Sequence Analysis of *bla*_{CTX-M} Genes Carried by Clinically Significant *Escherichia coli* Isolates in Kuwait Hospitals

G. Al Hashem N. Al Sweih W. Jamal V.O. Rotimi

Department of Microbiology, Faculty of Medicine, Health Sciences Centre, Kuwait University, Jabriya, Kuwait

Key Words

Sequence analysis • *bla*_{CTX-M} • *Escherichia coli* • Kuwait

Abstract

Objective: To investigate the extent, distribution and sequence analysis of *bla*_{CTX-M} genes carried by *Escherichia coli* isolated from patients admitted to all government hospitals in Kuwait. **Methods:** Extended-spectrum β -lactamase (ESBL)-producing *E. coli* isolates were collected from the 8 major hospitals in Kuwait. CTX-M ESBLs were analyzed by PCR and sequenced. Clonality of the positive isolates was determined for genetic relatedness using pulsed-field gel electrophoresis (PFGE) with *Xba*I digestion of the genomic DNA. **Results:** Of the 136 ESBL-positive isolates, 106 (77.9%) harbored *bla*_{CTX-M} genes. Among these, *bla*_{CTX-M-15} was the most frequent with a prevalence rate of 84.1%, followed by *bla*_{CTX-M-14} (6.8%), *bla*_{CTX-M-14b} (5.7%) and *bla*_{TOHO-1} (3.4%). Ninety-three (87.7%) were isolated from Kuwaiti (35.9%), Egyptian (31.1%) and Indian (20.8%) nationals; the majority of isolates positive for *bla*_{CTX-M-15} were mainly from these 3 nationalities. PFGE analysis did not demonstrate any clustering of positive isolates in any particular hospital. **Conclusion:** This study confirms an explosive emergence of CTX-M-15 β -lactamase among *E. coli* isolates in Kuwait and shows that the strains were clonally heterogeneous with no evidence of inter- or intra-hospital spread. Thus Kuwait may represent an important source of CTX-M-15-positive *E. coli*.

Copyright © 2011 S. Karger AG, Basel

Introduction

CTX-M-type β -lactamases constitute a novel group of enzymes encoded by transferable plasmid. Throughout the world, plasmid-mediated extended-spectrum β -lactamases (ESBLs) are becoming increasingly frequent among clinical isolates of members of the family Enterobacteriaceae, including *Escherichia coli*. 'CTX-M' is called like this because it actively hydrolyzes cefotaxime [1, 2]. These enzymes confer high-level resistance to cefotaxime, ceftriaxone and aztreonam with little or no effect on the minimum inhibitory concentrations (MICs) of ceftazidime against isolates belonging to the family Enterobacteriaceae [1]. However, some reports have shown that 2 novel point mutants derived from CTX-M-9, CTX-M-16 and CTX-M-19 to a great extent do hydrolyze ceftazidime [3–5]. A study by Poirel et al. [6] also showed that another CTX-M-type (CTX-M-15) enzyme has catalytic efficiency for ceftazidime.

The preponderance of evidence in the literature appears to show that dissemination of CTX-M ESBL is pandemic [6–8]. The production of this enzyme is mediated by the *bla*_{CTX-M} gene, which confers resistance to the third-generation cephalosporins mainly in *E. coli* and *Klebsiella* spp. [9]. The *bla* genes are often associated with transferable plasmids, and some of them are parts of transposon or constitute cassettes in the integrons. Insertion sequences like *ISEcp1*, which mobilize β -lactamase-encoding genes, may lead to their dissemination and ex-

pression [10]. Resistance to the third-generation cephalosporins by *E. coli* is on the increase in Kuwait and in a recent study the predominance of CTX-M ESBL was demonstrated among *E. coli* and *Klebsiella* spp. isolated from patients cared for at one of the teaching hospitals in Kuwait [11, 12]. Anecdotal reports suggest that genes encoding CTX-M enzymes may be widespread in *E. coli* isolates in Kuwait.

This study was designed to investigate the distribution and sequence analysis of *bla*_{CTX-M}-carrying *E. coli* isolated from patients admitted to the main government hospitals in Kuwait.

