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UK epidemic *Escherichia coli* strains A–E, with CTX-M-15 β-lactamase, all belong to the international O25:H4-ST131 clone

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***Objectives*: Uropathogenic and invasive *Escherichia coli* O25:H4-ST131 isolates producing CTX-M-15 extended-spectrum β-lactamase (ESBL) enzymes have recently been shown to be disseminated across the globe. In the UK, many CTX-M-15 ESBL-producing *E. coli* strains have been previously defined as belonging to the epidemic strains A–E, as determined by PFGE. The present study was carried out to define the relationship between these two groups of pathogenic *E. coli*.**

***Methods*: Multilocus sequence typing and PFGE were used for molecular characterization of a collection of 61 ESBL-producing *E. coli* isolates from across the UK.**

***Results*: Strains A to E all belonged to the ST131 clone, further underscoring the epidemiological importance of this lineage.**

***Conclusions*: The future spread of the ST131 clone, and its UK variants, should be monitored closely and the pathogenic mechanisms explaining their success should be investigated.**

Keywords: multilocus sequence typing, MLST, molecular epidemiology, uropathogenic *Escherichia coli*, UPEC, extended-spectrum β-lactamases, PFGE

Introduction

Escherichia coli is one of the two most common causes of bacteraemia in England and Wales[1] and causes the majority of urinary tract infections (UTIs) worldwide. The widespread use of antibiotics has selected for drug-resistant strains, with current concern centred on those with fluoroquinolone resistance and extended-spectrum β-lactamases (ESBLs). ESBL-producing *E. coli*—principally with CTX-M-type enzymes, particularly CTX-M-15—increasingly cause UTIs in the community and long-term care and hospital settings in the UK.[2,3] Five major epidemic *E. coli* strains (A–E) with CTX-M-15 β-lactamase were identified in the UK, based on their PFGE banding patterns, along with many clonally diverse producers of the enzyme. Epidemic strain A is nationally distributed, being particularly dominant in parts of Lancashire, Shropshire, Hampshire and Ulster.[2]

Most strain A–E isolates are resistant to oxyimino-cephalosporins and, through co-production of OXA-1 β-lactamase, also to β-lactamase inhibitor combinations; in addition, most isolates are resistant to fluoroquinolones, trimethoprim, tetracyclines and amikacin. Strain A is susceptible to gentamicin, the others are generally resistant. A few strain A isolates in the north-west (NW) of England possess plasmid-mediated AmpC β-lactamase and consequently have enhanced resistance to cephamycins and cephalosporin/β-lactamase inhibitor combinations.[4]

This rise in *E. coli* with CTX-M enzymes is not unique to the UK and several recent studies using multilocus sequence typing (MLST) have identified a globally disseminated O25:H4-ST131

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clone with CTX-M-15 β-lactamase, causing significant morbidity and mortality.[5–7] The aim of the work presented here was to study the genetic relationship of the UK epidemic strains A–E, which are known to be serotype O25, with the international O25:H4-ST131 *E. coli* lineage.

Materials and methods

Bacterial isolates

Thirty-two *E. coli* isolates with CTX-M β-lactamases were selected from among those referred to the Health Protection Agency Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL) from UK centres: 24 had previously been assigned to the UK epidemic lineages A–E based on PFGE; the remaining eight were diverse. These 32 isolates were compared, using MLST and PFGE, with 29 *E. coli* isolates that had been previously reported as part of a larger group from the NW region of England.[6] These latter 29 isolates comprised 10 *E. coli* from hospital A and 19 from hospital B (Table 1) and were chosen to represent the diversity of sequence types (STs) circulating in the region, with ST131 dominating.[6] They were recovered from separate patients with UTI, between November 2004 and October 2005.[6]

Typing of isolates

Isolation of DNA and MLST analysis were performed as described previously.[6] Isolates were also compared by PFGE separation of *Xba*I-digested genomic DNA, as described previously.[8] Profiles were compared using Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium).

Results

Characterization of E. coli isolates by MLST

MLST analysis identified 12 different STs among the total of 61 isolates studied (Table 1); of these 12 STs, 10 occurred only once in the data set and 2 (ST683, ST684) were novel.

Among the 32 isolates referred to ARMRL, all of the 24 previously assigned by PFGE to the five major UK epidemic strains (A–E) belonged to ST131, as did 2 further isolates, distinct by PFGE. The remaining six strains from the ARMRL collection, all with distinct PFGE patterns, belonged to STs unrelated to ST131 (Table 1).

Among the 29 isolates from NW England, the predominant lineage was ST131; all of the 10 isolates from hospital A were identified as ST131, as were 12/19 among those from hospital B.

