PREVALENCE OF EXTENDED-SPECTRUM BETA-LACTAMASE AND CLASS 1 INTEGRON INTEGRASE GENE *INTI1* IN *ESCHERICHIA COLI* FROM THAI PATIENTS AND HEALTHY ADULTS

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Abstract. Among 120 *Escherichia coli* isolates from Thai patients, 37 and 9 isolates were extended-spectrum beta-lactamase (ESBL) and suspected ESBL producers respectively while 5 *E. coli* isolates from 120 Thai healthy adults were suspected ESBL producers. Integrase (*intI1*) gene was detected in 99% of the clinical and 87% of the non-clinical isolates. Among 37 ESBL producers, percent recovery of bla_{TEM}, bla_{CTX-M}, bla_{SHV} and bla_{VEB} was 78%, 78%, 8% and 8%, respectively. Twenty-five isolates of ESBL producers carried both bla_{TEM} and bla_{CTX-M}, 2 isolates carried 3 genes (bla_{TEM}, bla_{CTX-M}, and bla_{SHV}) and 3 showed no detectable *bla* gene. Among the 14 suspected ESBL producers, *intI1* and bla_{TEM} were detected in 13 isolates. ESBL producers from clinical samples were resistant to most of the tested antimicrobial agents compared to non-ESBL producers and isolates from healthy adults with about half of the latter susceptible to all tested antimicrobial agents. Only one clinical isolate was resistant to imipenem. Susceptibility to trimethoprim/sulfamethoxazole among the clinical isolates in ESBL producer group (27%) and non-producer group (33%) were comparable, whereas the percent susceptibility of the non-clinical isolates was about twice that of the clinical isolates.

INTRODUCTION

Although *Escherichia coli* is normally considered as a non harmful member of the colon flora, however, it has been associated with a wide range of infections, including nosocomial. For empirical treatment of the infection, clinicians often recommend beta-lactam antimicrobial agents even in the midst of increasing beta-lactam resistance. The most common mechanism of resistance is the carriage on mobile genetic elements of resistance genes, especially ones encoding for extended-spectrum beta-lactamase (ESBL) (Sabate *et al*, 2002; Yu *et al*, 2003, 2004; Machado *et al*, 2005; Skurnik *et al*, 2005).

At present, there are many types and derivatives of ESBL including TEM, SHV, CTX-M and VEB beta-lactamases (Rasheed *et al*, 1997; Ryoo *et al*, 2005). However, there have been very few reports concerning ESBL-producing *E. coli* and prevalence of mobile genetic elements in Thailand (Girlich *et al*, 2001; Ingviya *et al*, 2003). Thus, this study of ESBL-producing *E. coli,* ESBL types, frequency of the integrase gene *intI1*, a marker of multiple antibiotic resistance class 1 integron elements, and antimicrobial susceptibility of ESBL- and

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non ESBL-producing *E. coli* was performed on *E. coli* isolated from clinical samples from Siriraj Hospital, Bangkok, Thailand and healthy adults.

MATERIALS AND METHODS

Bacterial isolates

A total of 120 *E. coli* isolates were obtained from 120 clinical specimens: 30 from blood, 30 from pus and 60 from urine, from Siriraj Hospital, Mahidol University, Bangkok, Thailand during the period of June to August 2004. One hundred and twenty isolates were obtained from stools of healthy adults. *E. coli* ATCC 25922 was used as control. All isolates were identified according to standard methods (Farmer, 2003).

Detection of ESBL-producing *E. coli*

ESBL-producing *E. coli* isolates were detected using an initial screening test and phenotypic confirmatory test (NCCLS, 2004). In brief, 5 antimicrobial disks containing cefpodoxime (10 μg), ceftazidime (30 μg), ceftriaxone (30 μg), cefotaxime (30 μg), and aztreonam (30 μg) were used for the screening test, and combination disks containing beta-lactamase inhibitor clavulanic acid and cefpodoxime plus clavulanic acid, ceftazidime and ceftazidime plus clavulanic acid, and cefotaxime and cefotaxime plus clavulanic acid were used for the confirmatory test.

Detection of *IntI1* by dot-blot hybridization

Detection of *intI1* was performed using the dot-blot hybridization method of Gene Images Random Prime Labeling Module and CDP-Star Detection Module (Amersham Biosciences). *IntI1* positive control was 471 base pairs of *intI1* inserted in the pCTF202 plasmid (Tribuddharat, 1999).