Materials and Methods

Bacterial Isolates

Eight government and teaching hospitals, namely Adan, Amiri, Fawaniya, Ibn Sina, Jahra, Maternity, Mubarak and Sabah hospitals, that contributed the isolates used for an earlier national susceptibility surveillance study were invited to participate in this study. They were asked to submit all ESBL-positive *E. coli* identified among the first 100 consecutive isolates obtained from clinical specimens in their laboratories during a 6-month period, January to May 2008; Adan Hospital submitted 11 isolates, Amiri 11, Fawaniya 12, Ibn Sina 35, Jahra 18, Maternity 4, Mubarak 5, and Sabah 10. For uniformity, all were required to carry out ESBL identification by both VITEK 2 (bioMerieux, Marcy-l'Etoile, France) and ESBL Etest (AB Biodisk, Sweden) methods that were available in all participating laboratories. Replicate isolates were omitted from the study. The ESBL-positive isolates were then sent to the Anaerobe and Hospital Infection Laboratory in the Department of Microbiology, Faculty of Medicine, Health Sciences Centre, Kuwait University, for confirmation and molecular analysis. Detailed biodata, including age, sex and nationality, were carefully recorded for each patient.

Confirmation of ESBL Production and Susceptibility Testing

Upon receipt, all isolates were immediately retested for production of ESBL by the ESBL Etest method. For this phenotypic characterization, the Etest ESBL method, using both ceftazidime/ceftazidime combined with clavulanic acid and cefotaxime/cefotaxime combined with clavulanic acid strips (AB Biodisk), was carried out and results interpreted according to the manufacturer's instructions. In-house ESBL-producing *E. coli* strain K31 [11] and ESBL-negative strain were included in the test runs as positive and negative controls, respectively. Susceptibility testing was performed on all ESBL-positive isolates using the Etest method according to the manufacturer's instructions and results were interpreted according to the criteria recommended by the Clinical and Laboratory Standards Institute [13].

PCR Confirmatory Testing and Sequence Analysis

ESBL-positive isolates were confirmed by PCR. Briefly, DNA extraction was performed using the NucleoSpin tissue kit (Macherey-Nagel). PCR amplification was then carried out to detect the presence of *bla*_{CTX-M}, using the following published primer

pairs: MA-1 5'-SCS ATG TGC AGY ACC AGT AA-3' and MA-2 5'-CCG CRA TAT GRT TGG TGG TG-3' [10]. The presence of TEM and SHV β -lactamases was detected by PCR using previously described primer sets [10]. The size of amplicons was 550 bp. *Salmonella* strains 971 and C600 were used as positive and negative controls, respectively. Strains with PCR amplicons positive for *bla*_{CTX-M} were sequenced and purified using a NucleoSpin extract kit (Macherey-Nagel) and then sequenced using BigDye Terminator (Applied Biosystem). The nucleotides were analyzed by comparing them with previously described sequences in the GenBank database with software available on the website <http://www.ncbi.nlm.nih.gov/blast>.

Detection of ISEcp1

The genetic organization of the *bla*_{ISEcp1} was investigated by sequencing this short segment using the following primers: ISEcp1A (5'-GCA GGT CTT TTT CTG CTC C-3') and ISEcp1B (5'-ATT TCC GCA GCA GCA CCG TTT GC-3') [10]. PCR was performed as previously described [14]. The PCR products were analyzed by electrophoresis in a 1% agarose gel. *Salmonella* strain 971 was the positive control and distilled water the negative control.

Genotyping of Isolates

The CTX-M-15-positive strains were evaluated for genetic relatedness using pulsed-field gel electrophoresis (PFGE) with *Xba*I digestion of the genomic DNA separated by electrophoresis in a 1.2% agarose gel, as previously described [15]. The strains were compared based on the differences in the number and mobility of the bands.

Results

The total number of *E. coli* with ESBL phenotypes obtained from participating hospitals and confirmed by PCR were 136. Of the 136, 106 (77.9%) produced CTX-M-type ESBL. These were obtained from patients aged 19–74 years (mean 59 years); 58 (54.7%) were females and 48 (45.3%) males, with a female-to-male ratio of 1.2:1. Eighty (75.5%) and 26 (24.5%) of these were isolates taken from patients on admission and patients managed at the outpatient clinics, respectively. The majority (94 of 106; 88.7%) were from adult and 12 (11.3%) from pediatric patients. As shown in table 1, the commonest source of isolates harboring CTX-M ESBL was predominantly urine (67%), followed by wound swabs (21.7%) and respiratory specimens (7.5%). Only 1.9% came from blood.

