Original Article

The Prevalence and Susceptibility Patterns of ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* in Chonburi Hospital

Jirachai Waiwarawooth, M.D.*,
Kamonwon Jutiworakul, M.D.*,
Wacharee Joraka, M.Sc.**

ABSTRACT

Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae especially *Escherichia coli* and *Klebsiella pneumoniae* are a worldwide problem in hospitalized patients. The prevalence among clinical isolates of these organisms varies between countries. They are usually resistant to several classes of antibiotics including β-lactams (except carbapenems and cephamycins), aminoglycosides, and fluoroquinolones. This study aimed to determine the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* and the succeptibility patterns of these isolates in Chonburi Hospital, Chonburi, Thailand, from January to December 2005. Overall, 38.21 percent of *E. coli* and 50.90 percent of *K. pneumoniae* were ESBL-producing strains. The prevalence of ESBL-producing strains was significantly higher among those isolated from patients hospitalized for more than 48 hours than isolated from patients hospitalized within 48 hours ($p < 0.001$). ESBL-producing *E. coli* were recovered most frequently from the urine (38.73%), the pus (27.52%), and the sputum (17.29%). ESBL-producing *K. pneumoniae* were recovered most frequently from the sputum (44.69%), the urine (21.60%), and the pus (18.24%). The susceptibility to third-generation cephalosporins, gentamicin, fluoroquinolones, and β-lactam-β-lactamase inhibitors varied greatly between ESBL-nonproducing and ESBL-producing *E. coli* and *K. pneumoniae*, but showed a little or no difference in amikacin and fosfomycin, or carbapenem, respectively.

In conclusion, there is a high prevalence of ESBL-producing *K. pneumoniae* and *E. coli* in Chonburi Hospital, especially in those organisms isolated from patients hospitalized for more than 48 hours. Most ESBL-producing organisms were resistant to several classes of antibiotics including third-generation cephalosporins, β-lactam-β-lactamase inhibitors, fluoroquinolones, and gentamicin. (*J Infect Dis Antimicrob Agents 2006;23:57-65.*)

*Deparment of Medicine, Chonburi Hospital, Chonburi 20000, Thailand.

**Deparment of Microbiology, Chonburi Hospital, Chonburi 20000, Thailand.

Received for publication: April 18, 2006.

Reprint request: Jirachai Waiwarawooth, M.D., Deparment of Medicine, Chonburi Hospital, Chonburi 20000, Thailand.

Keywords: ESBL-producing *K. pneumoniae*, ESBL-producing *E. coli*, extended-spectrum β-lactamase (ESBL)

INTRODUCTION

An emergence of pathogens resistant to antibiotics often occurs because of an inappropriate use of antibiotics. The production of β-lactamase enzymes, which is the most important and common mechanism of resistance to β-lactam antibiotics.[1] β-lactamase enzymes can hydrolyze virtually all β-lactam antibiotics except cephamycins and carbapenems, and are generally inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam, or tazobactam.[2,3] To date, a wide variety of β-lactamase enzymes continue to be identified, partly due to a wide use of β-lactam antibiotics. Extended-spectrum β-lactamases (ESBLs) are one of the most common type of β-lactamase enzymes. ESBL-producing strains have emerged among the Enterobacteriaceae, prominently in *Escherichia coli* and *Klebsiella pneumoniae*. They were first isolated in Germany in 1983[4], and a rapid dissemination has been responsible for numerous outbreaks of infections throughout the world, and are now a major problem in many clinical setting especially in a tertiary-care medical center.[5]

The prevalence of ESBL-producing Enterobacteriaceae especially *K. pneumoniae* and *E. coli*, among clinical isolates varies according to the type of hospital and country. For example, the prevalence of ESBL-producing *K. pneumoniae* was highest in isolates from Latin America (45.4%), the Western Pacific region (24.6%), and Europe (22.6%), and lowest from the United States (7.6%), and Canada (5.9%).[6] The prevalence of ESBL-producing *E. coli* was 8.5 percent, 7.9 percent, 5.3 percent, 3.3 percent, and 4.2 percent in isolates from Latin America, the Western Pacific region, Europe, the United States, and Canada, respectively.[6]

