Molecular Characterization of Extended Spectrum Beta-Lactamase among Clinical Isolates *Escherichia coli* and *Klebsiella pneumoniae*

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Background: *Resistance to β-lactams has been increasing in the treatment of infections caused by Escherichia coli and Klebsiella pneumoniae. The production of extended-spectrum β-lactamases (ESBLs), that hydrolyze extended- spectrum cephalosporins, is the major cause of β-lactam resistance.*

Objective: *To determine the prevalence and characterize of ESBLs produced by E. coli and K. pneumoniae from clinical specimens.*

Material and Method: *ESBLs were determined by disk diffusion test, double disk synergy test, and E-test ESBLs. All ESBLs producing isolates were investigated for the presence of bla_{TEM}, $bla_{SHV,}$ bla_{CTX-M}, and bla_{VEB} genes by polymerase chain reaction (PCR). Nucleotide sequencing of bla_{TEM} and bla_{SHV} were performed. E. coli and K. pneumoniae were isolated from clinical specimens of patients in King Chulalongkorn Memorial Hospital between February and May 2002. Of the 270 isolates, 212 were E. coli and 58 were K. pneumoniae.*

Results: *ESBL production was detected in 17% (36/212) of E. coli and 34.5% (20/58) of K. pneumoniae isolates. Of the 20 K. pneumoniae isolates, the β-lactamase genes were bla_{SHV} (18/20, 90%), bla_{TEM} (10/20, 50%), $bla_{VEB-like}$ (6/20, 30%) and $bla_{CTX-M-like}$ (3/20, 15%). Thirty-six E. coli isolates carried bla_{TEM}, $bla_{CTX-M-like}$ and $bla_{VEB-like}$ genes in 72.2% (26/36), 52.8% (19/36) and 16.7% (6/36), respectively. Bla_{SHV} was not detected in ESBL-producing E. coli, whereas it predominated in K. pneumoniae. Of the 56 ESBL producing isolates, 30 (53.6%) coharboured at least two different bla genes. All TEM identified were TEM-1B, which is not an ESBL. CTX-M ESBLs were the most common in E. coli.*

Conclusion: *The double disk diffusion test should be added routinely in the antibiotic susceptibility test for the Enterobacteriaceae. It is simple to perform, easy to interpret, and economical. The presence of bla_{CTX-M} and bla_{VEB} in ESBL-producing E. coli and K. pneumoniae indicates the high prevalence of these genes in Thailand.*

Keywords: *Extended-spectrum beta-lactamases, ESBLs, Disk diffusion test, Double disk synergy test, E-test, Polymerase chain reaction, Polymerase chain reaction, Nucleotide sequencing*

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Extended-spectrum β-lactamases (ESBLs) are enzymes that confer resistance to oxyimino-cephalosporins, such as cefotaxime, ceftazidime and ceftriaxone and to monobactams, such as aztreonam, resulting in resistance to these drugs. ESBLs are predominantly derivatives of plasmid-mediated TEM or SHV β-lactamases, arise through a mutation or mutations that result in one or more amino acid substitutions. These mutations alter the configuration or binding properties of the active site, thereby expanding the hydrolytic spectrum of the enzyme. Though these enzymes, especially TEM and SHV-derived ESBLs, are most commonly detected in *Klebsiella pneumoniae* and *Escherichia coli* and have been reported worldwide[1].

Detection of ESBL producing organisms remains a challenge for the microbiology laboratory. The problem is that routine methods for monitoring a decrease in susceptibility to oxyimino-cephalosporins and aztreonam have not been sensitive enough to detect ESBL-producing strains. Minimum inhibitory concentrations (MIC) may be raised only slightly and fail to reach the level of accepting breakpoint resistance.

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The current National Committee for Clinical Laboratory Standards[2] recommendations for detection of ESBLs in *K. pneumoniae* and *E. coli* include an initial screening test with following β-lactam antibiotics, ceftazidime, aztreonam, cefotaxime, ceftriaxone, or cefpodoxime. A second test has been recommended for the detection of ESBL activity. The double-disk synergy test is most widely used due to its simplicity and ease of interpretation. The E-test ESBL screening test is another useful test for detection of ESBL activity by measuring the MIC scale.