Detection of *IntI1* and beta-lactam antibiotic resistant genes by PCR

Primers specific for integrase (*intI1*), bla_{TEM}, bla_{SHV}, bla_{VEB} , and bla_{CTX-M} genes used in this study are shown in Table 1. The PCR reaction was performed by denaturing the genomic templates at 94ºC for 5 minutes, followed by 30 cycles of denaturing, annealing, and extension at 94ºC, 1 minute, 55ºC, 1 minute, and 72ºC, 1 minute, respectively.

Antimicrobial susceptibility test

Antimicrobial susceptibility tests of all isolates included *E. coli* ATCC 25922 against 10 antimicrobial agents that are commonly used in the treatment of *E. coli* infections were performed according to the disk diffusion method (NCCLS, 2004). These antimicrobial agents

Table 1
Sequence of primer and size of PCR product.

Specific for	Primer name	Primer sequence	Product size(bp)	Reference
intI1	INT1F	5′-AAGGATCGGGCCTTGATGTT-3′	471	This study
	INT1R	5′-CAGCGCATCAAGCGGTGAGC-3′		
bla_{TEM}	TEM-for	5′-ATGAGTATTCAACATTTCCG-3′	863	Rasheed *et al,* 1997
	TEM-rev	5′-CTGACAGTTACCAATGCTTA-3′		
bla_{SHV}	SHV-for	5′-GGTTATGCGTTATATTCGCC-3′	867	Racheed *et al,* 1997
	SHV-rev	5′-TTAGCGTTGCCAGTGCTC-3′		
bla_{CTX-M}	CTXM-MA1	5′-SCSATGTGCAGYACCAGTAA-3′	544	Eckert *et al,* 2004
	CTXM-MA2	5′-CCGCRATATGRTTGGTGGTG-3′		
bla_{VEB}	VEB1F	5′-CGACTTCCATTTCCCGATGC-3′	643	This study
	VEB1R	5′-GGACTCTGCAACAAATACGC-3′		

included ampicillin (10 μg), amoxicillin/clavulanic acid (20/10 μg), cefazolin (30 μg), cefoxitin (30 μg), cetazidime (30 μg), cefotaxime (30 μg), imipenem (10 μg), gentamicin (10 μg), norfloxacin (10 μg), and trimethoprim/sulfamethoxazole (1.25 and 23.75 μg).

RESULTS

Identification of ESBL-producing *E. coli* isolated from patients and healthy adults

Thirty-seven out of 120 clinical isolates (31%) were confirmed as ESBL producers, while none was identified from the healthy adults (Table 2). Most ESBL producers were from urine (19%), while only 6% were from either blood or pus. Only nine and five of the clinical and non-clinical isolates respectively were identified as suspected ESBL producers.

Prevalence of *intI1* and ESBL genes in *E. coli* from clinical samples and from stool of healthy adults

As shown in Table 3, almost all (99%) *E. coli* from the clinical isolates carried *intI1* integrase gene, but 87% of the isolates from healthy adults also carried this gene. Among the 37 confirmed ESBL producers, 29 (78%), 3 (8%), 3 (8%), 29 (78%) and 36 (97%) showed the presence of bla_{TEM}, bla_{SHV}, bla_{VEB}, bla_{CTX-M}, and *intI1*, respectively. Three isolates carried no detectable ESBL gene. Among the

Table 2
Extended-spectrum beta-lactamase (ESBL) producing *E. coli* isolated from the patients and healthy adults by phenotypic tests.

Subject	Type of specimen	Number of the ESBL producing *E. coli* (%)	
		Initial screen test	Confirmatory test
Patient	Urine (*n* = 60)	27 (22)	23 (19)
	Blood (*n* = 30)	7 (6)	7 (6)
	Pus (*n* = 30)	12 (10)	7 (6)
Healthy adult	Feces (*n* = 120)	5 (4)	0 (0)

Table 3
Prevalence of *IntI1* and ESBL genes of *E. coli* isolates.

Type of *E. coli* (number)	Source	*Intl 1*	ESBL gene[a] (%)			
		No. (%)	bla_{TEM}	bla_{SHV}	bla_{VEB}	bla_{CTX-M}
ESBL producers (37)	Clinical	36 (97)	29 (78)	3 (8)	3 (8)	29 (78)
Suspected ESBL producers (9)	Clinical	9 (100)	9 (100)	0 (0)	0 (0)	0 (0)
Non-ESBL producers (74)	Clinical	74 (100)	ND	ND	ND	ND
Suspected ESBL producers (5)[b]	Non-clinical	4[c] (80)	4 (80)	0 (0)	0 (0)	0 (0)
Non-suspected ESBL producers (115)	Non-clinical	101 (88)	ND	ND	ND	ND

[a]Exact genotype was not determined, as amplicon was amplified using universal primers.
[b]Isolates showing positive result when tested by phenotypic screening test.
[c]These 4 isolates also carried bla_{TEM} gene.
ND = not done.

suspected ESBL producers, 9 (100%) isolated from clinical samples and 4 (80%) from the healthy adults carried bla_{TEM} and *intI1*. The presence of combined genes among the 37 ESBL producers is shown in Table 4. Two ESBL producers (5%) carried 3 genes (bla_{TEM}, bla_{SHV}, and bla_{CTX-M}) and 28 (76%) carried 2 genes, the majority being bla_{TEM} and bla_{CTX-M} .

