J Antimicrob Chemother 2015; **70**: 2223–2227
doi:10.1093/jac/dkv114 Advance Access publication 9 May 2015

Journal of Antimicrobial Chemotherapy

Emergence and spread of O16-ST131 and O25b-ST131 clones among faecal CTX-M-producing *Escherichia coli* in healthy individuals in Hunan Province, China

Yi-Ming Zhong[1], Wen-En Liu[1]*, Xiang-Hui Liang[1], Yan-Ming Li[1], Zi-Juan Jian[1] and Peter M. Hawkey[2,3]

[1]Department of Clinical Laboratory, Xiangya Hospital, Central South University, Changsha 410008, Hunan Province, China; [2]PHE Public Health Laboratory Birmingham, Heart of England NHS Foundation Trust, Birmingham B9 5SS, UK; [3]Institute of Microbiology and Infection, University of Birmingham, Birmingham B15 2TT, UK

*Corresponding author. Tel: +86-731-84327437; Fax: +86-731-84327332; E-mail: wenenliu@163.com

Received 19 December 2014; returned 14 January 2015; revised 3 April 2015; accepted 4 April 2015

Objectives: The objectives of this study were to determine CTX-M-producing *Escherichia coli* ST131 strain prevalence in stool specimens from healthy subjects in central China and to molecularly characterize clonal groups.

Methods: From November 2013 to January 2014, stool specimens from healthy individuals in Hunan Province were screened for ESBL-producing *E. coli* using chromogenic medium and CTX-M genotypes and phylogenetic groups were determined. ST131 clonal groups were detected by PCR and characterized for antibiotic resistance, *fimH*, *gyrA* and *parC* alleles, plasmid-mediated quinolone resistance determinants, virulence genotypes and PFGE patterns.

Results: Among 563 subjects, 287 (51.0%) exhibited the presence of faecal ESBL-producing *E. coli*, all of which produced CTX-M enzymes. The most common CTX-M genotypes were CTX-M-14 (48.4%), CTX-M-15 (27.5%) and CTX-M-27 (15.0%). Of the 287 CTX-M-producing isolates, 32 (11.1%) belonged to the ST131 clone. O16-ST131 isolates were dominant (75%) and contained the *fimH*41 allele. The remaining eight (25%) ST131 isolates were of the O25b subgroup and contained *fimH*30 or *fimH*41. Ciprofloxacin resistance was found in 100% of the O25b-ST131 isolates, whereas only 8% of the O16-ST131 isolates were resistant. All of the O25b-ST131 isolates except one showed *gyrA*1AB and *parC*1aAB mutations; most of the O16-ST131 isolates had *gyrA*1A and *parC*1b mutations. The virulence genotypes of O16-ST131 resembled those of the O25b-ST131 isolates. The 32 ST131 isolates formed one large group at the 64% similarity level. They comprised 15 PFGE groups (defined at ≥85% similarity).

Conclusions: O16-ST131 isolates have emerged as the predominant type of ST131 isolate in faecal CTX-M-producing *E. coli* in healthy individuals in China.

Keywords: *E. coli*, faecal carriage, ESBLs, genotypes

Introduction

CTX-M-producing *Escherichia coli* are the most common type of ESBL-producing *E. coli*, causing hospital-associated and community infections with extensive dissemination in the environment.[1] There are few data on the community carriage of CTX-M ESBL-producing *E. coli* in China[2] and two studies describe greatly different carriage rates: 7% and 50.5%.[3,4] ST131 is the predominant extraintestinal pathogenic *E. coli* (ExPEC) clone and frequently carries CTX-M.[5]

The gut is the main reservoir of ExPEC,[5] although there are only a few studies of colonization by ST131, with differences in carriage rates.[5,6] Studies on ST131 carriage, with one exception,[7] either failed to distinguish the O16-ST131 clade from the more common O25b-ST131[8] or missed the O16 subgroup.[4] Therefore, further studies are necessary to characterize O16-ST131 colonization of the digestive tracts of healthy subjects.