The prevalence of ESBL production varies greatly from country to country and institution to institution. In the present study, the authors determined the molecular epidemiology of genes associated with TEM-type and SHV-type β-lactamases and to detect the possible existence of new TEM and SHV enzymes, which were produced by *K. pneumoniae* and *E. coli* in King Chulalongkorn Memorial Hospital.

The authors also determined the prevalence of strains producing ESBLs among *K. pneumoniae*. and *E. coli*.

Material and Method

Bacterial strains

Non-duplicate isolates of *E. coli* and *K. pneumoniae* isolated consecutively from clinical specimens were collected at Department of Microbiology in King Chulalongkorn Memorial Hospital between February and May 2002. Two hundred seventy isolates (212 *E. coli*, 58 *K. pneumoniae*) were obtained. Most of them (246 isolates, 91.1%) were isolated from urine. Among the 212 isolates of *E. coli*, 197 (92.9%) isolates were isolated from urine, 11 (5.2%) isolates from body fluid and four (1.9%) isolates from sputum. While the 58 *K. pneumoniae* were isolated from various specimens, 49 (84.8%) isolates from urine, five (8.6%) isolates from sputum, three (5.2%) isolates from pus and one (1.7%) isolate from body fluid. Each isolate was identified according to conventional procedures.

Reference strains of *E. coli* ATCC 25922, *E. coli* ATCC 35218 and *K. pneumoniae* ATCC 700603 were used for quality control in the disk diffusion test, double disk synergy test and E-test. Reference strains used as positive control strains in PCR amplification step were *E. coli* C600 (Pcff04) carrying $bla_{\text{TEM-3}}$, *E. coli* C600 (pUD) carrying $bla_{\text{TEM-4}}$, *K. pneumoniae* ATCC 700603[3] carrying $bla_{\text{SHV-12}}$, *K. pneumoniae* KU204-0345 carrying $bla_{\text{VEB-1-like}}$ and *E. coli* EU269-0445 carrying $bla_{\text{CTX-M-15}}$ were obtained for the present study.

Antimicroicrobial agents

Antimicrobial susceptibility disks, ceftazidime (CAZ 30 μg), ceftriaxone (CRO 30 μg), cefotaxime (CTX 30 μg) and aztreonam (ATM 30 μg), amoxicillin/clavulanate (AMC 20 μg/10 μg), were obtained commercially (BBL, Becton Dickinson and Company, Cockeysville, MD, USA). E-test ESBL strips (AB Biodisk) are double-ended strips with antibiotic and antibiotic/inhibitor gradients. These strips yield the MIC as well as the MIC ratio, which determines the presence of an ESBL. The strips used in the present study were ceftazidime + clavulanic acid (TZ/TZL) and cefotaxime + clavulanic acid (CT/CTL), for which the recommended ratio value indicates the presence of an ESBL.

Detection of ESBLs

Disk diffusion test

The test was performed according to the NCCLS[2]. Reduced zones of inhibition around third-generation β-lactam disks were interpreted as positive results. The β-lactams and breakpoints used were ceftazidime, ≤ 22 mm, cefotaxime, ≤ 27 mm, ceftriaxone, ≤ 25 mm and aztreonam, ≤ 27 mm. Each isolate was tested on three separate occasions.

Double disk synergy test

The test was performed as described by Jarlier et al[4]. Briefly, Ceftazidime (30 μg), cefotaxime (30 μg), ceftriaxone (30 μg) and aztreonam (30 μg) disks were placed 25 mm (center to center) away from a 20 μg amoxicillin/10 μg clavulanic acid disk before incubation. Enhancement of the inhibition zone of any of the antibiotics towards the disk containing clavulanic acid was interpreted as positive for ESBL production.

E-test

The test was performed according to the guidelines from the manufacturer. A greater than eight-fold reduction in the ratio of MIC with and without clavulanate was taken as positive for ESBL production.

PCR amplification

The primers TEM-A, TEM-B, SHV-A and SHV-B were performed as described by Yan et al[5]. Primer specific for the $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-3}}$ genes, CTX-A and CTX-B were described by Bonnet et al[6]. The synthesis of primer VEB-A and VEB-B were performed with the program Primer 3, which available

at the website http://wwwgenome.wi.mit.edu/cgibin/primer/primer 3_www.cgi/egi[7] (bla_{VEB-1} numbering from accession number AF010416), which corresponded to the conserved regions of VEB ESBL gene as demonstrated in Table 1.

