Eur J Clin Microbiol Infect Dis (2013) 32:431–436
DOI 10.1007/s10096-012-1764-x

ARTICLE

An abbreviated MLVA identifies *Escherichia coli* ST131 as the major extended-spectrum β-lactamase-producing lineage in the Copenhagen area

J. B. Nielsen · A. Albayati · R. L. Jørgensen · K. H. Hansen · B. Lundgren · K. Schønning

Received: 5 July 2012 / Accepted: 9 October 2012 / Published online: 6 November 2012

Abstract Rapid bacterial typing is a valuable and necessary tool in the prevention and detection of outbreaks. The purpose of this study was to adapt a multilocus variable number of tandem repeats analysis (MLVA) for analysis on a benchtop capillary electrophoresis instrument and compare the modified assay with multilocus sequence typing (MLST) for typing cefpodoxime-resistant *Escherichia coli* (*E. coli*). Further, we identified the causative resistance mechanisms and epidemiological type of infection for isolates producing extended-spectrum β-lactamases (ESBLs). A collection of *E. coli* resistant to cefpodoxime was typed by MLST and a modified MLVA assay using a benchtop capillary electrophoresis instrument. Resistance mechanisms were identified by polymerase chain reaction (PCR) and sequencing. Patient history was examined to establish the epidemiological type of infection for ESBL-producing *E. coli*. MLVA yielded typing results homologous with MLST and it correctly identified *E. coli* sequence type (ST) 131 that was accounting for 45 % of all ESBL-producing isolates in the sample collection. The majority (76.7 %) of ESBL-producing isolates was healthcare-related and only 23.3 % of the ESBL-producing isolates were community-onset infections (COI), regardless of the ST. Patients with COI were significantly more often of female gender and younger age compared to healthcare-associated infections (HCAI) and hospital-onset infections (HOI). In conclusion, the modified MLVA is a useful tool for the rapid typing of *E. coli* and it identified ST131 as the predominating ESBL-producing lineage in Copenhagen. Healthcare-related infections were the predominant infection setting of ESBL-producing *E. coli* and the demographic characteristics differed between patients with COI and healthcare-related infections.

Introduction

Bacterial typing is a valuable tool in both outbreak investigation and epidemiological surveillance. Multilocus sequence typing (MLST) is an excellent tool for longitudinal epidemiological surveillance [1]. The method is, however, laborious. Multilocus variable number of tandem repeats analysis (MLVA) provides many of the benefits of MLST at lesser economic and labor cost, and still fulfils essential criteria for typing methods, such as high discriminatory power, high typability, and reproducibility [2]. Several MLVA protocols have been developed for the typing of enterohemorrhagic *Escherichia coli* (*E. coli*) O157 [3, 4], as well as MLVA protocols designed to detect all serogroups of *E. coli* [5, 6]. However, all the typing methods described rely on fragment analysis using sequencing apparatuses. Benchtop capillary electrophoresis instruments are increasingly used as an alternative to agarose gel electrophoresis. This method allows automation and offers increased accuracy of size determination and high throughput compared to gel electrophoresis, and may provide an alternative to more expensive sequence apparatuses in MLVA protocols.

Electronic supplementary material The online version of this article (doi:10.1007/s10096-012-1764-x) contains supplementary material, which is available to authorized users.

J. B. Nielsen (✉) · A. Albayati · R. L. Jørgensen · K. H. Hansen · B. Lundgren · K. Schønning
Department of Clinical Microbiology 445, Hvidovre Hospital, Kettegaard Alle 30,
2650 Hvidovre, Denmark
e-mail: jesper.boye.nielsen@regionh.dk

Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs) constitute a major global health concern [7]. *E. coli* sequence type (ST) 131 expressing *bla*CTX-M-15 is of particular concern, as this lineage has been established worldwide and has been reported as a pathogen capable of efficient transmission both within the community and in care-providing settings [8–11]. Further, ESBL-producing *E. coli* has been described to be an important cause of community-onset infections (COI) [12].

