Molecular characterization and antimicrobial susceptibility of extended-spectrum β -lactamase-producing *Enterobacteriaceae* isolates at a tertiary-care centre in Monterrey, Mexico

Elvira Garza-González,^{1†} Sandra Iveth Mendoza Ibarra,^{1†}
Jorge M. Llaca-Díaz² and Gloria M. Gonzalez¹

Correspondence

Elvira Garza-González
elvira_garza_gzz@yahoo.com

¹Departamento de Microbiología, Facultad de Medicina, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, Mexico

²Hospital Universitario 'Dr José Eleuterio González', Universidad Autónoma de Nuevo León, Monterrey, Nuevo Leon, Mexico

Our objective was to analyse phenotypic and genetic data of extended-spectrum β -lactamase (ESBL)-producing *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Escherichia coli* and *Serratia marcescens* that cause infections in our hospital. Over a 3 year period, 342 randomly selected clinical *Enterobacteriaceae* isolates were tested for ESBL production and evaluated for the presence of the β -lactamase genes *bla*_{SHV}, *bla*_{TEM}, *bla*_{CTX-M} and *bla*_{TLA-1}. The antibiotic susceptibilities of these isolates were also determined, and the clonality of the isolates was assessed by PFGE. Based on our analyses, 33/92 (35.9 %) *K. pneumoniae*, 31/87 (35.6 %) *Enterobacter cloacae*, 24/80 (30 %) *E. coli* and 17/83 (20.5 %) *S. marcescens* were identified as ESBL producers. The presence of TEM, SHV or CTX ESBL types was detected in 99/105 (94 %) of the isolates. TLA-1 was not detected in any of the 105 isolates. The dominant ESBL types were *bla*_{SHV-5} (*n*=33), *bla*_{SHV-12} (*n*=31) and *bla*_{CTX-M-15} (*n*=30). The predominant ESBL identified in *E. coli* and *Enterobacter cloacae* isolates was CTX-M-15, whereas in *K. pneumoniae* and *S. marcescens* the predominant types were SHV-12 and SHV-5, respectively. PFGE genotyping revealed two main genetic patterns in the *K. pneumoniae* isolates, types SHV-12 and TEM-1 + SHV-5. An outbreak caused by *Enterobacter cloacae* SHV-5 + CTX-M-15 was detected. In contrast, most ESBL-producing isolates of *E. coli* and *S. marcescens* did not have similar PFGE banding patterns and thus were not genetically similar. *Enterobacteriaceae* are a concern in our hospital, especially *K. pneumoniae* and *Enterobacter cloacae*. Our results confirm that the CTX-M-15 ESBL type has spread rapidly in the hospital, and thus requires careful monitoring.

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INTRODUCTION

Enterobacteriaceae rendered resistant to penicillins, broad-spectrum cephalosporins and monobactams, by the production of extended-spectrum β -lactamases (ESBLs), are recognized as major causes of both nosocomial and community-acquired infections (Bradford, 2001; Cantón *et al.*, 2008). ESBLs are found predominantly in *Klebsiella* species and *Escherichia coli*, but have been described in other species as well, including *Enterobacter*, *Serratia*, *Citrobacter*, *Proteus* and *Salmonella* (Paterson & Bonomo, 2005).

†These authors contributed equally to this work.

Abbreviations: CSF, cerebrospinal fluid; ESBL, extended-spectrum β -lactamase.

The prevalence of ESBL-expressing bacteria varies across different geographical regions. Fewer than 10 % of isolated strains express ESBLs in Sweden, Japan and Singapore, compared to rates higher than 30 % in Portugal, Italy, New York and some Latin American countries. In Turkey, as many as 58 % of isolated strains express ESBLs (Paterson & Bonomo, 2005).