The aim of this study was to determine the prevalence and genotype distribution of CTX-M-producing *E. coli* and the

prevalence of ST131 and the O16/O25b subgroups in healthy subjects across a province in China.

Methods

Participants

A cross-sectional survey was conducted on healthy subjects who had routine health screening at community health service centres (CHSCs) in Hunan Province between November 2013 and January 2014. A total of 11 CHSCs were selected using a multistage stratified sampling method. In the first stage, Hunan Province was divided into four different areas (northern, eastern, western and southern Hunan) according to economic development levels and geographical location. In the second stage, 11 counties were identified, representative by population, and a single CHSC from each was selected for study. Healthy individuals (≥18 years of age) who were examined at the 11 CHSCs from November 2013 to January 2014 were given an attendee list number, which was used to identify participants on the day of visit. Every day, one individual from each centre was selected by matching one computer-generated random number to the attendance list at the centre and was invited to participate in the study. The proportion of refusals was 2.3%. Individuals were excluded if they had digestive tract diseases or had been exposed to a hospital environment or treated with antibiotics within 3 months prior to sample collection. The study was approved by the Ethics Committee of the Xiangya Hospital of Central South University (reference number ECCT 201310096). Participants provided fresh stools and informed consent on the day of visit.

ESBL-producing *E. coli* identification, CTX-M type and phylogenetic group

Faecal samples were plated within 24 h onto chromogenic ESBL selective medium (ChromID, bioMérieux, Marcy-l'Étoile, France). *E. coli* were identified using API 20E (bioMérieux). Three to five pure colonies were examined for ESBL production by the double-disc synergy test according to CLSI guidelines.[9]

ESBL-producing *E. coli* isolates were investigated for the presence of $bla_{\text{CTX-M}}$ genes by multiplex PCR and sequencing as previously described.[10,11] The phylogenetic groups of *E. coli* were determined by quadruplex PCR.[12]

Identification of ST131

The ST131 clonal group and O16/O25b variants were identified as previously described.[13] MLST was used to resolve discrepant results (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli).

Antimicrobial susceptibility testing

For all of the ST131 isolates, three to five pure colonies were tested for antibiotic susceptibility by disc diffusion and the results were interpreted according to the breakpoints recommended by the CLSI.[9] The following antimicrobial agents were tested: ampicillin, ampicillin/sulbactam, cefazolin, ceftriaxone, ceftazidime, gentamicin, ciprofloxacin, piperacillin/tazobactam, imipenem, minocycline, nitrofurantoin, chloramphenicol and trimethoprim/sulfamethoxazole. *E. coli* ATCC 25922 was used as a quality control strain. Intermediate susceptibility was considered as resistant. The resistance score was calculated as previously described.[7] Isolates that were considered MDR were those resistant to at least three antimicrobial categories.

Molecular characterization of ST131

The alleles of *fimH*, *gyrA* and *parC*, the *H*30-Rx subclones, plasmid-mediated quinolone resistance (PMQR) determinants [*qnrA*, *qnrB*, *qnrC*, *qnrS*, *aac(6′)-Ib-cr* and *qepA*], virulence factor genes ($n=32$) and virotypes were determined as previously described.[14–17]

ST131 isolates were subjected to PFGE analysis using XbaI digestion. A PFGE dendrogram was constructed with BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium) according to the unweighted pair group method based on Dice coefficients. Isolates with a Dice similarity index ≥85% were considered to belong to the same PFGE group.

Statistical analysis

Comparisons of proportions were performed using Fisher's exact test. Comparisons of antibiotic resistance scores were performed using the Mann–Whitney *U*-test. A P value <0.05 was considered to be statistically significant.