Bacterial DNA was prepared by suspending one or two fresh colonies in 50 µl of sterile distilled water and heating at 95°C for 5 minutes. Supernatant was used as PCR template as described by Chanawong et al[8]. PCR amplifications of the bla_{SHV} was done under the following conditions, 95°C for three minutes followed by 30 cycles of 95°C for 30 seconds (denaturation), 65°C for one minute (annealing), 65°C for one minute (extension), with final extension at 72°C for one minute 30 seconds. PCR amplifications of the bla_{TEM}, $bla_{CTX-M-1}$, $bla_{CTX-M-3}$ and bla_{VEB} were carried out under the following conditions, 95°C for three minutes followed by 35 cycles of 95°C for 30 seconds, 56°C for 30 seconds and 72°C for one minute 30 seconds and finally 72°C for one minute 30 seconds. Amplified products were separated by electrophoresis in a 1% agarose gel and visualized by UV light trans-illumination following ethidium bromide staining.

DNA Sequencing

The PCR products of 962 bp bla_{TEM} and 1,012 bp bla_{SHV} were purified by QIAquick PCR purification kit as described by the manufacturer (QIAGEN, Max-Volmer-StraBE4, Hilden, Germany). Sequencing reactions were performed with the PCR primers TEM-A, TEM-B, SHV-A and SHV-B[5] primer TEM-C (5'CTGACAACGATCGGAGGA-3'), TEM-D (5'-AAGG CGAGTTACATGATC-3'), SHV-C (5'-GCCTTTTTG CGCCAGAT-3') and SHV-D(5'-ATTCAG TTCCGTT TCCCA-3') were synthesized from position 414-432, 486-468, 447-464 and 510-492 respectively, which corresponded to the conserved regions of bla_{TEM} and bla_{SHV} gene. PCR products were sequenced with an ABI prism 310 automated sequencer (Perkin Elmer Corporation, ABI prism, Applied Biosystem, Foster city, USA) according to the manufacturer's instructions.

The nucleotide sequence and the deduced protein sequence were analyzed with the software available over the Internet at the National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov[9]. Multiple sequence alignment and pairwise comparisons of sequences were analyzed by Chromas and ClustalW, version 1.74. All amino acid sequences were compared with the published data at website http://www.lahey.org/studies/webt.htm[1].

Results

Comparing the results of positive ESBLs isolates of *E. coli* and *K. pneumoniae* performed by disk diffusion test, double disk synergy test and E-test ESBL, strains giving positive results with at least two of the three methods were designated as ESBL producers. The results described into five categories (Table 2).

The results of the positive ESBLs producing isolates screened by three methods were shown in Table 2. Of the 270 isolates, 67 (24.8%) isolates were positive for disk diffusion test. Among the 67 positive isolates of initial screening 49 (18.2%) isolates were positive by double disk synergy test and 53 (19.6%) isolates were confirmed to be ESBL producer by E-test ESBL. Of the 212 clinical *E. coli* isolates, 43 (20.3%) isolates were positive for initial screening by disk diffusion. Subsequent confirmation of ESBL production among the *E. coli* isolates revealed that 31 (14.6%) isolates demonstrated a clavulanic acid effect by double disk synergy, whereas 36 (17.0%) isolates were identified as ESBL producer by E-test ESBL. Of the 58 clinical *K. pneumoniae* isolates, 24 (41.2%) isolates were positive for initial screening by disk diffusion. Subsequent confirmation of ESBL

Table 1. Primer sets used in characterizing beta-lactamases

Gene sought	Primer	Primer sequence	Location	Product size (bp)
bla_{TEM}	TEM-A	5'-CCCCTATTTGTTTATTTTTC-3'	112-130	962
	TEM-B	5'-GACAGTTACCAATGCTTAATCA-3'	1,074-1,053	
bla_{SHV}	SHV-A	5'-GCCGGGTTATTCTTATTTGTCGC-3'	55-77	1,012
	SHV-B	5'-TCTTTCCGATGCCGCCGCCAGTCA-3'	1,067-1,044	
bla_{VEB}	VEB-A	5'-CCTTTTGCCTAAAACGTGGA-3'	460-479	216
	VEB-B	5'-TGCATTTGTTCTTCGTTTGC-3'	675-656	
bla_{CTX}	CTXM-A	5'-CGCTTTGCGATGTGCAG-3'	264-280	550
	CTXM-B	5'-ACCGCGATATCGTTGGT-3'	814-798	

