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Escherichia coli bacteraemia: 2 years of prospective regional surveillance (2010–12)

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Objectives: To determine the prevalence of antibiotic resistance and the epidemiology of *Escherichia coli* bacteraemia isolates across the Yorkshire and Humber National Health Service region over 2 years.

Methods: Ten percent of all *E. coli* blood culture isolates were collected per month from 14 laboratories across the Yorkshire and Humber region. Individual laboratories submitted antibiotic susceptibility data and isolates were re-tested centrally using the VITEK2® system (bioMérieux, France). Isolates were also characterized using PCR to test for the presence of sequences encoding extended-spectrum β-lactamases (ESBLs) and genotyped using amplified fragment length polymorphism (AFLP). Selected isolates were further characterized using multilocus sequence typing.

Results: Between July 2010 and June 2012, 770 isolates were examined: 63%, 40%, 14% and 7% of isolates were non-susceptible to ampicillin, trimethoprim, ciprofloxacin and gentamicin, respectively. Eight percent of isolates ($n=63$) were ESBL positive; CTX-M group 1 enzymes were the most common (68%). There was a fluctuating trend in the prevalence of resistance to amoxicillin/clavulanic acid (MIC >8 mg/L): July–September 2010, 16%; July–September 2011, 38%; and April–June 2012, 22%. AFLP identified 106 types. The majority of isolates belonged to one of two AFLP types: AFLP 1 [sequence type (ST) 131; 17%] and AFLP 2 (ST73; 18%). ST131 and ST73 were both associated with hospital- and community-onset bacteraemia, and with urinary, hepatobiliary and gastrointestinal sources of infection. ESBL-positive isolates were predominantly ST131 (60%).

Conclusions: Continued surveillance of antibiotic resistance among *E. coli* bacteraemia isolates is necessary to enhance these early baseline data. The variable prevalence of resistance to amoxicillin/clavulanic acid raises concerns, as both *E. coli* bacteraemia and empirical use of this antibiotic are common.

Keywords: antibiotic susceptibility, epidemiology, genetic background

Introduction

Escherichia coli is currently the most common cause of bacteraemia in England.[1] Between 2007 and 2011, voluntary surveillance data generated from laboratory reports in England, Wales and Northern Ireland indicated a 35% increase in the number of *E. coli* bacteraemia reports.[2] In June 2011, the Department of Health introduced mandatory reporting of *E. coli* bacteraemia by all National Health Service (NHS) acute trust hospitals in England.[3] Between June 2011 and September 2012, >40 000 reports of *E. coli* bacteraemia were submitted to the mandatory surveillance scheme.[4]

Preliminary analysis of 21 553 cases of *E. coli* bacteraemia reported to the mandatory surveillance scheme between June 2011 and January 2012 indicates that rates increase with age, with the highest prevalence occurring in male patients of most age groups; the exception being the female 15–54 age group, probably due to the higher prevalence of urinary tract infections (UTIs) in this cohort.[5] Genitourinary infections were the most common presumed primary source for *E. coli* bacteraemia ($n=9961$, 46%). Patients with *E. coli* bacteraemia were most commonly admitted from home ($n=15965$, 74%) and 58% ($n=12482$) of patients had blood cultures that were positive for *E. coli* taken on the day of admission. Reported rates of *E. coli* bacteraemia varied markedly across regions of England. The highest and lowest rates were reported from the North East NHS region (79.61 cases per 100 000 population) and the East of England region (47.95 cases per 100 000 population), respectively.

The Yorkshire and Humber region (Figure 1, left-hand side), along with the North West region, had the second highest rates of *E. coli* bacteraemia (>70 cases per 100 000 population). Further analysis is required to determine whether these variations in reported rates of *E. coli* bacteraemia are subject to ascertainment bias.

At the time of the study, the Yorkshire and Humber NHS region covered >15 000 km^2 and comprised a population of approximately 5 million.[6] The region included five major cities (Leeds, Sheffield, Bradford, Hull and York) but also spanned large rural locations such as Airedale, Harrogate and Scarborough.[6] We introduced a surveillance scheme to understand the epidemiology of *E. coli* causing bacteraemia in the Yorkshire and Humber region.

Methods

Bacterial isolates

Each of the 14 diagnostic microbiology laboratories in the Yorkshire and Humber region (Figure 1, right-hand side) was invited to send on a monthly basis the first 10% (consecutive and non-duplicate) of *E. coli* isolates from blood cultures (range 1–8 isolates) to the central testing laboratory based at the Department of Microbiology, Leeds General Infirmary (see Table 1). Antibiotic susceptibility data and demographic and clinical details were submitted with each isolate. Isolates were cultured from the slope or swab of receipt onto a cystine lactose electrolyte-deficient agar plate to check the purity of the organism received. Organism identification was not confirmed unless a discrepancy in antibiotic susceptibilities determined locally and centrally was noted. The following control organisms were used: NCTC13441 [*E. coli* with CTX-M15, sequence type (ST) 131, PFGE strain A], NCTC13351 (*E. coli* with TEM-3) and NCTC13368 (*Klebsiella pneumoniae* with SHV-18).

Antibiotic susceptibility testing

An automated, standardized susceptibility testing method (VITEK2®) with AST-N142 cards (July 2010–April 2012) or AST-N206 cards (May–June 2012) (bioMérieux, France) was used to test susceptibility to the following antibiotics: amikacin, amoxicillin/clavulanic acid, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, cefoxitin, cefuroxime, cefalotin, ciprofloxacin, ertapenem, gentamicin, meropenem, piperacillin (AST-N142 only), piperacillin/tazobactam, tigecycline, tobramycin and trimethoprim. Susceptibility results were interpreted by the Advanced Expert System (AES) software of the VITEK2 using EUCAST breakpoints.