Distribution of *bla*_{CTX-M} by Hospital

The distribution of these genes among the isolates from different hospitals in Kuwait is shown in table 2. The majority of *E. coli* carrying *bla*_{CTX-M} among the ESBL-positive isolates came from Ibn Sina Hospital (33%) followed by Jahra Hospital (17%); the least from Maternity Hospital (3.4%).

Sequence analysis of these *bla*_{CTX-M} genes revealed a preponderance of *bla*_{CTX-M-15} genes found in 89 (84%) of the 106 isolates, followed by *bla*_{CTX-M-14} in 7 (6.6%), *bla*_{CTX-M-14b} in 6 (5.7%), and *bla*_{TOHO-1} in 4 (3.8%). The predominant gene in all hospitals was *bla*_{CTX-M-15}. The highest proportion of isolates harboring *bla*_{CTX-M-15} was detected in 4 of 4 (100%) isolates positive for CTX-M β -lactamase in Maternity Hospital, followed by 9 of 10 (90%) in Sabah Hospital, 16 of 18 (88.9%) in Jahra Hospital, 30 of 35 (85.7%) in Ibn Sina Hospital, 10 of 12 (83.3%) in Fawaniya Hospital and 4 of 5 (80%) in Mubarak Hospital. In these hospitals, *bla*_{CTX-M-14} genes were relatively uncommon among the isolates. In only 3 hospitals, i.e. Fawaniya Hospital, Ibn Sina Hospital and Jahra Hospital, were isolates carrying *bla*_{CTX-M-14b} detected. Those harboring *bla*_{TOHO-1} were confined to 2 hospitals, i.e. Amiri Hospital and Ibn Sina Hospital.

A total of 71 of the 106 (67%) isolates positive for *bla*_{CTX-M} carried *bla*_{ISEcp1} upstream *bla*_{CTX-M}. The prevalence of *bla*_{ISEcp1} among the different types of the CTX-M ESBL-positive isolates was: 4 of 4 (100%) TOHO-1, 6 of 7 (85.7%) CTX-M-14, 59 of 89 (66.3%) CTX-M-15, and 2 of 6 (33.3%) CTX-M-14b.

Seventy-eight (73.6%) of the 106 CTX-M-positive isolates harbored genes for TEM or SHV β -lactamases while 4 and 2 non-CTX-M ESBL producers contained TEM and SHV and SHV β -lactamases, respectively. Only 3 isolates harbored the 3 genes, *bla*_{CTX-M}, *bla*_{SHV} and *bla*_{TEM} together; these were urinary isolates. A combination of *bla*_{SHV} and *bla*_{CTX-M} genes was found in 6 isolates, all cultured from urine, and all from Amiri Hospital, Jahra Hospital and Sabah Hospital. The combination of *bla*_{TEM} and *bla*_{SHV} genes without *bla*_{CTX-M} was detected only in 4 isolates; all were also isolated from urine of patients in Amiri Hospital, Adan Hospital, and Jahra Hospital.

The ESBL Etest for ESBL production detected all the CTX-M, SHV and TEM-positive isolates from which confirmatory testing with PCR was performed. It was noted that testing both cefotaxime and ceftazidime was necessary for the detection of CTX-M-positive isolates. As shown in table 3, the presence of CTX-M-15 could not be inferred by mere inspection of the MICs of the third-generation cephalosporins, as their MIC₉₀ values for strains positive for CTX-M-14, CTX-M-14b and TOHO-1 were >256 μ g/ml, except for ceftazidime whose MIC₉₀ values were in the susceptible or intermediate ranges. However, inspection of the MICs for piperacillin-tazobactam together with ceftazidime could possibly predict CTX-M-15-positive and not CTX-M-14-, CTX-M-14b- and TOHO-1-positive isolates. All CTX-M-positive iso-

Table 1. Distribution of CTX-M ESBL-producing *E. coli* isolated from clinical specimens

Source of ESBL	ESBL isolates positive for CTX-M from each source	Overall percentage of CTX-M-positive strains (n = 106)
Urine (n = 90)	71 (78.9%)	67.0
Wound swabs (n = 30)	23 (76.7%)	21.7
Respiratory (n = 8)	8 (100%)	7.6
Blood (n = 5)	2 (40%)	1.9
Eye (n = 1)	0	0
Fluids (n = 1)	1 (100%)	0.9
Stool (n = 1)	1 (100%)	0.9
Total (n = 136)	106	100