ESBLs arise mainly due to mutations in β -lactamases encoded by the *bla*_{SHV}, *bla*_{TEM}, and *bla*_{CTX-M} genes. At present, more than 300 different ESBL variants have been described (Paterson & Bonomo, 2005). Though TEM and SHV variants are the most common ESBLs, during the past decade strains expressing CTX-M ESBLs have begun to emerge in many countries (Bonnet, 2004) and are now the most frequent non-TEM, non-SHV ESBL type. CTX-M

β -lactamases are characterized by selective hydrolysis of cefotaxime rather than ceftazidime, though some CTX-M types, such as CTX-M-15, may actually hydrolyse ceftazidime (Poirel *et al.*, 2001). Other ESBL enzymes, such as OXA, VEB, PER and GES, are less frequently encountered (Paterson & Bonomo, 2005). In Mexico, several studies have reported the high prevalence of SHV-5 (Espinosa de los Monteros *et al.*, 2008; Miranda *et al.*, 2004). A novel ESBL type, TLA-1 (Silva *et al.*, 2000), has been associated with SHV-5 (Alcantar-Curiel *et al.*, 2004). In this report, we present a comprehensive phenotypic and genetic analysis of ESBL-producing infectious *Klebsiella pneumoniae*, *Enterobacter cloacae*, *E. coli* and *Serratia marcescens* isolated from a tertiary care hospital in Mexico.

METHODS

Clinical isolates. A total of 342 randomly selected *K. pneumoniae* ($n=92$), *E. coli* ($n=80$), *Enterobacter cloacae* ($n=87$) and *S. marcescens* ($n=83$) isolates were collected from the tertiary-care Hospital Universitario 'Dr José Eleuterio González' in Monterrey, Mexico, from January 2006 to January 2009. Information was collected about the patients from whom strains were isolated, including age, sex and the hospital unit where the patient stayed. Clinical specimens included those from the lower respiratory tract ($n=138$), wounds ($n=43$), blood ($n=38$), fluids other than cerebrospinal fluid (CSF) and blood ($n=34$), abscesses ($n=32$), catheters ($n=30$), CSF ($n=9$) and urine ($n=18$). Only one isolate per patient was included in our analyses.

Clinical isolates were identified using standard biochemical methods (Murray *et al.*, 2007). When needed, API 20E galleries were used according to the manufacturer's instructions, and results were interpreted using API 20 E V4.1 identification software. Bacterial isolates were stored in *Brucella* broth containing 15% glycerol at -70°C .

ESBL production and characterization. The ESBL phenotypic detection test was performed using the combination disc method, using ceftazidime, cefotaxime and the inhibitory effect of clavulanic acid recommended by the Clinical and Laboratory Standards Institute (CLSI, 2009). When an ESBL phenotype was detected, PCR assays were conducted to test isolates for the presence of the *bla*_{SHV}, *bla*_{TEM}, *bla*_{CTX-M} and *bla*_{TLA-1} genes. To obtain template DNA, 3–5 colonies from each isolate were boiled in sterile water for 5 min. Samples were centrifuged and 2 μl supernatant was used as the DNA template. The PCRs were carried out using published primers (Berçot *et al.*, 2010; Coque *et al.*, 2002; Siu *et al.*, 1999). PCR products were purified using the Wizard PCR preps DNA purification system (Promega). All bidirectional nucleotide sequencing was performed at the Instituto de Biotecnología, Universidad Nacional Autónoma de México (Cuernavaca, Morelos, Mexico), using the above PCR primers. Nucleotide sequences and the derived amino acid sequences were compared with previously described sequences (GenBank database <http://www.ncbi.nlm.nih.gov/> and www.lahey.org/studies, respectively).

Antibiotic susceptibility testing was performed on all ESBL producers using the broth microdilution method as described by the CLSI (2009). Six β -lactam antibiotics were tested: ceftriaxone, ceftazidime, cefepime, ampicillin, piperacillin and meropenem. Isolates were also tested against non- β -lactam antibiotics, including amikacin, levofloxacin and tigecycline.

PFGE. Isolates were genotyped using PFGE. Genomic DNA from each strain was isolated in an agarose-embedded form and subjected to in-gel enzymic digestion using 50 U *Xba*I. The agarose plug was

subsequently placed in the preformed wells of an agarose gel. PFGE was performed for 23 h with a CHEF Mapper system (Bio-Rad) using $0.5 \times \text{TBE}$ buffer ($10 \times \text{TBE}$: 890 mM Tris-borate, 890 mM boric acid, 20 mM EDTA). PFGE profiles were visualized under UV after staining with ethidium bromide (0.5 mg ml^{-1}). Patterns were interpreted using the Tenover criteria (Tenover *et al.*, 1995).