DNA extraction

DNA from isolates was extracted using the QIAcube™ automated spin column extraction platform (Qiagen, UK) according to the manufacturer's instructions. The final concentration of DNA (ng/μL) was measured using a spectrophotometer (Jenway Genova MK3, Bibby Scientific Limited, UK).

Amplification of bla_{TEM}, bla_{SHV} and bla_{CTX-M} alleles

Isolates suggested to have an extended-spectrum β-lactamase (ESBL) resistance phenotype by the VITEK2 system were tested by PCR for the presence of sequences encoding common ESBL genes. Previously published methods were used to amplify sequences encoding the following enzymes: TEM,[7] SHV[7] and CTX-M groups 1, 2 and 9.[8] Specific TEM and SHV enzymes were not identified; it was not therefore possible to determine which enzymes produced were ESBLs. Amplification of the conserved *adk* (adenylate kinase) gene served as a control for DNA extraction.[9]

Genotyping using amplified fragment length polymorphism (AFLP)

AFLP, based on a modified method of Arnold *et al*.,[10] was used to genotype isolates. Isolates were tested in duplicate and the control strain NCTC13441 was included with each batch of isolates tested to ensure the reproducibility of results. Restriction enzymes were sourced from New England BioLabs (UK) Ltd, and primers and PCR adaptors were sourced from Metabion International AG, Germany.

A combined restriction enzyme and ligase mixture was prepared (total volume 50 μL): 5 U of MseI, 2.0 μL of 10× MseI buffer, 0.2 μL of 1 mg/mL BSA, 0.5 μL of RNase I, 5 U of EcoRI, 1.68 μL of Tris HCl 0.5 M (pH 7.6), 2.1 μL of 0.5M NaCl, 40 U of T4 DNA ligase, 5 μL of 10× T4 ligase buffer, 5 μM of EcoRI adaptor (EcoRI-F 5′pho-CTC GTA GAC TGC GTA CC, 5′pho-AAT TGG TAC GCA GTC TAC) and 50 μM MseI adaptor (MseI-F 5′pho-GAC GAT GAG TCC TGA G, MseI-R 5′pho-TAC TCA GGA CTC AT). Fifty microlitres of enzyme mix was dispensed into wells of a 96-well microplate. The relevant DNA (approximately 50 ng) was added to the correct well and the microplate was sealed with aluminium foil. The contents of the plate were mixed by inversion and incubated for a minimum of 2 h at 37°C using a Veriti® thermal cycler (Life Technologies Limited, Paisley, UK).

A PCR mix was prepared and dispensed into wells of a 96-well microplate (total volume 22.5 μL): 12.5 μL of 2× Taq PCR mastermix [New England BioLabs (UK) Ltd], 6.34 μL of molecular grade water, 1.5 μL of $MgCl_2$, 1.0 μL of 1 mg/mL BSA, 16.6 pmol of FAM-EcoRI forward primer (5′ fam-GAC TGC GTA CCA ATT C), 100 pmol of MseI reverse primer (5′-GAT GAG TCC TGA GTA ATA). Ligated product (2.5 μL) was added to the appropriate wells. A touchdown PCR program was used to amplify DNA using a Veriti thermal cycler: 72°C for 2 min; 94°C for 20 s, 66–58°C for 30 s, 72°C for 2 min for 9 cycles, followed by 94°C for 20 s, 56°C for 30 s,72°C for 2 min for 20 cycles; finally, 60°C for 30 min. Resultant AFLP fragments were sized using a genetic analyser (Applied Biosystems, UK). AFLP profiles were analysed using BioNumerics software (Applied Maths, Belgium).

Multilocus sequence typing (MLST)

Representative isolates belonging to each AFLP type were selected for MLST using the MLST scheme available from the University College Cork *E. coli* MLST web site (http://mlst.ucc.ie/mlst/dbs/Ecoli).[9] The control strain NCTC13441 was included with each batch of isolates tested to ensure the reproducibility of results. Once an AFLP type had been confirmed by MLST, other isolates in the type were inferred to have the same ST.

Results

Between July 2010 and June 2012, 812 isolates were submitted for testing; 42 isolates were excluded from the analysis (19 were sent from the same patient and 15 isolates were additional to the 10% required). Eight isolates were not *E. coli*, four isolates were sent in error from a single laboratory and four isolates were identified following discrepant susceptibility results during testing. Thus, 770 isolates were suitable for analysis.

On initiation of the surveillance, the number of isolates estimated to represent 10% of the monthly *E. coli* bacteraemia total was determined for each laboratory in the Yorkshire and Humber region (Table 1) and the number of isolates submitted per laboratory remained the same over the 2 year period. All 14 laboratories participated in the surveillance at some time during the 2 year period; however, some laboratories participated more than others. Using data submitted to the Department of Health mandatory surveillance scheme to represent the actual rates of *E. coli* bacteraemia per trust, we calculated the actual percentage of isolates

