MOLECULAR CHARACTERIZATION OF EXTENDED-SPECTRUM BETA-LACTAMASES AND ITS CORRELATION WITH CLINICAL LABORATORY STANDARDS INSTITUTE INTERPRETIVE CRITERIA FOR DISK DIFFUSION SUSCEPTIBILITY TESTING IN ENTEROBACTERIACEAE ISOLATES IN THAIALND

Teerawit Tangkoskul, Surapee Tiengrim, Supiluck Onsomang, Naratchaphan Pati, Nalinee Aswapokee, Visanu Thamlikitkul and Methee Chayakulkeeree

Division of Infectious Diseases and Tropical Medicine, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Abstract. We performed extended-spectrum β-lactamase (ESBL) phenotypic testing and molecular characterization of three ESBL genes (TEM, SHV and CTX-M) and susceptibility testing by Clinical Laboratory Standards Institute (CLSI) disk diffusion method against three cephalosporins (ceftriaxone, ceftazidime, cefepime) and a cephamycin (cefoxitin) among 128 Thai *Escherichia coli* and 84 Thai *Klebsiella pneumoniae* clinical isolates. ESBL production was discovered in 62% of *E. coli* and 43% of *K. pneumoniae* isolates. All isolates susceptible to ceftriaxone were ESBL-negative. Nearly all isolates non-susceptible to ceftriaxone, ceftazidime and cefepime produced ESBL; the presence of CTX-M genes in the isolates correlated with a ceftriaxone non-susceptible phenotype. Thirty-nine of 83 isolates (47%) of ceftazidime-susceptible *E. coli* and 50 of 99 isolates (50.5%) of cefepime-susceptible *E.coli* were ESBL-producing. SHV-type β-lactamase genes were more prevalent among *K. pneumoniae* than *E. coli* isolates. CTX-M was the major ESBL gene harbored by ESBL-producers in both *E. coli* and *K. pneumoniae* isolates. Non-CTX-M ESBL-producers were found only among *K. pneumoniae* isolates. This study reveals an increase in ESBL-producing Enterobacteriaceae among Thai isolates and demonstrates gaps in the current CLSI disk diffusion susceptibility guidelines; it indicates the results of ceftazidime and cefepime disk diffusion susceptibility testing using CLSI criteria should be interpreted with caution.

Keywords: ESBL, Enterobacteriaceae, extended-specteum beta-lactamase, disk diffusion, CLSI

Correspondence: M Chayakulkeeree, Division of Infectious Diseases and Tropical Medicine, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700, Thailand.
Tel: +66 (0) 2419 7784; Fax: +66 (0) 2419 7783
E-mail: methee.cha@mahidol.ac.th

INTRODUCTION

The mechanisms for β-lactam resistance among gram-negative bacteria are mainly the production of β-lactam hydrolyzing enzymes (β-lactamases), whereas penicillin-binding protein (PBP)

modifications or substitutions are mainly found among gram-positive pathogens (Chambers and Hackbarth, 1987). Over 950 unique β-lactamase enzymes have been described so far and nearly half of them are extended-spectrum β-lactamases (ESBL) (Bush, 2010). ESBL are β-lactamases that can hydrolyze extended-spectrum cephalosporins, such as ceftazidime and ceftriaxone as well as aztreonam, but not cephamycins or carbapenems, and are susceptible to β-lactamase inhibitors (Babic *et al*, 2006). The first ESBLs described in 1983 were penicillinase mutants, TEM-1 and SHV-1, usually found among *Escherichia coli* and *Klebsiella* spp. Since 1995, the predominant ESBL has been CTX-M, which can be classified into 5 subgroups: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 (Falagas and Karageorgopoulos, 2009; Hawkey and Jones, 2009; Livermore, 2009). The dramatic change in ESBL epidemiology is a result of escape of chromosomal β-lactamase genes from *Kluyvera* spp facilitated by insertion sequences (Poirel *et al*, 2005).

