Long-Term Study of *Escherichia coli* and *Klebsiella pneumoniae* Isolates Producing Extended-Spectrum Beta-Lactamases

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Objective: *To evaluate the prevalence and susceptibility pattern of Escherichia coli and Klebsiella pneumoniae isolates producing extended-spectrum beta-lactamases (ESBLs) in Thailand from 2000 to 2005.*
Material and Method: *Data on the WHONET, from 28 hospitals participated in the National Antimicrobial Resistance Surveillance, Thailand surveillance program, were reviewed and analyzed for the prevalence and susceptibility pattern.*
Results: *During the five-year surveillance from 2000 to 2005, the prevalence of ESBL-producing E. coli detected by ceftazidime screening test was 17%, 21.3%, 23.2%, 20.4%, 23.1%, and 25.0%; as well as detected by cefotaxime screening test was 20.8%, 65.9%, 69.3%, 69.3%, 68.3%, and 33.8%, respectively. The prevalence of ESBL-producing K. pneumoniae detected by ceftazidime screening test was 30.9%, 34.7%, 32.5%, 34.4%, 37.2%, and 39.2%; as well as detected by cefotaxime screening test 38.4%, 39.3%, 40.1%, 41.0%, 42.8%, and 40.4%, respectively.*
Conclusion: *From 2000 to 2005, the prevalence of ESBL-producing organisms in Thailand was high. ESBL-producing E. coli was most commonly isolated from sputum, followed by blood and urine specimens. ESBL-producing K. pneumoniae had not been increasingly isolated from sputum, blood, and urine.*

Keywords: *Escherichia coli, Klebsiella pneumoniae, Beta-lactamases, Thailand*

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An increase in bacterial resistance to beta-lactam antibiotics has been observed worldwide, probably due to the spread of the plasmid-mediated extended-spectrum beta-lactamases (ESBLs). These enzymes have been evolving with stepwise mutation in their structural genes resulting in single or multiple amino acid changes in the encoded enzymes. The enzymes are predominantly present in *Escherichia coli* and *Klebsiella pneumoniae*[1],but recently they were discovered in other genera of the family Enterobacteriaceae including *Enterobacter*, *Serratia*, *Morganella*, and *Citrobacter*[2]. Since their first discovery in Germany in 1983, ESBL-producing organisms have been reported in several countries[3]. To date, official information has not been available on the overall occurrence of ESBL-producing isolates in most Thai hospitals. Thus, the authors aimed to determine the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* from the National Antimicrobial

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Resistance Surveillance Thailand (NARST) program from 2000 to 2005.

The data of ESBL-producing *E. coli* and *K. pneumoniae* isolated from clinical specimens submitted to microbiology laboratories enrolled in the NARST program from 2000 to 2005 were reviewed.

Material and Method

Setting

In 1998, NARST of the Department of Medical Sciences, Thailand's Ministry of Public Health, was established. The activities of NARST included a description of antimicrobial resistance situations of various microorganisms, strengthening and standardization laboratory practices related to antimicrobial susceptibility, and collecting antimicrobial susceptibility data using the WHONET software program. All of these activities are supported by the World Health Organization (WHO). Previously, there were 33 hospitals in the surveillance network. All data from each hospital such as types of specimen, bacterial isolation, and diameter of antimicrobial inhibition zone, were sent to NARST. Only 28 hospitals had all data available for analysis during the five-year surveillance period; *i.e.* 9 small hospitals (fewer than 500 beds) and 19 large hospitals (equal or more than 500 beds). The distribution of the participating hospitals included 13 hospitals in central Thailand, 6 in the Northeast, 5 in the North, and 4 in the South of Thailand. In each hospital, the sampling specimens were sent for a species confirmatory test by biochemistry, and for a susceptibility test by the double-disk or combined-disk methods.

Microbiology

All *E. coli* and *K. pneumoniae* isolates from significant clinical specimens were tested for ESBL production. The ESBL-producing phenotypes of all isolates were determined by the screening Kirby-Bauer disk diffusion method at each hospital. A confirmatory test of ESBL-production was performed at NARST center with the double-disk or combined-disk method. The zones of inhibition of each isolate were tested on Mueller-Hinton agar plates with the disks containing 30 μg of ceftazidime and cefotaxime alone and in combination with 10 μg of clavulanic acid, respectively. An organism was classified as having an ESBL-producing phenotype if the zone of inhibition produced by at least one combination disk was more than 5 mm larger than that produced by the corresponding antimicrobial disk without clavulanic acid[4]. At each hospital laboratory, the identification and susceptibility testing of the isolates were performed using standard conventional methods as recommended by the Clinical Laboratory Standards Institute (CLSI) [formerly National Committee for Clinical Laboratory Standards (NCCLS)].