Table 2. Distribution of *E. coli* isolates positive for different *bla*_{CTX-M} by hospital

Hospital	Isolates positive for the production of				
	CTX-M-15 (n = 89)	CTX-M-14 (n = 7)	CTX-M-14b (n = 6)	TOHO-1 (n = 4)	All CTX-M (n = 106)
ADH	8 (72.7)	3 (27.3)	0 (0)	0 (0)	11 (10.4)
AMH	8 (72.7)	1 (9.1)	0 (0)	2 (18.2)	11 (10.4)
FAH	10 (83.3)	0 (0)	2 (16.7)	0 (0)	12 (11.3)
ISH	30 (85.7)	0 (0)	3 (8.6)	2 (5.7)	35 (33.0)
JAH	16 (88.9)	1 (5.6)	1 (5.6)	0 (0)	18 (17.0)
MAH	4 (100)	0 (0)	0 (0)	0 (0)	4 (3.8)
MKH	4 (80.0)	1 (20.0)	0 (0)	0 (0)	5 (4.7)
SAH	9 (90.0)	1 (10.0)	0 (0)	0 (0)	10 (9.4)

ADH = Adan Hospital; AMH = Amiri Hospital; FAH = Fawaniya Hospital; ISH = Ibn Sina Hospital; JAH = Jahra Hospital; MAH = Maternity Hospital; MKH = Mubarak Al Kabir Hospital; SAH = Sabah Hospital. Figures in parentheses indicate percentages.

lates, with or without SHV and TEM, were susceptible to amikacin, imipenem, meropenem and tigecycline.

Distribution of CTX-M-Positive Isolates by Nationality

The distribution of the 106 CTX-M-positive isolates by nationality is shown in table 4. CTX-M-positive isolates were found mainly among Kuwaitis, Egyptians and Indians, representing 93 (87.7%); 38 (35.9%) Kuwaitis, 33 (31.1%) Egyptians and 22 (20.8%) Indians. Isolates carrying *bla*_{CTX-M-15} were mainly from these 3 nationalities; Kuwaitis 32 (30.2%), Egyptians 29 (27.4%) and Indians

Table 3. MICs of β -lactamase and other antibiotics for *E. coli* harboring *bla*_{CTX-M} genes

Antibiotic	MIC (μ g/ml) for CTX-M-producing isolates							
	CTX-M-15		CTX-M-14		CTX-M-14b		TOHO-1	
	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀
Amikacin	4	12	2	4	1.5	2	2	2
Amoxicillin-CA	8	12	6	8	6	6	6	6
Ampicillin	>256	>256	256	>256	>256	>256	256	256
Cefepime	96	>256	256	>256	24	>256	256	256
Cefotaxime	256	>256	256	>256	256	>256	256	256
Cefoxitin	4	12	4	16	4	4	6	6
Ceftazidime	32	>256	8	16	2	2	16	16
Ceftriaxone	>256	>256	256	>256	256	>256	256	256
Cefuroxime	>256	>256	256	>256	>256	>256	256	256
Ciprofloxacin	>32	>32	>32	>32	0.32	4	>32	>32
Imipenem	0.19	0.38	0.19	0.25	0.19	0.25	0.19	0.19
Meropenem	0.047	0.25	0.032	0.32	0.032	0.047	0.047	0.047
Piperacillin-TZ	12	>256	3	3	3	3	3	3
Tigecycline	0.25	0.5	0.125	0.5	0.06	0.125	0.125	0.25

CA = Clavulanic acid; TZ = tazobactam.

Table 4. Distribution of CTX-M ESBL types by nationality

Nationality	Positive for ESBL (n = 136)	ESBL-positive strains for CTX-M (n = 106)	CTX-M producers positive for			
			CTX-M-15 (n = 89)	CTX-M-14 (n = 7)	CTX-M-14b (n = 6)	TOHO-1 (n = 4)
Kuwaiti	48	38	32	5	1	1
Egyptian	42	33	29	1	2	1
Indian	26	22	18	1	1	2
SE Asian	5	4	2	0	1	0
Syrian	6	5	4	0	1	0
Bedouin	5	4	4	0	0	0
Saudi	4	0	0	0	0	0

18 (17%). Worthy of note, all 4 CTX-M-positive isolates from Bedouins harbored *bla*_{CTX-M-15} as well as 4 out of 5 (80%) from Syrians. The hitherto unreported and rare *bla*_{CTX-M-14b} and *bla*_{TOHO-1} in *E. coli* isolates from this region were detected in isolates from Kuwaitis, Indians, SE Asians, Egyptians and Syrians, and Kuwaitis, Indians and Egyptians, respectively.

