2014; **58**: 4997–5004.
- Stoesser N, Sheppard AE, Pankhurst L et al. Evolutionary history of the global emergence of the *Escherichia coli* epidemic clone ST131. *mBio* 2016; **7**: e02162–15.
- Price LB, Johnson JR, Aziz M et al. The epidemic of extended-spectrum- $\beta$ -lactamase-producing *Escherichia coli* ST131 is driven by a single highly pathogenic subclone, H30-Rx. *mBio* 2013; **4**: e00377–13.
- Kallonen T, Brodrick HJ, Harris SR et al. Systematic longitudinal survey of invasive *Escherichia coli* in England demonstrates a stable population structure only transiently disturbed by the emergence of ST131. *Genome Res* 2017; **27**: 1437–49.
- Gladstone RA, McNally A, Pöntinen AK et al. Emergence and dissemination of antimicrobial resistance in *Escherichia coli* causing bloodstream infections in Norway in 2002–17: a nationwide, longitudinal, microbial population genomic study. *Lancet Microbe* 2021; **2**: e331–41.
- Johnson JR, Thuras P, Johnston BD et al. The pandemic H30 subclone of *Escherichia coli* sequence type 131 is associated with persistent



- infections and adverse outcomes independent from its multidrug resistance and associations with compromised hosts. *Clin Infect Dis* 2016; **62**: 1529–36.
- 21** Van Walraven C, Austin PC, Jennings A *et al.* A modification of the Elixhauser comorbidity measures into a point system for hospital death using administrative data. *Med Care* 2009; **47**: 626–33.
- 22** Ben Zakour NL, Alsheikh-Hussain AS, Ashcroft MM *et al.* Sequential acquisition of virulence and fluoroquinolone resistance has shaped the evolution of *Escherichia coli* ST131. *mBio* 2016; **7**: e00347-16.
- 23** Baltas I, Stockdale T, Tausan M *et al.* Impact of antibiotic timing on mortality from Gram-negative bacteraemia in an English district general hospital: the importance of getting it right every time. *J Antimicrob Chemother* 2021; **76**: 813–9.
- 24** Evans RN, Pike K, Rogers CA *et al.* Modifiable healthcare factors affecting 28-day survival in bloodstream infection: a prospective cohort study. *BMC Infect Dis* 2020; **20**: 545.
- 25** Goswami C, Fox S, Holden M *et al.* Genetic analysis of invasive *Escherichia coli* in Scotland reveals determinants of healthcare-associated versus community-acquired infections. *Microb Genomics* 2018; **4**: e000190.
- 26** Trotter CL, Chandra M, Cano R *et al.* A surveillance network for meningococcal disease in Europe. *FEMS Microbiol Rev* 2007; **31**: 27–36.
- 27** Olesen B, Frimodt-Møller J, Leihof RF *et al.* Temporal trends in antimicrobial resistance and virulence-associated traits within the *Escherichia coli* sequence type 131 clonal group and its H30 and H30-Rx subclones, 1968 to 2012. *Antimicrob Agents Chemother* 2014; **58**: 6886–95.
- 28** Vidal L, Gafter-Gvili A, Borok S *et al.* Efficacy and safety of aminoglycoside monotherapy: systematic review and meta-analysis of randomized controlled trials. *J Antimicrob Chemother* 2007; **60**: 247–57.
- 29** Hawkey PM, Warren RE, Livermore DM *et al.* Treatment of infections caused by multidrug-resistant Gram-negative bacteria: report of the British Society for Antimicrobial Chemotherapy/Healthcare Infection Society/British Infection Association Joint Working Party. *J Antimicrob Chemother* 2018; **73**: iii2–78.
- 30** Palacios-Baena ZR, Gutiérrez-Gutiérrez B, Calbo E *et al.* Empiric therapy with carbapenem-sparing regimens for bloodstream infections due to extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae: results from the INCREMENT cohort. *Clin Infect Dis* 2017; **65**: 1615–23.
- 31** Shankar-Hari M, Rubenfeld GD. Race, ethnicity, and sepsis: beyond adjusted odds ratios. *Crit Care Med* 2018; **46**: 1009–10.
- 32** Ayoubkhani D, Nafilyan V, White C *et al.* Ethnic-minority groups in England and Wales—factors associated with the size and timing of elevated COVID-19 mortality: a retrospective cohort study linking census and death records. *Int J Epidemiol* 2021; **49**: 1951–62.