Results

Prevalence and genotype distribution of faecal CTX-M-producing *E. coli*

A total of 563 faecal samples, one sample from each participant, were analysed. The age range of the individuals (299 males and 264 females) recruited into the study was 18–83 (mean 44.3) years. Of the 563 samples collected, 287 were identified as ESBL-producing *E. coli*, with all strains harbouring $bla_{\text{CTX-M-type}}$ genes. The prevalence of CTX-M-producing *E. coli* was thus 51.0% (287/563 samples). Of the 287 isolates, 139 (48.4%) produced CTX-M-14, 79 (27.5%) CTX-M-15, 43 (15.0%) CTX-M-27, 11 (3.8%) CTX-M-65, 10 (3.5%) CTX-M-3, 2 (0.7%) CTX-M-24, 2 (0.7%) CTX-M-104 and 1 (0.3%) CTX-M-101.

Phylogenetic analysis of the 287 CTX-M-producing *E. coli* strains showed that 101 (35.2%) belonged to phylogroup D, 66 (23.0%) to phylogroup A, 63 (22.0%) to phylogroup B2, 36 (12.5%) to phylogroup B1, 11 (3.8%) to phylogroup F, 7 (2.4%) to phylogroup C, 2 (0.7%) to phylogroup E and 1 (0.3%) to clade I.

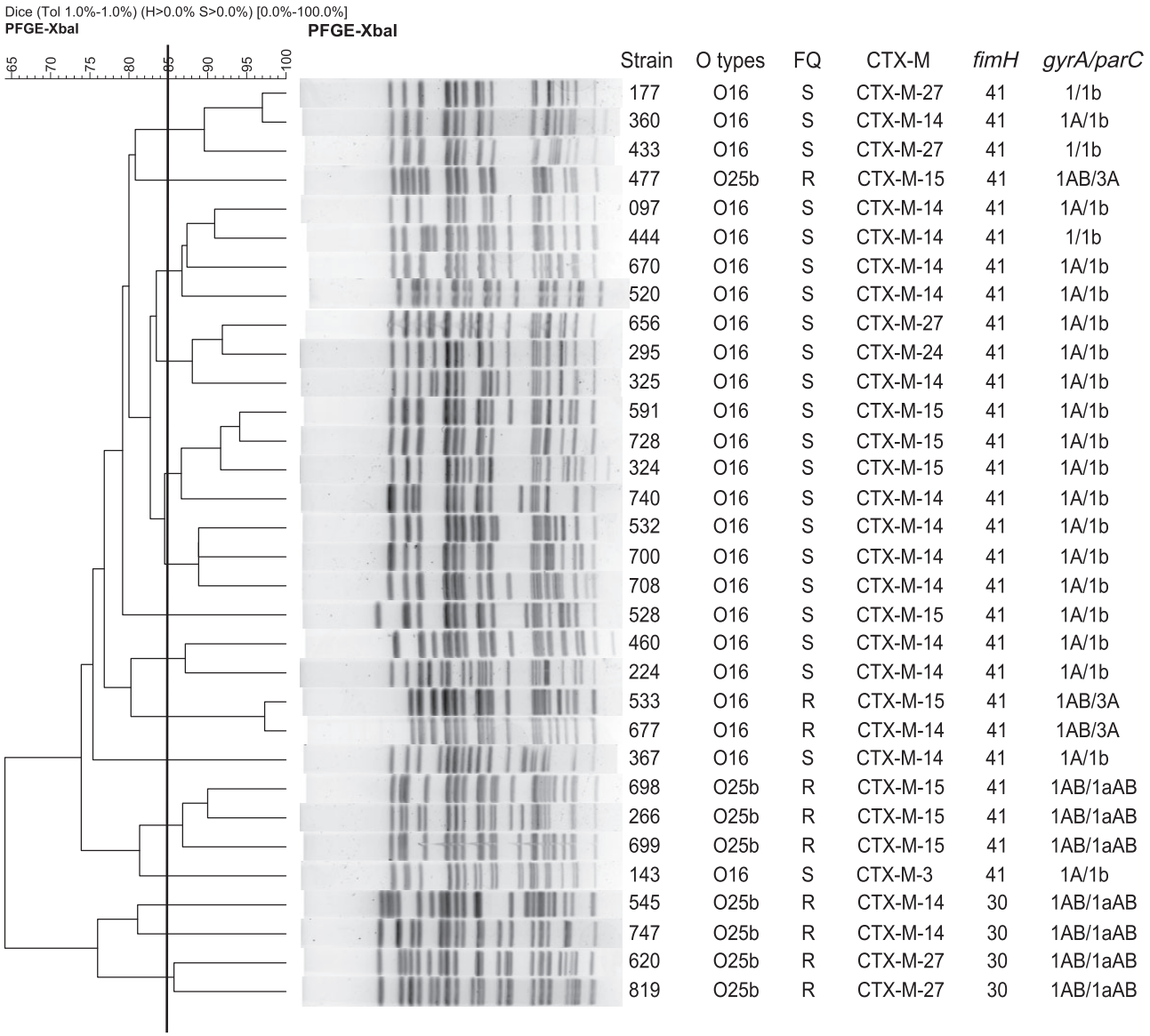
E. coli ST131 clonal group and antimicrobial susceptibility

