Occurrence of Extended-spectrum β-lactamase in Clinical isolates of *Klebsiella pneumoniae* in a University Hospital, Thailand

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The occurrence and antimicrobial susceptibility of extended-spectrum β-lactamase (ESBL)-producing Klebsiella pneumoniae in patients attending Siriraj Hospital in Bangkok from August 2000 to January 2001 were determined. ESBL-producing isolates were screened with four different methods: disk diffusion according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines, Etest ESBL (CT/CTL and TZ/TZL), Oxoid combination discs and MIC Etest strip. Antimicrobial susceptibility testing were determined by a microdilution automatic method (VITEX system, bioMerieux). Of 22,178 clinical specimens, 400 (1.8%) K. pneumoniae were isolated. Of 26% (104/400) of these isolates were suspected to be ESBL-producing. Rates of detection of ESBL-producing K. pneumoniae were 18.67%, 30% and 23.78% for blood, sputum and urine samples, respectively. Susceptibility testing has revealed that all 70 tested isolates including 53 isolates from blood and sputum and 17 isolates from urine samples were susceptible to imipenem (MIC d" 4 mg/L). None of the tested isolates were susceptible to cephalosporins, cephamycin and aztreonam. Rate of susceptibility to ciprofloxacin, levofloxacin, gentamicin and tobramycin were 60%, 64%, 28% and 9%, respectively, for isolates from blood and sputum; 71%, 71%, 18% and 6% for urinary isolates. The present findings revealed a high occurrence rate of multi-drug resistance ESBL-producing K. pneumoniae in patients attending the university hospital. Imipenem was highly active against ESBL-producing K. pneumoniae.

Keywords : *Klebsiella pneumoniae, Extended-spectrum β-lactamase (ESBL), MIC*

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Extended-spectrum β-lactamases (ESBLs) are plasmid-mediated β-lactamases which have the ability to hydrolyze β-lactam antibiotics containing an oxyimino group (e.g. ceftazidime, ceftriaxone, cefotaxime or aztreonam). They are most commonly present in *Klebsiella pneumoniae*. The vast majority of ESBLs are derivatives of TEM-1 or SHV-1. Both can activate ampicillin but not the third-generation cephalosporins; mutation of the gene encoding TEM-1 or SHV-1 extends the spectrum of activity of the β-lactamases so that inactivation of third-generation cephalosporins and aztreonam occurs[1]. Since the first discovery of ESBLs in Germany[2], ESBL-producing *K. pneumoniae* has been reported in many countries[3]. Risk factors for infection by ESBL-producing organisms included long hospital stay, ventilatory care or catheterization in ICU patients and exposure to antibiotics especially extended-spectrum cephalosporins[4,5]. Serious infections with ESBL-producing isolates are usually hospital-acquired, and could not be treated with cephalosporins which are the antibiotics of choice for many serious infections. The detection in clinical microbiology laboratory of ESBL production by *K. pneumoniae* is, therefore, of great importance. How-

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ever, lack of clear information on ESBL-producing *K. pneumoniae* infections was evident from the outcomes of the national drug resistance surveillance in Thailand during 1998-2001 (National Antimicrobial Resistance Surveillance Center, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand, personal communication). Thirty of 33 hospital laboratories taking part in this program did not carry a routine screening test for ESBL-producing organisms. The insufficiently monitored movement of ESBL-producing *K. pneumoniae* infected or colonized patients within a hospital may cause a very wide spread of particular ESBL phenotypes and their producer strains. The authors carried out a prospective study to assess the occurrence and the antibiotic susceptibility of extended-spectrum β-lactamase producing *K. pneumoniae* in one university hospital in Thailand.

Material and Method

Specimens

From August 2000 to January 2001, 22,178 clinical specimens were collected from patients attending Siriraj Hospital, Mahidol University, in Bangkok for bacterial culture. These included 4,757 sputum, 8,346 urine and 9,075 blood specimens. All clinical isolates of *K. pneumoniae* were identified by conventional biochemical tests[6]. Only one isolate of *K. pneumoniae* per patient was collected, to avoid repetition of isolates.