Statistical analysis

Descriptive statistics were presented in terms of number and percentage.

Results

A total of 158,352 *E. coli* and 67,263 *K. pneumoniae* isolates were recovered from 2000 to 2005. Among *E. coli*, the ESBL production detected by ceftazidime screening test was 17%, 21.3%, 23.2%, 20.4%, 23.1%, and 25.0% from 2000 to 2005, respectively. Cefotaxime screening test revealed such organism in 20.8%, 65.9%, 69.3%, 69.3%, 68.3%, and 33.8% from 2000 to 2005, respectively. The confirmatory test by double-disk or combined-disk method were performed on some of these isolates and confirmed the presence of ESBL in 84% to 100% of isolates tested (Table 1). Therefore, around 28% to 69% of *E. coli* during the study period were ESBL producers (Table 1). Among *K. pneumoniae*, ESBL production detected by ceftazidime screening test was 30.9%, 34.7%, 32.5%, 34.4%, 37.2%, and 39.2% from 2000 to 2005, respectively, and detected by cefotaxime screening test 38.4%, 39.3%, 40.1%, 41.0%, 42.8%, and 40.4% from 2000 to 2005, respectively. The confirmatory test showing ESBL production in 99.3% (453 specimens), 99.5% (781 specimens), 99.3% (686 specimens), 100% (1,564 specimens), 92.6% (3,026 specimens), and 90.8% (4,812 specimens) of isolates tested from 2000 to 2005, respectively (Table 1).

ESBL-producing *E. coli* strains, as screened by ceftazidime and cefotaxime screening test, had increasingly been isolated from 2000 to 2005 (Fig. 1). From 2000 to 2005, the percentage of ESBL-producing *E. coli* isolates obtained from the sputum specimens had been increasing most, followed by these isolated from the blood and the urine specimens (Fig. 1). The percentage of ESBL-producing *K. pneumoniae* isolates had not increased, whether isolated from the sputum, blood, and urine specimens(Fig. 2).

Discussion

ESBL-producing Enterobacteriaceae are among the most problematic multidrug-resistant bacteria worldwide, and have been isolated with

Table 1. Extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Thai hospitals from 2000 to 2005

Year of isolation	% (No. of *E. coli*)			% (No. of *K. pneumoniae*)		
	Positive screen testing		Positive confirmatory test (double-disk or combined-disk method)	Positive screen testing		Positive confirmatory test (double-disk or combined-disk method)
	CAZ (less than or equal 22 mm)	CTX (less than or equal 27 mm)		CAZ (less than or equal 22 mm)	CTX (less than or equal 27 mm)	
2000	17.0 (13,892)	20.8 (11,026)	99.0 (392)	30.9 (9,000)	38.4 (6,486)	99.3 (453)
2001	21.3 (15,055)	65.9 (31,965)	98.2 (702)	34.7 (9,921)	39.3 (8,788)	99.5 (781)
2002	23.2 (14,636)	69.3 (29,625)	98.0 (891)	32.5 (9,613)	40.1 (7,724)	99.3 (686)
2003	20.4 (13,517)	69.3 (30,899)	100.0 (1,524)	34.4 (11,680)	41.0 (8,643)	100 (1,564)
2004	23.1 (19,895)	68.3 (35,584)	86.3 (3,318)	37.2 (12,782)	42.8 (10,326)	92.6 (3,026)
2005	25.0 (22,481)	33.8 (19,253)	84.4 (5,221)	39.2 (14,267)	40.4 (11,192)	90.8 (4,812)