PCR analysis showed that 32 (11.1%) of the 287 CTX-M-producing isolates belonged to the ST131 clonal group, comprising 24 O16-B2-ST131 and 8 O25b-B2-ST131 isolates; only 2 isolates required full MLST.

All of the 32 ST131 isolates were found to be resistant to ceftriaxone, cefazolin and ampicillin, 17 (53.1%) to ceftazidime, 10 (31.3%) to ciprofloxacin, 14 (43.8%) to gentamicin, 28 (87.5%) to ampicillin/sulbactam, 20 (62.5%) to trimethoprim/sulfamethoxazole, 8 (25.0%) to minocycline, 2 (6.3%) to piperacillin/tazobactam and 4 (12.5%) to chloramphenicol. None of the isolates was resistant to imipenem or nitrofurantoin.

E. coli ST131 characterization

Characterization of the 32 *E. coli* ST131 isolates is shown in Figure 1. The *fimH* gene was carried by all 24 O16-ST131 isolates, which were *H*41 lineage. In contrast, only half of the eight



Strain	O types	FQ	CTX-M	*fimH*	*gyrA/parC*
177	O16	S	CTX-M-27	41	1/1b
360	O16	S	CTX-M-14	41	1A/1b
433	O16	S	CTX-M-27	41	1/1b
477	O25b	R	CTX-M-15	41	1AB/3A
097	O16	S	CTX-M-14	41	1A/1b
444	O16	S	CTX-M-14	41	1/1b
670	O16	S	CTX-M-14	41	1A/1b
520	O16	S	CTX-M-14	41	1A/1b
656	O16	S	CTX-M-27	41	1A/1b
295	O16	S	CTX-M-24	41	1A/1b
325	O16	S	CTX-M-14	41	1A/1b
591	O16	S	CTX-M-15	41	1A/1b
728	O16	S	CTX-M-15	41	1A/1b
324	O16	S	CTX-M-15	41	1A/1b
740	O16	S	CTX-M-14	41	1A/1b
532	O16	S	CTX-M-14	41	1A/1b
700	O16	S	CTX-M-14	41	1A/1b
708	O16	S	CTX-M-14	41	1A/1b
528	O16	S	CTX-M-15	41	1A/1b
460	O16	S	CTX-M-14	41	1A/1b
224	O16	S	CTX-M-14	41	1A/1b
533	O16	R	CTX-M-15	41	1AB/3A
677	O16	R	CTX-M-14	41	1AB/3A
367	O16	S	CTX-M-14	41	1A/1b
698	O25b	R	CTX-M-15	41	1AB/1aAB
266	O25b	R	CTX-M-15	41	1AB/1aAB
699	O25b	R	CTX-M-15	41	1AB/1aAB
143	O16	S	CTX-M-3	41	1A/1b
545	O25b	R	CTX-M-14	30	1AB/1aAB
747	O25b	R	CTX-M-14	30	1AB/1aAB
620	O25b	R	CTX-M-27	30	1AB/1aAB
819	O25b	R	CTX-M-27	30	1AB/1aAB