Detection of ESBLs

Four screening methods for detection of ESBL were used in the present study. ESBL production by *K. pneumoniae* was initially screened by using disk diffusion of cefotaxime, ceftazidime and ceftriaxone according to the National Committee for Clinical Laboratory Standards (NCCLS) recommendations[7]. The isolates which showed inhibition zone ≥27 mm for cefotaxime and ≥22 mm for ceftazidime and ≥ 25 mm for ceftriaxone were suspected of producing ESBL enzyme. The suspected isolates were further determined by Etest ESBL of cefotaxime/cefotaxime+clavulanic acid and ceftazidime/ceftazidime+clavulanic acid (CT/CTL and TZ/TZL, AB Biodisk, Solna, Sweden). The three combination discs (Oxoid Ltd., Basingstoke, UK) with and without clavulanic acid of cefpodoxime (CD01/CPD10), ceftazidime (CD02/CAZ30) and cefotaxime (CD03/CTX30) were also used to determine ESBLs in suspected *K. pneumoniae* isolates. A positive result was indicated by a zone size difference of ≤ 5 mm between the combination disc and the corresponding standard antibiotic disc. Minimal inhibitory concentrations (MICs) of cefotaxime, ceftazidime and ceftriaxone were tested against all isolates of *K. pneumoniae* with positive results by those three methods[8].

Susceptibility tests

In vitro antibiotic susceptibility of ESBL-producing *K. pneumoniae* isolates was determined by a microdilution automatic method (VITEX system, bioMerieux). The gram-negative susceptibility card of the Vitex GNS-120 was used for testing isolates from blood and sputum. This card contained 14 antibiotics: ampicillin, aztreonam, cefazolin, cefepime, cefotetan, ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, imipenem, levofloxacin, piperacillin/tazobactam, tobramycin and trimethoprim/sulfamethoxazole. The Vitex GNS-121 was used for testing isolates from urine. Fifteen antibiotics were included in the GNS-121 card, these were amikacin, amoxicillin/clavulanic acid, ampicillin, cefazolin, cefotetan, ceftazidime, ceftriaxone, ciprofloxacin, gantamicin, imipenem, levofloxacin, nitrofurantoin, piperacillin/tazobactam, tobramycin and trimethoprim/sulfamethoxazole. By using this Vitex system, ESBL could also be detected.

Quality control

Standard strains of *K. pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 35218 were used as internal controls in each susceptibility determination.

Results

All the 400 isolates of *K. pneumoniae* recovered from sputa, urines and bloods at Siriraj Hospital, Thailand from August 2000 to January 2002 were studied. On initial screening by NCCLS-recommended disk diffusion method, suspected ESBL-producing *K. pneumoniae* were observed for 104 (26%) isolates with the inhibition zone ≥27 mm for cefotaxime, ≥22 mm for ceftazidime and ≥ 25 mm for ceftriaxone. Of the 104 isolates, 100 (96.15 %) were positive by the combination discs while 97 (93.27%) were positive by the Etest ESBL method (Table 1). All suspected ESBL-producing isolates showed the MICs of cefotaxime, ceftazidime and ceftriaxone of ≤2 mg/L except 2 isolates showed MICs of cefotaxime at 1.5 mg/L (Table 2). The authors, therefore, classified all 104 suspected isolates as ESBL-producing *K. pneumoniae*.

The *in vitro* susceptibility of 53 ESBL-producing *K. pneumoniae* isolated from blood and sputum against 14 antibiotics is shown in Table 3. All

Table 1. Percentage of ESBL-producing *K. pneumoniae* detected by 3 different methods

Source (no. of specimen)	No. of positive *K. pneumoniae* (%)	No. of ESBL-producing *K. pneumoniae* (%)		
		Screening test	Combination discs	Etest ESBL
Blood (9,075)	75 (0.83)	14 (18.67)	14 (18.67)	14 (18.67)
Sputum (4,757)	140 (2.94)	42 (30.00)	42 (30.00)	42 (30.00)
Urine (8,346)	185 (2.21)	48 (25.95)	44 (23.78)[a]	41 (22.16)[b]
Total (22,178)	400 (1.80)	104 (26.00)	100 (25.00)	97 (24.25)

[a] Two isolates gave negative results to 2 combination discs (CD01/CPD10 and CD02/CAZ30) and another 2 isolates gave negative results to all 3 combination discs (CD01/CPD10, CD02/CAZ30 and CD03/CTX30)

[b] MIC values of 7 isolates of *K. pneumoniae* were above the test ranges; interpretation could not be made

Table 2. MICs of the third-generation cephalosporins against suspected ESBL-producing *K. pneumoniae*

Source (no. of isolates)	MICs 2 mg/L, determined by Etest (%)		
	cefotaxime	ceftazidime	ceftriaxone
Blood (14)	14 (100)	14 (100)	14 (100)
Sputum (42)	42 (100)	42 (100)	42 (100)
Urine (48)	46 (95.83) [a]	48 (100)	48 (100)
Total (104) [b]	102 (98.08)	104 (100)	104 (100)

[a] Two isolates showed MICs 1.5 mg/L

[b] All isolates were suspected of ESBL-producing *K. pneumoniae* detected by screening test

Table 3. Susceptibility of 53 ESBL-producing *K. pneumoniae* isolated from blood and sputum against 14 antibiotics