In Denmark, we have observed that the frequency of ESBL-producing *E. coli* and *Klebsiella pneumoniae* (*K. pneumoniae*) has risen during the last decade [13]. Earlier, we described an outbreak of ESBL-producing *K. pneumoniae*, where two clones that both carried *bla*CTX-M-15 were responsible for 77 % of all infections caused by ESBL-producing *K. pneumoniae* [14]. In contrast, little information is available on whether the increase observed in *E. coli* is caused by specific lineages or several unrelated clones, or on the epidemiological setting in which these infections are acquired.

In the present study, we evaluated an MLVA assay for the rapid typing of cefpodoxime-resistant *E. coli* using a benchtop capillary electrophoresis instrument for size determination. We compared the typing results obtained using MLVA with the distribution obtained using MLST. Furthermore, mechanisms accounting for cefpodoxime resistance were identified in all isolates using polymerase chain reaction (PCR) and sequencing. Finally, patient histories were reviewed to establish the epidemiological setting of infections with ESBL-producing *E. coli*.

Materials and methods

Strain collection

All cefpodoxime-resistant *E. coli* were collected at the Department of Clinical Microbiology, Hvidovre Hospital, providing diagnostic services for five hospitals and general practice in the Copenhagen area, in October 2009 (N=67). Only a single isolate from each patient was included in the strain collection. All isolates originated from urine samples (n=66), except for one that originated from a blood culture. *E. coli* resistant to cefpodoxime was identified using the disk diffusion method and all cefpodoxime-resistant isolates were further confirmed to be ESBL- or AmpC-producers using Mast D68C disks (Mast Group), following the directions of the manufacturer.

Epidemiology of ESBL-producing isolates

Patient history was reviewed using laboratory databases, the hospital information system, and demographic data. On this basis, infections were classified as community onset (COI), hospital onset (HOI), or healthcare-associated (HCAI) [15]. HOI was defined by the culture of an ESBL-producing *E. coli* from a sample obtained from a patient hospitalized for 48 h or longer. HCAI was defined by the culture of an ESBL-producing *E. coli* from a sample obtained from a patient in general practice or within 48 h after hospital admission and one or more of the following criteria have been fulfilled by the patient: (1) admitted to hospital for more than 2 days within the preceding 3 months; (2) attended an outpatient clinic within the preceding 30 days; (3) received specialized nursing care at home; (4) residence in a nursing home or long-term care facility. COI was defined by the culture of an ESBL-producing *E. coli* obtained from a patient in general practice or within 48 h after hospital admission where none of the criteria for HCAI were met [15].

For 15 patients, cultures prior to the one included in this study had yielded ESBL-producing *E. coli*. In these cases, epidemiological classification was done from the first culture yielding an ESBL-producing *E. coli*. The median interval between the first culture yielding an ESBL-producing *E. coli* and the isolate included in this study was 96.5 days (range 15–965 days).

β-Lactamase characterization

Phenotypic ESBL-producing isolates were screened by PCR for the presence of *bla*CTX-M, *bla*SHV, and *bla*TEM. PCR and sequencing were performed to identify specific genes, as previously described [16]. Phenotypic AmpC-producing isolates were screened for the presence of plasmid-mediated *ampC* (pAmpC) genes by multiplex PCR [17], followed by sequencing to identify the exact genotype. The promoter of the chromosomal *ampC* (cAmpC) gene was sequenced in all isolates with an AmpC phenotype and for which a pAmpC gene could not be detected by PCR in order to identify mutations associated with hyperproduction, as previously described [16].

MLVA

All isolates were typed using a modified MLVA assay originally described by Manges et al. [5]. This MLVA is based on multiplex PCR detection and size determination of eight variable number of tandem repeats (VNTR) loci using labeled primers, followed by size determination on a sequencing instrument. In this study, PCR for all targets were performed as described [5], with the exception that all reactions were done as singleplex PCR amplifications using unlabeled primers and the size of the amplified fragments was determined using an automated benchtop capillary electrophoresis system (QIAxcel, Qiagen), using a

high-resolution cartridge and the accompanying BioCalculator software.

