CTX-M EXTENDED-SPECTRUM β-LACTAMASES AMONG CLINICAL ISOLATES OF ENTEROBACTERIACEAE IN A THAI UNIVERSITY HOSPITAL

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Abstract. This study presents updates on molecular epidemiology of extended-spectrum β-lactamases (ESBLs) in clinical isolates of Enterobacteriaceae from Srinagarind Hospital, Khon Kaen University, Thailand. All isolates were screened for the presence of ESBL genes, bla_{TEM}, bla_{SHV}, bla_{VEB} and bla_{CTX-M}, using PCR followed by nucleotide sequence determination. The results revealed that β-lactamase genes among 48 isolates collected between 1998 and 1999 were bla_{SHV} (79%), $bla_{CTX-M-9}$ (52%), bla_{TEM-1} (48%) and bla_{VEB} (33%), whereas those found in 52 isolates collected in 2003 were bla_{TEM-1} (79%), $bla_{CTX-M-15}$ (44%), bla_{SHV} (36%), bla_{VEB} (36%), $bla_{CTX-M-14}$ (11%) and $bla_{CTX-M-9}$ (10%). In addition, 45 isolates carried at least two different ESBL genes. Using PCR, part of insertion sequence IS*Ecp1* was found in the upstream regions of $bla_{CTX-M-14}$ and $bla_{CTX-M-15}$. ERIC-PCR analysis revealed that most ESBL-producing isolates were of different strains. This is the first report of CTX-M-9, CTX-M-14 and CTX-M-15 β-lactamase genes in Enterobacteriaceae in Thailand.

INTRODUCTION

Extended-spectrum cephalosporins (ESCs), such as ceftazidime, cefotaxime and ceftriaxone, are drugs of choice for treatment of serious infections caused by members of family Enterobacteriaceae. After clinical use of the ESCs, resistant isolates have emerged in many countries (Bradford, 2001). Drug inactivation by extended-spectrum β-lactamases (ESBLs) is the most important mechanism of resistance to the ESCs among these bacteria. ESBL genes are usually encoded on plasmids, which can easily be transferred between isolates (Jacoby and Medeiros, 1991). ESBL-producing Enterobacteriaceae have also been found in Thailand since 1994 with the prevalence of 26% (Lulitanond and Kaewkes, 1999). The predominant ESBLs in Thai isolates are SHV-12 and VEB-1 β-lactamases (Chanawong *et al*, 2001; Girlich *et al*, 2001). Since then, there has been no more information regarding ESBL types in Thailand. In addition, CTX-M β-lactamases have become the most common ESBL during the past decade (Canton and Coque, 2006). Insertion of IS*Ecp1* element frequently found in the upstream region of the bla_{CTX-M} genes plays an important role in mobilization of these genes (Poirel *et al*, 2003). We therefore characterized CTX-M genes and looked for their IS*Ecp1* inserts in clinical isolates of Enterobacteriaceae collected between 1998 and 1999 and in 2003.

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MATERIALS AND METHODS

Bacterial isolates

A total of 100 clinical isolates of Enterobacteriaceae from Srinagarind Hospital, Khon Kaen University, Thailand, were used in this study. They included 48 isolates obtained between 1998 and 1999 and 52 isolates collected in 2003. They were isolated from various clinical specimens, viz. respiratory tract (39%), urinary tract (37%), pus (16%), body fluids (4%) and blood (4%). All isolates produced ESBLs as screened by a double-disk diffusion test (Jarlier *et al*, 1988). Using conventional methods (Kelly *et al*, 1985), the isolates obtained between 1998 and 1999 included 27 *Klebsiella pneumoniae*, 11 *Klebsiella* spp, 4 *Enterobacter* spp, 3 *Escherichia coli* and 3 *Serratia* spp, while those collected in 2003 included 23 *K. pneumoniae*, 19 *E. coli*, 5 *Enterobacter* spp, 2 *Proteus mirabilis* and one isolate of *Klebsiella oxytoca*, *Klebsiella* spp and *Providencia* spp. All isolates were kept at -70ºC in skimmed milk with 20% glycerol until analysis.