Table 2. ESBL- producing *E. coli* and *K. pneumoniae* according to screen test

Categories	DT	DDST	E-test	No. of *E. coli* isolates	No. of *K. pneumoniae* isolates	Total
1*	+	+	+	31	15	46
2*	+	+	ND	0	3	3
3*	+	-	+	5	2	7
4	+	-	ND	2	3	5
5	+	-	-	5	1	6
Total				43	24	67

DT = disk diffusion test; DDST = double disk diffusion test; ND = non-determinable
* Categories 1, 2, 3 indicated the isolates producing ESBLs

production among the *K. pneumoniae* isolates revealed that 18 (31.0%) strains demonstrated a clavulanic acid effect by double disk synergy whereas 17 (29.3%) isolates were identified as ESBL producer by E-test ESBL.

Among 67 isolates, which confirmed by both double disk synergy test and E-test ESBL, indicated that 56 of 270 (20.7%) isolates produced ESBL. Thirty-six of 212 (17%) isolates were *E. coli* and 20 of 58 (34.5%) isolates were *K. pneumoniae*.

PCR amplification for detection of ESBLs

PCR amplification of TEM and SHV genes were performed for all positive isolates by screening in the present study. However, many isolates showed a phenotype of ESBL production, but neither SHV nor TEM ESBLs was detected. Since CTX-M and VEB ESBLs have been reported from Asian countries, screening for the presence of these ESBLs was included in the present study.

Of 56 positive ESBLs producing isolates of *E. coli* and *K. pneumoniae*, 36 (64.3%) isolates were TEM positive, 22 (39.3%) were CTX-M positive, 18 (32.1%) isolates were SHV positive and 12 (21.4%) isolates were VEB positive. Among 36 of *E. coli* ESBLs producing isolates, 26 (72.2%) isolates produced TEM, six (16.7%) produced VEB, 19 (52.8%) produced CTX-M ESBLs, four isolates were unidentified, and none of SHV was found. In the 20 ESBL-producing *K. pneumonia*e TEM, SHV, VEB, and CTX-M ESBLs, were produced by 10 (50%) isolates, 18 (90%) isolates, six (30%) isolates, and three (15%) isolates, respectively (Fig. 1).

Among 36 of *E. coli* ESBL producing isolates, 14 (38.9%) isolates co-harboring both TEM and CTX-M ESBL were the most common. Of the 20 of *K. pneumoniae* ESBLs producing isolates, seven (35.0%) isolates harboring SHV were the most common. It was noteworthy that 30 (53.6%) out of 56 were co-harboring at least two enzymes.

Type of enzymes identification by PCR and DNA sequencing

Sequencing of bla_{TEM} and bla_{SHV} genes, using primers TEM-A, -B, -C and -D and SHV-A, -B, -C and -D, were performed in the present study. The nucleotide sequences were determined by sequencing both strands of positive isolates including reference strain of *E. coli* C600 (pCFF04) and *K. pneumoniae* ATCC 700603 by automated sequencing method. The results were described into five categories according to the screening results.

The sequencing results revealed that all 45 positive TEM-PCR isolates were identical to the wild-type TEM-1B, a narrow-spectrum β-lactamase. While sequencing of the SHV-type ESBL revealed five mutations located at positions 7, 35, 238, 240 and unknown type 254. Among 18 of 20 SHV ESBL-positive isolates, SHV-1, -2, -11, -12, -28 and the unknown type N254H were identified. Three (15%) out of 20 isolates