The exact sizes of the flanking regions of each locus were determined by the sequencing of MLVA PCR products from each VNTR region from selected isolates. Tandem repeats were identified by manual inspection of sequences or by the use of the tandem repeats finder [18]. The number of tandem repeats was calculated using the following equation: number of repeats = (amplicon size − flanking region)/repeat size. The isolates were sorted manually into groups according to their number of repeats. The same number of repeats in each individual locus was assigned the same value, and this was done for all loci, hereby, generating a numeric MLVA code for each isolate.

MLST

All isolates were also typed by MLST, as previously described [19]. Allele profiles and STs were assigned at the *E. coli* MLST homepage (http://mlst.ucc.ie/mlst/dbs/Ecoli).

Results

Validation of MLVA

PCR amplifications of all eight MLVA alleles were carried out and the size of the resulting PCR products was determined using the QIAxcel capillary electrophoresis system. For each locus, the distribution of size determinations was analyzed using frequency plots and the results were sorted into seemingly normally distributed data. The coefficient of variation (CV) for the largest clusters of data showed CV ranging between 0.7 % for VNTR-5 (mean size for the cluster: 368 bp) and 1.2 % for VNTR-1 (mean size for the cluster: 210 bp). From this analysis, it was apparent that the accuracy of the size determination using the QIAxcel system was inadequate for determining repeat numbers for locus O157-11 (repeat size: 6 bp) and O157-56 N (repeat size: 5 bp). These loci were, therefore, excluded from further analysis. The remaining six loci had repeat sizes between 12 and 95 bp.

MLVA

Using this abbreviated MLVA (a-MLVA) scheme to type the strain collection, 67/67 (100 %) isolates were typable. In all, 27 different a-MLVA types were identified and the largest cluster contained 25 isolates (37.3 %) (Table 1). Within this cluster, 22 isolates contained *bla*CTX-M-15, and the remaining three isolates contained *bla*CTX-M-14, *bla*CTX-M-27, and hyperproduced cAmpC, respectively (Online resource 1).

Table 1 Typing results of cefpodoxime-resistant *Escherichia coli* typed by abbreviated multilocus variable number of tandem repeats analysis (a-MLVA) and multilocus sequence typing (MLST)

Sequence type (ST)	a-MLVA code	Number of isolates (N=67)
ST10	132251[a]	n=2
	132214	n=1
ST12	266562	n=1
ST38	173052	n=1
ST59	161370	n=1
ST69	173287	n=2
ST88	132261[a]	n=2
ST101	131261[a]	n=1
ST131	153562	n=25
	153526	n=3
ST144	224652	n=1
ST167	132251[a]	n=1
ST394	171787	n=1
ST401	132231	n=1
ST405	121250	n=1
	121252	n=4
	121254	n=1
ST410	132261	n=1
ST443	131217	n=1
ST448	131216	n=2
	131261[a]	n=2
ST517	131291	n=1
	131217	n=1
ST538	152366	n=1
ST624	161162	n=1
ST648	161160	n=1
	161166	n=1
ST657	253263	n=1
ST998	224665	n=1
ST1193	124645	n=1
ST2574	151432	n=2
ST2673	132261[a]	n=1

[a] Signifies a-MLVA codes shared between different STs

MLST

MLST was performed on the collected isolates. Using this method, 67/67 (100 %) isolates were typed and the results are summarized in Table 1. The isolates belonged to 25 different STs. The largest sequence type, ST131, contained 28 isolates (41.8 %). A number of smaller clusters were also identified. Of these, the three largest were ST405, ST448, and ST10, containing six, four, and three isolates, respectively.

When comparing a-MLVA and MLST, homologous results were found (Table 1). The major ST131 cluster identified by MLST included all the isolates identified in

the major a-MLVA cluster (153562), together with three isolates, all with another a-MLVA code (153526). These two a-MLVA codes were found exclusively within the ST131 cluster. Eighteen of 25 STs had unique a-MLVA codes, i.e., the a-MLVA code was not shared with isolates with another ST. Three isolates of ST10 displayed two different a-MLVA codes; two isolates shared their a-MLVA code with the single isolate of ST167. ST167 is a single-locus variant of ST10. Two isolates of ST88 shared a-MLVA codes with the single ST410 isolate and the single ST2673 isolate. ST410 is a single-locus variant of ST88 and ST2673 is a double-locus variant of ST88. Finally, four ST448 isolates displayed two different a-MLVA codes. Two of the isolates shared their a-MLVA code with the single ST101 isolate. ST101 and ST448 are unrelated by MLST, sharing only a single allele.