Susceptibility testing

Minimum inhibitory concentrations (MICs) of cefotaxime, ceftazidime, cefoperazone and ceftriaxone (Sigma Chemical, St Louis, USA) were determined by an agar dilution method (CLSI, 2005). Clavulanic acid (Smithkline Beecham, UK) at a fixed concentration of 4 μg/ml was added to either cefotaxime or ceftazidime in MIC determination. Susceptibility testing by a disk diffusion method (CLSI, 2005) was also performed for 23 isolates of *K. pneumoniae* and 19 isolates of *E. coli* collected in 2003. Antibiotic disks (Oxoid, Basingstoke, Hampshire, England) contained aminoglycosides (amikacin, gentamicin and netilmicin), β-lactams (cefepime, cefoxitin, cefpirome, imipenem and meropenem), β-lactams/β-lactamase inbibitor (amoxicillin/clavulanic acid, cefoperazone/sulbactam and piperacillin/tazobactam), fluoroquinolones (ciprofloxacin and ofloxacin) and trimethoprim/sulfamethoxazole. *E. coli* ATCC 25922 was used as a drug-sensitive control.

PCR amplification and nucleotide sequence determination

All isolates were screened for the presence of bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{VEB} genes using previously described PCR protocols (Mabilat *et al*, 1990; Hibbert-Rogers *et al*, 1994; M'Zali *et al*, 1996; Gniadkowski *et al*, 1998; Naas *et al*, 1999). Oligonucleotide primers (QIAGEN, Hilden, Germany) used are shown in Table 1. Part of the insertion sequence IS*Ecp1* of the bla_{CTX-M} genes was also detected using primer C 8 as forward primer and either primer CTXM1A or C 4 as reverse primer. Bacterial DNA was prepared by suspending one or two fresh colonies in 50 μl of sterile distilled water and heating at 95ºC for 5 minutes. Deoxynucleosidetriphosphates (dNTP) and *Taq* DNA polymerase were supplied by Promega (Medison, WI, USA) and New England Biolabs (Ontario, Canada), respectively.

PCR products were used as templates for nucleotide sequence determination. The entire genes of bla_{TEM}, bla_{SHV} and bla_{CTX-M} and part of IS*Ecp1* of bla_{CTX-M} were determined as described previously (Mabilat *et al*, 1990; Chanawong *et al*, 2001, 2002). Primer CTXCF derived from the upstream region of the $bla_{CTX-M-14}$ gene (GenBank accession no. AF252622) and primer C4 were also used for amplification of the entire bla_{CTX-M} gene. The PCR products were purified with QIAquick PCR purification kit (QIAGEN, Hilden, Germany) and the nucleotide sequences of both strands were determined using an ABI PRISM automated sequencing machine (model 3100, version 3.7, Applied Biosystems, Foster, CA, USA), according to the manufacturer's instructions.

ERIC-PCR typing

All isolates were typed by an enterobacterial repetive intergenic consensus (ERIC)-PCR method using ERIC2 primer (Versalovic *et al*, 1991). DNA templates were prepared as

described above except those from two isolates of *Serratia* spp that were prepared using DNA Purification Kit (Promega). PCR was carried out as described by Versalovic *et al* (1991) except that dimethylsulfoxide was not included and the annealing step was performed at 50ºC. The amplification products were analyzed by electrophoresis in a 1% (w/v) agarose gel (Promega). A 1-kb DNA ladder (New England Biolabs) was used as a standard DNA size marker. ERIC-PCR fingerprints of all isolates were compared by visual inspection and considered to be the same type when all visible bands from two isolates had the same apparent migration distance; variation in intensity or shape of band was not considered. Fingerprint profiles were interpreted without prior knowledge of clinical or laboratory data.

