

# Extended-spectrum Beta-lactamases in *Escherichia coli* Isolated from Patients with Community-acquired Urinary Tract Infection at Songklanagarind Hospital, Thailand

Wanutsanun Tunyapanit, M.Sc.,  
Pornpimol Pruekprasert, M.D.

## ABSTRACT

Extended-spectrum beta-lactamases (ESBL)-producing *Escherichia coli* resulting in  $\beta$ -lactam antimicrobial resistance are commonly observed in nosocomial infection. To date, there have been a few studies of community-acquired ESBL-producing *E. coli*.

In this study, we determined the prevalence and the susceptibility patterns of ESBL-producing *E. coli* isolated from patients with community-acquired urinary tract infection at Songklanagarind Hospital, Hat Yai, southern Thailand from July 2003 to January 2004. ESBL-producing *E. coli* were detected in six of 107 (6%) urine isolates; all of which were resistant to ampicillin, cefazolin, and cefuroxime. Of these six isolates, 67 percent, 50 percent, and 50 percent were resistant to gentamicin, cefotaxime, and norfloxacin, respectively. ESBL-nonproducing isolates were detected in 101 of 107 (94%) isolates, in which 76 percent, 30 percent, 6 percent, 8 percent, 0 percent, and 3 percent were resistant to ampicillin, norfloxacin, cefazolin, cefuroxime, cefotaxime, and gentamicin respectively. The minimal inhibitory concentration<sub>50</sub> (MIC<sub>50</sub>) of cefazolin, cefuroxime, cefotaxime, gentamicin, and norfloxacin against ESBL-producing isolates were 32 to 256 times higher than in ESBL-nonproducing isolates. (*J Infect Dis Antimicrob Agents* 2006;23:51-6.)

## INTRODUCTION

Extended-spectrum beta-lactamases (ESBLs) are the enzymes produced by bacteria mostly in the Enterobacteriaceae family (mainly *Klebsiella pneumoniae* and *Escherichia coli*). These enzymes are capable of hydrolyzing penicillins, broad-spectrum cephalosporins (e.g. cefotaxime, ceftriaxone, and

ceftazidime), and monobactams (e.g. aztreonam).<sup>1-4</sup> Many ESBL-producing organisms are also frequently resistant to aminoglycosides, trimethoprim-sulfamethoxazole, and quinolones.<sup>5</sup> ESBLs are generally derived from TEM- and SHV-type enzymes, and are often located on plasmids that are transferable from strain to strain and between bacterial species.<sup>1,4,6,7</sup>

Department of Pediatrics, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.

Received for publication: February 3, 2006.

Reprint request: Tunyapanit W, M.Sc., Department of Pediatrics, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.

**Keywords:** extended-spectrum beta-lactamases, ESBLs, *E. coli*, community

In various parts of the world, 10-40 percent of *E. coli* and *K. pneumoniae* are ESBL-producing isolates.<sup>8</sup> Although ESBL-producing *E. coli* resulting in beta-lactam antimicrobial resistance are commonly detected in nosocomial infection, a recent study has reported the emergence of community-acquired infections caused by ESBL-producing *E. coli* in Canada, France, Israel, Spain, Italy, and the United Kingdom.<sup>5,9,10</sup>

The aims of this study were to determine the prevalence and the susceptibility patterns of ESBL-producing *E. coli* isolated from patients with community-acquired urinary tract infection (UTI) at Songklanagarind Hospital, Hat Yai, southern Thailand.

## MATERIALS AND METHODS

### Bacterial isolates

Isolates were collected from urine specimens of outpatients or inpatients hospitalized less than 48 hours and who had clinical evidence of community-acquired UTIs from July 2003 to January 2004. All patients reported no hospitalization or catheterization during the two months preceding this illness.<sup>11-13</sup> UTI were defined as the culture of a single organism from a midstream urine specimen at  $> 10^5$  colony-forming units per milliliter. Only one specimen per patient was processed. Identification and confirmation of *E. coli* isolates were performed according to the standard procedures at the Microbiology Laboratory of the Department of Pathology, Prince of Songkla University, Hat Yai, southern Thailand.