RESULTS AND DISCUSSION

ESBL production and resistance rates

Using the phenotypic assay, 33/92 (35.9%) of *K. pneumoniae*, 31/87 (35.6%) of *Enterobacter cloacae*, 24/80 (30%) of *E. coli* and 17/83 (20.5%) of *S. marcescens* were identified as ESBL producers. All of the ESBL producers tested positive for at least one of the β -lactamase encoding genes by PCR. However, ESBLs could be sequenced in only 99 of the 105 isolates. The six other isolates contained *bla*_{TEM-1}, which is not an ESBL (Table 1). The dominant ESBL types detected were *bla*_{SHV-5} ($n=33$), *bla*_{SHV12} ($n=31$) and *bla*_{CTX-M-15} ($n=30$).

A particularly interesting result was the finding that 66.7% of the *E. coli* and 38.7% of the *Enterobacter cloacae* isolates contained CTX-M-15. In contrast to other CTX-M types, this ESBL type may hydrolyse ceftazidime (Poirel *et al.*, 2001). CTX-M-15 has been detected in *Salmonella* sp. (Rotimi *et al.*, 2008) and is believed to be the dominant ESBL type in some Asian countries (Bonnet, 2004), occurring in 99.6% of *E. coli* and 99.2% of *K. pneumoniae* isolates in Thailand (Kiratisin *et al.*, 2008). The only report of the CTX-M-15 type in Mexico was made recently in *E. coli* (Rocha-Gracia *et al.*, 2010). Our study confirms that the CTX-M-15 ESBL has spread in *Enterobacteriaceae*, because it was the most prevalent ESBL in the *E. coli* and *Enterobacter cloacae* isolates analysed in this study.

To our knowledge, CTX-M ESBLs are not the predominant type in Mexican hospitals. SHV ESBLs, along with a new ESBL named TLA-1, are more commonly reported in these institutions (Espinosa de los Monteros *et al.*, 2008; Garza-Ramos *et al.*, 2009; Silva *et al.*, 2000). None of the isolates in this study contained TLA-1.

The dominant ESBL in *K. pneumoniae* was SHV-12 (51.5%) and in *S. marcescens* it was SHV-5 (47.1%). The isolation of *S. marcescens* SHV-5 is somewhat relevant because it represents a serious threat in the hospital, as was shown by a previous outbreak caused by a cephalosporin-resistant *S. marcescens* SHV-5 producing strain reported in a Mexican hospital. In this outbreak, the strain was isolated from patients, from medical staff and from chlorhexidine disinfectant solutions (Espinosa de los Monteros *et al.*, 2008).

High resistance rates were observed for levofloxacin and, as expected, for ampicillin. In contrast, low resistance was observed for amikacin and tigecycline, and none of the strains was resistant to meropenem. The most highly resistant ESBL strains were *Enterobacter cloacae* SHV-5 + CTX-M-15, *Enterobacter cloacae* TEM-1 + CTX-M-15,

Table 1. Distribution of the *bla*_{TEM}, *bla*_{SHV} and *bla*_{CTX} ESBLs and antimicrobial susceptibility

All isolates had 0 % resistance to meropenem.

