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Population structure of *Escherichia coli* causing bacteraemia in the UK and Ireland between 2001 and 2010

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Objectives: *Escherichia coli* is the most common agent of bacteraemia, bacterial gastroenteritis and urinary tract infections (UTIs). Lineages causing UTIs and gastrointestinal disease are well defined, but less is known about those causing bacteraemia. We therefore investigated the population structure of *E. coli* from bacteraemia in the UK and Ireland between 2001 and 2010.

Methods: *E. coli* isolates ($n=2166$) were submitted to the BSAC Bacteraemia Surveillance Programme from 18 UK and Irish centres from 2001 to 2010. Genotypes were analysed by MLST using the Achtman scheme; MICs, $bla_{CTX\text{-}M}$ group and patient demographics were previously determined in the BSAC surveillance.

Results: Four hundred and forty-eight STs were identified, but five of these, and their associated clonal complexes (CCs), accounted for 58.4% (1264 of 2166) of isolates: CC73 was the most common (20.7%), followed by CC131 (13.9%), CC95 (11.3%), CC69 (6.9%) and CC12 (5.5%). All these, except CC69 (group D), belong to phylogenetic group B2. CC131 isolates were much more often MDR than other STs were: they rose from 2.9% of isolates in 2001 to 20.5%–20.7% in 2007–08 and then declined to 14.3% in 2010. Resistance rates to cephalosporins, aminoglycosides and fluoroquinolones remained below 10% in other major CCs throughout.

Conclusions: The five most prevalent bacteraemia STs have all been associated previously with UTIs. They dominated in all years, but their proportions fluctuated, most notably for ST131, a globally disseminated high-risk clone that is often MDR.

Introduction

The incidence of *Escherichia coli* bacteraemia has recently increased progressively and, by 2013, the species accounted for 32% of all bacteraemias reported to Public Health England in the UK except Scotland.[1] Reporting became mandatory in England in June 2011, with 34 275 cases in fiscal 2013–14.[2] Mortality is 10%–30%,[3] rising to 60% for MDR infections.[4]

Understanding the distribution of *E. coli* strains in bacteraemia could elucidate routes to improve control, for example by tailoring antibiotic use to the strain, or developing vaccines against major lineages. Here, we studied the population structure of bloodstream *E. coli* from 18 hospitals in England, Ireland, Northern Ireland and Wales over a decade.

Materials and methods

Bacterial strains

E. coli isolates ($n=2166$) from patients with bacteraemia were collected under the aegis of the BSAC Bacteraemia Surveillance Programme.[5,6] They comprised all the *E. coli* submitted from 18 centres that participated in the surveillance throughout, with ~10 isolates per centre in 2001–07, 20 per centre in 2008 and 2009 and 14 per centre in 2010. MICs and $bla_{CTX\text{-}M}$ groups were determined by published methods,[7,8] and supplied by the BSAC along with basic demographic information. Susceptibility data were reviewed against 2014 EUCAST/BSAC breakpoints, which may differ from those when the isolates were collected. Amoxicillin/clavulanate had been tested as a 2:1 ratio and was reviewed against the previous 8+4 mg/L breakpoint, not the current 8+2 (fixed) mg/L criterion.

DNA extraction

DNA extraction was performed using a QIAxtractor robot, with DX reagents and plasticware, according to the manufacturer's instructions (QIAxtractor; Qiagen, Crawley, UK).

Phylotyping and MLST

Isolates were assigned to the major phylogenetic groups of *E. coli* (A, B1, B2 and D) by multiplex PCR.[9] MLST was performed as described.[10] Data were assembled and analysed in BioNumerics (v6.1; Applied Maths, Keistraat, Belgium); isolates were assigned allele numbers and STs via the Warwick database.[11]

Statistical analysis

Clonal complexes (CCs) were grouped as CC73, CC131, CC95, CC69, CC12 and 'others' for analysis, with CC73 (the largest group) as 'baseline'. Factors predicting CC were explored in multinomial logistic regression models. Intermediate and resistant isolates were combined as 'non-susceptible' for analysis. Candidate models were compared by Akaike information criterion. The final models used cluster-robust standard errors to allow for clustering by collection centre.