### Media and antimicrobial agents

Media used were Mueller-Hinton agar, Mueller-Hinton broth, MacConkey agar, and tryptic soy broth, manufactured by Difco (Maryland, USA). The disks used in the detection of ESBL production included amoxicillin/clavulanic acid (20/10  $\mu\text{g}$ ), ceftazidime (30  $\mu\text{g}$ ), cefotaxime (30  $\mu\text{g}$ ), ceftriaxone (30  $\mu\text{g}$ ),

cefepodoxime (10  $\mu\text{g}$ ), cefepodoxime/clavulanic acid (10/1  $\mu\text{g}$ ), ceftazidime/clavulanic acid (30/10  $\mu\text{g}$ ), and cefotaxime/clavulanic acid (30/10  $\mu\text{g}$ ) (Oxoid, Hampshire, England). The six standard laboratory powder of antimicrobial agents used were ampicillin (Aldrich, Steinheim, Germany), cefazolin, cefuroxime, cefotaxime, gentamicin, and norfloxacin (Sigma, Steinheim, Germany).

### Extended-spectrum beta-lactamase production detection and susceptibility test

The production of ESBLs was detected by the double-disk synergy test.<sup>14</sup> Briefly, after plating bacterial inoculum on a Mueller-Hinton plate, a disk of amoxicillin/clavulanic acid was placed in the middle and four antimicrobial disks (ceftazidime, cefotaxime, ceftriaxone, and cefepodoxime) were placed 30 mm apart (center to center) around the amoxicillin/clavulanic acid disk. After overnight incubation, an enhancement of the inhibition zone between the amoxicillin/clavulanic acid disk and any one of the four antimicrobial disks indicated the presence of an ESBL. A confirmation of ESBL production was determined by the combination disk diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS) recommendations.<sup>15</sup>

The minimum inhibitory concentration (MIC) and the susceptibility of the isolates to six antimicrobial agents were determined by the standard agar dilution method.<sup>16</sup>

Quality control was conducted using the reference strains, *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603.

## RESULTS

### Patient information

From July 2003 to January 2004, there were 107 urine isolates of *E. coli* from patients with community-

acquired UTI. The mean age of the enrolled patients was 48 years (19 days to 83 years). Twelve (11%) patients were children under 15 years old. The male-to-female ratio was 1:4. All patients had clinical signs and symptoms of UTI. Six (6%) patients were infected with ESBL-producing *E. coli*.

ESBL-producing *E. coli* were isolated in two (10%) of 20 and four (5%) of 87 patients, with and without a history of antibiotic treatment within three months prior to the study, respectively. However, the difference was not statistically significant ( $P=0.3$ ). ESBL-producing isolates were recovered in one (6%) of 17 and five (6%) of 90 patients with and without preexisting urinary tract disease, respectively. Even though five (29%) of 17 patients with preexisting urinary tract disease had a history of antibiotic treatment, compared to 15 (17%) of 90 patients without preexisting urinary tract disease ( $P=0.3$ ).

### The prevalence and susceptibility patterns of community-acquired ESBL-producing *E. coli*

ESBL-producing *E. coli* were detected in six of 107 (6%) isolates; all of which were resistant to ampicillin, cefazolin, and cefuroxime. The susceptibility of *E. coli* isolates against six antimicrobial agents

(ampicillin, cefazolin, cefuroxime, cefotaxime, gentamicin, and norfloxacin) was determined by the agar dilution method. Of these six isolates, 67 percent, 50 percent, and 50 percent were resistant to gentamicin, cefotaxime, and norfloxacin, respectively (Table 1). Of ESBL-producing *E. coli*, two isolates were resistant to all six antimicrobial agents, two isolates were resistant to three agents, and two isolates were resistant to five agents (Table 2). Non-ESBL-producing *E. coli* were detected in 101 (94%) of 107 isolates. The resistance to ampicillin, norfloxacin, cefuroxime, cefazolin, and gentamicin was detected in 76 percent, 30 percent, 8 percent, 6 percent, and 3 percent of isolates, respectively (Table 3). However, all isolates were susceptible to cefotaxime.