CAZ: ceftazidime, CTX: cefotaxime

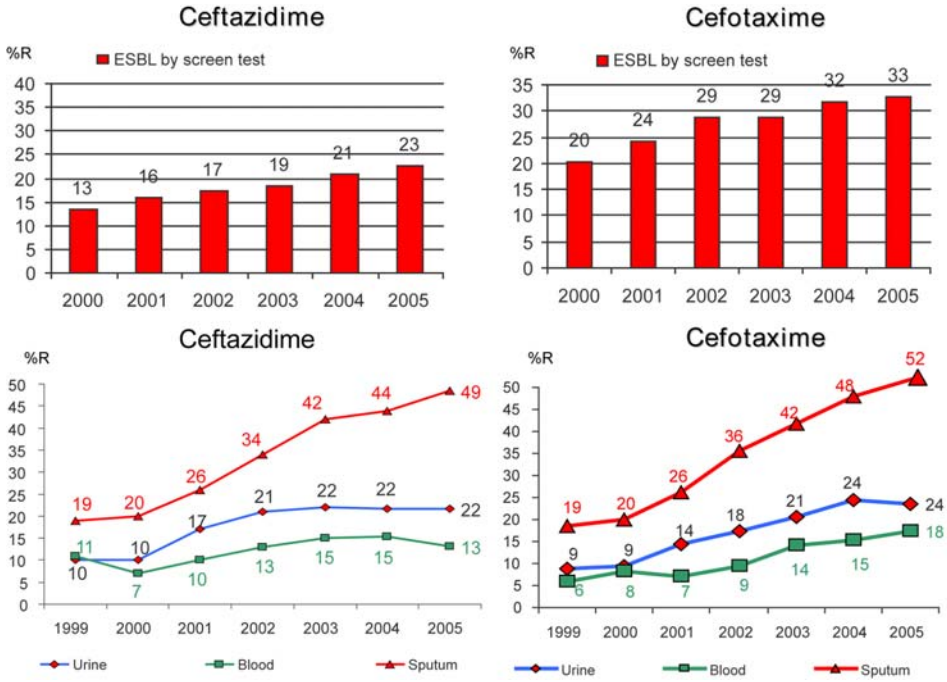

Fig. 1 Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* by the screening test

increased frequency[5,6]. In the present study, the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* isolates in different hospitals in Thailand was determined. The prevalence of ESBL-producing *E. coli* by the screening ceftazidime disk test ranged from 17.0% to 25.0% (17%, 21.3%, 23.2%, 20.4%, 23.1% and 25.0% from 2000 to 2005) and by screening cefotaxime disk test ranged from 20.8% to 69.3% (20.8%, 65.9%, 69.3%, 69.3%, 68.3%, and 33.8% from 2000 to 2005, respectively). From 2000 to 2005, the prevalence of ESBL-producing *E. coli* in Thailand was higher than in other countries such as the Netherlands (less than 1% in 1999)[7] and Spain (1.7% in 2005)[8]. The prevalence of ESBL-producing *K. pneumoniae* by the

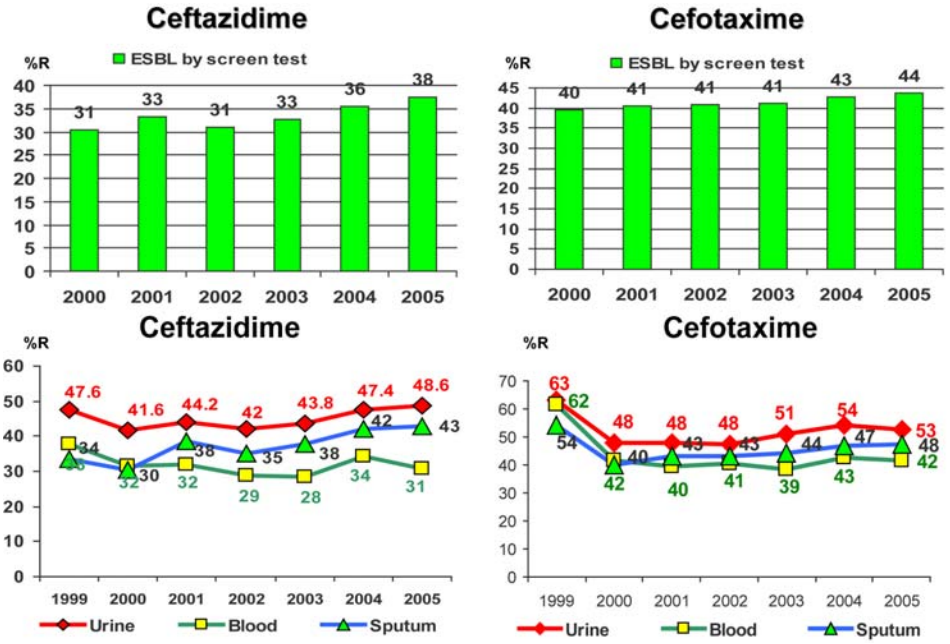

Fig. 2 Extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae* by the screening test

screening ceftazidime test ranged from 30.9% to 39.2% (30.9%, 34.7%, 32.5%, 34.4%, 37.2%, and 39.2% from 2000 to 2005, respectively); and by the screening cefotaxime test ranged from 38.4% to 42.8% (38.4%, 39.3%, 40.1%, 41.0%, 42.8%, and 40.4% from 2000 to 2005, respectively). Similar to *E. coli*, the prevalence of ESBL-producing *K. pneumoniae* in Thailand was higher than Spain (4.0% in 2005)[8].