Antibiotic	% isolates		
	Susceptible	Intermediate	Resistant
Ampicillin	0	0	100
Aztreonam	0	0	100
Cefazolin	0	0	100
Cefepime	0	0	100
Cefotetan	0	0	100
Ceftazidime	0	0	100
Ceftriaxone	0	0	100
Ciprofloxacin	60.38	7.55	32.08
Gentamicin	28.30	33.96	37.74
Imipenem	100	0	0
Levofloxacin	64.15	3.77	32.08
Piperacillin/ tazobactam	0	66.04	33.96
Tobramycin	9.43	9.43	81.13
Trimethoprim/ sulfamethoxazole	50.94	0	49.06

ESBL-producing *K. pneumoniae* isolated from blood and sputum were resistant, according to NCCLS criteria, to ampicillin, aztreonam, cefazolin, cefepime, cefotetan, ceftazidime, and ceftriaxone but susceptible to ciprofloxacin (60.38%), gentamicin (28.30%), imipenem (100%), levofloxacin (64.15%), tobramycin (9.43%) and trimethoprim/sulfamethoxazole (50.94%). However, a resistance to piperacillin/tazobactam was seen in 33.96%. All ESBL-producing isolates from urine were resistant to ampicillin and cephalosporins but susceptible to amikacin (76.47%), ciprofloxacin (70.59%), gantamicin (17.65%), imipenem (100%), levofloxacin (70.59%), nitrofurantoin (29.41%), tobramycin (5.88%) and trimethoprim/sulfamethoxazole (35.29%). While a resistance to amoxicillin/clavulanic acid and piperacillin/tazobactam was observed in 47.06% and 35.29%, respectively (Table 4).

Imipenem showed the greatest activity of all of the antibiotics tested against all isolates of ESBL-producing *K. pneumoniae* with MIC 4 mg/L. MICs for ciprofloxacin and levofloxacin were ranging from 0.5 to 4 mg/L and 1 to 8 mg/L, respectively, while those of

Table 4. Susceptibility of 17 ESBL-producing *K. pneumoniae* isolated from urine against 15 antibiotics

Antibiotic	% isolates		
	Susceptible	Intermediate	Resistant
Amikacin	76.47	17.65	5.88
Amoxicillin/ Clavulanic acid	0	52.94	47.06
Ampicillin	0	0	100
Cefazolin	0	0	100
Cefotetan	0	0	100
Ceftazidime	0	0	100
Ceftriaxone	0	0	100
Ciprofloxacin	70.59	0	29.41
Gentamicin	17.65	5.88	76.47
Imipenem	100	0	0
Levofloxacin	70.59	0	29.41
Nitrofurantoin	29.41	29.41	41.18
Piperacillin/ Tazobactam	0	64.71	35.29
Tobramycin	5.88	11.76	82.35
Trimethoprim/ Sulfamethoxazole	35.29	0	64.71

gentamicin and tobramycin were ranging from 0.5 to 16 mg/L.

Discussion

Infection caused by ESBL-producing organisms has increased worldwide in recent years. The occurrence rate varies from country to country and from hospital to hospital. From the SENTRY Antimicrobial Surveillance Program in 1997-99 from all over the world, showed that ESBL-producing *K. pneumoniae* may account for about 45% in Latin America, 25% in the Western Pacific, 23% in Europe and 8% in USA[9]. In 1999, there was a survey involving 14 hospitals from 11 regions of France, ESBLs were detected in 9.5% of *K. pneumoniae* isolates. An ESBL survey was performed in 28 hospitals in South Korea in 1999 and revealed a frequency of 18.1% ESBL-producing among *K. pneumoniae* isolates[4]. Comparable to studies in other parts of the world, the present result showed a high frequency of ESBL producing in clinical isolates of *K. pneumoniae* with 26 % of occurrence rate. Factors involving such high ESBL-producing *K. pneumoniae* infection rates may be similar to other teaching hospitals and tertiary care hospitals which included severely ill patients, a large number of ICU patients and widely-used antibiotics especially cephalosporins.

The NCCLS recommends that, for all ESBL-producing strains, the test result should be reported as resistant for all penicillins, cephalosporins, and aztreonam[7]. As expected, the present results showed that all cephalosporins (e.g. cefazolin, cefepime, ceftazidime and ceftriaxone), cephamycin (cefotetan), ampicillin and aztreonam were not active (100% resistant) against all tested ESBL- producing *K. pneumoniae*. Those isolates might be producing various beta-lactamases other than ESBLs such as AmpC or OXA-type enzymes which are hardly blocked by clavulanic acid or tazobactam. Further study on the characteristics of ESBL produced by the isolates obtained from the present study should be performed before any conclusions about the types of enzymes can be made.