Having a method for detecting ESBL-producing Enterobacteriaceae in the clinical microbiology laboratory is essential. Before 2010, the Clinical Laboratory Standards Institute (CLSI) recommended performing an ESBL phenotypic confirmatory test by determining the synergism of extended-spectrum cephalosporins and β-lactamase inhibitors in four organisms: *E. coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Proteus mirabilis* (CLSI, 2009). However, in 2010 the CLSI guidelines increased the zone diameter breakpoint for cefotaxime, ceftriaxone, ceftazidime and ceftizoxime for the disk diffusion test and eliminated the necessity for performing ESBL testing (CLSI, 2010). In this study, we determined the ESBL genes among *E. coli* and *K. pneumoniae* isolates along with phenotypic ESBL characterization to evaluate the accuracy of the current CLSI disk diffusion susceptibility criteria for predicting ESBL production among those isolates.

MATERIALS AND METHODS

Bacterial isolates

We used 128 *E. coli* and 84 *K. pneumoniae* non-redundant isolates for this study. The isolates were recovered from clinical specimens sent for culture and sensitivity testing at the Infectious Diseases Laboratory, Department of Medicine, Siriraj Hospital, Thailand. The specimens were obtained from respiratory, urinary and gastrointestinal tracts.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed following the standard disk diffusion technique found in the 2009/2010 CLSI guidelines (CLSI, 2009). Mueller-Hinton (MH) agar (Oxoid, Cambridge, UK) was used as a medium for susceptibility testing. *E. coli* ATCC 25922 was used as a drug-sensitive control.

Phenotypic ESBL testing

Phenotypic ESBL production testing was performed using the double disk synergy test. Briefly, the bacteria were plated onto MH agar and tested for sensitivity to amoxicillin-clavulanic acid (AMC), cefoxitin (FOX), ceftriaxone (CRO), ceftazidime (CAZ) and cefepime (FEP) (Monno *et al*, 2007). The enhanced inhibition zone difference between AMC and CRO, CAZ or FEP indicated ESBL production. Resistance to cefoxitin was considered suspicious for AmpC β-lactamase production.

Molecular characterization of β-lactamases

One hundred sixteen *E. coli* and 72 *K. pneumonia* isolates susceptible to cefoxitin were evaluated for the presence of three

common β-lactamase genes. Bacterial DNA was prepared by suspending 200 µl of the overnight culture in 800 µl sterile distilled water and heating to 95°C for 10 minutes prior to centrifuging at 10,000 rpm for 1 minute. The supernatant was used as a template for the PCR. Multiplex PCR of 3 β-lactamase genes (TEM, SHV, and CTX-M) was performed using primers previously described with minor modifications (Monstein *et al*, 2007). The PCR was performed using 1 µl DNA template, 0.5 U of Qiagen TopTaq DNA polymerase (Qiagen, Valencia, CA), and 10 pmol each of the following primers: SHV.SE (5′-ATGCGTTATATTCGCCTGTG-3′), SHV.AS (5′-TGCTTTGTTATTCGGGCCAA-3′), TEM-164.SE (5′-TCGCCGCATA CACTATTCTCAGAATGA-3′), TEM-165.AS (5′-ACGCTCACCGGCTCCAGATT TAT-3′), CTX-M-U1 (5′-ATGTGCAGYACCAGTAARGTKATGGC-3′) and CTX-M-U2 (5′-TGGGTRAARTARGTSACCAGAAYCAGCGG-3′) in a final volume of 20 µl. The multiplex PCR was carried out for 15 minutes at 95°C, 30 seconds at 94°C, 30 seconds at 60°C, and 2 minutes at 72°C for 30 cycles, followed by a final extension for 10 minutes at 72°C. The PCR products were analyzed with 2.0% (wt/vol) agarose gel electrophoresis, using a standard DNA ladder, by staining with ethidium bromide (0.5µg/ml). The amplicon sizes for SHV, TEM, and CTX-M were 747, 445, and 593 bp, respectively.