Figure 1. The XbaI PFGE-based dendrogram for the 32 *E. coli* ST131 isolates. The dendrogram was generated according to the unweighted pair group method based on Dice coefficients. O types, serogroup; FQ, fluoroquinolone phenotype (R, resistant; S, susceptible); *fimH*, allele of *fimH* (type 1 fimbrial adhesin); *gyrA*/*parC*, allele of *gyrA* and *parC* (encoding DNA gyrase and topoisomerase, respectively); 1/1b, *gyrA*1 (WT) combined with *parC*1b (one silent mutation); 1A/1b, *gyrA*1A (one replacement mutation, Ser83Leu) combined with *parC*1b; 1AB/3A, *gyrA*1AB (two replacement mutations, Ser83Leu and Asp87Asn) combined with *parC*3A (a recombined variant containing one replacement mutation Ser80Ile); 1AB/1aAB, *gyrA*1AB combined with *parC*1aAB (*parC*1a plus the replacement mutations Ser80Ile and Glu84Val). The 32 ST131 isolates formed one large group at the 64% similarity level. They comprised 15 PFGE groups (defined at ≥85% similarity).

O25b-ST131 isolates belonged to the *H*41 lineage. The remaining four isolates harbouring *fimH* belonged to the *H*30 lineage, with none representing the *H*30-Rx sublineage. All of the O25b-ST131 isolates except one showed *gyrA*1AB and *parC*1aAB mutations; most of the O16-ST131 isolates had *gyrA*1A and *parC*1b mutations. None of the PMQR determinants was detected in any of the 32 ST131 isolates. Nine PFGE clusters with a similarity index of ≥85% were identified. Unique PFGE profiles were seen in six isolates (Figure 1).

The virulence genotypes of O16-ST131 resembled those of the O25b-ST131 isolates (Table S1, available as Supplementary data at *JAC* Online). All of the ST131 isolates were found to belong to virotype C.

Association of O16 and O25b subgroups with antimicrobial resistance and CTX-M type

As indicated in Table 1, the O25b-ST131 isolates had a significantly higher prevalence of resistance to ciprofloxacin compared with O16-ST131. The O16-*H*41 isolates were more likely to be positive for $bla_{\text{CTX-M-14}}$. All of the O25b-*H*41 isolates produced CTX-M-15, which was not found in the O25b-*H*30 isolates.

Table 1. Antimicrobial resistance and CTX-M genotypes of the 32 *E. coli* ST131 isolates

	No. (%) of ST131 isolates			*P* value		
		O25b				
	O16-*H*41 (*n*=24)	*H*30 (*n*=4)	*H*41 (*n*=4)	O16-*H*41 versus O25b-*H*30	O16-*H*41 versus O25b-*H*41	O25b-*H*30 versus O25b-*H*41
Antimicrobial resistance						
ciprofloxacin	2 (8)	4 (100)	4 (100)	0.001	0.001	—
gentamicin	12 (50)	2 (50)	0	1.000	0.113	0.429
minocycline	4 (17)	1 (25)	3 (75)	1.000	0.038	0.486
piperacillin/tazobactam	1 (4)	1 (25)	0	0.270	1.000	1.000
ampicillin/sulbactam	22 (92)	2 (50)	4 (100)	0.086	1.000	0.429
trimethoprim/sulfamethoxazole	16 (67)	4 (100)	0	0.295	0.024	0.029
imipenem	0	0	0	—	—	—
nitrofurantoin	0	0	0	—	—	—
chloramphenicol	1 (4)	0	3 (75)	1.000	0.005	0.143
cefazolin	24 (100)	4 (100)	4 (100)	—	—	—
ceftriaxone	24 (100)	4 (100)	4 (100)	—	—	—
ceftazidime	10 (42)	3 (75)	4 (100)	0.311	0.098	1.000
ampicillin	24 (100)	4 (100)	4 (100)	—	—	—
MDR	11 (46)	4 (100)	3 (75)	0.102	0.596	1.000
Resistance score	3.4 (2–6)[a]	4.5 (3–6)[a]	4.5 (3–5)[a]	0.269	0.055	0.760
CTX-M genotype						
CTX-M-14	14 (58)	2 (50)	0	1.000	0.098	0.429
CTX-M-15	5 (21)	0	4 (100)	1.000	0.006	0.029
CTX-M-27	3 (13)	2 (50)	0	0.135	1.000	0.429
other CTX-M[b]	2 (8)	0	0	1.000	1.000	—

[a]The value denotes the mean and range.
[b]CTX-M-3 was found in one O16-ST131 isolate and CTX-M-24 was found in one O16-ST131 isolate.