Clonal Relatedness of the Isolates Positive for CTX-M-15 ESBL

The *Xba*I PFGE fingerprints for 16 randomly selected CTX-M-15-positive *E. coli* isolates from different hospi-

tals (2/hospital) are shown in figure 1. All were genetically heterogeneous, as these isolates did not fall within a particular cluster, having been distributed among the entire dendrogram.

Discussion

Our current experience in Kuwait lends credence to the dramatic worldwide increase in prevalence of CTX-M-type β -lactamases, which appear to be the most widely spread enzymes produced among the family of En-

Fig. 1. PFGE pattern of *E. coli* isolates positive for only CTX-M-15 β -lactamase from different hospitals (a), and *E. coli* isolates producing multiple ESBL β -lactamases (b). Strain No. 20 was positive only for CTX-M-14b. ADH = Adan Hospital; AMH = Amiri Hospital; FAH = Fawaniya Hospital; ISH = Ibn Sina Hospital; JAH = Jarah Hospital; MAH = Maternity Hospital; MKH = Mubarak Al Kabir Hospital; SAH = Sabah Hospital.

terobacteriaceae. This assertion is supported by finding *bla*_{CTX-M}, the gene that mediates CTX-M enzyme production, in approximately 78% of the ESBL-producing isolates either alone or in combination with other known ESBL-mediating genes, *bla*_{SHV} or *bla*_{TEM}. The dominant type representing 84% of all *bla*_{CTX-M} genes was *bla*_{CTX-M-15}. Thus, CTX-M-15 is the most common CTX-M type detectable in *E. coli* in Kuwait. This confirmed the earlier report by Ensor et al. [11], which found this enzyme to be the most common type among ESBL-producing strains of *E. coli* and *Klebsiella* spp. encountered in 1

single hospital in Kuwait. Over the last few years, different studies conducted in North America [9, 16, 17], Europe [8, 18] and South America [19] have detected the emergence and increasing prevalence of CTX-M-15 enzymes among CTX-M-producing *E. coli* isolates. This enzyme type has also been found to be responsible for community outbreaks of multidrug-resistant *E. coli* infections in the UK and other locations [20, 21].

Our results confirmed and extended the findings of Ensor et al. [11] in that our isolates from the entire country were also predominantly from urinary tract infec-

tions (67%). This is in contrast to a study in Pennsylvania [22] in which the isolates were mostly non-urinary bacteria (69%), although the proportion of CTX-M-15-positive isolates among their collection was almost the same as ours. The types and distributions of *bla*_{CTX-M} gene among our ESBL-producing isolates are similar to that which has been reported in some European countries, with a high percentage of isolates from urinary tract infections [3, 20]. A previous report from Kuwait also found a high prevalence of CTX-M-15 β -lactamase in strains of non-typhoidal and typhoidal *Salmonella* spp., including *S. enterica* serotype Typhi [14]. This report together with Ensor et al.'s [11] and our present findings demonstrate the explosive nature of this enzyme in Kuwait. Thus, there is reason to be concerned in our setting, just as many other clinical microbiologists and infectious diseases experts are in the UK and other European countries [23].

Our results revealed that Ibn Sina Hospital has the highest prevalence of *bla*_{CTX-M-15} in ESBL-producing *E. coli* followed by Jahra Hospital. Ibn Sina Hospital is unique for its richness in multidrug-resistant organisms because of the nature of its care. This hospital is the only tertiary referral hospital for burn, plastic surgery and also oncology patients for whom cocktails of antibiotics are usually administered. Jahra Hospital is a large general hospital with a great number of geriatric bed-bound patients for whom uncontrolled antibiotics are also usually prescribed. We noted that the situation in the other hospitals, which are general hospitals with more acute care patients, is different since they had less prevalence of this enzyme.