RESULTS

ESBL phenotype and cephalosporin susceptibility of bacterial isolates

Using the double disk synergy technique, 79 of 128 *E. coli* isolates (61.7%) and 36 of 84 *K. pneumoniae* isolates (42.9%) were identified as ESBL-producers. The susceptibilities of these isolates to ceftriaxone, ceftazidime, cefepime, and cefoxitin are shown in Table 1. All of the ESBL producing isolates were non-susceptible (either intermediately susceptible or resistant) to ceftriaxone, 7 of 49 *E. coli* isolates (14.3%) and 4 of 48 *K. pneumoniae* isolates (8.3%) that were not ESBL producing were non-susceptible to ceftriaxone, 40 of 79 *E. coli* isolates (50.6%) and 31 of 36 *K. pneumoniae* isolates (86.1%) that were ESBL producing were non-susceptible to ceftazidime using CLSI disk diffusion susceptibility testing criteria. Five *E. coli* isolates (10.2%) and 1 *K. pneumoniae* isolate (2.1%) that were not producing ESBL were non-susceptible to ceftazidime. None of the isolates that did not produce ESBL exhibited cefepime non-susceptibility. Twenty-nine of the *E. coli* isolates (36.7%) and 25 of the *K. pneumoniae* isolates (69.4%) that produced ESBL were non-susceptible to cefepime. Twelve *E. coli* isolates were resistant to cefoxitin, 5 of those isolates produced ESBL. Twelve *K. pneumoniae* isolates were resistant to cefoxitin, 7 of those isolates produced ESBL. Since overexpression of AmpC β-lactamase may cause resistance to extended-spectrum cephalosporins, all 24 cefoxitin-resistant isolates were excluded from further experiments.

Using ceftriaxone, ceftazidime and cefepime to detect ESBL producing strains

Since 2010, CLSI guidelines state ESBL testing is not recommended for routine laboratory testing and the zone diameter breakpoints for the disk diffusion test for some cephalosporins, such as ceftriaxone and ceftazidime, have been increased. The sensitivity, specificity, and accuracy of using ceftriaxone, ceftazidime, and cefepime susceptibilities following current CLSI disk diffusion testing in order to predict ESBL-producing *E. coli* and *K. pneumoniae* are shown in Table 2. The sensitivities for

Table 1
ESBL phenotypes and susceptibilities against ceftriaxone, ceftazidime, cefepime, and cefoxitin among 128 *E. coli* and 84 *K. pneumoniae* isolates.

ESBL phenotype	Non-susceptible isolates against cephalosporins by disk diffusion method (CLSI 2010/2011) (No, %)			
	Ceftriaxone	Ceftazidime	Cefepime	Cefoxitin
E. coli (*N*=128)				
ESBL producer (*n*=79)	79 (100)	40 (50.6)	29 (36.7)	5 (6.3)
Non-ESBL producer (*n*=49)	7 (14.3)	5 (10.2)	0 (0)	7 (14.3)
K. pneumoniae (*N*=84)				
ESBL producer (*n*=36)	36 (100)	31 (86.1)	25 (69.4)	7 (19.4)
Non-ESBL producer (*n*=48)	4 (8.3)	1 (2.1)	0 (0)	5 (10.4)

Table 2
Sensitivity, specificity and accuracy of using ceftriaxone, ceftazidime and cefepime susceptibilities as determined by the disk diffusion test (2010/2011 CLSI) for detecting ESBL-producing *E. coli* (EC) and *K. pneumoniae* (KP).