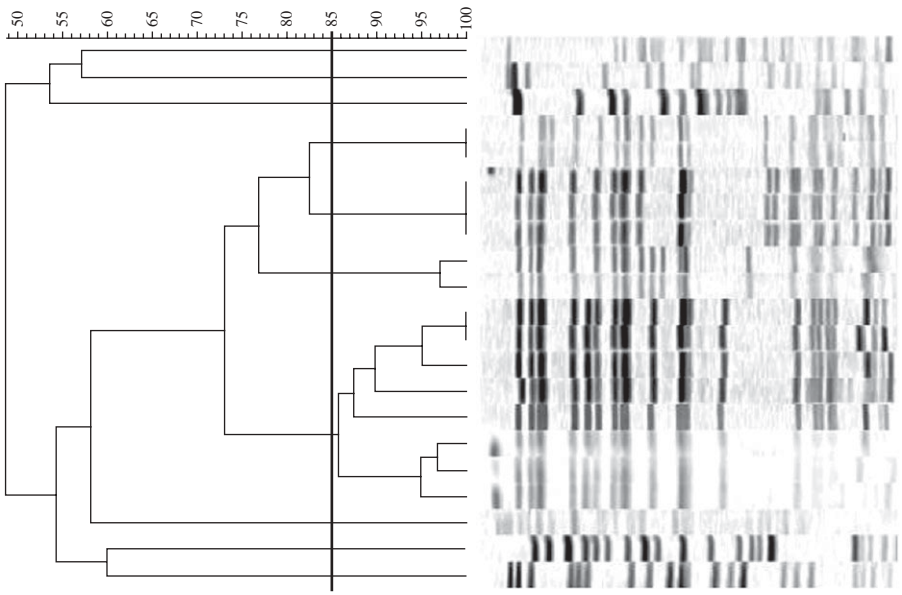
PFGE characterization of E. coli isolates

PFGE was performed on the 29 isolates from NW England. All of the 10 ST131 *E. coli* isolates from hospital A, 7 of them with both AmpC and group 1 CTX-M enzymes, had the PFGE profile characteristic of the UK epidemic strain A (Table 1). The 12 ST131 isolates from hospital B comprised two distinct PFGE types (2 and 3) (Table 1 and Figure 1). The seven non-ST131

Table 1. Comparison of MLST STs and PFGE types of 61 *E. coli* isolates from the UK

Resistance phenotype	Resistance genotype[a]	Number of isolates	Source	Sequence type	PFGE type
ESBL	CTX-M-15	11	ARMRL	131	A
ESBL	CTX-M group 1	3	ARMRL	131	B
ESBL	CTX-M group 1	2	ARMRL	131	C
ESBL	CTX-M-15	5	ARMRL	131	D
ESBL	CTX-M group 1	3	ARMRL	131	E
ESBL	CTX-M group 1	2	ARMRL	131	unique
ESBL	CTX-M group 1	1	ARMRL	167	unique
ESBL	CTX-M group 1	1	ARMRL	405	unique
ESBL	CTX-M group 1	1	ARMRL	648	unique
ESBL	CTX-M groups 1 (1) and 9 (1)	2	ARMRL	683	unique
ESBL	CTX-M group 1	1	ARMRL	684	unique
ESBL and AmpC	CTX-M group 1 and CIT type (7)	7	hospital A	131	A
AmpC	CIT type (3)	3	hospital A	131	A
ESBL	CTX-M group 1 (6)	10	hospital B	131	2
ESBL	CTX-M group 1 (1)	2	hospital B	131	3
ESBL	ND	1	hospital B	23	unique
ESBL	CTX-M group 9 (1)	1	hospital B	95	unique
ESBL	ND	1	hospital B	95	unique
ESBL	ND	1	hospital B	394	unique
ESBL	CTX-M group 1 (1)	1	hospital B	410	unique
ESBL	ND	1	hospital B	457	unique
ESBL	ND	1	hospital B	458	unique

ARMRL, Antibiotic Resistance Monitoring and Reference Laboratory; ND, not determined.
[a]Numbers in parentheses indicate the number of organisms tested.



ST	CTX-M/PFGE	Source
23	Unique	Hospital B
394	Unique	Hospital B
410	Unique	Hospital B
131	CTX-M-Gp1/2	Hospital B
131	CTX-M-Gp1/2	Hospital B
131	CTX-M-15/D	ARMRL
131	CTX-M-15/D	ARMRL
131	CTX-M-15/D	ARMRL
131	CTX-M-Gp1/3	Hospital B
131	CTX-M-Gp1/3	Hospital B
131	CTX-M-15/A	ARMRL
131	CTX-M-15/A	ARMRL
131	CTX-M-15/A	ARMRL
131	CTX-M-15/A	ARMRL
131	CTX-M -ve/A	Hospital A*
131	CTX-M-15/A	ARMRL
131	CTX-M-15/A	ARMRL
131	CTX-M-15/A	ARMRL
457	Unique	Hospital B
458	Unique	Hospital B
95	Unique	Hospital B

Figure 1. Dendrogram showing the relationship of ESBL-producing *E. coli* isolates from the NW region of England to representatives of the UK epidemic strains A (CTX-M-15/A) and D (CTX-M-15/D). Genotypic identification of NW England isolates was carried out to CTX-M group (Gp) level. PFGE profiles were generated by *Xba*I digestion of genomic DNA. The dendrogram was constructed using the Dice coefficient. The thick black line indicates 85% banding pattern similarity, used to define strains. The asterisk indicates that the strain is not an ESBL producer but carries an AmpC determinant.

isolates from hospital B showed unique PFGE profiles within this data set (Table 1); six of these are shown in Figure 1.