In the present study, a mobile genetic element, *ISEcp1*, which is a single-copy insertion sequence responsible for mobilization of *bla* genes and located upstream of *bla*_{CTX-M} genes [24], was found to coexist in 67% of our CTX-M ESBL-positive strains. This finding calls for concern because of the ability of *ISEcp1* to facilitate the spread of resistance.

A very mixed population of ethnicities lives in Kuwait, and there is a constant movement of people to and from different parts of the world. Kuwait is a small country with a single metropolitan area and has a population of approximately 2.8 million people, of which about 1.3 (46.3%) are non-nationals. Frequent importation of many infectious diseases from Asia and Africa into Kuwait is common [14]. It is of great interest to note in this study that *bla*_{CTX-M-15}-positive *E. coli* isolates were mostly detected among Kuwaiti and other Arab populations, especially Egyptians, since this gene is said to be clonal and originating in India [23]. However, we also noted that a

substantial number of isolates from Indian nationals carried the *bla*_{CTX-M-15} gene. The fact that many of the latter work as maids, drivers and cleaners and also in restaurants may in part explain the likelihood of introduction of *bla*_{CTX-M-15}-positive isolates into the population over time. Analysis of the PFGE pattern of the *bla*_{CTX-M} genes demonstrates the heterogeneous nature of the enzymes, as there is no evidence of any clonal clustering within a single hospital or clonal spread across hospitals in Kuwait. Finding other types of CTX-M ESBLs, such as CTX-M-14, associated mainly with strains from China, and TOHO-1, found in Japan, further demonstrates the dynamic and fluid nature of the expatriate workers among the Kuwait population since nationals from these 2 countries are also well represented in Kuwait.

Conclusion

Our data demonstrate an explosive emergence of isolates positive for *bla*_{CTX-M-15} gene that appears to be highly prevalent in all hospitals in Kuwait and found in patients belonging to the predominant nationalities residing in Kuwait. We also detected no evidence of intra- or inter-hospital clonal spread.

Acknowledgements

This work was supported by a Kuwait University Research Grant No. YM 07/06, which is gratefully acknowledged. Ms. May Shahin is greatly appreciated for her technical support.

References

- 1 Tzouveleakis LS, Tzelepi E, Tassios PT, Legakis NJ: CTX-M-type β -lactamases: an emerging group of extended-spectrum enzymes. *Int J Antimicrob Agents* 2000;14: 137-142.
- 2 Bauernfeind A, Stemmlinger I, Jungwirth R, Ernst S, Casellas JM: Sequences of β -lactamase genes encoding CTX-M-1 (MEN-1) and CTX-M-2 and relationship of their amino acid sequences with those of other β -lactamases. *Antimicrob Agents Chemother* 1996;40:509-513.
- 3 Bonnet R, Dutour C, Sampalo JL, Chanal C, Sirot D, Labia R, De Champs C, Sirot J: Novel cefotaximase (CTX-M-16) with increased catalytic efficiency due to substitution Asp-240 to Gly. *Antimicrob Agents Chemother* 2001;45:2269-2275.