Table 4
Prevalence of combined ESBL genes in the 37 ESBL producers.

Combined genes	Number of isolates (%)
bla_{TEM} , bla_{CTX-M} and bla_{SHV}	2 (5)
bla_{TEM} and bla_{CTX-M}	25 (68)
bla_{TEM} and bla_{SHV}	1 (3)
bla_{TEM} and bla_{VEB}	1 (3)
bla_{CTX-M} and bla_{VEB}	1 (3)
bla_{CTX-M} and bla_{SHV}	0 (0)
bla_{CTX-M} and bla_{SHV}	0 (0)
bla_{SHV} and bla_{VEB}	0 (0)
No bla_{TEM} , bla_{CTX-M}, bla_{SHV}, or bla_{VEB}	3 (8)

Antimicrobial susceptibility of *E. coli* isolates

Antimicrobial agents used to determine the susceptibility of the *E. coli* isolates were divided into 7 groups as follows: 1) restricted spectrum group, namely penicillin (ampicillin), amoxicillin/clavulanic acid; 2) cephalosporin group, namely cefazolin, ceftazidime, cefotaxime; 3) cephamycin group, namely cefoxitin; 4) carbapenem group, namely imipenem; 5) antimetabolite group, namely trimethoprim/sulfamethoxazole; 6) aminoglycoside group, namely gentamicin; 7) quinolone group, namely norfloxacin (Table 5). Among the 37 ESBL producers, 24%, 40%, and 16% were resistant to group 4, 5 and 6, respectively. One isolate (3%) was resistant to all 7 groups. Among the 83 non-ESBL producers from clinical samples, 17%, 25%, 23% and 16% were resistant to group 1, 2, 3 and 4, respectively. For the 120 non-ESBL producers obtained from the healthy adults, half of them were susceptible to all 7 groups.

Antimicrobial susceptibility of the 37 ESBL producers showed a low percent susceptibility to most antimicrobial agents tested except for imipenem (97%) and cefoxitin

Table 5
Antimicrobial resistance of *E. coli* isolates.

Source and number of *E. coli* isolates	Number of isolates resistant to multiple groups of antimicrobial agents[a] (%)							
	0	1	2	3	4	5	6	7
Patients (120)								
ESBL producers	0 (0)	0 (0)	2 (5)	4 (101)	9 (24)	15 (40)	6 (16)	1 (23)
Non-ESBL producers	8 (10)	14 (17)	21 (25)	19 (23)	13 (16)	7 (8)	1 (1)	0 (0)
Total	8 (7)	14 (12)	23 (19)	23 (19)	22 (18)	22 (18)	7 (6)	1 (1)
Healthy adults (120)								
Non-ESBL producers	61 (51)	24 (20)	24 (20)	6 (5)	3 (2)	1 (1)	1 (1)	0 (0)

[a]Multiple groups of antimicrobial agents: 0) all tested antimicrobial agent group; 1) restricted spectrum group: penicillin (ampicillin, amoxycillin/clavulanic acid); 2) cephalosporin group: cefazolin, ceftazidime, cefotaxime; 3) cephamycin group: cefoxitin; 4) carbapenem group: imipenem; 5) antimetabolite group: trimethoprim/sulfamethoxazole; 6) aminoglycoside group: gentamicin; 7) quinolone group: norfloxacin.

Table 6
Antimicrobial susceptibility of *E. coli* isolated from patients and healthy adults.

Antimicrobial agent	Clinical isolate		Non-clinical isolate
	ESBL producer (%) *n* = 37	Non-ESBL producer (%) *n* = 83	Non-ESBL producer (%) *n* = 120
Ampicillin	1 (3)	13 (16)	72 (60)
Amoxicillin/clavulanic acid	4 (11)	51 (61)	102 (85)
Cefazolin	1 (3)	59 (71)	115 (96)
Cefoxitin	25 (68)	74 (92)	116 (97)
Ceftazidime	17 (46)	74 (92)	118 (98)
Cefotaxime	3 (8)	71 (85)	117 (97)
Imipenem	36 (97)	83 (100)	120 (100)
Trimethoprim/sulfamethoxazole	10 (27)	28 (34)	76 (63)
Gentamicin	5 (13)	68 (82)	114 (95)
Norfloxacin	12 (32)	69 (83)	116 (97)

(68%). More than 80% of the 83 non ESBL-producers and 60-100% of the 120 non clinical isolates showed susceptible to various antimicrobial agents (Table 6).