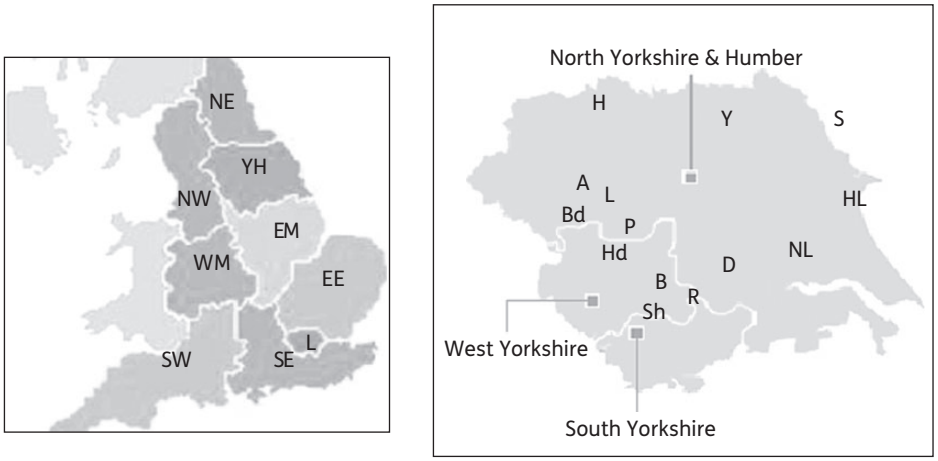

Figure 1. Geographical location of the Yorkshire and Humber NHS region in England (left-hand side). Approximate locations of the participating hospital trusts in the Yorkshire and Humber NHS region (right-hand side). Courtesy of Public Health England (http://www.hpa.org.uk). NE, North East; NW, North West; YH, Yorkshire and Humber; EM, East Midlands; WM, West Midlands, EE, East of England; L, London; SE, South East; SW, South West; A, Airedale NHS Foundation Trust; B, Barnsley Hospital NHS Foundation Trust; Bd, Bradford Teaching Hospitals NHS Foundation Trust; Hd, Calderdale and Huddersfield NHS Foundation Trust; D, Doncaster and Bassetlaw Hospitals NHS Foundation Trust; H, Harrogate and District NHS Foundation Trust; HL, Hull and East Yorkshire Hospitals NHS Trust; L, Leeds Teaching Hospitals NHS Trust; P, Mid Yorkshire Hospitals NHS Trust; NL, North Lincolnshire and Goole NHS Foundation Trust; R, Rotherham NHS Foundation Trust; S, Scarborough and North East Yorkshire NHS Trust; Sh, Sheffield Teaching Hospitals NHS Foundation Trust; Y, York Hospitals NHS Foundation Trust.

submitted to the regional surveillance during the same period (June 2011–June 2012), corresponding with the second year of surveillance). We identified that some trusts were under-represented ($n=6$) in the regional surveillance whereas others were over-represented ($n=3$).

Antibiotic susceptibility

The non-susceptibility results for the antibiotics tested are presented per quarterly period (Figure 2). The analysis excluded cefoxitin, which is used as an indicator for the AmpC resistance phenotype; cefepime and piperacillin, which are not licensed for use in the UK; carbapenems, which had levels of non-susceptibility <1%, and piperacillin/tazobactam following advice from the manufacturer of the AST cards (bioMérieux, France).

Averaged over all the quarterly periods, the prevalence of non-susceptibility to ampicillin, trimethoprim, amoxicillin/clavulanic acid, cefalotin and ciprofloxacin was 63%, 40%, 26%, 16% and 14%, respectively. The prevalence of non-susceptibility to third-generation cephalosporins and gentamicin was <10% (Figure 2). Only one isolate was determined to be non-susceptible to carbapenem agents (0.1%); this isolate was confirmed to be OXA-48 by the Antimicrobial Resistance and Healthcare-Associated Infections Reference Unit (AMRHAI), Colindale, London. All isolates tested were susceptible to tigecycline.

In order to determine the level of resistance to piperacillin/tazobactam in the absence of data from the VITEK2, susceptibilities submitted by the original laboratory were interrogated. A proportion of isolates ($n=581$) had been tested against piperacillin/tazobactam and 44 (8%) isolates were non-susceptible to this antibiotic. There was no change in piperacillin/tazobactam susceptibility over the study period.

No sustained, significant changes in antibiotic susceptibility were identified over the 2 year period. The prevalence of resistance to amoxicillin/clavulanic acid (MIC >8 mg/L) did increase between July–October 2010 (16%) and July–September 2011 (38%) but had returned to 22% by April–June 2012 (Figure 2). This fluctuation was not due to a change in susceptibility testing method or breakpoint during the study.

Participating laboratories in the region provide services for different sizes of hospitals, which was taken into account when analysing antibiotic susceptibility data. There were no significant differences between the prevalence of antibiotic resistance in small, district general hospitals (<500 beds) compared with medium-sized (500–999 beds) or large hospitals (≥1000 beds) or teaching hospitals (Leeds and Sheffield) (data not shown). Data relating to local antibiotic usage or prescriptions were not available/provided by the regional laboratories during the study period; however, this information would be useful to place the antibiotic susceptibility data in context.

Detection of bla_{TEM}, bla_{SHV} and bla_{CTX-M} alleles

Sixty-three isolates (8%) were ESBL positive; CTX-M group 1 (68%) and TEM (49%) enzymes were common (Figure 3). The prevalence of ESBL-positive isolates ranged from 5% (July–September 2010) to 13% (July–September 2011) (Figure 2). ESBL-positive isolates were identified from all 14 laboratories. The prevalence of isolates with an AmpC resistance phenotype or an inhibitor-resistant TEM phenotype was low (0.5% and 6%, respectively). Nine isolates (14%) were negative for common ESBL genes. Six of these isolates were also noted to be ESBL positive by the original laboratory, probably owing to enzymes not detected by the current PCR scheme. Three isolates were likely to have the AmpC resistance phenotype, as the isolates had a cefoxitin MIC ≥64 mg/L and were susceptible to cefepime ≤1 mg/L.