RESULTS

Susceptibility testing

From the MIC values for ceftazidime, cefotaxime, ceftriaxone and cefoperazone, most isolates were of intermediate susceptibility or resistant to these β-lactams (Table 2). Approximately 80% of the isolates obtained between 1998 and 1999 had higher MICs for ceftazidime than for cefotaxime, whereas the isolates collected in 2003 showed two predominant MIC patterns, higher MICs for ceftazidime than for cefotaxime (48%), and vice versa (42%) (data not shown). Using the disk diffusion test, all *K. pneumoniae* and *E. coli* isolates collected in 2003 were susceptible to imipenem and meropenem (data not shown). Most of them were susceptible to cefoxitin (84-90%), amikacin (85%) and netilmicin (71-74%). *E. coli* isolates were also susceptible to piperacillin/tazobactam (79%) and cefoperazone/sulbactam (74%), whereas only 40-50% of the *K. pneumoniae* isolates were susceptible to these antimicrobial agents. Both *E. coli* and *K. pneumoniae* isolates were resistant to trimethoprim/sulfamethoxazole (95%), gentamicin (75-90%), amoxicillin/clavulanic acid (75%), cefpirome

Table 1
Oligonucleotide primers used.

Genes	Primers	Oligonucleotide sequence (5′→3′)	References
bla_{TEM}	TEMA	ATA AAA TTC TTG AAG AC	Mabilat *et al*, 1990
	TEMB	TTA CCA ATG CTT AAT CA	Mabilat *et al*, 1990
	TEMH	AGG AAG AGT ATG AGT AT	Hibbert-Rogers *et al*, 1994
bla_{SHV}	S4	TCA GCG AAA AAC ACC TTG C	M'Zali'*et al*, 1996
	S5	TCC CGC AGA TAA ATC ACC A	M'Zali'*et al*, 1996
	S6	CTT TAC TCG CCT TTA TCG	M'Zali'*et al*, 1996
	S8	AGT CAT ATC GCC CGG CAC	Chanawong *et al*, 2001
	F240	CCG ATA AGA CCG GAG TTC GC	Chanawong *et al*, 2001
bla_{CTX-M} and IS*Ecp1*	CTX-M-1A	GCG ATG TGC AGC ACC AGT AA	Gniadkowski *et al*, 1998
	CTX-M-1B	GGT TGA GGC TGG GTG AAG TA	Gniadkowski *et al*, 1998
	P1C	TCG TCT CTT CCA GA	Gniadkowski *et al*, 1998
	P2D	CAG CGC TTT TGC CGT CTA AG	Gniadkowski *et al*, 1998
	C8	CAC TCA CCT CAC AAG CAA CGA A	Chanawong *et al*, 2002
	C4	GTT GTC GGG AAG ATA CGT GA	Chanawong *et al*, 2002
	CTXCF	GAG AAG CAG TCT AAA TTC TTC GTG	This study
bla_{VEB-1} typing	VEBF	CGA CTT CCA TTT CCC GAT GC	Naas *et al*, 1999
	VEBB	GGA CTC TGC AAC AAA TAC GC	Naas *et al*, 1999
	ERIC2	AAG TAA GTG ACT GGG GTG AGC G	Versalovic *et al*, 1991

Table 2
MIC of β-lactam antibiotics determined for ESBL-producing Enterobacteriaceae isolates.