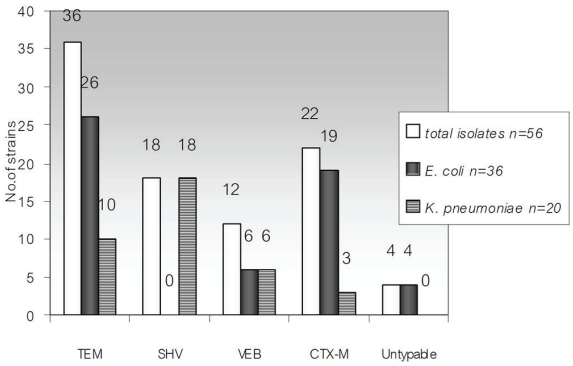

Fig. 1 Frequencies of *bla* genes in the 56 ESBL producing *E. coli* and *K. pneumoniae* isolates

produced SHV-1, 5 (25%) isolates produced SHV-11, 7 (35%) isolates produced SHV-12, 1 (5%) isolate each produced SHV-2, SHV-28 and the unknown type N254H (Table 3).

The authors found 16 positive unusual phenotype, which confer positive screening but none of ESBLs were found, nine of these were TEM-1B, one was SHV-1, two were co-harboring between TEM-1B and SHV-11, and four un-typable. Moreover, the authors also found two ESBLs producing isolates that have no synergistic effects, one produced SHV-12, CTX-M and TEM-1B and another produced TEM-1B and VEB (Table 4).

Discussion

The prevalence of ESBL production among isolates of *Enterobacteriaceae* varies greatly from country to country. In Asia the percentage of ESBL production in *E. coli* and *K. pneumoniae* varies, from 4.8% in Korea to 8.5% in Taiwan and up to 12% in Hong Kong[5,10,11]. In the present study, the authors found that 20.7% of clinical isolates were ESBLs-producing isolates a higher rate than other studies in Asia. However, 16.98% of *E. coli* produced ESBLs with similar rate to those found in the multicenter study in Thailand (15.7%),whereas the prevalence of ESBL producing *Klebsiella* spp. were 34.48%, which was lower than those found in a previous report (45.6%)[12].

In 2001, Leelarasamee et al[13] described the ESBL producing strains in nosocomial infection in Thailand to be 40% of *E. coli* and 30.2% of *K. pneumoniae*. Our study had 27.7% and 50% respectively. Most of the isolates were obtained from urine. Therefore, ESBLs producing isolates

Table 3. ESBL-types identified by PCR and sequencing in an ESBL producing isolates

ESBL-types by PCR	Genotype by DNA sequencing	No. of isolates (%)		
		Total	*E. coli*	*K. pneumoniae*
TEM	TEM-1B(9)**	9 (16.1%)	8 (22.2%)	1 (5.0%)
SHV	SHV-1(1), SHV-2(1), SHV-12 (4), Unknown type N254H(1)	7 (12.5%)	-	7 (35.0%)
VEB*		2 (3.6%)	2 (5.6%)	-
CTX-M*		4 (7.1%)	4 (11.0%)	-
SHV, VEB*	SHV-1(1), SHV-12(1), SHV-28(1)	3 (5.6%)	-	3 (15.0%)
TEM, SHV	SHV-11(2), SHV-12(1)	3 (5.6%)	-	3 (15.0%)
TEM, VEB*	TEM-1B(4)	4 (7.1%)	3 (8.3%)	1 (5.0%)
TEM, CTX-M*	TEM-1B(14)	14 (25.0%)	14 (38.9%)	-
TEM, SHV, VEB*	TEM-1B(2), SHV-1(1), SHV-12(1)	2 (3.6%)	-	2 (10.0%)
TEM, SHV, CTX-M*	TEM-1B(3), SHV-11(3)	3 (3.6%)	-	3 (15.0%)
TEM, VEB*, CTX-M*	TEM-1B(1)	1 (1.8%)	1 (2.8%)	-
Non-TEM,SHV, VEB, CTX-M		4 (7.1%)	4 (11.0%)	-
Total		56	36	20

* Not performed by sequencing, ** ( ) = number of isolates

Table 4. ESBL-types identified by PCR and sequencing in non ESBL producing isolates

ESBL-types by PCR	Genotype by DNA sequencing	No. of isolates		
		Total	*E. coli*	*K. pneumoniae*
TEM-B	TEM-1B(5)**	5	5	-
TEM-1B, SHV	TEM-1B(2), SHV-11(2)	2	-	2
TEM-1B, VEB*	TEM-1B(1)	1	-	1
TEM-1B, SHV, CTX-M*	TEM-1B(1), SHV-12(1)	1	-	1
Non-TEM, -SHV, -VEB, -CTX-M	-	2	2	-
Total	-	11	7	4

* Not performed by sequencing, ** ( ) = number of isolates

distribution in other clinical specimens cannot be evaluated.