	Ceftriaxone		Ceftazidime		Cefepime	
	EC	KP	EC	KP	EC	KP
Sensitivity	97.3%	90.6%	100%	92.8%	100%	100%
Specificity	100%	100%	51.8%	93.2%	54.4%	79.6%
Accuracy	98.3%	95.8%	66.4%	93.1%	57.8%	84.7%

using the ceftriaxone disk diffusion test for predicting ESBL-producing *E. coli* and *K. pneumoniae* were 97.3 and 90.6%, respectively, and the specificities were both 100%. The sensitivity and specificity of the ceftazidime disk diffusion test for detecting ESBL producing *K. pneumoniae* were 92.8 and 93.2%, respectively. The sensitivity and specificity of the ceftazidime disk diffusion test for detecting ESBL producing *E. coli* were 100 and 51.8%, respectively. The sensitivity and specificity of the cefepime disk diffusion test for detecting ESBL producing *E. coli* were 100 and 54.4%, respectively, and for detecting ESBL producing *K. pneumoniae* were 100 and 79.6%, respectively. The accuracies for using the ceftriaxone, ceftazidime, and cefepime disk diffusion tests for detecting ESBL producing *E. coli* were 98.3, 66.4, and 57.8%, respectively, and for detecting ESBL producing *K. pneumoniae* were 95.8, 93.1, and 84.7%, respectively.

Molecular characterization of TEM, SHV, and CTX-M β-lactamase genes

The 3 β-lactamase genes TEM, SHV and CTX-M were detected by multiplex PCR followed by agarose gel electrophoresis in 116 *E. coli* isolates (74 ESBL-positive and 42 ESBL-negative) and 72 *K. pneumoniae* isolates (29 ESBL-positive and 43 ESBL-negative). The TEM, SHV and CTX-M genes were detected in 69.8,

Table 3
Correlation by β-lactamase genotype and phenotype susceptibilities against ceftriaxone, ceftazidime and cefepime.

β-lactamase genotypes	Susceptibilities against cephalosporins (CLSI 2010/2011) (No, %)					
	Ceftriaxone		Ceftazidime		Cefepime	
	Susceptible	Non-susceptible	Susceptible	Non-susceptible	Susceptible	Non-susceptible
E. coli (*N*=128)						
ESBL producers						
CTX-M (*n*=28)		28 (100)	18 (64.3)	10 (35.7)	21 (75)	7 (25)
CTX-M, TEM (*n*=46)		46 (100)	21 (45.7)	25 (54.3)	27 (58.7)	19 (41.3)
Non-ESBL producers						
TEM (*n*=33)	31 (93.9)	2 (6.1)	33 (100)		33 (100)	
TEM, SHV (*n*=2)	2 (100)		2 (100)		2 (100)	
Not identified (*n*=7)	7 (100)		7 (100)		7 (100)	
K. pneumoniae (*N*=29)						
ESBL producers						
CTX-M (*n*=1)		1 (100)		1 (100)	1 (100)	
SHV (*n*=4)		4 (100)		4 (100)	2 (50)	2 (50)
CTX-M, TEM (*n*=1)		1 (100)		1 (100)		1 (100)
CTX-M, SHV (*n*=5)		5 (100)	3 (60)	2 (40)	2 (40)	3 (60)
SHV, TEM (*n*=2)		2 (100)		2 (100)	2 (100)	
SHV, TEM, CTX-M (*n*=16)		16 (100)		16 (100)	4 (25)	12 (75)
Non-ESBL producers						
TEM (*n*=1)	1 (100)		1 (100)		1 (100)	
SHV (*n*=17)	15 (88.2)	2 (11.8)	16 (94.1)	1 (5.9)	17 (100)	
TEM, SHV (*n*=23)	23 (100)		23 (100)		23 (100)	
SHV, TEM, CTX-M (*n*=2)	1 (50)	1 (50)	1 (50)	1 (50)	2 (100)	