Year	Organism (n)	MIC (μg/ml)			
		CAZ [a]	CTX	CFP	CRO
1998-1999	*K. pneumoniae* (27)	≤4–>128	≤4–128	≤4–>128	≤4>128
	MIC_{50}	>128	16	128	16
	MIC_{90}	>128	64	>128	64
	Klebsiella spp (11)	128->128	8->128	32->128	16->128
	MIC_{50}	>128	64	>128	64
	MIC_{90}	>128	>128	>128	>128
	Enterobacter (4)	128->128	32->128	16->128	16->128
	E. coli (3)	128->128	8–64	32-128	8-64
	Serratia (3)	128->128	8->128	16->128	≤4->128
2003	*K. pneumoniae* (23)	4–>128	2->128	8->128	4->128
	MIC_{50}	128	64	>128	128
	MIC_{90}	>128	>128	>128	>128
	E. coli (19)	1->128	2->128	8->128	2->128
	MIC_{50}	32	128	>128	128
	MIC_{90}	64	>128	>128	>128
	Enterobacter (5)	64->128	8-32	8-32	4-32
	P. mirabilis (2)	8-64	4-8	16-64	2-8
	Providencia (1)	>128	16	32	16
	K. oxytoca (1)	>128	64	>128	64
	Klebsiella spp (1)	128	>128	>128	>128

[a]CAZ, ceftazidime; CFP, cefoperazone; CRO, ceftriaxone; CTX, cefotaxime.

(60-75%) and cefepime (60%). Approximately 95% of *E. coli* and 57% of *K. pneumoniae* were resistant to fluoroquinolones.

CTX-M genes and IS*Ecp1* inserts

β-Lactamase genes detected from the 48 isolates obtained between 1998 and 1999 were bla_{SHV} (38 isolates, 79%), bla_{CTX-M} (25 isolates, 52%), bla_{TEM} (23 isolates, 48%) and bla_{VEB} (16 isolates, 33%), while those found in the 52 isolates collected in 2003 were bla_{TEM} (41 isolates, 79%), bla_{CTX-M} (34 isolates, 65%), bla_{SHV} (19 isolates, 36%) and bla_{VEB} (19 isolates, 36%) (Table 3). No amplification products were obtained from an isolate of *K. pneumoniae*. Nucleotide sequence determination revealed that the bla_{TEM} genes from 2 representative isolates were bla_{TEM-1}, whereas bla_{SHV} genes from 3 representative isolates were bla_{SHV-12} (2 isolates) and bla_{SHV-5}. Using primers P1C and P2D and CTXCF and C4 for amplification of the entire bla_{CTX-M} genes from all bla_{CTX-M} carrying isolates, 23 gave amplification product with primers P1C and P2D, 6 with primers CTXCF and C4, but no amplification products were detected in the 30 remaining isolates. Nucleotide sequence analysis of the bla_{CTX-M} genes from 5 representative isolates (2 positive with primers P1C and P2D, 1 positive with primers CTXCF and C4 and 2 negative with both pairs of primers) showed that they were $bla_{CTX-M-15}$, $bla_{CTX-M-14}$ and $bla_{CTX-M-9}$ respectively. Therefore, the bla_{CTX-M} genes from all 25 isolates (52%) obtained between 1998 and 1999 were $bla_{CTX-M-9}$, whereas those from the 34 isolates (65%) collected in 2003 were $bla_{CTX-M-15}$ (23 isolates, 44%), $bla_{CTX-M-14}$ (6

Table 3
β-Lactamase genes present in ESBL-producing Enterobacteriaceae isolates.

bla genes	No. of isolates collected in 1998-1999					2003						
	K. pneumoniae	*Klebsiella* spp	*Enterobacter* spp	*E. coli*	*Serratia* spp	*K. pneumoniae*	*E. coli*	*Enterobacter* spp	*P. mirabilis*	*Providencia* spp	*K. oxytoca*	*Klebsiella* spp
CTX-M-9					2							
CTX-M-14						1						
CTX-M-15							1					
SHV	9					3						
TEM-1							1					
VEB			1			1			2	1		
CTX-M-9/SHV	6	1	1									
CTX-M-9/VEB								1				
CTX-M-14/TEM-1						1	3					
CTX-M-15/TEM-1						3	8					
SHV/TEM-1	2	1	1			2						
SHV/VEB	2	1										
TEM-1/VEB				3	1		3	3				
CTX-M-9/SHV/TEM-1	5	3										
CTX-M-9/SHV/VEB		1				1						
CTX-M-9/TEM-1/VEB	1		1					1			1	
CTX-M-14/TEM-1/VEB							1					
CTX-M-15/SHV/TEM-1						8	1					1
CTX-M-15/TEM-1/VEB							1					
SHV/TEM-1/VEB	1					2						
CTX-M-9/SHV/TEM-1/VEB		4				1						
Non-CTX-M/SHV/TEM-1/VEB	1											
Total	27	11	4	3	3	23	19	5	2	1	1	1