Resistance mechanisms

Eleven different mechanisms of cefpodoxime resistance were identified (Table 2). Of these, *bla*CTX-M-15 and *bla*CTX-M-14 were the most commonly detected in 42/67 (62.7 %) and 7/67 (10.4 %) of the isolates, respectively. AmpC enzymes accounted for resistance in 7/67 (10.5 %) of the isolates, with cAmpC being more common [5/67 (7.5 %)] than *bla*CMY-2 [(2/67 (3.0 %)] (Table 2).

Epidemiology of ESBL-producing isolates

Infections caused by ESBL-producing *E. coli* were classified as HOI, HCAI, and COI. In all, 42/60 (76.7 %) infections were classified as healthcare-related (HCAI or HOI) and only 18/60 (23.3 %) were classified as COI (Table 3). Patients with COI were significantly younger (median age 38 years; $p<0.001$, Mann–Whitney *U*-test) and more often female (13/14; $p<0.01$, Fisher's exact probability test) than patients with HCAI (median age 77 years and 12/28 females) or HOI (median age 78 years and 11/18 females) (Online resource 1). If stratified into infections caused by *E. coli* ST131 and *E. coli* non-ST131, the two groups did not differ in the epidemiological type of infection (Table 3).

Table 2 Resistance genes identified and their distribution on sequence types (STs)

Resistance gene	Number of isolates (*N*=67)	STs
CTX-M-1	2 (3 %)	ST10[a]
CTX-M-9	1 (1.5 %)	ST38
CTX-M-14	7 (10.4 %)	ST10[a], ST131[a], ST144, ST167, ST538, ST2574
CTX-M-15	42 (62.7 %)	ST12, ST69, ST131[a], ST394, ST405[a], ST410, ST443, ST448, ST517, ST648[a]
CTX-M-27	3 (4.5 %)	ST131[a], ST405[a]
CTX-M-55/57/79	1 (1.5 %)	ST648[a]
SHV-2	1 (1.5 %)	ST101
SHV-12	2 (3 %)	ST88[a], ST401
TEM-52	1 (1.5 %)	ST624
CMY-2	2 (3 %)	ST657, ST1193
cAmpC	5 (7.5 %)	ST59, ST131[a], ST998, ST88[a], ST2673

[a] Signifies that different resistance genes are present within this ST

Discussion

Typing methods that provide rapid and reliable typing results are valuable and necessary tools in the prevention and detection of outbreaks. However, many of today's typing methods, such as pulsed-field gel electrophoresis (PFGE) and MLST, are laborious [14]. The a-MLVA described here can be used as a high-throughput assay and can provide rapid typing results compared to MLST and PFGE.

The exclusion of two loci affected the discriminatory power of the MLVA assay, making it less discriminatory than the original assay. When comparing a-MLVA typing results with MLST results, similar typing patterns were obtained, although, occasionally, more than one ST was found within an a-MLVA code. Including additional VNTR loci of sufficient repeat size can increase the discriminatory power of the MLVA assay and could possibly resolve these discrepancies. In a recently published MLVA protocol, the addition of three VNTR loci to a total of ten loci increased the number of different genotypes from 296 to 507 in a collection of 794 *E. coli* [20].

The a-MLVA divided the predominant ST131 lineage into two subtypes that were unique for ST131. The subdivision of ST lineages was not unexpected, as studies comparing more discriminative methods, such as PFGE with MLST, have identified several different PFGE clones within the same ST [21, 22]. As ST131 is highly prevalent, typing

Table 3 Epidemiological classification of infections with extended-spectrum β-lactamase (ESBL)-producing *E. coli*

	HOI	HCAI	COI	Total
ST131	9	14	4	27 (45.0 %)
Non-ST131	9	14	10	33 (55.0 %)
Total	18 (30.0 %)	28 (46.7 %)	14 (23.3 %)	60 (100 %)

The type of infection was classified as hospital-onset infections (HOI), healthcare-associated infections (HCAI), or community-onset infections (COI)

methods that are capable of subdividing this ST are desirable from an epidemiological point of view.