The data from the National Antimicrobial Resistance Surveillance Thailand (NARST) showed the prevalence ESBL-producing *K. pneumoniae* and *E. coli* in Thailand were 30.5 percent and 19.9 percent, respectively, from 2002 to 2003.[7] The data from Songklanagarind Hospital found 32 percent and 19 percent of ESBL-producing *K. pneumoniae* and *E. coli* in 2002.[8]

Clinical Microbiology Laboratory of Chonburi Hospital has tested for the ESBL production for three years. We have found an increasing prevalence of ESBL-producing *K. pneumoniae* and *E. coli*, especially in nosocomial infections. The aim of this study was to determine the prevalence and the susceptibility patterns of ESBL-producing *K. pneumoniae* and *E. coli* among clinical isolates in Chonburi Hospital, a tertiary care center, Chonburi, Thailand.

MATERIALS AND METHODS

Bacterial isolation

From January to December 2005, all clinical isolates were obtained from the patients hospitalized in Chonburi Hospital. Bacteria were identified by the standard methods used in the microbiology laboratory. The organisms were categorized into two groups including those isolated within and more than 48 hours of hospitalization. *K. pneumoniae* and *E. coli* isolates were collected and identified in Clinical Microbiology Laboratory of Chonburi Hospital.

Susceptibility testing and ESBL detection

All *K. pneumoniae* and *E. coli* isolates were tested for the antimicrobial susceptibility. The antimicrobial agents used in the susceptibility testing included cefotaxime, ceftriaxone, ceftazidime, amoxicillin-clavulanate, cefoperazone-sulbactam, piperacillin-tazobactam, imipenem, meropenem, levofloxacin, ciprofloxacin, gentamicin, amikacin, and fosfomycin. The reference strain *E. coli* ATCC 25922 was used as the quality control strain for the susceptibility testing.

Antimicrobial susceptibility testing of all antibiotics was carried out using the Kirby-Bauer disk diffusion method according to the recommendation of the National Committee for Clinical Laboratory Standards (NCCLS).[9] A phenotypic confirmatory test for the ESBL production was performed according to the recommendation of the NCCLS.[9] Four disks containing cefotaxime (30 μg), cefotaxime-clavulanic acid (30 μg/ 10 μg), ceftazidime (30 μg), ceftazidime-clavulanic acid (30 μg/10 μg) were used in this confirmatory test. The ESBL production was determined when there was a more than five-mm increase in an inhibition zone for each antimicrobial agent in combination with clavulanic acid, compared with its zone when tested without clavulanic acid.

Statistical analysis

A chi-square test was used to compare the prevalence of ESBL-producing strains between isolates within 48 hours of hospitalization and more than 48 hours after hospitalization. The percentage of susceptibility rate of ESBL producers and ESBL non-producers was compared, and the differences in the susceptibility to antimicrobial agents used by the 95-percent exact binomial confidence intervals (CI) are presented as the difference calculated as the percentage of susceptibility rate for ESBL-nonproducing isolates minus the percentage of susceptibility rate for ESBL-producing isolates. All p-values were two-tailed with those less than 0.05 considered to be statistically significant.

RESULTS

ESBL production

A total of 15,530 bacterial isolates from 6,192 hospitalized patients were collected from January to December, 2005. 1,589 isolates (10.23%) were *E. coli*, and 1,055 isolates (6.79%) were *K. pneumoniae*. All *E. coli* and *K. pneumoniae* isolates were tested for the ESBL production and the antimicrobial susceptibility. 38.20 percent (607 of 1,589) of *E. coli* and 50.90 percent (537 of 1,055) of *K. pneumoniae* produced ESBLs.