isolates, 11%) and $bla_{CTX\text{-}M\text{-}9}$ (5 isolates, 10%). In addition, part of insertion sequence IS*Ecp1* was found in the upstream region of both $bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}15}$ as confirmed by nucleotide sequencing. There were 38 isolates carrying two different ESBL genes and 7 isolates harboring three different ESBL genes. The dominant ESBL genes found between 1998 and 1999 were SHV, CTX M 9 and VEB, whereas those found in 2003 were CTX-M-15, VEB and SHV.

ERIC-PCR typing

ERIC-PCR analysis identified 35 distinct types among 50 *K. pneumoniae* isolates, 14 types in 22 *E. coli*, 8 types in 13 *Klebsiella* spp, 2 types in 3 *Serratia* spp and one type in 2 *P. mirabilis*, whereas all isolates of *Enterobacter* spp gave different ERIC-PCR patterns (data not shown).

DISCUSSION

ESBL-producing *E. coli* have been found to increase when compared with the previous report (9.6%) in 1994 and 1996 (Lulitanond and Kaewkes, 1999). Most ESBL producers in 1994 and 1996 have a phenotype that confers more *in vitro* resistance to ceftazidime than to cefotaxime and SHV 12 enzyme was the most prevalent ESBL (Chanawong *et al*, 2001). In 2003, isolates with ESBL phenotype of higher MIC for cefotaxime than for ceftazidime were seen to increase, suggesting the presence of CTX-M ESBLs. Molecular characterization of the ESBL-producing isolates obtained between 1998 and 1999 and in 2003 revealed the high prevalence of CTX-M ESBL genes. The predominance of CTX-M β-lactamases may be due the selective pressure, from an increased use of ceftriaxone in Srinagarind Hospital. This report also confirmed the emergence of CTX-M ESBLs in Thailand.

Variants of CTX-M-1 and CTX-M-9 groups are the most common CTX-M types in Asian countries (Cao *et al*, 2002; Chanawong *et al*, 2002; Yu *et al*, 2002; Yamasaki *et al*, 2003; Munday *et al*, 2004; Ho *et al*, 2005; Izumiya *et al*, 2005; Kim *et al*, 2005). This is the first report of CTX-M-9, CTX-M-14 and CTX-M-15 ESBL genes in Enterobacteriaceae in Thailand. However, there may be other variants of CTX-M among these isolates since the $bla_{\text{CTX-M}}$ genes were sequenced from only five representative isolates. In the present study, the CTX-M genes were found in various genera of Enterobacteriaceae and the insertion sequence IS*Ecp1* was also found in the upstream region of both $bla_{\text{CTX-M-14}}$ and $bla_{\text{CTX-M-15}}$. ERIC-PCR analysis of the CTX-M-containing isolates revealed that most of them were of different types. These findings support the notion that dissemination of these resistance genes in different strains occurs via mobile elements such as IS*Ecp1* (Poirel *et al*, 2005).

SHV ESBLs found in the present study were SHV-12 and SHV-5 as described previously (Chanawong *et al*, 2001). In addition, TEM ESBLs have not yet been discovered in Thailand. The prevalence of VEB ESBLs among Enterobacteriaceae isolates has increased from 2.3% in 1994 and 1996 (Chanawong *et al*, 2001) to 33-36% in the present study. This gene was detected in various genera of Enterobacteriaceae, including *Klebsiella*, *Escherichia, Enterobacter*, *Serratia*, *Proteus* and *Providencia*, indicating a horizontal gene transfer. Girlich *et al* (2001) also reported a high prevalence of VEB ESBLs in Thai isolates obtained in 1999 from another hospital. Therefore, SHV, VEB and CTX-M are the most common ESBLs in Thailand.