The major cause of cefpodoxime resistance in the study collection was *bla*CTX-M-15. This gene has also been shown to be the major cause of ESBL production in *K. pneumoniae* in the same region [14], and has been described worldwide to be a dominating ESBL gene [12, 23–26]. Interestingly, several cases were found where different resistance mechanisms were identified within the same STs, which could support that several different subclones within the same ST are circulating.

The epidemiological data of this study indicated the presence of two different transmission routes. Healthcare-related infections (HOI and HCAI) accounted for over three out of four ESBL-producing *E. coli* infections. This distribution is in contrast to a recent Canadian study, where it was reported that 130 of 209 isolates (62 %) were submitted from community collection sites, 63 (30 %) were from hospitals, and 16 (8 %) were from nursing homes [12]. However, this study did not investigate the patient history data to determine if the patients had prior hospital association, which could, together with the different geographical settings, explain the discrepancy in observed infection settings.

The different demographic characteristics of COI and healthcare-related infections may indicate that different risk factors are present in the two settings. Risk factors for infection due to ESBL-producing *E. coli*, such as hospitalization within the previous 3 months, age ≥65 years, male gender, comorbidity, and prior receipt of antibiotics, have been described [27–29]. However, these risk factors are mainly healthcare-related and other risk factors may be relevant for COI. Foreign travel has been identified as a risk factor for subsequent colonization and infection with ESBL-producing *E. coli* [30, 31]. A significant difference in the frequency of fecal carriage of ESBL-producing *E. coli* among different ethnic populations has also been reported [32]. This may be caused by high travel rates to and from regions with high rates of ESBL-producing *E. coli* [33] or differences in lifestyle or dietary habits. Studies investigating COI may, therefore, likely identify other risk factors and exposure routes than studies conducted in a hospital setting.

Acknowledgment This work was supported by Fondation Idella (3.3.5/2008/II to K.S.), Fonden til Lægevidenskabens Fremme (10–316 to K.S.), and Hvidovre Hospitals Forskningsfond (grant to J.B.N.).