Species and ESBL type*	No of isolates (%)	Percentage resistant to:							
		AMK	LVX	CRO	CAZ	PIP	FEP	AM	TIG
<i>Enterobacter cloacae</i> (n=31)									
TEM-1 + SHV-12	8 (7.6)	25	13	50	75	13	13	13	13
TEM-1 + CTX-M15	6 (5.7)	17	50	100	83	0	67	100	0
SHV-5 + CTX-M15	5 (4.8)	100	20	100	60	20	20	100	0
TEM-1	4 (3.8)	0	25	50	100	25	25	100	25
TEM-1 + SHV-5	4 (3.8)	25	0	75	75	50	50	100	0
SHV-2	2 (1.9)	0	0	0	50	0	0	100	0
TEM-1 + SHV-5 + CTX-M15	1 (1)	0	0	100	0	0	0	100	0
TEM-1 + SHV-2	1 (1)	0	100	0	100	0	0	100	0
<i>Escherichia coli</i> (n=24)									
TEM-1 + CTX-M15	11 (10.5)	9	100	100	27	0	73	100	0
CTX-M15	5 (4.8)	0	100	20	60	20	40	100	20
SHV-2	2 (1.9)	0	100	100	50	0	50	100	0
TEM-1 + SHV-5	2 (1.9)	0	50	50	0	0	0	100	0
SHV-5	2 (1.9)	0	100	100	0	0	50	100	0
TEM-1 + SHV-12	1 (1)	0	100	0	0	0	0	100	0
TEM-1	1 (1)	0	100	0	0	0	0	100	0
<i>Klebsiella pneumoniae</i> (n=33)									
SHV-12	17 (16.2)	35	76	53	82	35	24	94	6
TEM-1 + SHV-5	11 (10.5)	27	27	55	73	18	9	91	9
TEM-1 + CTX-M15	2 (1.9)	0	100	50	50	0	0	100	0
SHV-2	2 (1.9)	0	50	50	50	0	0	100	0
TEM-1	1 (1)	100	100	100	100	100	0	100	0
<i>Serratia marcescens</i> (n=17)									
SHV-5	8 (7.6)	50	0	38	50	38	38	100	13
SHV-12	5 (10.5)	0	60	60	40	100	20	100	20
SHV-2	4 (3.8)	0	0	50	0	0	0	50	0

AM, Ampicillin; AMK, amikacin; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; LVX, levofloxacin; PIP, piperacillin; TIG, tigecycline.

*TEM-1 is not an ESBL.

E. coli TEM-1 + CTX-M-15, *K. pneumoniae* SHV-12 and *S. marcescens* SHV-12. These strains were more than 50 % resistant to four of the nine antibiotics tested. The high resistance rates observed for these isolates are not surprising, as bacteria that produce CTX-M-type β -lactamases typically have high cefotaxime MICs, of greater than 64 $\mu\text{g ml}^{-1}$. In particular, CTX-M-15 may actually hydrolyse ceftazidime, with an MIC as high as 256 $\mu\text{g ml}^{-1}$ (Baraniak *et al.*, 2002; Poirel *et al.*, 2002). Additionally, cefepime MICs for the CTX-M ESBL types are greater than those observed for bacteria that produce other ESBL types (Yu *et al.*, 2002). Furthermore, the SHV-12 type has been associated with high-level resistance to ceftazidime (Tansawai *et al.*, 2009).

Molecular epidemiology

The majority of ESBL producers were isolated from lower respiratory tract specimens ($n=44$), followed by catheter

($n=15$), blood ($n=14$), wound ($n=11$), fluids other than CSF and blood ($n=8$), abscesses ($n=7$), urine ($n=5$) and CSF ($n=2$). A pulsotype was defined as a group of isolates that displayed PFGE banding patterns ≥ 85 % similar (Singh *et al.*, 2006). Eighty-two pulsotypes could be described from the PFGE patterns of the isolates tested.

Our analyses revealed that 78.3 % (18/23) of the typable *K. pneumoniae* isolates could be separated into two clones (Fig. 1, Table 2). A total of 37 % (10/27) of *Enterobacter cloacae* isolates could be grouped into two clones, of which one clone (clone 3) was an SHV-5 + CTX-M-15 producer. Though SHV-5-producing *Enterobacter cloacae* strains have been described in Mexico (Garza-Ramos *et al.*, 2009), this is, to the best of our knowledge, the first report of an *Enterobacter cloacae* strain isolated in Mexico that produces both SHV-5 and CTX-M15.

PFGE analysis revealed a high genomic heterogeneity among ESBL-producing *E. coli* and *S. marcescens* isolates.