In summary, the epidemiology of ESBLs in this hospital indicates a change of ESBL types, with a higher prevalence of CTX-M and VEB ESBLs. In addition, the presence of multiple ESBL genes in single strains and the occurrence of different ESBL-producing strains suggest that resistance in these bacteria may be due to dissemination of resistance determinants via plasmids or mobile elements. Therefore, policies regarding not only improved control of infection but also judicious use of antibiotic are urgently needed in Thailand.

ACKNOWLEDGEMENTS

This work was supported by Faculty of Medicine, Khon Kaen University. We are grateful to staff of Clinical Microbiology Unit, Srinagarind Hospital, for collecting clinical isolates. We thank Dr J Xiong for providing CTX-M β lactamase-producing strains; Mr K Punpieng, Ms R Triworawat, Mr A Sookkhammay, Ms U Burana, Ms K Duen-ngye, Ms A Techasen and Ms J Songsri for technical assistance; and Center for Research and Development of Medical Diagnostic Laboratories, Khon Kaen University, Khon Kaen, Thailand for their support.

REFERENCES

Bradford PA. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev* 2001; 14: 933-51.

Canton R, Coque TM. The CTX-M β-lactamase pandemic. *Curr Opin Microbiol* 2006; 9: 466-75.

Cao V, Lambert T, Nhu DQ, *et al*. Distribution of extended-spectrum β-lactamases in clinical isolates of *Enterobacteriaceae* in Vietnam. *Antimicrob Agents Chemother* 2002; 46: 3739-43.

Clinical and Laboratory Standards Institute (CLSI)/ NCCLS. Performance standards for antimicrobial susceptibility test: Fifteenth informational supplement. CLSI/NCCLS document M100-S15 [ISBN 1-56238-556-9]. Pennsylvania: Clinical and Laboratory Standards Institute, 2005.

Chanawong A, M'Zali FH, Heritage J, Lulitanond A, Hawkey PM. SHV-12, SHV-5, SHV-2a and VEB-1 extended-spectrum β-lactamases in Gram-negative bacteria isolated in a university hospital in Thailand. *J Antimicrob Chemother* 2001; 48: 839-52.

Chanawong A, M'Zali FH, Heritage J, Xiong JH, Hawkey PM. Three cefotaximases, CTX-M-9, CTX-M-13, and CTX-M-14, among *Enterobacteriaceae* in the People's Republic of China. *Antimicrob Agents Chemother* 2002; 46: 630-7.

Girlich D, Poirel L, Leelaporn A, *et al*. Molecular epidemiology of the intergron-located VEB-1 extended-spectrum β-lactamases in nosocomial enterobacterial isolates in Bangkok, Thailand. *J Clin Microbiol* 2001; 39: 175-82.

Gniadkowski M, Schneider I, Palucha A, Jungwirth R, Mikiewicz B, Bauernfeind A. Cefotaxime-resistant *Enterobacteriaceae* isolates from a hospital in Warsaw, Poland: identification of a new CTX-M-3 cefotaxime-hydrolyzing β-lactamase that is closely related to the CTX-M-1/MEN-1 enzyme. *Antimicrob Agents Chemother* 1998; 42: 827-32.

Hibbert-Rogers LCF, Heritage J, Todd N, Hawkey PM. Convergent evolution of TEM 26, a β-lactamase with extended-spectrum activity. *J Antimicrob Chemother* 1994; 33: 707-20.