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Maiden MCJ (2006) Multilocus sequence typing of bacteria. Annu Rev Microbiol 60:561–588
2. van Belkum A, Tassios PT, Dijkshoorn L, Haeggman S, Cookson B, Fry NK, Fussing V, Green J, Feil E, Gerner-Smidt P, Brisse S, Struelens M; European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group on Epidemiological Markers (ESGEM) (2007) Guidelines for the validation and application of typing methods for use in bacterial epidemiology. Clin Microbiol Infect 13(Suppl 3):1–46
3. Keys C, Kemper S, Keim P (2005) Highly diverse variable number tandem repeat loci in the *E. coli* O157:H7 and O55:H7 genomes for high-resolution molecular typing. J Appl Microbiol 98:928–940
4. Noller AC, McEllistrem MC, Pacheco AG, Boxrud DJ, Harrison LH (2003) Multilocus variable-number tandem repeat analysis distinguishes outbreak and sporadic *Escherichia coli* O157:H7 isolates. J Clin Microbiol 41:5389–5397
5. Manges AR, Tellis PA, Vincent C, Lifeso K, Geneau G, Reid-Smith RJ, Boerlin P (2009) Multi-locus variable number tandem repeat analysis for *Escherichia coli* causing extraintestinal infections. J Microbiol Methods 79:211–213
6. Lindstedt BA, Brandal LT, Aas L, Vardund T, Kapperud G (2007) Study of polymorphic variable-number of tandem repeats loci in the ECOR collection and in a set of pathogenic *Escherichia coli* and *Shigella* isolates for use in a genotyping assay. J Microbiol Methods 69:197–205
7. Pitout JD, Laupland KB (2008) Extended-spectrum beta-lactamase-producing *Enterobacteriaceae*: an emerging public-health concern. Lancet Infect Dis 8:159–166
8. Pitout JD, Gregson DB, Campbell L, Laupland KB (2009) Molecular characteristics of extended-spectrum-beta-lactamase-producing *Escherichia coli* isolates causing bacteremia in the Calgary Health Region from 2000 to 2007: emergence of clone ST131 as a cause of community-acquired infections. Antimicrob Agents Chemother 53:2846–2851
9. Rooney PJ, O'Leary MC, Loughrey AC, McCalmont M, Smyth B, Donaghy P, Badri M, Woodford N, Karisik E, Livermore DM (2009) Nursing homes as a reservoir of extended-spectrum beta-lactamase (ESBL)-producing ciprofloxacin-resistant *Escherichia coli*. J Antimicrob Chemother 64:635–641
10. Rogers BA, Sidjabat HE, Paterson DL (2011) *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. J Antimicrob Chemother 66:1–14
11. Van der Bij AK, Peirano G, Pitondo-Silva A, Pitout JD (2012) The presence of genes encoding for different virulence factors in clonally related *Escherichia coli* that produce CTX-Ms. Diagn Microbiol Infect Dis 72:297–302
12. Peirano G, Richardson D, Nigrin J, McGeer A, Loo V, Toye B, Alfa M, Pienaar C, Kibsey P, Pitout JD (2010) High prevalence of ST131 isolates producing CTX-M-15 and CTX-M-14 among extended-spectrum-beta-lactamase-producing *Escherichia coli* isolates from Canada. Antimicrob Agents Chemother 54:1327–1330
13. Hansen DS, Frimodt-Møller N; DANRES Study Group (2009) Extended spectrum beta-lactamase producing bacteria in Danish pigs, Danish and imported retail meat and human patients. DANMAP 2009, pp. 19–21. Available online at: http://www.danmap.org/Downloads/Reports.aspx
14. Nielsen JB, Skov MN, Jørgensen RL, Heltberg O, Hansen DS, Schønning K (2011) Identification of CTX-M15-, SHV-28-producing *Klebsiella pneumoniae* ST15 as an epidemic clone in the Copenhagen area using a semi-automated Rep-PCR typing assay. Eur J Clin Microbiol Infect Dis 30:773–778
15. Friedman ND, Kaye KS, Stout JE, McGarry SA, Trivette SL, Briggs JP, Lamm W, Clark C, MacFarquhar J, Walton AL, Reller LB, Sexton DJ (2002) Health care-associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. Ann Intern Med 137:791–797
16. Jørgensen RL, Nielsen JB, Friis-Møller A, Fjeldsøe-Nielsen H, Schønning K (2010) Prevalence and molecular characterization