Results and discussion

Molecular typing

The 2166 isolates belonged to 448 different STs (Table S1, available as Supplementary data at *JAC* Online), 230 of which had new alleles or new allelic combinations not described in the Warwick database. Nevertheless 47% (1020 of 2166) belonged to five major STs, namely ST73 (16.8%; $n=363$), ST131 (12.0%; $n=260$), ST95 (8.5%; $n=184$), ST69 (5.4%; $n=117$) and ST12 (4.4%; $n=96$). If single locus variants and double locus variants were included, the proportion belonging to the five major CCs rose to 58.4% (1264 of 2166) (Table 1).

CC12, CC73, CC95 and CC131 isolates all belonged to phylogenetic group B2, and CC69 isolates belonged to group D. More generally, phylogroups B2 (68.4%; 1481 of 2166) and D (19.0%; 412 of 2166) dominated the entire collection and are the *E. coli* groups typically associated with virulent extra-intestinal infections.

Overall, the proportion of isolates belonging to the five major CCs rose from 46.5% (80 of 172) in 2001 to 63.3% (150 of 237) in 2010 ($P=0.001$ for trend). The prevalence of the five individual major CCs fluctuated, most noticeably for CC131, which rose from 2.9% (5 of 172 isolates) in 2001 to a peak of 20.5%–20.7% (37 of 179 and 71 of 346) in 2007 and 2008 (Figure 1). CC73 was most prevalent between 2001 and 2006, but was surpassed by CC131 in 2007–08, only to become the most prevalent CC again in 2009 and 2010. The decline of ST131 may reflect the change in prescribing practice in the UK away from cephalosporins and quinolones.[12]

Antibiotic resistance

Except for amoxicillin and amoxicillin/clavulanate there was little resistance (≤10%) to tested antimicrobials in CC12, CC69, CC73 or CC95, but resistance to cephalosporins, fluoroquinolones and aminoglycosides was prevalent in CC131 (Table 1). Other CCs showed smaller, but still significant ($P\leq0.02$), differences from CC73, which served as the reference; thus CC69 and 'other' CC isolates were more often resistant to ciprofloxacin and gentamicin, and CC69 and CC12 isolates were more often resistant to amoxicillin, whereas CC95 isolates were less often resistant to β-lactams and their inhibitor combinations (Table 1).

Non-susceptibility generally rose from 2001 to 2006–08, including for amoxicillin/clavulanate, cefotaxime, ceftazidime, ciprofloxacin and gentamicin, though with some subsequent decline in 2009–10 for cephalosporins and ciprofloxacin, as outlined elsewhere.[12,13] These trends were not detected within any of the five major CC groups or 'others', but, rather, reflected the fluctuating proportion of the frequently MDR CC131 isolates.

ESBL phenotypes were seen for 144 (6.6%) isolates. Among these, 86.8% (125 of 144) had $bla_{CTX\text{-}M}$ genes, 96.0% (120 of 125)

Table 1. Non-susceptibility to antimicrobials in relation to CC

CC	Total no.	Patient sex (%)			Age (years) (%)				Onset (%)		Non-susceptibility (%)									β-Lactamase (%)				
		N/R	female	male	N/R	0–19	20–49	50–69	70+	N/R	≤48 h	>48 h	AMX	AMC	TZP	CTX[a]	CAZ	CIP	GEN	TGC[a]	IPM	CTX-M other[b]	CTX-M-1	CTX-M-9	ESBL, non-CTX-M	non-ESBL
73	449	0.2	59.5	40.3	0.0	3.6	12.5	20.7	63.3	1.3	64.4	34.3	55.9	27.8	9.1	1.5	1.8	1.3	1.6	0.2	0.0	0.0	0.0	0.0	1.1	98.9
131	302	0.3	42.7	57.0	0.7	3.0	6.6	25.8	63.9	0.0	**50.0**	**50.0**	83.4	59.3	22.2	35.0	29.5	64.2	20.2	0.0	0.0	0.3	32.5	0.3	1.0	65.9
95	245	0.0	63.7	36.3	0.4	9.4	13.5	23.7	53.1	0.8	64.5	34.7	**45.3**	13.9	**2.9**	0.0	0.0	0.4	2.4	0.0	0.0	0.0	0.0	0.0	0.0	100
69	149	0.0	**72.5**	**27.5**	0.0	2.7	18.1	23.5	55.7	0.7	65.8	33.6	81.9	32.2	10.1	1.4	2.0	6.7	**4.0**	0.7	0.0	0.0	0.7	0.0	0.0	99.3
12	119	0.0	49.6	50.4	0.8	3.4	11.8	22.7	61.3	0.0	68.9	31.1	**71.4**	28.6	7.6	1.8	5.0	0.8	3.4	0.0	0.0	0.0	0.8	0.0	1.7	97.5
Other[c]	902	0.2	**50.4**	**49.3**	0.4	4.5	11.2	27.3	56.5	1.1	**57.0**	**41.9**	60.9	27.7	9.3	**5.3**	**5.3**	15.2	6.4	0.1	0.2	0.2	**2.2**	0.1	1.0	96.5
Total	2166	0.2	54.2	45.6	0.4	4.5	11.6	24.8	58.8	0.9	59.6	39.5	63.3	30.9	10.3	7.9	7.1	16.1	6.6	0.1	0.1	0.1	5.5	0.1	0.9	93.4