Table 1 and 2 show that the prevalence of ESBL-producing isolates occurred significantly and more frequently ($p < 0.001$) in organisms isolated in patients hospitalized for more than 48 hours, compared with those isolated from patients within 48 hours of hospitalization. This prevalence was 55.90 percent and 22.54 percent in *E. coli* isolated from patients hospitalized for more than and within 48 hours, respectively. In addition, the prevalence was 66.02 percent and 23.80 percent in *K. pneumoniae* isolated from patients hospitalized for more than and within 48 hours, respectively.

ESBL-producing *E. coli* were recovered most frequently from the urine (38.73%), followed by the pus (27.52%), the sputum (17.29%), the blood (12.35%), and the sterile body fluids (4.11%) (Table 3). ESBL-producing *K. pneumoniae* were recovered most frequently from the sputum (44.69%), followed by the urine (21.60%), the pus (18.24%), the blood (10.28%), and the sterile body fluids (0.93 %) (Table 3).

Susceptibility patterns

The percentage of susceptibility rate of *E .coli* and *K. pneumoniae* isolated within 48 hours of hospitalization was higher than those isolated more than 48 hours after hospitalization (Table 4 and 5). The susceptibility to third-generation cephalosporins, β-lactam-β-lactamase inhibitors, and fluoroquinolones varied greatly between ESBL-nonproducing and ESBL-producing isolates, but showed a little or no difference in susceptibility to amikacin and fosfomycin, or carbapenems, respectively.

Table 1. The prevalence of ESBL-producing *E. coli* isolated within 48 hours of hospitalization and more than 48 hours after hospitalization.

E. coli isolates	Isolates after hospitalization		Total (isolates, patients)
	≤ 48 hours (isolates, patients)	> 48 hours (isolates, patients)	
ESBL-producing	190 (160)	417 (297)	607 (457)
ESBL-nonproducing	653 (526)	329 (228)	982 (754)
Total	843 (686)	746 (525)	1,589 (1,211)
% ESBL	22.54%*	55.90%*	38.20%

*p < 0.001 for between ESBL-nonproducing and ESBL-producing *E. coli*

Table 2. The prevalence of ESBL-producing *K. pneumoniae* isolated within 48 hours of hospitalization and more than 48 hours after hospitalization.

K. pneumonia isolates	Isolates after hospitalization		Total (isolates, patients)
	≤ 48 hours (isolates, patients)	> 48 hours (isolates, patients)	
ESBL-producing	90 (74)	447 (344)	537 (418)
ESBL-nonproducing	288 (249)	230 (165)	518 (414)
Total	378 (323)	677 (509)	1,055 (832)
% ESBL	23.80%*	66.02 %*	50.90%

*p < 0.001 for between ESBL-nonproducing and ESBL-producing *K. pneumoniae*

Table 3. ESBL-producing *K. pneumoniae* and *E. coli* isolated from all clinical specimens.

Specimen	*E. coli* isolates (%)	*K pneumoniae* isolates (%)
Urine	235 (38.73)	116 (21.60)
Pus	167 (27.52)	98 (18.24)
Sputum	105 (17.29)	240 (44.69)
Blood	75 (12.35)	58 (10.80)
Sterile fluid	25 (4.11)	5 (0.93)
Total	607 (100)	537 (100)

Table 4. ***In vitro*** **susceptibility patterns the ESBL-producing and ESBL-nonproducing** ***E. coli*****.**

			Percentage of susceptibility rate												
E. coli **isolates**		**No. of isolates**	**CRO**	**CTX**	**CAZ**	**AMC**	**CPZ**	**TZP**	**AMK**	**GEN**	**LVX**	**CIP**	**IPM**	**MEM**	**FOS**
<48 hours of hospitalization	ESBL-nonproducing	653	89	90	94	70	95	87	100	74	71	74	100	100	98
	ESBL-producing	190	0	0	O	42	56	66	100	28	34	33	100	100	89
	Difference 95% CI*		89 (86.60, 91.40)	90 (87.70, 92.30)	94 (92.18, 95.82)	28 (20.31, 35.69)	39 (31.92, 46.08)	21 (13.95, 28.05)	0 (0.00, 0.00)	46 (38.93, 53.07)	37 (29.53, 44.47)	41 (33.67, 48.33)	0 (0.00, 0.00)	0 (0.00, 0.00)	9 (4.54, 13.46)
>48 hours after hospitalization	ESBL-nonproducing	329	97	97	99	82	96	92	98	84	73	72	100	100	99
	ESBL-producing	417	0	0	0	46	60	73	94	50	25	25	100	100	97
	Difference 95% CI*		97 (95.18, 98.81)	97 (95.18, 98.81)	99 (97.18, 100.06)	36 (29.66, 42.34)	36 (30.81, 41.19)	19 (13.85, 24.15)	4 (1.26, 6.74)	34 (27.77, 40.23)	48 (41.68 54.32)	47 (40.64, 53.36)	0 (0.00, 0.00)	0 (0.00, 0.00)	2 (0.09, 3.96)