of clinical isolates of *Escherichia coli* expressing an AmpC phenotype. J Antimicrob Chemother 65:460–464
17. Pérez-Pérez FJ, Hanson ND (2002) Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 40:2153–2162
18. Benson G (1999) Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res 27:573–580
19. Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MC, Ochman H, Achtman M (2006) Sex and virulence in *Escherichia coli*: an evolutionary perspective. Mol Microbiol 60:1136–1151
20. Løbersli I, Haugum K, Lindstedt BA (2012) Rapid and high resolution genotyping of all *Escherichia coli* serotypes using 10 genomic repeat-containing loci. J Microbiol Methods 88:134–139
21. Novais A, Pires J, Ferreira H, Costa L, Montenegro C, Vuotto C, Donelli G, Coque TM, Peixe L (2012) Characterization of globally spread *Escherichia coli* ST131 isolates (1991 to 2010). Antimicrob Agents Chemother 56:3973–3976
22. Lau SH, Kaufmann ME, Livermore DM, Woodford N, Willshaw GA, Cheasty T, Stamper K, Reddy S, Cheesbrough J, Bolton FJ, Fox AJ, Upton M (2008) UK epidemic *Escherichia coli* strains A–E, with CTX-M-15 beta-lactamase, all belong to the international O25:H4-ST131 clone. J Antimicrob Chemother 62:1241–1244
23. Severin JA, Mertaniasih NM, Kuntaman K, Lestari ES, Purwanta M, Lemmens-Den Toom N, Duerink DO, Hadi U, van Belkum A, Verbrugh HA, Goessens WH; Study Group 'Antimicrobial Resistance in Indonesia: Prevalence and Prevention' (AMRIN) (2010) Molecular characterization of extended-spectrum beta-lactamases in clinical *Escherichia coli* and *Klebsiella pneumoniae* isolates from Surabaya, Indonesia. J Antimicrob Chemother 65:465–469
24. Kiratisin P, Apisarnthanarak A, Laesripa C, Saifon P (2008) Molecular characterization and epidemiology of extended-spectrum-beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates causing health care-associated infection in Thailand, where the CTX-M family is endemic. Antimicrob Agents Chemother 52:2818–2824
25. Önnberg A, Mölling P, Zimmermann J, Söderquist B (2011) Molecular and phenotypic characterization of *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum β-lactamases with focus on CTX-M in a low-endemic area in Sweden. APMIS 119:287–295
26. Lascols C, Hackel M, Hujer AM, Marshall SH, Bouchillon SK, Hoban DJ, Hawser SP, Badal RE, Bonomo RA (2012) Using nucleic acid microarrays to perform molecular epidemiology and detect novel β-lactamases: a snapshot of extended-spectrum β-lactamases throughout the world. J Clin Microbiol 50:1632–1639
27. Ben-Ami R, Rodríguez-Baño J, Arslan H, Pitout JD, Quentin C, Calbo ES, Azap OK, Arpin C, Pascual A, Livermore DM, Garau J, Carmeli Y (2009) A multinational survey of risk factors for infection with extended-spectrum beta-lactamase-producing enterobacteriaceae in nonhospitalized patients. Clin Infect Dis 49:682–690
28. Rodríguez-Baño J, Picón E, Gijón P, Hernández JR, Cisneros JM, Peña C, Almela M, Almirante B, Grill F, Colomina J, Molinos S, Oliver A, Fernández-Mazarrasa C, Navarro G, Coloma A, López-Cerero L, Pascual A (2010) Risk factors and prognosis of nosocomial bloodstream infections caused by extended-spectrum-beta-lactamase-producing *Escherichia coli*. J Clin Microbiol 48:1726–1731
29. Cornejo-Juárez P, Pérez-Jiménez C, Silva-Sánchez J, Velázquez-Acosta C, González-Lara F, Reyna-Flores F, Sánchez-Pérez A, Volkow-Fernández P (2012) Molecular analysis and risk factors for *Escherichia coli* producing extended-spectrum β-lactamase bloodstream infection in hematological malignancies. PLoS One 7:e35780
30. Freeman JT, McBride SJ, Heffernan H, Bathgate T, Pope C, Ellis-Pegler RB (2008) Community-onset genitourinary tract infection due to CTX-M-15-producing *Escherichia coli* among travelers to the Indian subcontinent in New Zealand. Clin Infect Dis 47:689–692
31. Tängdén T, Cars O, Melhus A, Löwdin E (2010) Foreign travel is a major risk factor for colonization with *Escherichia coli* producing CTX-M-type extended-spectrum beta-lactamases: a prospective study with Swedish volunteers. Antimicrob Agents Chemother 54:3564–3568
32. Wickramasinghe NH, Xu L, Eustace A, Shabir S, Saluja T, Hawkey PM (2012) High community faecal carriage rates of CTX-M ESBL-producing *Escherichia coli* in a specific population group in Birmingham, UK. J Antimicrob Chemother 67:1108–1113
33. Hawser SP, Bouchillon SK, Hoban DJ, Badal RE, Hsueh PR, Paterson DL (2009) Emergence of high levels of extended-spectrum-beta-lactamase-producing gram-negative bacilli in the Asia-Pacific region: data from the Study for Monitoring Antimicrobial Resistance Trends (SMART) program, 2007. Antimicrob Agents Chemother 53:3280–3284