The present study focused on the distribution of SHV and TEM ESBLs. They are distributed worldwide and limited data concerning these enzymes has been published in Thailand. However, many isolates with a phenotype of ESBL production had neither SHV nor TEM ESBLs. Therefore CTX-M and VEB ESBLs have been further included since there were reports on the detection of these ESBL enzymes in Asia.

CTX-M ESBL, which preferentially hydrolyzes cefotaxime, has mainly been found in isolates of *Salmonella enterica* serovar Typhimurium and *E. coli*. They have been identified in numerous countries of Africa, South America, Asia, and Europe during the last decade[14]. Several institutions in the areas with the outbreaks of nosocomial infection reported that the CTX-M ESBLs were the most frequently isolated ESBLs among clinical isolates[14,15]. The authors found that CTX-M ESBLs were the most common ESBL (52.8%) detected in ESBLs producing *E. coli*, but no significant information concerning these enzymes has been reported in Thailand.

SHV-1 is an endogenously chromosomal gene in *K. pneumoniae*[16,17]. Similar to the present study, SHV ESBLs were the most common ESBL in *K. pneumoniae* (90%). The authors found that in 18 SHV producing *K. pneumoniae* SHV-12 was the most common (35%). SHV-12 have been reported in Thailand[8] and in China, Korea, Japan and Taiwan[5,18-20]. SHV-12 b-lactamase is the most common ESBL, emerging apparently as the best-adapted ESBL for the prevailing antibiotic selection pressure in the Far East Asia. The authors' findings augment others concerning the occurrence of SHV ESBLs in the former report in the Far East Asia.

The authors found VEB ESBLs at 16.7% and 30% of *E. coli* and *K. pneumoniae,* respectively. VEB-1 was first found in a single isolate of *E. coli* in a patient from Vietnam, but was subsequently found in a *P. aeruginosa* isolate from a patient from Thailand[19]. In 2001, Girlich et al [20] reported that VEB ESBLs seemed to be highly prevalent in Thai isolates as it accounted for 60% of the ESBL-possessing *Enterobacteriaceae* isolates in their study.

The false positive of screening tests can occur with any possess an ESBL. Several groups have reported that the high-level expression of TEM-1 or SHV-1 plasmid-encoded β-lactamases in *E. coli* and *K. pneumoniae* were responsible for resistance to inhibitor and can cause the MIC of ceftazidime to rise to levels at which an ESBL would be suspected[22,23]. Rasheed et al[3,24] also reported that the production of SHV-1 in a strain of *K. pneumoniae* lacking an outer membrane porin protein caused a false-positive in ESBL detection tests.

Moreover, confirmation of ESBL production by clavulanic acid inhibition can be difficult in some isolates, not only the activity of the β-lactamases varies with different substrates, but also organisms may contain additional resistance mechanisms that can mask the presence of ESBL activity. Our negative ESBL strains by screening test, one produced SHV-12, CTX-M and TEM-1B and another produced TEM-1B and VEB with no synergistic effects. These could include AmpC-type enzymes, porin changes, TEM and SHV β-lactamases that are no longer inhibited by clavulanic acid due to mutations in the coding sequences[23,25].

In conclusion, it is important for clinical laboratories to detect these enzymes as effectively and rapidly as is needed. The clinicians will be able to select empiric antibiotic regimens for serious infections in patients hospitalized in Thailand and for prevention of nosocomial infection. The double disk diffusion test should be added routinely in the antibiotic susceptibility test for the *Enterobacteriaceae.* It is simple to perform, easy to interpret, and economical. On the other hand, the E-test ESBL is the method of choice. However, some positive ESBLs strains were unable to interpret the high concentration MIC and rather expensive. It was demonstrated that VEB and CTX-M -ESBL were playing role in resistant associate ESBL in *E. coli* and *K. pneumoniae* in Thailand. The unknown type SHV enzymes, which had mutation at position 254, have been detected. Further study in the characterization of this new enzyme and sequencing of bla_{VEB} and bla_{CTX-M} genes of these isolates should be performed.