CRO: ceftriaxone, CTX: cefotaxime, CAZ: ceftazidime, AMC: amoxicillin-clavulanate, CPZ: cefoperazone-sulbactam, TZP: piperacillin-tazobactam, AMK: amikacin, GEN: gentamicin, LVX: levofloxacin, CIP: ciprofloxacin, IPM: imipenem, MEM: meropenem, and FOS: fosfomycin.

*The 95 percent exact binomial confidence intervals (CI) are presented as the difference calculated as the percentage susceptibility rate for ESBL-nonproducing isolates obtained minus the percentage susceptibility rate for ESBL-producing isolates.

Table 5. ***In vitro*** **susceptibility patterns of ESBL-producing and ESBL-nonproducing** ***K. pneumoniae*****.**

			Percentage susceptibility												
K. pneumoniae **isolates**		**No. of isolates**	**CRO**	**CTX**	**CAZ**	**AMC**	**CPZ**	**TZP**	**AMK**	**GEN**	**LVX**	**CIP**	**IPM**	**MEM**	**FOS**
<48 hours of hospitalization	ESBL-nonproducing	288	98	98	99	94	90	90	100	97	94	88	100	100	98
	ESBL-producing	90	0	0	O	27	52	20	100	3	74	26	100	100	80
	Difference 95% CI*		98 (96.26, 99.74)	98 (96.26, 99.74)	99 (97.76, 100.24)	67 (57.97, 76.03)	38 (27.52, 48.48)	70 (61.32, 78.68)	0 (0.00, 0.00)	94 (90.04, 97.96)	47 (36.78, 57.22)	62 (52.50, 71.500)	0 (0.00, 0.00)	0 (0.00, 0.00)	18 (9.97, 26.03)
>48 hours after hospitalization	ESBL-nonproducing	230	96	96	97	91	99	92	100	91	95	86	100	100	96
	ESBL-producing	447	0	0	0	17	34	18	91	17	51	24	100	100	76
	Difference 95% CI*		96 (96.26, 98.26)	96 (96.26, 98.26)	97 (95.03, 98.97)	74 (70.7, 77.3)	65 (60.32, 69.68)	74 (69.17, 78.83)	9 (6.26, 11.74)	74 (70.70, 77.30)	44 (38.59 49.41)	62 (56.28, 67.72)	0 (0.00, 0.00)	0 (0.00, 0.00)	20 (15.33, 24.67)

CRO: ceftriaxone, CTX: cefotaxime, CAZ: ceftazidime, AMC: amoxicillin-clavulanate, CPZ: cefoperazone-sulbactam, TZP: piperacillin-tazobactam, AMK: amikacin, GEN: gentamicin, LVX: levofloxacin, CIP: ciprofloxacin, IPM: imipenem, MEM: meropenem, and FOS: fosfomycin.

*The 95 percent exact binomial confidence intervals (CI) are presented as the difference calculated as the percentage of susceptibility rate for ESBL-nonproducing isolates obtained minus the percentage of susceptibility rate for ESBL-producing isolates.