Table 1. Expected versus actual number of *E. coli* isolates submitted to the regional surveillance scheme compared with the number of cases reported to the Department of Health mandatory surveillance scheme (June 2011 – June 2012)[23]

Hospital trust	Number of isolates requested to represent 10%	Actual number of isolates received	Number of *E. coli* bacteraemia cases reported to the mandatory surveillance scheme	Actual percentage of *E. coli* bacteraemia cases sent to the regional surveillance
Airedale NHS Foundation Trust	1	12	134	9
Barnsley Hospital NHS Foundation Trust	2	0	155	0^{-}
Bradford Teaching Hospitals NHS Foundation Trust	3	35	232	15^{+}
Calderdale and Huddersfield NHS Foundation Trust	2	24	248	9.6
Doncaster and Bassetlaw Hospitals NHS Foundation Trust	3	37	346	10.6
Harrogate and District NHS Foundation Trust	1	12	104	11.5
Hull and East Yorkshire Hospitals NHS Trust	3	14	408	3.4^{-}
Leeds Teaching Hospitals NHS Trust	8	104	701	14.8^{+}
Mid Yorkshire Hospitals NHS Trust	2	25	390	6.4^{-}
North Lincolnshire and Goole NHS Foundation Trust	5	52	286	18^{+}
Rotherham NHS Foundation Trust	3	10	220	4.5^{-}
Scarborough and North East Yorkshire NHS Trust	1	10	158	6.3^{-}
Sheffield Teaching Hospitals NHS Foundation Trust	4	39	677	5.6^{-}
York Hospitals NHS Foundation Trust	2	23	259	8.8

$^{+}$Laboratories that were over-represented in the regional surveillance.
$^{-}$Laboratories that were under-represented in the regional surveillance.

Genetic background of isolates

Seven hundred and sixty-eight isolates produced fragments by AFLP; two isolates did not (one belonging to ST131, the other to ST69). In order to define each AFLP type, 130 (17%) non-duplicate isolates were tested by MLST. Using an 85% similarity cut-off, as determined by MLST, there were 106 different AFLP types; 9 types comprised ≥20 isolates [ST73 (n=143), ST131 (n=129), ST69 (n=81), ST95 (n=73), ST12 (n=31), ST404 (n=27), ST10 (n=26), ST127 (n=27) and ST141 (n=20)] (Table 2) and there were 29 AFLP types that had only a single representative isolate. There were over 77 different STs, with another 19 STs that were unidentified. Representatives of ST131, ST73 and ST95 were submitted by all 14 laboratories. Isolates sent from one laboratory (Leeds Teaching Hospitals NHS Trust) showed the greatest diversity, with at least 36 different AFLP types, although this is likely to be related to the high number of isolates submitted for analysis compared with other laboratories (Table 1). Analysis by quarter did not indicate an increase in any particular genotype during the study period (data not shown).

Relationship between genotype and antibiotic susceptibility

Isolates belonging to ST131 were most associated with ESBL carriage and multiple antibiotic resistance (Table 2). In both ESBL-positive and ESBL-negative isolates of ST131, antibiotic resistance was above the mean for trimethoprim, ciprofloxacin, amoxicillin/clavulanic acid and gentamicin (Table 2). Isolates belonging to ST10 also showed appreciable antibiotic resistance, with 15% carrying ESBLs and levels of non-susceptibility to trimethoprim,

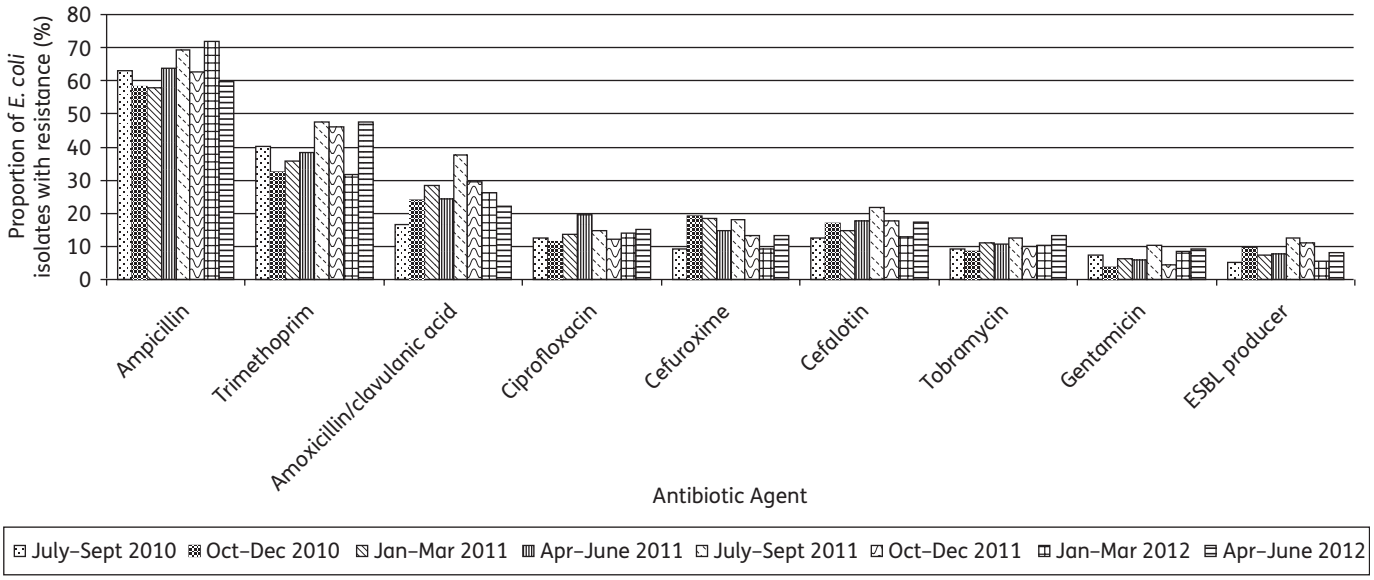

Figure 2. Proportion of isolates ($n=770$) with non-susceptibility to individual antibiotic agents, July 2010–June 2012.