1.7, and 63.8% of *E. coli* isolates and 62.5, 95.8 and 34.7% of *K. pneumoniae* isolates, respectively. All the ESBL producing *E. coli* (*n*=74) had the CTX-M gene; 46 isolates (62.1%) also co-harbored the TEM gene (Table 3). A good correlation was seen between ceftriaxone resistance and the presence of the CTX-M gene among ESBL producing *E. coli*. Thirty-five isolates (47.3%) and 26 isolates (35.1%) of *E. coli* with the CTX-M gene were non-susceptible to ceftazidime and cefepime, respectively. Of the *E. coli* isolates not producing ESBL, 33 (78.6%) had the TEM gene, 2 (4.8%) had both the TEM and SHV genes, and the other 7 (16.7%) were undetermined. All but 2 of the *E. coli* isolates not producing ESBL were susceptible to ceftriaxone, ceftazidime and cefepime. Of the 29 *K. pnenumoniae* isolates producing ESBL, 16 (55.2%) had all 3 CTX-M, TEM and SHV genes, 4 (13.8%) had the SHV gene only and 1 (3.4%) had the CTX-M gene only. The other ESBL producing *K. pneumoniae* isolates harbored a combination of any two genes of TEM, SHV or

CTX-M (Table 3). Similar to ESBL producing *E. coli*, all ESBL producing *K. pneumoniae* showed resistance to ceftriaxone. For ESBL non-producing *K. pneumoniae*, 42 isolates (97.7%) possessed the SHV gene. Surprisingly, there were 2 ESBL non-producing *K. pneumoniae* isolates that carried the CTX-M gene; one isolate was susceptible to ceftriaxone and ceftazidime, while another was not. Similar to *E. coli*, all the ESBL non-producing *K. pneumoniae* isolates were susceptible to cefepime, whereas 18 ESBL producing isolates (62%) were non-susceptible to cefepime.

DISCUSSION

Inappropriate use of antibiotics and transferable genetic elements are important factors contributing to antimicrobial resistance among bacteria. The problem of resistance began with a few narrow-spectrum β-lactamase enzymes, followed by worldwide spread of ESBL in 1980s, and currently there is a spread of carbapenemases in 2000s (Pfeifer *et al*, 2010). From the Tigecycline Evaluation and Surveillance Trial (TEST) global surveillance database, the rate of ESBL producing *K. pneumoniae* isolates in the Asia-Pacific Rim was 22.4% (Reinert *et al*, 2007). A large scale surveillance study during 1997-2003 demonstrated the overall prevalences of ESBL producing *E. coli* and *K. pneumoniae* in the Asia-Pacific region were 14.2 and 28.2%, respectively (Turner, 2005). A short survey at Siriraj Hospital in 2003 revealed increased resistance to various antibacterial agents among ESBL producers compared with non-producing isolates (Chayakulkeeree *et al*, 2005). The prevalences of ESBL producing *E. coli* and *K. pneumoniae* at the same hospital during 2004 were 12.6 and 12.8%, respectively, and an increase in resistance of 3 times since then (Kiratisin *et al*, 2008). Our study demonstrated a high prevalence of ESBL producing *E. coli* isolates (61.7%) and *K. pneumoniae* isolates (42.9%), highlighting the need for urgent intervention to prevent the spread of these multi-drug resistant organisms.

Our results support previous studies that found the SHV gene was more prevalent among *K. pneumoniae* than among *E. coli* (Philippon *et al*, 1989; Babini and Livermore, 2000; Tarnberg *et al*, 2009). In this study, CTX-M genes were found among all ESBL producing *E. coli* isolates (100%) and 23 of 29 ESBL producing *K. pneumoniae* isolates (79.3%). Since 2010, the CLSI has increased the zone diameter breakpoints of some cephalosporins for the disk diffusion test and suggested that ESBL testing is no longer necessary (CLSI, 2010). Our study found significant differences between ESBL phenotype testing and the disk diffusion test using current CLSI inhibition zone size criteria. Some of the ESBL non-producing isolates were non-susceptible to cephalosporins with the disk susceptibility test; these are considered as false non-susceptible. Thirty-nine isolates (52.7%) which were false susceptible against ceftazidime were identified as ESBL-producing *E. coli*. These results may be explained by a previous study by Kiratisin *et al* (2008) who found 80% of CTX-M genes in *E. coli* isolates at our hospital were CTX-M-15 and CTX-M-14 genotypes. These CTX-M genes were classified as CTX-M subgroups 1 and 9, respectively. CTX-M-15 carriers were extremely resistant to ceftazidime (MIC ≥ 256 μg/ml) but CTX-M-14 carries were susceptible to ceftazidime (Babic *et al*, 2006; Chanawong *et al*, 2007). When either CTX-M subgroup 1 or 9 was co-harbored with other enzymes, it resulted in an increase in MICs against ceftriaxone