- 4 Poirel L, Naas T, Le Thomas I, Karim A, Bingen E, Nordmann P: CTX-M-type extended-spectrum β -lactamase that hydrolyzes ceftazidime through a single amino acid substitution in the omega loop. *Antimicrob Agents Chemother* 2001;45:3355–3361.
- 5 Bonnet R: Growing group of extended spectrum β -lactamases: the CTX-M enzymes. *Antimicrob Agents Chemother* 2004;48:1–14.
- 6 Poirel L, Gniadkowski M, Nordmann P: Biochemical analysis of the ceftazidime-hydrolysing extended-spectrum β -lactamase CTX-M-15 and of its structurally related β -lactamase CTX-M-3. *J Antimicrob Chemother* 2002;50:1031–1034.
- 7 Pournaras S, Ikonomidis A, Kristo I, Tsakris A, Maniatis AN: CTX-M enzymes are the most common extended-spectrum β -lactamases among *Escherichia coli* in a tertiary Greek hospital. *J Antimicrob Chemother* 2004;54:574–575.
- 8 Woodford N, Ward ME, Kaufmann ME, Turton J, Fagan EJ, James D, Johnson AP, Pike R, Warner M, Cheasty T, Pearson A, Harry S, Leach JB, Loughrey A, Lowes JA, Warren RE, Livermore DM: Community and hospital spread of *Escherichia coli* producing CTX-M extended-spectrum beta-lactamases in the UK. *J Antimicrob Chemother* 2004;54:735–743.
- 9 Boyd D, Tyler S, Christianson S, McGeer A, Muller MP, Willey BM, Bryce E, Gardman M, Nordmann P, Mulvey MR: Complete nucleotide sequence of a 92-kilobase plasmid harbouring the CTX-M-15 extended-spectrum β -lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. *Antimicrob Agents Chemother* 2004;48:3758–3764.
- 10 Cao V, Lambert T, Courvalin P: CoIE1-like plasmid pIP843 of *Klebsiella pneumoniae* encoding extended-spectrum β -lactamase CTX-M-17. *Antimicrob Agents Chemother* 2002;46:1212–1217.
- 11 Ensor VM, Jamal W, Rotimi VO, Evans JT, Hawkey PM: Predominance of CTX-M-15 extended-spectrum β -lactamases in diverse *Escherichia coli* and *Klebsiella pneumoniae* from hospital and community patients in Kuwait. *Int J Antimicrob Agents* 2009;33:487–489.
- 12 Jamal WY, Al Hashem G, Khodakhast F, Rotimi VO: Comparative in vitro activity of tigecycline and nine other antibiotics against Gram-negative bacterial isolates, including ESBL-producing strains. *J Chemother* 2009;21:261–266.
- 13 Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Susceptibility Testing: 16th Informational Supplement Document M100-S16. Wayne, Clinical and Laboratory Standards Institute, 2006.
- 14 Rotimi VO, Jamal W, Pal T, Sonnevend A, Albert MJ: Emergence of CTX-M-15 type extended-spectrum β -lactamase-producing *Salmonella* spp. in Kuwait and the United Arab Emirates. *J Med Microbiol* 2008;57:881–886.
- 15 CDC: Standardized Molecular Subtyping of Foodborne Bacterial Pathogens by Pulsed-Field Gel Electrophoresis. Atlanta, National Center for Infectious Diseases, 2002.
- 16 Lewis JS, Herrera M, Wickers B, Patterson JE, Jorgensen JH: First report of the emergence of CTX-M-type extended-spectrum beta-lactamases (ESBLs) as the predominant ESBL isolated in a US Health Care System. *Antimicrob Agents Chemother* 2007;51:4015–4021.
- 17 Castanheira M, Mendes RE, Rhomberg PR, Jones RN: Rapid emergence of *bla*_{CTX-M} among *Enterobacteriaceae* in US medical centers: molecular evaluation from the MYSTIC Program (2007). *Microb Drug Resist* 2008;14:211–216.
- 18 Kassis-Chikhani N, Vimont S, Asselat K, Triville C, Minassian B, Sengelin C, Gautier V, Mathieu D, Dussaix E, Arlet G: CTX-M beta-lactamase-producing *Escherichia coli* in long-term care facilities, France. *Emerg Infect Dis* 2004;10:1697–1698.
- 19 Radice M, Power P, Di Conza J, Gutkind G: Early dissemination of CTX-M-derived enzymes in South America. *Antimicrob Agents Chemother* 2002;46:602–603.
- 20 Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, Ayala J, Coque TM, Kern-Zdanowicz I, Luzzaro F, Poirel L, Woodford N: CTX-M: changing the face of ESBLs in Europe. *J Antimicrob Chemother* 2007;59:165–174.
- 21 Rossolini GM, D'Andrea MM, Mugnaioli C: The spread of CTX-M-type extended-spectrum beta-lactamases. *Clin Microbiol Infect* 2008;14(suppl 1):33–41.
- 22 McGettigan SE, Hu B, Andreacchio K, Nachamkin I, Edelstein PH: Prevalence of CTX-M β -lactamases in Philadelphia, Pennsylvania. *J Clin Microbiol* 2009;47:2970–2974.
- 23 Livermore DM, Hawkey PM: CTX-M: changing face of ESBLs in the UK. *J Antimicrob Chemother* 2005;56:451–454.
- 24 Walther-Rasmussen J, Hoiby N: Cefotaximases (CTX-M-ases), an expanding family of extended-spectrum β -lactamases. *Can J Microbiol* 2004;50:137–165.