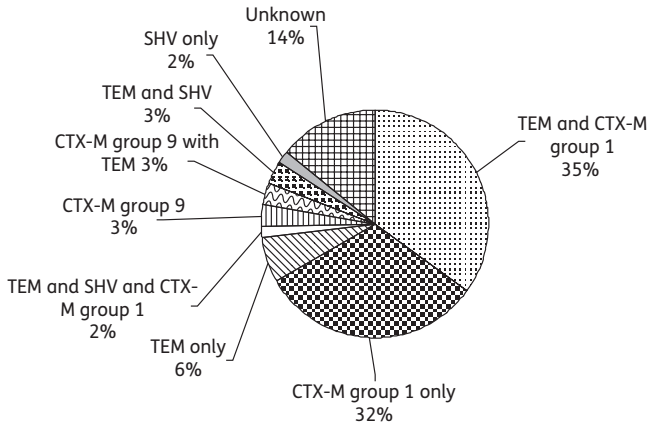

Figure 3. PCR detection of genes encoding common ESBLs (TEM, SHV and CTX-M groups 1, 2 and 9) ($n=63$ isolates).

ciprofloxacin and gentamicin above the mean. Non-susceptibility to trimethoprim was associated with isolates of ST69, an association that has been reported previously.[11] ESBL-positive isolates were a single predominant AFLP type ($n=39$, 62%), confirmed by MLST to be ST131 (Figure 4). CTX-M group 1 enzymes were most commonly identified in these isolates ($n=29$), along with the TEM enzyme ($n=14$). Another 13 STs were associated with ESBL carriage: ST648 ($n=4$), ST10 ($n=4$), ST73 ($n=3$), ST38 ($n=3$), two isolates of ST617 ($n=2$), and one of each of the following ST59, ST95, ST69, ST104, ST155 ST404, and ST767. One isolate had an unknown MLST.

Relationship between genotype and presumed source of infection

Information submitted by the participating laboratories regarding the presumed source of bacteraemia was limited; only 249 (32%) isolate submissions provided this information. In addition, the information submitted may have been based only on the clinical details sent with the blood culture request (e.g.?UTI, right flank pain). Therefore, information submitted to the mandatory *E. coli* surveillance scheme about the presumed source of infection following medical assessment was considered to be more objective and was used to assess the relationship between genotype and presumed source of infection. Such data were available for isolates submitted by three laboratories [Leeds ($n=97$ reports); Sheffield ($n=24$ reports) and Doncaster and Bassetlaw ($n=24$ reports)]. The urinary tract was the presumed source of infection in 39% of cases, compared with a hepatobiliary source in 18% and a gastrointestinal source (non-hepatobiliary) in 10%. ST73 was associated with 23%, 19% and 6% of urinary, hepatobiliary and gastrointestinal sources, respectively; corresponding rates for ST131 were 19%, 15% and 13%.

Relationship between genotype and presumed onset of bacteraemia

The onset of bacteraemia may be categorized as community onset (diagnosis within 48 h of admission to hospital) or hospital onset (diagnosis >48 h after hospital admission).[12,13] A large proportion of individuals, however, require frequent contact with hospital/healthcare establishments and may thus encounter interventions that predispose them to developing *E. coli* bacteraemia. A subset of surveillance data submitted by Leeds Teaching Hospitals Trust (LTHT) ($n=101$, July 2010–June 2011) was assessed using the traditional definition of community onset and by interrogation of hospital admission data. More cases of *E. coli* bacteraemia were categorized as community onset when the traditional definition was applied (65%) compared with 47% cases from patients who had no record of hospital admission prior to the *E. coli*-positive blood culture.

Table 2. Antibiotic non-susceptibility according to sequence type (ST)