and ceftazidime to > 256 µg/ml (Kiratisin *et al*, 2008). Since the 2010/2011 CLSI disk diffusion zone size criteria for cefepime remained unchanged, 48 ESBL producing *E. coli* isolates (64.9%) showed a false susceptibility to cefepime. Therefore, one should use caution with interpreting cefepime susceptibility testing in *E. coli* isolates without ESBL testing. Of the 42 isolates of ESBL non-producing *E. coli*, 2 isolates (4.7%) were identified as falsely non-susceptible to ceftriaxone. This phenomenon may be caused by expression of non-TEM β-lactamases or other resistance mechanisms. With *K. pneumoniae*, false susceptibilities to ceftriaxone and ceftazidime were also found, although the prevalence was lower than that for *E. coli*. Previous studies showed ceftazidimases, such as SHV-12, SHV-5, SHV-2a, and VEB-1, were disseminated among *K. pneumoniae* over a decade ago in Thailand and Asia (Chanawong *et al*, 2001; Girlich *et al*, 2001; Chanawong *et al*, 2007; Niumsup *et al*, 2008). Therefore, SHV-derived or other ceftazidimases co-harbored with CTX-M in *K. pneumoniae* may reduce the false susceptibility rate of *K. pneumoniae* to ceftazidime. We found 38% of SHV genes harbored in *K. pneumoniae* were ESBLs (data not shown).

We found 9 to 12% cefoxitin resistance among isolates in our study, even among ESBL-producers, which indicates the potential spread of AmpC β-lactamase enzymes. Further study of these cefoxitin resistant isolates is important because overexpression of either ESBL or AmpC in combination with porin loss or an efflux pump could cause carbapenem resistance (Jacoby *et al*, 2004; Hawkey and Jones, 2009).

In conclusion, we report the increasing prevalence of ESBL producing *E. coli* and *K. pneumoniae* at our hospital, which is caused mainly by dissemination of CTX-M genes. SHV genes were discovered only in *K. pneumoniae* but not in *E. coli* isolates. Among ESBL producing isolates, 53% of *E. coli* isolates and 10.3 % of *K. pneumoniae* isolates were falsely susceptible to ceftazidime with the disk diffusion test. All ESBL producing isolates were non-susceptible to ceftriaxone. Ceftazidime susceptibility testing using the current 2010/2011 CLSI disk diffusion test does not correlate with ESBL production in *E. coli* isolates, and may give a false susceptibility result. This may lead to inappropriate antimicrobial use. The ceftriaxone disk diffusion test provides a better correlation with ESBL production in both *E. coli* and *K. pneumoniae* isolates.

ACKNOWLEDGEMENTS

This work was supported by the Siriraj Research Development Fund.

REFERENCES

Babic M, Hujer AM, Bonomo RA. What's new in antibiotic resistance? Focus on beta-lactamases. *Drug Resist Update* 2006; 9: 142-56.

Babini GS, Livermore DM. Are SHV beta-lactamases universal in *Klebsiella pneumoniae*? *Antimicrob Agents Chemother* 2000; 44: 2230.

Bush K. Alarming beta-lactamase-mediated resistance in multidrug-resistant Enterobacteriaceae. *Curr Opin Microbiol* 2010; 13: 558-64.

Chambers HF, Hackbarth CJ. Effect of NaCl and nafcillin on penicillin-binding protein 2a and heterogeneous expression of methicillin resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1987; 31: 1982-88.