Onset ≤48 h considered as community onset and onset >48 h considered as hospital onset.
Values shaded grey are significantly different ($P\leq0.0001$) compared with the baseline CC73 group.
Values in bold are significant ($P\leq0.05$) compared with the baseline CC73 group.
N/R, not reported; AMX, amoxicillin; AMC, amoxicillin/clavulanate; TZP, piperacillin/tazobactam; CTX, cefotaxime; CAZ, ceftazidime; CIP, ciprofloxacin; GEN, gentamicin; TGC, tigecycline; IPM, imipenem.
[a]Cefotaxime and tigecycline were tested in 2002–10 only.
[b]'CTX-M other' indicates an unidentified CTX-M group.
[c]'Other' CCs includes 902 isolates representing 443 STs, with no more than 65 isolates belonging to any CC.

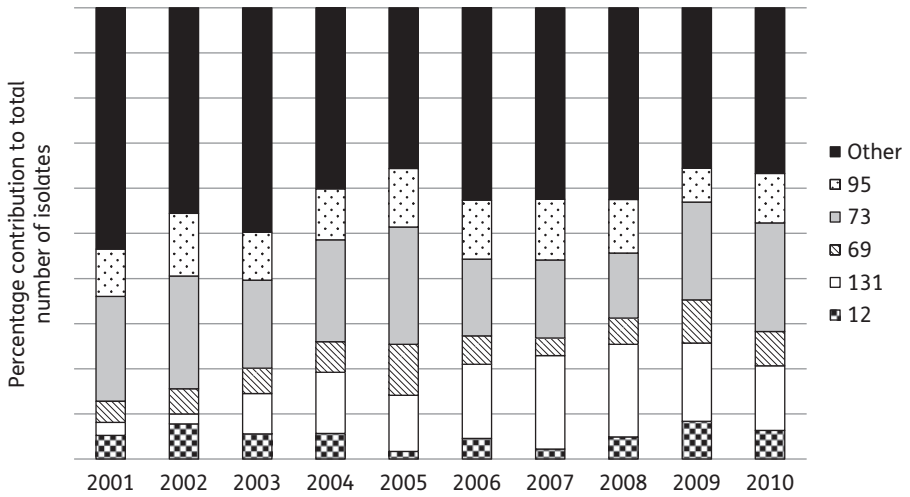

Figure 1. Proportions of different CCs among bloodstream *E. coli* over time. 'Other' CCs includes 902 isolates representing 443 STs, with no more than 65 isolates belonging to any CC and with 96 isolates being sole representatives of unique STs.

of them had $bla_{\text{CTX-M-group-1}}$ types. Predictably, non-susceptibility to other agents was more prevalent in ESBL producers than those without ESBLs. $bla_{\text{CTX-M-group-1}}$ genes were present in 98 of 302 CC131 isolates (32.5%) (Table 1), with CC131 accounting for 81.7% (98 of 120) of all $bla_{\text{CTX-M-group-1}}$ genes. The remaining 22 isolates with $bla_{\text{CTX-M-group-1}}$ genes were spread across 11 CCs, with CC23 (5 of 120; 4.2%) and CC10 (4 of 120; 3.3%) accounting for the largest (but still small) shares. Much less common were $bla_{\text{CTX-M-group-9}}$ genes, found in single ST131 and ST405 isolates, and non-CTX-M ESBLs, with 19 producers spread across 11 CCs, but most often in CC73 (5 isolates) (Table 1). Only two isolates were non-susceptible to imipenem, with MICs of 8 and 16 mg/L; both belonged to CC405 and were from different hospitals in southern England, they were isolated in 2006 and had a CTX-M ESBL with impermeability.[13] No carbapenemase producers were collected.