In view of the present *in vitro* data and the failure of cephalosporin therapy in serious infections due to ESBL-producing *K. pneumoniae* in recent studies[4,10], clinical microbiology laboratories should test all clinically significant isolates of *K. pneumoniae* against bata-lactam drugs. Isolates that demonstrate reduced susceptiblility or resistance to three of cefotaxime, ceftazidime, ceftriaxone, cefuroxime, cefpodoxime, cefotetan or aztreonam are considered as potential producers of ESBLs. In addition, selective testing for ESBL production should be considered for *K. pneumoniae* or other gram-negative enteric bacilli isolated from normally sterile body sites. Avoidance of using extended-spectrum cephalosporins in serious infections in big university hospitals or tertiary referral hospitals may decrease the rates of emergence of ESBL-producing organisms.

In conclusion, a high proportion of the clinical isolates of *K. pneumoniae* were ESBL- producing strains. Imipenem was the most active drug against ESBL-producing *K. pneumoniae*. In contrast, reduced activity was observed with fluoroquinolones and aminoglycosides. More prudent use of antibiotics and control of the spread of these resistant *K. pneumoniae* are necessary in countries where antibiotics are easily available at drug stores without prescription. Further identification of ESBL-producing strains requires molecular techniques for specific control interventions.

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อัตราการตรวจพบเชื้อ Klebsiella pneumoniae ที่สร้าง extended-spectrum b-lactamase ในตัวอย่างผู้ป่วยโรงพยาบาลหนึ่งแห่งของมหาวิทยาลัยในประเทศไทย

มยุรา กุสุมภ์, ศิริพรรณ วงศ์วานิช, เชิดศักดิ์ ธีระบุตร, พิณทิพย์ พงษ์เพ็ชร, เพ็ญพรรณ แน่นหนา

ได้ตรวจหาเชื้อ Klebsiella pneumoniae ที่สร้าง extended-spectrum β-lactamase (ESBL) จากตัวอย่างผู้ป่วยในโรงพยาบาลศิริราช กรุงเทพมหานคร ระหว่างเดือนสิงหาคม 2540 ถึงเดือนมกราคม 2541 โดยใช้วิธีทดสอบคัดกรอง 4 วิธีได้แก่ disk diffusion ของ NCCLS guidelines, Etest ESBL (CT/CTL and TZ/TZL), Oxoid combination discs และ MIC Etest strip สำหรับการทดสอบหาความเข้มข้นต่ำสุดของยาที่ยับยั้งการเจริญของเชื้อ (MIC) ใช้ microdilution automatic method (VITEX system, bioMerieux) จากการศึกษาในตัวอย่างผู้ป่วย 22,178 ตัวอย่าง พบเชื้อ K. pneumoniae 400 ตัวอย่าง (ร้อยละ 1.8) ในจำนวนนี้เป็นสายพันธุ์ที่แสดงว่าน่าจะสร้าง ESBL ร้อยละ 26 (104/400) โดยพบเป็น ESBL K. pneumoniaeในตัวอย่างเลือด เสมหะ และปัสสาวะของผู้ป่วยในอัตราร้อยละ 18.67, 30 และ 23.78 ตามลำดับ จากการนำเชื้อรวมจำนวน 70 สายพันธุ์ที่แยกได้จากเลือดและเสมหะรวม 53 สายพันธุ์ และจากปัสสาวะ 17 สายพันธุ์ มาทดสอบหาค่า MIC พบว่าเชื้อที่ทดสอบทั้งหมดไวต่อยา imipenem (MIC 4 mg/L) แต่ไม่พบสายพันธุ์ที่ไวต่อยาในกลุ่ม cephalosporins, cephamycin, cefomycin และ aztreonam อัตราความไวของเชื้อต่อยา ciprofloxacin, levofloxacin, gentamicin และ tobramycin ของเชื้อที่แยกได้จากเลือดและเสมหะคิดเป็นร้อยละ 60, 64, 28 และ 9 ตามลำดับ ส่วนของเชื้อที่แยกได้จากปัสสาวะคิดเป็นร้อยละ 71, 71, 18 และ 6 ตามลำดับ การศึกษานี้แสดงอัตราการตรวจพบเชื้อ K. pneumoniae สายพันธุ์ที่ดื้อยาหลาย ๆ ชนิด ที่สร้าง ESBL ในกลุ่มผู้ป่วยที่มารักษาที่โรงพยาบาลของมหาวิทยาลัย อย่างไรก็ตามยา imipenem ยังแสดงคุณสมบัติที่ยับยั้งเชื้อ ESBL K. pneumoniae ได้ดีมาก