### The MIC<sub>50</sub> of commonly used antimicrobial agents

We determined the MIC values of six antimicrobial agents (ampicillin, cefazolin, cefuroxime, cefotaxime, gentamicin, and norfloxacin) commonly used in the treatment of UTI caused by *E. coli* by the agar dilution method. The MIC<sub>50</sub> values of six antimicrobial agents against ESBL-producing *E. coli* are shown in Table 4. Even though the MIC<sub>50</sub> levels of ampicillin, cefazolin,

**Table 1. Activities of six antimicrobial agents against ESBL-producing isolates.**

Antimicrobial agent	Number of isolates (%)		
	Susceptible*	Intermediately resistant*	Resistant*
Ampicillin	-	-	6 (100)
Cefazolin	-	-	6 (100)
Cefotaxime	2 (33)	1 (17)	3 (50)
Cefuroxime	-	-	6 (100)
Gentamicin	2 (33)	-	4 (67)
Norfloxacin	3 (50)	-	3 (50)

Note: \*Based on susceptibility breakpoints defined by the NCCLS:

Ampicillin, cefazolin, and cefuroxime: susceptible < 8 µg/ml, intermediately resistant 16 µg/ml, resistant > 32 µg/ml

Cefotaxime: susceptible < 8 µg/ml, intermediately resistant 16-32 µg/ml, resistant > 64 µg/ml

Gentamicin and norfloxacin: susceptible < 4 µg/ml, intermediately resistant 8 µg/ml, resistant > 16 µg/ml

cefuroxime, cefotaxime, and gentamicin in ESBL-producing *E. coli* were high, and the MIC<sub>50</sub> levels of norfloxacin were low. Of ESBL-nonproducing *E. coli*, the MIC<sub>50</sub> and MIC<sub>90</sub> levels of ampicillin were

as high as in ESBL-producing *E. coli*. However, the MIC<sub>50</sub> and MIC<sub>90</sub> levels of cefazolin, cefuroxime, cefotaxime, gentamicin, and norfloxacin were lower than those in ESBL-producing isolates (Table 5).

**Table 2. Susceptibility patterns for six antimicrobial agents tested against six ESBL-producing isolates.**

Pattern	Susceptibility patterns						Number of isolate
	Ampicillin	Cefazolin	Cefotaxime	Cefuroxime	Gentamicin	Norfloxacin	
1	R	R	S	R	S	S	1
2	R	R	I	R	S	S	1
3	R	R	S	R	R	R	1
4	R	R	R	R	R	S	1
5	R	R	R	R	R	R	2

Note: S=susceptible, I=intermediately resistant, R=resistant

**Table 3. Activities of six antimicrobial agents against ESBL-nonproducing isolates.**

Antimicrobial agent	Number of isolates (%)		
	Susceptible*	Intermediately resistant*	Resistant*
Ampicillin	24 (24)	-	77 (76)
Cefazolin	84 (83)	11 (11)	6 (6)
Cefotaxime	101 (100)	-	-
Cefuroxime	74 (73)	19 (19)	8 (8)
Gentamicin	98 (97)	-	3 (3)
Norfloxacin	68 (67)	3 (3)	30 (30)

Note: \*Based on susceptibility breakpoints defined by the NCCLS:

Ampicillin, cefazolin, and cefuroxime: susceptible < 8 µg/ml, intermediately resistant 16 µg/ml, resistant > 32 µg/ml

Cefotaxime: susceptible < 8 µg/ml, intermediately resistant 16-32 µg/ml, resistant > 64 µg/ml

Gentamicin and norfloxacin: susceptible < 4 µg/ml, intermediately resistant 8 µg/ml, resistant > 16 µg/ml