It is interesting that cefotaxime screening test yieled higher percentage of suspected ESBL positive *E. coli* strain than ceftazidime screening test. This apparent ceftazidime-susceptible, cefotaxime-resistant might reftect the endemicity of CTK-M type ESBL which have been circulated in the Far East and Southeast Asia in recent years. If this postulation is correct, then we needed to re-consider national antibiotic policy particularly the wide spread injudicious use of both parenteral of and oral third-generation cephalsporins in our community since it is a known risk factor for the emergence and spread of this type of ESBL. However, further study regarding epidemiology of each type of ESBL is needed to "fine-tune" the mentioned policy. Furthermore, both ceftazidime and cefotaxime should be used in the screening of ESBL production among important gram-negative bacteria.

There are several limitations of the present study. The major problems may be due to the lack of knowledge at what time ESBL-producing organisms should be tested and data should be obtained. Only the ceftazidime screening test was used to determine ESBL production in some hospitals while others did use both ceftazidime and cefotaxime screening test. The data sent from participating hospitals to NARST did not give information regarding both ESBL-producing and non-producing strains of *E. coli* and *K. pneumoniae*. The prevalence of ESBL-producing organisms may not be accurately determined. ESBL-producing *E. coli* has increasingly been isolated from many sites including blood, urine and sputum. The prevalence of ESBL-producing *K. pneumoniae* remains constantly high.

It is important for clinical laboratories to detect ESBL-producing strains because they may also resist to other classes of antibiotics[9-12] including aminoglycosides, trimethoprim-sulfamethoxazole, fluoroquinolones, cephalosporins, beta-lactam/beta-lactamase inhibitors except carbapenems.

Given the findings of the present study, continuous surveillance should be performed to evaluate the prevalence and antimicrobial susceptibility of ESBL-producing organisms overtime. The improvement in data collection such as space in data sheet for filling data of ESBL-producing and non-producing organisms in the WHONET program, increased screening disk both ceftazidime and

cefotaxime that appropriated in use and close monitoring of quality of participating hospitals should be enhanced.

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การศึกษาความชุกของเชื้อ E. coli และเชื้อ K. pneumoniae ซึ่งผลิตเอนไซม์เบต้าแลคตาเมสในประเทศไทยในปี พ.ศ. 2543-2548

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วัตถุประสงค์: *เพื่อศึกษาความชุกของเชื้อ E. coli และเชื้อ K. pneumoniae ซึ่งผลิตเอนไซม์เบต้าแลคตาเมสในประเทศไทยปี พ.ศ. 2543-2548*

วัสดุและวิธีการ: *รวบรวมข้อมูลของ 28 โรงพยาบาลซึ่งอยู่ในเครือข่ายการสำรวจข้อมูลแบคทีเรียดื้อยาของกรมวิทยาศาสตร์ กระทรวงสาธารณสุข ทำการบันทึกด้วยโปรแกรมคอมพิวเตอร์ WHONET นำมาวิเคราะห์หาความชุกของเชื้อ E. coli และเชื้อ K. pneumoniae ซึ่งผลิตเอนไซม์เบต้าแลคตาเมสในประเทศไทยปี พศ. 2543-2548 การทดสอบใช้วิธี disk diffusion method (Kirby Bauer)*

ผลการศึกษา: *พ.ศ. 2543-2548 เชื้อ E. coli ซึ่งผลิตเอ็นซัยม์เบต้าแลคตาเมส ตรวจโดยยา ceftazidime พบความชุกร้อยละ 17 21.3 23.2 20.4 23.1 และ 25.0 ตามลำดับ ตรวจโดยยา cefotaxime พบความชุก ร้อยละ 20.8 65.9 69.3 69.3 68.3 และ 33.8 ตามลำดับ เชื้อ K. pneumoniae ซึ่งผลิตเอนไซม์เบต้าแลคตาเมส ตรวจโดยยา ceftazidime พบความชุกร้อยละ 30.9 34.7 32.5 34.4 37.2 และ 39.2 ตามลำดับ ตรวจโดยยา cefotaxime พบความชุก ร้อยละ 38.4 39.3 40.1 41.0 42.8 และ 40.4 ตามลำดับ*

สรุป: *ระหว่างปี พ.ศ. 2543-2548 ความชุกของเชื้อ E. coli และเชื้อ K. pneumoniae ซึ่งผลิตเอนไซม์เบต้าแลคตาเมสในประเทศไทย อยู่ในระดับสูง พบเชื้อ E. coli ซึ่งผลิตเอนไซม์เบต้าแลคตาเมส สูงที่สุดและเพิ่มขึ้นในเสมหะ รองลงมาพบในเลือด และในปัสสาวะ ส่วนเชื้อ K. pneumoniae ซึ่งผลิตเอนไซม์เบต้าแลคตาเมส พบได้สูง แต่ไม่เพิ่มขึ้นในเสมหะ ในเลือด และในปัสสาวะ*