Ho PL, Shek RH, Chow KH, *et al*. Detection and characterization of extended-spectrum β-lactamases among bloodstream isolates of *Enterobacter* spp in Hong Kong, 2000-2002. *J Antimicrob Chemother* 2005; 55: 326-32.

Izumiya H, Mori K, Higashide M, *et al*. Identification of CTX-M-14 β-lactamase in a *Salmonella enterica* serovar Enteritidis isolate from Japan. *Antimicrob Agents Chemother* 2005; 49: 2568-70.

Jacoby GA, Medeiros AA. More extended-spectrum β-lactamases. *Antimicrob Agents Chemother* 1991; 35: 1697-704.

Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility pattern. *Rev Infect Dis* 1988; 10: 867-78.

Kelly MT, Brenner DJ, Farmer III JJ. *Enterobacteriaceae*. In: Lennette EH, Balows A, Hausler WJ, Shadomy HJ, eds. Manual of clinical microbiology. 5th ed. Washington DC: American Society of Microbiology, 1985: 263-77.

Kim J, Lim YM, Jeong YS, Seol SY. Occurrence of CTX-M-3, CTX M-15, CTX-M-14, and CTX-M-9 extended-spectrum β-lactamases in *Enterobacteriaceae* clinical isolates in Korea. *Antimicrob Agents Chemother* 2005; 49: 1572-5.

Lulitanond A, Kaewkes W. Prevalence of extended–spectrum β-lactamases (ESBLs) production in Gram-negative bacilli isolated from Srinagarind Hospital Thailand. *J Infect Dis Antimicrob Agents* 1999; 16: 115-9.

Mabilat C, Goussard S, Sougakoff W, Spencer RC, Courvalin P. Direct sequencing of the amplified structural gene and promoter for the extended spectrum β-lactamase TEM-9 (RHH-1) of *Klebsiella pneumoniae*. *Plasmid* 1990; 23: 27-34.

Munday CJ, Xiong J, Li C, Shen D, Hawkey PM. Dissemination of CTX-M type β-lactamases in *Enterobacteriaceae* isolates in the People's

Republic of China. *Int J Antimicrob Agents* 2004; 23: 175-80.

M'Zali FM, Gascoyne-Binzi DM, Heritage J, Hawkey PM. Detection of mutations conferring extended-spectrum activity on SHV β-lactamase using polymerase chain reaction single strand conformational polymorphism (PCR-SSCP). *J Antimicrob Chemother* 1996; 37: 797-802.

Naas T, Poirel L, Karim A, Nordmann P. Molecular characterization of In50, a class 1 integron encoding the gene for the extended-spectrum β-lactamase VEB-1 in *Pseudomonas aeruginosa*. *FEMS Microbiol Lett* 1999; 176: 411-9.

Poirel L, Decousser JW, Nordmann P. Insertion sequence IS*Ecp1B* is involved in expression and mobilization of a bla_{CTX-M} β-lactamase gene. *Antimicrob Agents Chemother* 2003; 47: 2938-45.

Poirel L, Lartigue MF, Decousser JW, Nordmann P. IS*Ecp1B*-mediated transposition of bla_{CTX-M} in *Escherichia coli*. *Antimicrob Agents Chemother* 2005; 49: 447-50.

Versalovic J, Koeuth T, Lupski JR. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res* 1991; 19: 6823-31.

Yamasaki K, Komatsu M, Yamashita T, *et al*. Production of CTX-M-3 extended-spectrum β-lactamase and IMP-1 metallo-β-lactamase by five Gram-negative bacilli: survey of clinical isolates from seven laboratories collected in 1998 and 2000, in the Kinki region of Japan. *J Antimicrob Chemother* 2003; 51: 631-8.

Yu WL, Winokur PL, Von Stein DL, Pfaller MA, Wang JH, Jones RN. First description of *Klebsiella pneumoniae* harboring CTX-M β-lactamases (CTX-M-14 and CTX-M-3) in Taiwan. *Antimicrob Agents Chemother* 2002; 46: 1098-100.