**Table 4. The minimal inhibitory concentration<sub>50</sub> (MIC<sub>50</sub>) of six ESBL-producing isolates.**

Isolates	MIC <sub>50</sub> (mg/ml)					
	Ampicillin	Cefazolin	Cefotaxime	Cefuroxime	Gentamicin	Norfloxacin
1	> 256	> 128	> 128	> 256	64	256
2	> 256	> 128	64	> 256	64	256
3	> 256	128	4	32	64	256
4	> 256	> 128	64	> 256	32	4
5	> 256	32	2	256	4	0.5
6	> 256	> 128	32	> 256	1	0.125

**Table 5. The MIC<sub>50</sub> and MIC<sub>90</sub> of six ESBL-producing isolates and 101 ESBL-nonproducing isolates.**

Antimicrobial agent	ESBL-producing isolates			ESBL-nonproducing isolates			<i>E. coli</i> ATCC 25922	
	Range	MIC (□g/ml)		Range	MIC (□g/ml)		Range	MIC (□g/ml)
		MIC <sub>50</sub>	MIC <sub>90</sub>		MIC <sub>50</sub>	MIC <sub>90</sub>		
Ampicillin	>256	>256	>256	1->256	>256	>256	2-8	4
Cefazolin	32->128	>128	>128	1-128	2	16	1-4	2
Cefotaxime	2->128	32	>128	0.125-2	0.125	0.25	0.03-0.12	0.125
Cefuroxime	32->256	>256	>256	1-256	8	16	2-8	8
Gentamicin	1-64	32	64	0.25-128	0.5	1	0.25-1	0.5
Norfloxacin	0.125-256	4	256	0.125->256	0.125	128	0.03-0.12	0.125

\*Range=acceptable quality control limits of MIC for nonfastidious organisms defined by the NCCLS

## DISCUSSION

Previous studies have shown that community-acquired ESBL-producing *E. coli* isolates were found in 1.4-10.5 percent of patients who have UTI.<sup>3,5,17-19</sup>

This study investigated the prevalence and the susceptibility patterns of ESBL-producing *E. coli* in patients who presented with community-acquired UTI at Songklanagarind Hospital.

Our results showed a six percent prevalence of community-acquired ESBL-producing *E. coli*. We found that all community-acquired ESBL-producing *E. coli* had multidrug-resistant patterns and high MIC<sub>50</sub> and MIC<sub>90</sub> values to several antimicrobials including cefazolin, cefuroxime, cefotaxime, gentamicin, and norfloxacin. Astal et al<sup>3</sup> reported that ESBL-producing *E. coli* were resistant to several antimicrobials and had high rates of resistance to cefuroxime, cefotaxime, and gentamicin. In contrast, Hryniewicz et al<sup>19</sup> found that most ESBL-producing *E. coli* were susceptible to gentamicin. The similarities and differences in antimicrobial resistance patterns between studies may be due to population (socio-demographical, socioeconomic, and socio-epidemiological) and clinical parameters.<sup>3</sup>

We found that all ESBL-nonproducing *E. coli*

were susceptible to cefotaxime, and most were susceptible to cefazolin, cefuroxime, gentamicin, and norfloxacin.

The prevalence of ESBL-producing *E. coli* isolated from patients with community-acquired UTI in this study was approximately one-third of that previously reported in hospitalized patients in Songklanagarind Hospital.<sup>20</sup> In addition, this study showed the community-acquired ESBL-producing *E. coli* isolates were resistant to several antimicrobials commonly used in the treatment of UTI. Therefore, antimicrobial control policies should be implemented in the community in order to minimize the development of antimicrobial resistance. Due to an increasing incidence of community-acquired UTI caused by ESBL-producing *E. coli*, there would be an urgent need of well-designed studies to determine the epidemiological and clinical data in accordance with treatment and outcome.

## ACKNOWLEDGEMENT

This work was partially supported by a grant from the Faculty of Medicine, Prince of Songkla University. We thank the microbiology personnel of the Department of Pathology, Faculty of Medicine, Prince of Songkla University for providing *E. coli* isolates.

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