DISCUSSION

The prevalence of ESBL-producing Enterobacteriaceae varies from country to country, and was increasing every year especially in tertiary hospitals. The infections caused by ESBL-producing *E. coli* and *K. pneumoniae* are considered an increased risk associated with the treatment failure.[10]

This study shows the high prevalence of ESBL-producing *K. pneumoniae* (50.90%) and *E. coli* (38.20%) in Chonburi Hospital, especially isolates from the patients with more than 48 hours of hospitalization, compared with those with less than 48 hours of hospitalization (66.02% and 55.90%, respectively).

Kusum and colleagues[11] as well as Taravichitikul and colleagues[12] reported that both ESBL-producing *K. pneumoniae* and *E. coli* were most frequently isolated from the sputum and urine. 49 percent of ESBL-producing *K. pneumoniae* were resistant to levofloxacin, compared to a previous study in 2002 which showed a 47-percent ciprofloxacin resistance in ESBL-producing *E. coli* and a 12-percent ciprofloxacin resistance in ESBL-producing *K. pneumoniae*.

In this study, among the three β-lactamase inhibitors (clavulanate, sulbactam, and tazobactam), piperacillin-tazobactam had the highest activity against ESBL-producing *E. coli* in comparison with cefoperazone-sulbactam and amoxicillin-clavulanate. Among ESBL-producing *K. pneumoniae*, cefoperazone-sulbactam had the highest activity, compared to the other two antibiotics. Despite a good *in vitro* activity of β-lactam-β-lactamase inhibitors, against ESBL-producing organisms, they are not recommended to be used in the clinical practice because of very large amounts of ESBL production[18], very high inoculum size of bacteria[19] or associated influx porin loss[20], resulting in vivo resistance. In addition, some clinical studies showed a failure after treatment with β-lactam-β-lactamase inhibitor.[21]

In this study, among non-β-lactam antibiotics, fluoroquinolones showed a much reduced susceptibility against ESBL-producing isolates.[10] Of this isolates from patients with more than 48 hours after hospitalization, 75 and 70 percent of ESBL-producing *E. coli* were resistant to levofloxacin and ciprofloxacin, respectively. In addition, 70 and 49 percent of ESBL-producing *K. pneumoniae* were resistant to ciprofloxacin and levofloxacin, respectively. In contrast to this study, a previous study in 2002 showed 47-percent and 12-percent ciprofloxacin resistance in ESBL-producing *E. coli* and *K. pneumoniae*, respectively.[8]

In this study, among aminoglycosides, amikacin had a better activity than gentamicin against these ESBL-producing organisms. This resistance pattern may be due the overuse of gentamicin in our hospital. Gentamicin resistance is usually associated with an enzymatic modification of the drug by *O*-nucleotidyl-transferase enzymes which cannot modify amikacin.[22]

In this study, fosfomycin had a good activity against *K. pneumoniae* and *E. coli* and there was no much difference in the susceptibility between the ESBL producers and ESBL nonproducers, similar to that reported from Maharaj Nakorn Chiang Mai.[12]

In this study, carbapenems, both imipenem and meropenem, had the best activity against *K. pneumoniae* and *E. coli*, and there was no difference in the susceptibility between the ESBL producers and ESBL nonproducers.

This study has some limitations. The phenotype of ESBLs is not determined in this study. Cefoxitin was not used in the susceptibility test, thus AmpC β-lactamase, another a common type of β-lactamases, could not be detected in this study.

In addition, the isolates obtained from patients within 48 hours of hospitalization may not be truly community-acquired strains because the patients may be recently discharge from the hospital or referred from another hospital. In contrast, the isolates obtained from patients with more than 48 hours of hospitalization may not be truly hospital-acquired strains.

The microbiology laboratory plays an important role in routinely detecting and reporting the isolation of

ESBL-producing isolates, as well as in providing the clinicians with reliable therapeutic options for a successful treatment.

In conclusion, the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* was very high in Chonburi Hospital, especially in those isolates from patients hospitalized for more than 48 hours. Most ESBL-producing organisms were resistant to several classes of antibiotics including third-generation cephalosporins, β-lactam-β-lactamase inhibitors, fluoroquinolones, and gentamicin.

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