Fig. 1. PFGE analysis of *K. pneumoniae* (A), *Enterobacter cloacae* (B), *E. coli* (C) and *S. marcescens* (D) isolates. The boxes indicate an 85 % banding pattern similarity, the standard used to define the clones.

Table 2. ESBL type and clonal origin

Lower respiratory tract specimens, 85 % mainly bronchial lavage.

Isolate and ESBL type	Clone	Isolate	Specimen	Isolation date	Patient		
					Age (years)	Gender	Hospital ward
<i>Klebsiella pneumoniae</i> TEM-1 + SHV-5	1	1146	Fluids	19/07/2006	0	M	Paediatrics
		1503	Blood	29/08/2006	0	M	N-ICU
		1504	Catheter	01/08/2006	NA	M	IM
		1517	Blood	14/09/2006	18	M	IM
		1716	Blood	14/09/2006	18	M	IM
		4837	Blood	08/08/2007	1	F	Paediatrics
<i>Klebsiella pneumoniae</i> SHV12	2 (A)	1262	Catheter	27/07/2006	64	M	IM
		1715	LR	14/09/2006	19	F	A-ICU
		1717	Catheter	14/09/2006	52	F	PS-ICU
		1783	Catheter	20/09/2006	38	F	PS-ICU
		2805	LR	24/11/2006	32	F	Surgery
		4529	Blood	13/07/2007	NA	M	PS-ICU
		4563	LR	18/07/2007	65	F	Pensioner
		5706	Blood	24/10/2007	18	F	IM
		1753	LR	18/09/2006	52	F	A-ICU
		2406	Blood	23/10/2006	16	F	Obstetrics
		3444	Blood	06/03/2007	48	M	IM
		5978	LR	27/11/2007	24	F	A-ICU
		7008	LR	20/05/2008	72	F	A-ICU
		7078	LR	23/05/2008	25	M	A-ICU
		5555	LR	04/10/2007	64	M	A-ICU
<i>Enterobacter cloacae</i> SHV-5 + CTX-M-15	3	5602	LR	11/10/2007	40	M	A-ICU
		5618	LR	12/10/2007	42	F	A-ICU
		6024	LR	04/12/2007	86	M	A-ICU
		540	Fluids	08/06/2006	0	F	N-ICU
<i>Enterobacter cloacae</i> TEM-1 + SHV-12	4	541	Fluids	08/06/2006	70	M	Pensioner
		542	Catheter	08/06/2006	15	M	Paediatrics
		1050	Blood	10/07/2006	0	F	N-ICU
		1151	LR	19/07/2006	19	F	A-ICU

A, Adult; F, female; ICU, Intensive Care Unit; IM, Internal Medicine; LR, lower respiratory tract specimens; M, male; N, neonate; NA, not available; PS, post surgery.

More than 70 % of these isolates could not be assigned to clonal groups. This is in contrast to published reports that describe a high degree of genetic relatedness among *E. coli* and *S. marcescens* strains isolated in other hospitals (Paterson & Bonomo, 2005).

In this study, we detected six isolates that contained TEM-1, which has been reported in many countries (Al-Obeid *et al.*, 2008; Severin *et al.*, 2010). However, TEM-1 is not an ESBL, and surveillance of strains carrying the TEM-1

β -lactamase is needed, as these strains may give rise to new ESBL types. Evidence that these strains can develop into new ESBLs was presented after an outbreak of a TEM-1-containing *Klebsiella oxytoca* strain. After patients were treated with ceftazidime, *K. oxytoca* TEM-12 was detected in the same neonatal unit (Du Bois *et al.*, 1995). In our study, TEM-1 was detected in *K. pneumoniae*, *E. coli* and *Enterobacter cloacae* (six isolates in total), which may increase the potential health risk of this non-ESBL type. In these isolates, the presence of other non-common ESBL

types, such as OXA, VEB, PER and GES, may be responsible for the ESBL phenotype observed for the isolates characterized in this study, though additional testing to detect the presence of genes encoding the less common ESBL types is needed to confirm this.