	ST131		ST131 ESBL positive		ST131 ESBL negative		ST73		ST69		ST95		ST12		ST127		ST404		ST10		ST141	
Resistance phenotype/antibiotic agent (average quarterly proportion non-susceptible)	*n* 129	%	*n* 39	%	*n* 90	%	*n* 143	%	*n* 81	%	*n* 73	%	*n* 31	%	*n* 27	%	*n* 27	%	*n* 26	%	*n* 20	%
ESBL positive (8%)	39	**30**	39	–	0	–	3	2	1	1	1	1	0	0	0	0	1	4	4	15	0	0
Ampicillin[a] (63%)	108	**84**	39	**100**	69	**77**	89	62	62	**77**	35	48	18	58	13	48	14	52	17	65	10	50
Cefalotin[a] (16%)	60	**47**	39	**100**	19	**21**	13	9	5	6	4	5	4	13	2	7	1	4	3	12	0	0
Amoxicillin/clavulanic acid (26%)	68	**53**	35	**90**	32	**36**	38	27	9	11	11	15	9	29	3	11	2	7	6	23	4	20
Cefuroxime[a] (14%)	48	**37**	39	**100**	15	17	18	13	2	2	3	4	3	10	2	7	1	4	2	8	0	5
Aztreonam[a] (6%)	28	**22**	38	**97**	2	2	2	1	0	0	0	0	0	0	0	0	1	4	1	4	0	0
Cefotaxime[a] (8%)	41	**32**	38	**97**	2	2	5	3	0	0	1	1	0	0	0	0	1	4	1	4	0	0
Ceftazidime[a] (5%)	40	**31**	38	**97**	1	1	3	2	0	0	0	0	0	0	0	0	1	4	1	4	0	0
Trimethoprim (40%)	81	**63**	28	**72**	52	**58**	37	26	46	**57**	20	27	11	35	1	4	8	30	12	46	4	20
Ciprofloxacin (14%)	75	**58**	38	**97**	36	**40**	5	3	2	2	2	3	0	0	0	0	1	4	4	15	0	0
Tobramycin (11%)	56	**43**	35	**90**	21	**23**	4	3	4	5	1	1	0	0	1	4	0	0	3	12	1	5
Gentamicin (7%)	33	**26**	17	**44**	15	**17**	3	2	3	4	1	1	0	0	1	4	0	0	3	12	1	5
Amikacin (<1%)	2	2	2	5	0	0	0	0	0	0	0	0	0	0	2	7	0	0	0	0	0	0

Numbers in bold type indicate those proportions that are above the average values for non-susceptibility to the antibiotic agents.
[a]Interpretation of the unaltered MIC values reported directly from the VITEK2 system rather than susceptibility that has been altered for compatibility with ESBL carriage. No isolates belonging to the STs listed had resistance to ertapenem, meropenem or tigecycline.

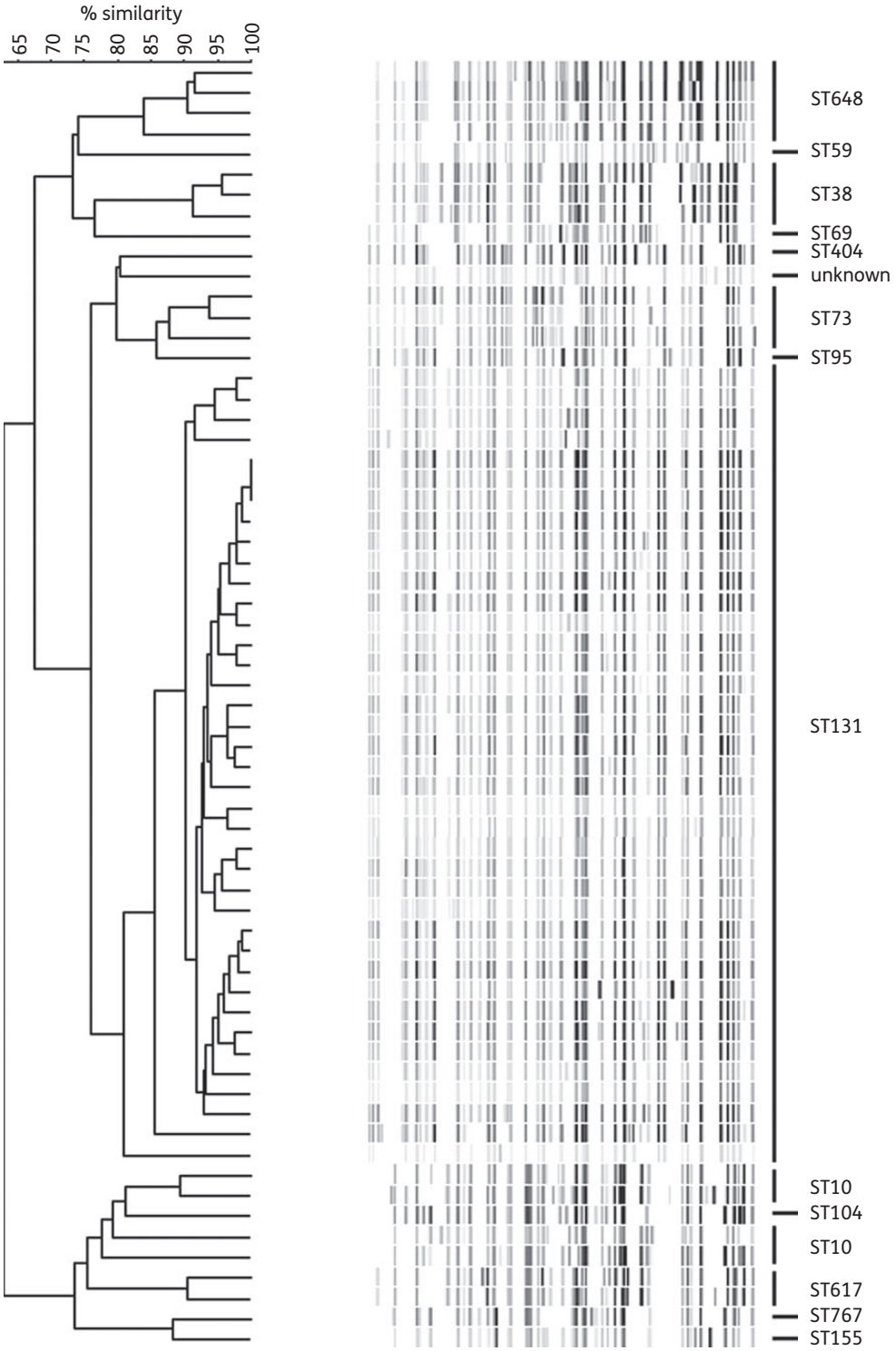

Figure 4. Dendrogram of the AFLP types associated with isolates positive for ESBL production ($n=63$).