Chanawong A, Lulitanond A, Kaewkes W, Lulitanond V, Srigulbutr S, Homchampa P. CTX-M extended-spectrum beta-lac-

tamases among clinical isolates of Enterobacteriaceae in a Thai university hospital. *Southeast Asian J Trop Med Public Health* 2007; 38: 493-500.

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

Chayakulkeeree M, Junsriwong P, Keerasuntonpong A, Tribuddharat C, Thamlikitkul V. Epidemiology of extended-spectrum beta-lactamase producing gram-negative bacilli at Siriraj Hospital, Thailand, 2003. *Southeast Asian J Trop Med Public Health* 2005; 36: 1503-9.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Nineteenth informational supplement M100-S19. Wayne, PA: CLSI, 2009.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Twentieth international supplement M100-S20. Wayne, PA: CLSI, 2010.

Falagas ME, Karageorgopoulos DE. Extended-spectrum beta-lactamase-producing organisms. *J Hosp Infect* 2009; 73: 345-54.

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

Hawkey PM, Jones AM. The changing epidemiology of resistance. *J Antimicrob Chemother* 2009; 64 (suppl 1): i3-10.

Jacoby GA, Mills DM, Chow N. Role of beta-lactamases and porins in resistance to ertapenem and other beta-lactams in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2004; 48: 3203-6.

Kiratisin P, Apisarnthanarak A, Laesripa C, Saifon P. 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* 2008; 52: 2818-24.

Kiratisin P, Chattammanat S, Sa-Nguansai S, *et al*. A 2-year trend of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Thailand: an alert for infection control. *Trans R Soc Trop Med Hyg* 2008; 102: 460-4.

Livermore DM. Has the era of untreatable infections arrived? *J Antimicrob Chemother* 2009; 64 (suppl 1): i29-36.

Monno R, Rizzo C, De Vito D, *et al*. Prevalence, antimicrobial resistance, and extended-spectrum beta-lactamases characterization of *Salmonella* isolates in Apulia, southern Italy (2001-2005). *Microb Drug Resist* 2007; 13: 124-9.

Monstein HJ, Ostholm-Balkhed A, Nilsson MV, Nilsson M, Dornbusch K, Nilsson LE. Multiplex PCR amplification assay for the detection of blaSHV, blaTEM and blaCTX-M genes in Enterobacteriaceae. *APMIS* 2007; 115: 1400-8.

Niumsup PR, Tansawai U, Boonkerd N, Polwichai P, Dejsirilert S. Dissemination of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli* in Thai hospitals. *J Infect Chemother* 2008; 14: 404-8.

Pfeifer Y, Cullik A, Witte W. Resistance to cephalosporins and carbapenems in Gram-negative bacterial pathogens. *Int J Med Microb* 2010; 300: 371-9.

Philippon A, Labia R, Jacoby G. Extended-spectrum beta-lactamases. *Antimicrob Agents Chemother* 1989; 33: 1131-6.

Poirel L, Lartigue MF, Decousser JW, Nordmann P. ISEcp1B-mediated transposition of blaCTX-M in *Escherichia coli*. *Antimicrob Agents Chemother* 2005; 49: 447-50.

Reinert RR, Low DE, Rossi F, Zhang X, Wattal C, Dowzicky MJ. Antimicrobial suscep-

tibility among organisms from the Asia/Pacific Rim, Europe and Latin and North America collected as part of TEST and the in vitro activity of tigecycline. *J Antimicrob Chemother* 2007; 60: 1018-29.

Tarnberg M, Nilsson LE, Monstein HJ. Molecular identification of (bla)SHV, (bla)LEN and (bla)OKP beta-lactamase genes in *Klebsiella pneumoniae* by bi-directional sequencing of universal SP6- and T7-sequence-tagged (bla)SHV-PCR amplicons. *Mol Cell Probes* 2009; 23: 195-200.

Turner PJ. Extended-spectrum beta-lactamases. *Clin Infect Dis* 2005; 41 (suppl 4): S273-5.