In conclusion, *K. pneumoniae* and *Enterobacter cloacae* were the species with the highest ESBL production, and multiple CTX-M-15 isolates were detected in this Mexican hospital. The results of this study describe the genetic characteristics and molecular epidemiology of ESBLs among *Enterobacteriaceae* isolates at a tertiary care hospital in Mexico and provide insight into the emergence of bacterial strains harbouring ESBL genes.

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REFERENCES

- Alcantar-Curiel, D., Tinoco, J., Gayosso, C., Carlos, A., Daza, C., Perez-Prado, M., Salcido, L., Santos, J. & Alpuche-Aranda, C. (2004). Nosocomial bacteremia and urinary tract infections caused by extended-spectrum β -lactamase-producing *Klebsiella pneumoniae* with plasmids carrying both SHV-5 and TLA-1 genes. *Clin Infect Dis* 38, 1067–1074.
- Al-Obeid, S., Bremont, S., Jabri, L., Massoudi, N. & Haddad, O. (2008). *Klebsiella pneumoniae* LO10 producing extended-spectrum β -lactamase SHV-12 in Saudi Arabia. *J Chemother* 20, 709–713.
- Baraniak, A., Fiett, J., Hryniewicz, W., Nordmann, P. & Gniadkowski, M. (2002). Ceftazidime-hydrolysing CTX-M-15 extended-spectrum β -lactamase (ESBL) in Poland. *J Antimicrob Chemother* 50, 393–396.
- Berçot, B., Poirel, L., Silva-Sanchez, J. & Nordmann, P. (2010). Association of the extended-spectrum β -lactamase gene *bla*_{TLA-1} with a novel ISCR element, ISCR20. *Antimicrob Agents Chemother* 54, 4026–4028.
- Bonnet, R. (2004). Growing group of extended-spectrum β -lactamases: the CTX-M enzymes. *Antimicrob Agents Chemother* 48, 1–14.
- Bradford, P. A. (2001). Extended-spectrum β -lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev* 14, 933–951.
- Cantón, R., Novais, A., Valverde, A., Machado, E., Peixe, L., Baquero, F. & Coque, T. (2008). Prevalence and spread of extended-spectrum β -lactamase-producing *Enterobacteriaceae* in Europe. *Clin Microbiol Infect* 14 (Suppl. 1), 144–153.
- CLSI (2009). *Performance Standards for Antimicrobial Susceptibility Testing*, 19th informational supplement, M100–S19. Wayne, PA: Clinical and Laboratory Standards Institute.
- Coque, T. M., Oliver, A., Pérez-Díaz, J. C., Baquero, F. & Cantón, R. (2002). Genes encoding TEM-4, SHV-2, and CTX-M-10 extended-spectrum β -lactamases are carried by multiple *Klebsiella pneumoniae* clones in a single hospital (Madrid, 1989 to 2000). *Antimicrob Agents Chemother* 46, 500–510.
- Du Bois, S. K., Marriott, M. S. & Amyes, S. G. (1995). TEM- and SHV-derived extended-spectrum β -lactamases: relationship between selection, structure and function. *J Antimicrob Chemother* 35, 7–22.
- Espinosa de los Monteros, L. E., Silva-Sanchez, J., Jiménez, L. V., Rojas, T., Garza-Ramos, U. & Valverde, V. (2008). Outbreak of infection by extended-spectrum β -lactamase SHV-5-producing *Serratia marcescens* in a Mexican hospital. *J Chemother* 20, 586–592.
- Garza-Ramos, U., Davila, G., Gonzalez, V., Alpuche-Aranda, C., López-Collada, V., Alcantar-Curiel, D., Newton, O. & Silva-Sanchez, J. (2009). The *bla*_{SHV-5} gene is encoded in a compound transposon duplicated in tandem in *Enterobacter cloacae*. *Clin Microbiol Infect* 15, 878–880.
- Kiratisin, P., Apisarnthanarak, A., Laesripa, C. & Saifon, P. (2008). Molecular characterization and epidemiology of extended-spectrum β -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.