When admission records were interrogated for those patients at LTHT who had an *E. coli* bacteraemia ($n=195$), 56% ($n=110$) of patients had a record of hospital admission within 4 weeks of a blood culture positive for *E. coli*. Thirty-seven STs were identified from the 195 isolates submitted by LTHT. Isolates from patients with a hospital admission represented 30 STs, whereas those with no record of hospital admission represented 23 STs. The majority of STs were identified in patients both with and without previous hospital admission; however, there were four STs (ST58, ST357, ST372 and ST405) associated only with patients without

hospital admission, compared with one ST (ST648) specific to patients with previous hospital admission.

Three STs appeared to be more frequently associated with patients without hospital admission ($n=85$): ST73, 18% ($n=15$); ST95, 11% ($n=9$) and ST69, 12% ($n=10$), compared with 15% ($n=17$), 6% ($n=7$) and 10% ($n=11$), respectively, in patients with a record of hospital admission; however, these associations were not statistically significant ($P>0.4$, χ^2 test). In those patients with evidence of previous hospital admission 22% ($n=24$) of isolates were ST131, compared with 16% ($n=14$) in patients with no record of hospital admission; this was not a statistically significant difference ($P=0.45$, χ^2 test). Even though ST12 was preponderant among those patients with hospital admission (7%, $n=8$) compared with those without hospital admission (2%, $n=2$), this association was not significant ($P=0.1$, Fisher's exact test). In summary, although the total number of isolates tested was large, the number of isolates becomes smaller on analysis of subgroups, such as that based on ST, which hinders statistical analysis and makes it difficult to draw accurate conclusions.

Discussion

These data represent a unique, contemporaneous picture of the epidemiology of *E. coli* bacteraemia in the Yorkshire and Humber NHS region over a 2 year period. Isolates were tested from multiple centres and were not selected based on patient group or resistance phenotype. As *E. coli* isolated from blood cultures represent invasive disease, the issue of sampling bias experienced by other clinical syndromes, such as a UTI, is avoided. This prospective surveillance took place at a time of high incidence of *E. coli* bacteraemia in England,[4] when the emergence of multiresistant Gram-negative bacteria, including those with resistance to carbapenem agents, was a serious threat to health. As such, this has been made a priority by the Chief Medical Officer.[14]

Approximately 10% of *E. coli* isolates from blood cultures were tested from each participating laboratory, a figure which was thought to be a fair representation of isolates circulating in the region. The surveillance scheme was voluntary, with no incentive or reimbursement for participation. Attempts were made to contact each laboratory if isolates were noted to be missing per month, and some isolates were sent retrospectively.

All 14 diagnostic microbiology laboratories in the region participated in the surveillance; however, participation was not equal. When compared with the number of *E. coli* bacteraemia cases reported to the mandatory surveillance scheme, six laboratories (Barnsley, Hull, Mid Yorkshire, Rotherham, Scarborough and Sheffield) had submitted <10% of total isolates to the regional surveillance scheme. Unequal participation may be explained by (i) isolates being sent from only one of two hospital sites when the mandatory bacteraemia figures included both hospital sites (Sheffield); (ii) probable underestimation of the number of *E. coli* bacteraemia cases at the beginning of the surveillance (Mid Yorkshire and Scarborough); and (iii) possible underparticipation in the surveillance due to pressure on the laboratory, such as staff and/or costs, associated with the surveillance (Barnsley, Hull, Rotherham).

These variations are unlikely to cause bias as the prevalence of antibiotic resistance and/or genotype did not vary significantly between laboratories in the region; however, in order to be a fair representation throughout the region the number of isolates submitted to the regional surveillance are likely to be amended to reflect the actual numbers of *E. coli* bacteraemia cases reported to the mandatory surveillance scheme.

Standardized, centralized susceptibility testing showed no sustained change in antimicrobial resistance over time and these data were reflected in the susceptibility information submitted by the original laboratories. The ESBL positivity rate remained constant (mean 8%). Isolates carrying genes encoding ESBLs were distributed across the region with no particular prevalence in a single centre. Notably, we found that resistance to amoxicillin/clavulanic acid was at least twice as common as measured ESBL expression. Surveillance of *E. coli* based in the Nottingham area (2008–09) observed comparable levels of non-susceptibility to trimethoprim (41%), lower rates of resistance to ampicillin (45% versus 63%) and higher rates of resistance to ciprofloxacin (21% versus 14%) and the first-generation cephalosporins tested in each study (cefradine, 26% versus cefalotin, 16%). Carriage of CTX-M ESBL genes was higher (11%) compared with the present study (6%).[15]

Although PFGE is a discriminatory method used to genotype *E. coli*,[16] it was not considered practical to genotype a large number of isolates from a regional surveillance scheme using this technique. AFLP proved to be a reproducible, high-throughput genotyping method that had good correlation with MLST. Investigation of the O25b subtype or presence of the FimH30 allele was not done but these are other methods of typing to be considered.