CC distribution in relation to patient demographics

There was an association between patient sex and CC ($P<0.0001$); specifically, and compared with CC73, CC131 and 'other' CCs were significantly more often associated with men, whereas CC69 was more associated with women, as noted previously by Gibreel *et al.*[14] Age, by contrast, had little association with CC after accounting for sex. Patients with CC131 isolates divided equally between those with onset ≤48 or >48 h after hospitalization, whereas onset at ≤48 h was twice as frequent for patients with CC12, 69, 73 or 95 isolates. Hospital onset thus was a significant predictor of CC ($P<0.0001$), with CC131 and 'others' being more associated with hospital-onset bacteraemias. Previous studies have shown greater 30 day mortality for hospital than community-acquired *E. coli* bacteraemias (28% versus 14%),[15] though it is unclear if this reflects the strains, their resistances or the underlying health status of hospital versus community cases.

Presumed-source-of-bacteraemia data were available for 1446 isolates (66.8%). Overall, and for each of the top five CCs individually, urinary tract infections were the most common source (66.4%; 960 of 1446) followed by intra-abdominal infection (17.6%; 254 of 1446). Presumed source (genitourinary versus other) was a strong predictor of CC ($P<0.0001$), with CC69 significantly more associated with a genitourinary source than CC73, whereas 'others' were significantly less associated with a genitourinary source.

Conclusions

Overall, these results show the dominance, for a decade, of the same five *E. coli* CCs as agents of bacteraemia in the UK and Ireland, namely CC73, CC131, CC95, CC69 and CC12. This dominance increased over time or, looked at another way, these five types accounted for much of the overall increase in *E. coli* bacteraemias during the decade. In addition, these five CCs have been associated with urinary tract infections in (i) north-west England (2007–09) and their proportions were 16.6% (ST73), 13.3% (ST131), 9% (ST69), 6% (ST95) and 4.3% (ST10),[14] and (ii) Yorkshire and Humber (2010–12) where ST131, ST73 and ST95 were predominant.[16] ST131 (59%)—which is an internationally distributed 'high-risk clone' notorious for fluoroquinolone resistance and producing CTX-M ESBLs—dominated among cephalosporin-resistant urinary isolates in north-west England in 2004/2005,[17] whereas, as here, resistance rates in ST73 and ST95 were low, both at 6.8%.

The association of bacteraemia with just a few *E. coli* lineages, and of MDR with just one of these (CC131), along with the increased mortality associated with resistance and treatment inadequacy, suggest that it may be both practicable and beneficial to identify strain lineages rapidly, predicting the likelihood of resistance to standard therapies. This could be done, e.g. by PCR from early growth in blood culture bottles,[18] allowing swifter adaptation of therapy than is possible based on conventional testing. The low rates of resistance in the other top CCs besides CC131 imply that their success reflects some other selective advantage and STs 131, 69, 12 and 127 have all been reported to have unusually high virulence scores.[14,19,20]

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Transparency declarations

D. M. L. is partly self-employed and consults for numerous pharmaceutical and diagnostic companies, including Achaogen, Adenium, Allecra, Astellas, AstraZeneca, Bayer, Basilea, bioMérieux, bioVersys, Cubist, Curetis, GSK, Longitude, Merck, Meiji Seika, Pfizer, Roche, Tetraphase, VenatoRx and Wockhardt, he holds grants from AstraZeneca, Basilea, Cubist, Meiji Seika, Merck and VenatoRx, he has received lecture honoraria or travel reimbursement from AstraZeneca, Curetis, GSK, J&J, Leo, Meiji, Merck, Novartis, Pfizer and Tetraphase, and he holds shares in Dechra, GSK, Merck and Pfizer, collectively amounting to <10% of portfolio value.

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All other authors: none to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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