Discussion

We have found the colonization prevalence of CTX-M-producing *E. coli* in healthy individuals to be 51.0%, which is much higher than that reported from European countries (0.6%–11.3%).[1,18] Although a high prevalence (27.5%) of CTX-M-15 has not been observed in previous studies of community carriage from China,[3,4] our findings suggest that this worldwide dominant genotype is displacing the current genotypes (CTX-M-14 and CTX-M-27) in China. Our observation correlates with a recent report on clinical *E. coli* isolates across China showing 14.5% CTX-M-15 and 19.7% CTX-M-55 (a single-locus variant of CTX-M-15).[19] This emphasizes the importance of studying the human faecal resistome as many strains causing infection are derived from the gut.

O25b:H4 was considered the dominant ST131 clone serotype in almost all previous studies,[5,6] whereas O16:H5, a recently identified serotype of ST131 isolates, accounted for a small subset (1%–12%) in a few countries.[5–7] However, data from China are lacking and the present study is the first to show the presence of O16-ST131 in this country. Surprisingly, we found that O16-ST131 was the predominant subset (75%) among ST131 isolates, whereas this type only accounted for 8% in France.[7] These disparities may be due to geographical or host population differences. To our knowledge, our study is the first to report that the O16 subset accounts for such a high proportion of the ST131 isolates. This finding implies that O16-ST131 may be poised to emerge as a major type of *E. coli* ST131 in the community in China.

All of the O16-ST131 isolates were found to belong to the *fimH*-based *H*41 subclone, as reported previously.[7,13] In contrast, the O25b-ST131 isolates carried the *fimH*30 or *H*41 allele. This is the first study to show the presence of O25b-ST131 with the *fimH*41 allele. Multiple previous studies have documented that most O25b-ST131 isolates contain the *H*30 allele, with a minority containing *H*22 and the remainder containing one of several rarer *fimH* alleles, but not *fimH*41.[6,13] The reason for the emergence of the *fimH*41 allele in O25b-ST131 is not yet clear, but may be due to the homologous recombination of *fimH* alleles in O25b-ST131 isolates.[20] Further studies are required to elucidate and monitor the evolution of O25b-ST131 with the *fimH*41 allele.

A limitation was that the small number of O25b-ST131 isolates restricted the broad generalizability of the characterization (especially statistical analyses) of O16-*H*41, O25b-*H*30 and O25b-*H*41 subgroups presented in this study. Thus, studies with larger sample sizes and more broadly distributed populations are required to further explore the characterization of these subgroups in the future.

In conclusion, to our knowledge, this is the first study to report O16-ST131 as the predominant type of clonal group in ST131. Our

findings indicate that future studies should focus more on O16-ST131 isolates, especially in high-incidence areas such as China.

Acknowledgements

We thank Zhigang Cui, Prof. Fangyou Yu, Xin Xia and Yun Luo for their technical assistance. We thank all staff in the Microbiology Department of Xiangya Hospital for their help with bacterial cultures.

Funding

This work was supported by the Fundamental Research Funds for the Central Universities of Central South University (2014zzts352) and by a grant from the Development and Reform Commission of Hunan Province ([2014]658).

Transparency declarations

None to declare.

Supplementary data

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

References

1 Hawkey PM, Jones AM. The changing epidemiology of resistance. *J Antimicrob Chemother* 2009; **64** Suppl 1: i3–10.

2 Woerther PL, Burdet C, Chachaty E *et al*. Trends in human fecal carriage of extended-spectrum β-lactamases in the community: toward the globalization of CTX-M. *Clin Microbiol Rev* 2013; **26**: 744–58.