As may be expected from a large geographical area such as the Yorkshire and Humber region, circulating *E. coli* strains were diverse, with 106 different AFLP types and at least 77 STs. No changes in the prevalence of any one genotype occurred during the study period. Four lineages of *E. coli* (ST73, ST131, ST69 and ST95), which are common strains of *E. coli* circulating in England known to be associated with UTI,[11,15] accounted for 55% of the isolates in the present study. In a collection of 150 uropathogenic *E. coli* isolates from a cohort of elderly patients (≥70 years) in the Nottingham area (October 2008–June 2009), the largest proportion of isolates were ST131 (22%), followed by ST73 (11%) and ST69 (9%).[15] In a collection of 300 uropathogenic *E. coli* from north-west England (June 2007 and June 2009), the most common STs were ST73 (17%), ST131 (12%), ST69 (9%), ST95 (6%), ST10 (4%) and ST127 (3%).[11]

Isolates of ST131 are associated with the carriage of ESBLs and multiple resistance,[11,15,17] and this lineage is therefore of particular interest.[18] The analysis of ST131 is often biased by location (i.e. results from a single centre), by situation (i.e. isolates collected during an outbreak), by resistance phenotype (i.e. ESBL positive) or by population (i.e. elderly or urinary isolates). The work presented here describes a collection of ST131 isolates from a non-selected population, albeit from cases of bacteraemia, dispersed over a wide geographical area of northern England. We identified high rates of resistance to ampicillin in ST131 (84%) but resistance to trimethoprim (63%) and ciprofloxacin was lower (58%) than previously reported.[15] ST131 was associated with a high $bla_{\text{CTX-M}}$ carriage rate (23%), which is comparable to the rate reported elsewhere (26%).[15] We identified isolates of ST131 that did not carry sequences encoding an ESBL but were nonetheless resistant to multiple antimicrobial agents: 77%, 58%, 40% and 17% were resistant to ampicillin, trimethoprim, ciprofloxacin and gentamicin, respectively. Nine isolates (14%) that were suggested to be positive for ESBL carriage by the VITEK2 system were negative when tested by PCR for common ESBL genes. Molecular detection of

plasmid-mediated *ampC* β-lactamase genes may help to confirm or identify isolates with this particular phenotype, although the number of isolates with the AmpC phenotype was low (0.5%). Given the above-average expression of resistance to ciprofloxacin in ST131, investigation of the underlying resistance mechanism is also of interest. The use of whole genome sequencing would enable detection of the presence of single nucleotide polymorphisms, in particular those relating to quinolone resistance.

Isolates tested in the present study were collected from hospitalized patients with invasive infections; however, these isolates may not represent the same strains that are circulating and causing non-invasive infections in the community or strains associated with asymptomatic carriage. It is necessary to identify those strains that are circulating to identify those strains that are specifically associated with invasive infection to determine whether these strains are more virulent; however, information comparing the natural population of *E. coli* found in the healthy population with *E. coli* associated with disease is limited. Further comparison could include *E. coli* strains circulating in the community versus a hospital population.

Commonly, two-thirds of cases of *E. coli* bacteraemia are classified as community onset using the 48 h rule;[12,13] however, using such a definition to categorize *E. coli* bacteraemia may be misleading. In the present study we used a broader definition for categorization of the onset of infection, and determined that a higher proportion (56%) of *E. coli* bacteraemia in LTHT were likely to be healthcare associated. This corresponds with data from Enoch *et al.*,[19] who identified that 70% of *E. coli* bacteraemias are likely to be healthcare associated.

The recent increase in the number of reports of *E. coli* bacteraemia may be related to (i) the ageing population associated with increased morbidity; (ii) advances in medical care leading to increases in the occurrence of invasive procedures such as catheterization; and (iii) an increase in the number of resistant *E. coli*.[19,20] The proportion of *E. coli* bacteraemias predicted to be preventable is <20%,[12,13,19] where preventable is defined as 'an unintended bacteraemia that was caused by healthcare management or as a failure to meet the reasonably expected standard of care.'[19] The National Safety Thermometer has been recently introduced, and aims to protect patients in the NHS from avoidable harm, including catheter-associated UTIs.[21] Given the current rates of *E. coli* bacteraemia, a 20% reduction would still result in 8000 fewer cases per annum across England. Given that 30 day mortality rates following *E. coli* bacteraemia have been reported to be 17%–32%,[22] such a reduction could result in 2000 fewer deaths per annum associated with such infections in England alone. This would still leave >32 000 cases per year, and other suitable interventions, in addition to improved catheter care, are required to achieve further reductions in the number of *E. coli* bacteraemia cases. Information about the presumed source or likely onset of infection may contribute to the identification of other suitable interventions. Unfortunately, owing to limited data submitted with each isolate, it was difficult to evaluate an association between *E. coli* genotype and presumed source or probable onset of infection in the present study. Given that the mandatory surveillance dataset is incomplete, data collection relating to presumed source of infection or probable onset of infection needs to be improved to enable statistical analysis of a large, reliably collected dataset.

In summary, this prospective surveillance of invasive *E. coli* isolates affecting a large regional population has confirmed the circulation of two dominant strains of *E. coli*: ST73 and ST131. It is reassuring that the prevalence of *E. coli* carrying sequences encoding ESBLs remains <10% and that carbapenemase-producing organisms were not frequently identified. However, we note the variable and concerning prevalence of resistance to amoxicillin/clavulanic acid, given the relative commonality of *E. coli* bacteraemia and empirical use of this antibiotic. These data highlight the need for continuing monitoring for the emergence of resistance and the potential requirement for modification of empirical prescribing. Interventions are needed to address the large health burden represented by *E. coli* bacteraemia. Prospective surveillance schemes are well placed to determine the effectiveness of such interventions.

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Transparency declarations

None to declare.

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