- Miranda, G., Castro, N., Leaños, B., Valenzuela, A., Garza-Ramos, U., Rojas, T., Solórzano, F., Chihu, L. & Silva, J. (2004). Clonal and horizontal dissemination of *Klebsiella pneumoniae* expressing SHV-5 extended-spectrum β -lactamase in a Mexican pediatric hospital. *J Clin Microbiol* 42, 30–35.
- Murray, P. R., Baron, J. L., Jorgensen, J. H., Landry, M. L. & Pfaller, M. A. (2007). *Manual of Clinical Microbiology*, 9th edn, vol. 1. Washington, DC: American Society for Microbiology.
- Paterson, D. L. & Bonomo, R. A. (2005). Extended-spectrum β -lactamases: a clinical update. *Clin Microbiol Rev* 18, 657–686.
- Poirel, L., Naas, T., Le Thomas, I., Karim, A., Bingen, E. & Nordmann, P. (2001). CTX-M-type extended-spectrum β -lactamase that hydrolyzes ceftazidime through a single amino acid substitution in the omega loop. *Antimicrob Agents Chemother* 45, 3355–3361.
- Poirel, L., Gniadkowski, M. & Nordmann, P. (2002). 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* 50, 1031–1034.
- Rocha-Gracia, R., Ruiz, E., Romero-Romero, S., Lozano-Zarain, P., Somalo, S., Palacios-Hernández, J., Caballero-Torres, P. & Torres, C. (2010). Detection of the plasmid-borne quinolone resistance determinant *qepA1* in a CTX-M-15-producing *Escherichia coli* strain from Mexico. *J Antimicrob Chemother* 65, 169–171.
- Rotimi, V. O., Jamal, W., Pal, T., Sovenned, A. & Albert, M. J. (2008). Emergence of CTX-M-15 type extended-spectrum β -lactamase-producing *Salmonella* spp. in Kuwait and the United Arab Emirates. *J Med Microbiol* 57, 881–886.
- Severin, J. A., Mertaniasih, N. M., Kuntaman, K., Lestari, E. S., Purwanta, M., Lemmens-Den Toom, N., Duerink, D. O., Hadi, U., van Belkum, A. & other authors (2010). Molecular characterization of extended-spectrum β -lactamases in clinical *Escherichia coli* and *Klebsiella pneumoniae* isolates from Surabaya, Indonesia. *J Antimicrob Chemother* 65, 465–469.
- Silva, J., Aguilar, C., Ayala, G., Estrada, M., Garza-Ramos, U., Lara-Lemus, R. & Ledezma, L. (2000). TLA-1: a new plasmid-mediated extended-spectrum β -lactamase from *Escherichia coli*. *Antimicrob Agents Chemother* 44, 997–1003.
- Singh, A., Goering, R., Simjee, S., Foley, S. & Zervos, M. (2006). Application of molecular techniques to the study of hospital infection. *Clin Microbiol Rev* 19, 512–530.
- Siu, L. K., Lu, P.-L., Hsueh, P.-R., Lin, F. M., Chang, S.-C., Luh, K.-T., Ho, M. & Lee, C.-Y. (1999). Bacteremia due to extended-spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in a pediatric oncology ward: clinical features and identification of different plasmids carrying both SHV-5 and TEM-1 genes. *J Clin Microbiol* 37, 4020–4027.

Tansawai, U., Boonkerd, N., Polwichai, P., Dejsirilert, S. & Niumsup, P. (2009). SHV-12 extended spectrum β -lactamase associated with high-level ceftazidime resistance in *Enterobacter cloacae* isolated from Thailand. *Southeast Asian J Trop Med Public Health* **40**, 148–154.

Tenover, F. C., Arbeit, R. D., Goering, R. V., Mickelsen, P. A., Murray, B. E., Persing, D. H. & Swaminathan, B. (1995). Interpreting chromoso-

mal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* **33**, 2233–2239.

Yu, W. L., Pfaller, M. A., Winokur, P. L. & Jones, R. N. (2002). Cefepime MIC as a predictor of the extended-spectrum β -lactamase type in *Klebsiella pneumoniae*, Taiwan. *Emerg Infect Dis* **8**, 522–524.