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Potential conflicts of interest

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ลักษณะทางโมเลกุลของ extended spectrum beta-lactamase สร้างโดย Escherichia coli และ Klebsiella pneumoniae ที่แยกได้จากสิ่งส่งตรวจของผู้ป่วย

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ภูมิหลัง: ปัจจุบันการดื้อยาในกลุ่ม beta-lactams ที่ใช้ในการรักษาโรคติดเชื้อจาก Escherichia coli และ Klebsiella pneumoniae มีอัตราสูงขึ้นมากกลไกการดื้อยาของเชื้อเหล่านี้ส่วนใหญ่เนื่องจากเชื้อสร้างเอนไซม์ extended-spectrum beta-lactamases (ESBLs) มีฤทธิ์ทำลายยาในกลุ่ม extended-spectrum cephalosporins

วัตถุประสงค์: เพื่อหาความชุกและจำแนกชนิดของเอนไซม์ extended-spectrum beta-lactamases (ESBLs) จากเชื้อ E. coli และ K. pneumoniae ที่แยกได้จากสิ่งส่งตรวจของผู้ป่วย

วัสดุและวิธีการ: เชื้อที่ใช้ในการศึกษาครั้งนี้แยกได้จากสิ่งส่งตรวจของผู้ป่วยที่เข้ารับการรักษาในโรงพยาบาลจุฬาลงกรณ์ ระหว่างเดือนกุมภาพันธ์ ถึง เดือนพฤษภาคม พ.ศ. 2545 จำนวน 270 สายพันธุ์ เป็น E. coli 212 สายพันธุ์ และ K. pneumoniae 58 สายพันธุ์ ทำการตรวจหา ESBLs ด้วยวิธี disk diffusion test, double disk synergy test และ E-test ESBL แล้วนำสายพันธุ์ที่สร้าง ESBLs มาตรวจหายีน bla_{TEM}, bla_{SHV}, bla_{CTX-M} และ bla_{VEB} ด้วยวิธี polymerase chain reaction (PCR) และหาลำดับเบสของยีน bla_{TEM} และ bla_{SHV} โดยวิธี nucleotide sequencing

ผลการศึกษา: พบ E. coli ที่สร้าง ESBL ร้อยละ 17 (36/212) และพบ K. pneumoniae ที่สร้าง ESBL ร้อยละ 34.5 (20/58) โดย K. pneumoniae 20 สายพันธุ์พบยีน bla_{SHV} ร้อยละ 90 (18/20), bla_{TEM} ร้อยละ 50 (10/20), $bla_{VEB-like}$ ร้อยละ 30 (6/20) และ $bla_{CTX-M-like}$ ร้อยละ 15 (3/20) สำหรับ E. coli 36 สายพันธุ์พบยีน bla_{TEM} ร้อยละ 72.2 (26/36), $bla_{CTX-M-like}$ ร้อยละ 52.8 (19/36) และ $bla_{VEB-like}$ ร้อยละ 16.7 (6/36) ตรวจไม่พบ bla_{SHV} ใน E. coli ขณะที่ bla_{SHV} พบมากที่สุดใน K. pneumoniae เชื้อที่สร้าง ESBL ตรวจพบ bla จีน อย่างน้อย 2 ชนิด ในสายพันธุ์เดียวกันจำนวนร้อยละ 53.6 (30/56) bla_{TEM} ที่ตรวจพบทั้งหมดเป็น bla_{TEM-1B} ซึ่งไม่ใช่ ESBL CTX-M เป็น ESBL ที่พบบ่อยที่สุดใน E. coli

สรุป: การตรวจหาเอนไซม์ ESBLs ที่ผลิตโดยเชื้อ E. coli และ K. pneumoniae ควรทำควบคู่ไปกับการทดสอบความไวของเชื้อต่อยาต้านจุลชีพโดยเฉพาะในกลุ่ม Enterobacteriaceae ของงานประจำ โดยเฉพาะวิธี double disk synergy test เป็นวิธีที่ทำได้ง่ายรวมทั้งการแปลผลง่ายและราคาไม่แพง และในการศึกษาครั้งนี้พบว่าเชื้อ E. coli และ K. pneumoniae ที่สร้าง ESBLs ในประเทศไทยส่วนใหญ่เป็นชนิด CTX-M และ VEB