3 Tian SF, Chen BY, Chu YZ *et al*. Prevalence of rectal carriage of extended-spectrum β-lactamase-producing *Escherichia coli* among elderly people in community settings in China. *Can J Microbiol* 2008; **54**: 781–5.

4 Li B, Sun JY, Liu QZ *et al*. High prevalence of CTX-M β-lactamases in faecal *Escherichia coli* strains from healthy humans in Fuzhou, China. *Scand J Infect Dis* 2011; **43**: 170–4.

5 Nicolas-Chanoine MH, Bertrand X, Madec JY. *Escherichia coli* ST131, an intriguing clonal group. *Clin Microbiol Rev* 2014; **27**: 543–74.

6 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.

7 Blanc V, Leflon-Guibout V, Blanco J *et al*. Prevalence of day-care centre children (France) with faecal CTX-M-producing *Escherichia coli* comprising O25b:H4 and O16:H5 ST131 strains. *J Antimicrob Chemother* 2014; **69**: 1231–7.

8 Nicolas-Chanoine MH, Gruson C, Bialek-Davenet S *et al*. 10-fold increase (2006–11) in the rate of healthy subjects with extended-spectrum β-lactamase-producing *Escherichia coli* faecal carriage in a Parisian check-up centre. *J Antimicrob Chemother* 2013; **68**: 562–8.

9 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-third Informational Supplement M100-S23*. CLSI, Wayne, PA, USA, 2013.

10 Woodford N, Fagan EJ, Ellington MJ. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum β-lactamases. *J Antimicrob Chemother* 2006; **57**: 154–5.

11 Grobner S, Linke D, Schutz W *et al*. Emergence of carbapenem-non-susceptible extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* isolates at the university hospital of Tubingen, Germany. *J Med Microbiol* 2009; **58**: 912–22.

12 Clermont O, Christenson JK, Denamur E *et al*. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. *Environ Microbiol Rep* 2013; **5**: 58–65.

13 Johnson JR, Clermont O, Johnston B *et al*. Rapid and specific detection, molecular epidemiology, and experimental virulence of the O16 subgroup within *Escherichia coli* sequence type 131. *J Clin Microbiol* 2014; **52**: 1358–65.

14 Johnson JR, Tchesnokova V, Johnston B *et al*. Abrupt emergence of a single dominant multidrug-resistant strain of *Escherichia coli*. *J Infect Dis* 2013; **207**: 919–28.

15 Banerjee R, Robicsek A, Kuskowski MA *et al*. Molecular epidemiology of *Escherichia coli* sequence type 131 and its H30 and H30-Rx subclones among extended-spectrum-β-lactamase-positive and -negative *E. coli* clinical isolates from the Chicago region, 2007 to 2010. *Antimicrob Agents Chemother* 2013; **57**: 6385–8.

16 Kim HB, Park CH, Kim CJ *et al*. Prevalence of plasmid-mediated quinolone resistance determinants over a 9-year period. *Antimicrob Agents Chemother* 2009; **53**: 639–45.

17 Blanco J, Mora A, Mamani R *et al*. Four main virotypes among extended-spectrum-β-lactamase-producing isolates of *Escherichia coli* O25b:H4-B2-ST131: bacterial, epidemiological, and clinical characteristics. *J Clin Microbiol* 2013; **51**: 3358–67.

18 Wickramasinghe NH, Xu L, Eustace A *et al*. High community faecal carriage rates of CTX-M ESBL-producing *Escherichia coli* in a specific population group in Birmingham, UK. *J Antimicrob Chemother* 2012; **67**: 1108–13.

19 Xia S, Fan X, Huang Z *et al*. Dominance of CTX-M-type extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolated from patients with community-onset and hospital-onset infection in China. *PLoS One* 2014; **9**: e100707.

20 Paul S, Linardopoulou EV, Billig M *et al*. Role of homologous recombination in adaptive diversification of extraintestinal *Escherichia coli*. *J Bacteriol* 2013; **195**: 231–42.