Discussion

The global rise of *E. coli* producing CTX-M β-lactamases, principally CTX-M-15, as agents of UTIs, often has major public health implications.[3] In the UK, this rise became apparent from 2003 when it was recognized that, aside from many diverse producers, there were five major strains (A–E) defined by PFGE. Members of these strains were more closely related to each other than diverse producers and all were of serotype O25.

The use of MLST in the present study showed that the UK epidemic strains A–E clustered with the globally disseminated O25:H4-ST131 lineage already reported from Europe, Africa, Asia and North America.[5–7] It was also shown that the previously reported ST131 isolates from NW England, some of them with both AmpC and group 1 CTX-M enzymes, belonged to the PFGE-defined strain A.

The finding that isolates of ST131 encompassed multiple PFGE profiles, including the A–E lineages as well as other minor types, suggests that genomic diversification has occurred within this lineage, presumably reflecting DNA rearrangements, mutations and the integration of insertion sequences and other mobile elements. The reported diversity in virulence determinants carried by ST131 isolates[5,9] further supports the suggestion of genomic plasticity, though it is possible that some of these factors may be encoded by extrachromosomal DNA. Nevertheless, although recombination is occurring in this lineage, it has not resulted in breakdown of the clonal structure.

MLST provides a robust and relatively simple means of unambiguously defining the relatedness of *E. coli* clones, though additional analyses, including PFGE, may be useful for increasing discrimination within lineages. Moreover, many antibiotic resistance genes in these strains are carried on plasmids, which have been shown to vary within and between different lineages.[10] Further characterization of plasmids encoding CTX-M-15 enzymes in ST131 and non-ST131 hosts is likely to be informative and should be employed for a deeper understanding of the genetic relationships among these *E. coli* strains. In addition, a full investigation of the antibiotic and virulence determinants carried by representatives of the O25:H4-ST131 lineage should be a priority for those concerned with the disease caused by this widely disseminated organism. Such full characterization of these antibiotic-resistant *E. coli* clones may facilitate development of intervention and prevention strategies to control their spread in community and hospital environments.

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References

1. Health Protection Agency. *Trends in Antimicrobial Resistance in England and Wales, 2004–2005*. HPA, London, UK, 2006. http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1196942165725?p=1158945066450.

2. Woodford N, Ward ME, Kaufmann ME *et al*. Community and hospital spread of *Escherichia coli* producing CTX-M extended-spectrum β-lactamases in the UK. *J Antimicrob Chemother* 2004; **54**: 735–43.

3. Livermore DM, Canton R, Gniadkowski M *et al*. CTX-M: changing the face of ESBLs in Europe. *J Antimicrob Chemother* 2007; **59**: 165–74.

4. Woodford N, Reddy S, Fagan EJ *et al*. Wide geographic spread of diverse acquired AmpC β-lactamases among *Escherichia coli* and *Klebsiella* spp. in the UK and Ireland. *J Antimicrob Chemother* 2007; **59**: 102–15.

5. Clermont O, Lavollay M, Vimont S *et al*. The CTX-M-15-producing *Escherichia coli* clone belongs to a highly virulent B2 phylogenetic subgroup. *J Antimicrob Chemother* 2008; **61**: 1024–8.

6. Lau SH, Reddy S, Cheesbrough J *et al*. A major uropathogenic *Escherichia coli* strain identified in the North West of England by multilocus sequence typing. *J Clin Microbiol* 2008; **46**: 1076–80.

7. Coque TM, Novais A, Carattoli A *et al*. Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum β-lactamase CTX-M-15. *Emerg Infect Dis* 2008; **14**: 195–200.

8. Kaufmann ME. Pulsed-field gel electrophoresis. In: Woodford N, Johnson AP, eds. *Molecular Bacteriology: Protocols and Clinical Applications*. Totowa, NJ, USA: Humana Press, 1998.

9. Karisik E, Ellington MJ, Livermore DM *et al*. Virulence factors in *Escherichia coli* with CTX-M-15 and other extended-spectrum β-lactamases in the UK. *J Antimicrob Chemother* 2008; **61**: 54–8.

10. Karisik E, Ellington MJ, Pike R *et al*. Molecular characterization of plasmids encoding CTX-M-15 β-lactamases from *Escherichia coli* strains in the United Kingdom. *J Antimicrob Chemother* 2006; **58**: 665–8.