DISCUSSION

The persistent exposure of bacteria to a multitude of beta-lactams has induced dynamic changes in terms of increasing production of beta-lactamases and mutations in their restricted spectrum enzymes to become ESBLs. The high prevalence of ESBL-production among *Enterobacteriaceae* were commonly observed in *Klebsiella pneumoniae* and *E. coli* in Asia (Bell *et al*, 2003; Munday *et al*, 2004; Hirakata *et al*, 2005; Kader and Kumar, 2005; Rossi *et al*, 2006; Ryoo *et al*, 2005). In Thailand, 15% of ESBL-producing *E. coli* isolates were observed during the period 2000 to 2003 (Dejsirilert *et al*, 2004). Increasing prevalence of ESBL producing *E. coli* was also reported in the present study (31%). All *E. coli* were isolated from Siriraj Hospital, Mahidol University, Bangkok, the largest tertiary university hospital in Thailand, where extensive usage of antibiotics is currently very common.

Molecular methods, particularly PCR, are widely used for confirmation and determination of ESBL genes, although there are some limitations as there are many more ESBL genes than TEM, SHV, VEB and CTX-M families, *viz*. genes for SFO, TOHO, SME, IMI, PER, and GES groups, which were not included in this study. We have selected only genes in the families of TEM, SHV, VEB and CTX-M, because they are reported to be highly prevalent in this region, whereas the other gene families are reported in other geographic regions (Tribuddharat and Fennewald, 1999, 2002; Bradford, 2001; Chanawong *et al*, 2001; 2007; Empel *et al*, 2007).

The studies on the distribution of bla_{TEM}, bla_{SHV}, bla_{VEB} and bla_{CTX-M} in all ESBL-producing enterobacteria as well as the presence of integrase (*intI1*) gene, a marker of multiple antibiotic resistance class1 integron elements, have been performed worldwide, but only few data have been published in Thailand (Tribuddharat and Fennewald, 1999, 2002; Chanawong *et al*, 2001, 2007). This study focused on *E. coli*, which is the most common

bacteria associated with nosocomial infections and is the predominant intestinal flora in healthy persons.

Nosocomial pathogens usually are bacteria that survive the high antibiotic selection pressure in hospitals, and therefore are multi-drug resistant. The bacteria of community origin, *ie* bacteria from healthy adults, should be less resistant due to low antibiotic selection pressure. The results of this study supports this hypothesis as nosocomial isolates showed multi-drug resistant characters, with 31% carrying ESBL gene(s) and 97% *intI1*. The unexpected result was the very high positive rate (87%) of integrase gene in *E. coli* from healthy adults. This could mean that healthy adults recently received these types of *E. coli* either from consuming contaminated food or from contact with hospitals.

E. coli may collect certain integron elements that can exist without much need of antibiotic selection, and a high prevalence of integron elements has a cumulative effect. *IntI1* in *E. coli* was reported in many countries, *viz*. 30.8% in Korea (Yu *et al*, 2004), 1% in France (Skurnik *et al*, 2005) and 67% in Spain (Machado *et al*, 2005). Although there is a chance of finding the presence of integron elements in the absence of the antibiotic selection pressure, the high percent positive rate of integron integrase observed in this study indicates that there was already a strong selective pressure in the community. Further study of the prevalence of integron elements and their antibiotic resistance gene cassettes from bacteria of community origin would be worthwhile. Many investigators have also identified genes as gene cassettes in integrons, *ie* bla_{VEB-1}, bla_{OXA-10}, *arr*-2, *qacEΔ*1, *dfr*, *sul*, and *aadA* (Girlich *et al*, 2001; Sabate *et al*, 2002; Yu *et al*, 2003). It may be worthwhile to investigate the most prevalent resistance gene in such integrons and to study why those resistance genes are more prevalent. The finding that nosocomial pathogens had more than 1 type of beta-lactamase genes may be the result of high antibiotic pressure from use of various different classes of antibiotics and/or from the higher bacterial survival chances from an antibiotic treatment due to presence of combination resistance mechanisms.

High prevalence of bla_{TEM} and bla_{CTX-M}, ESBL genes in ESBL-producing *E. coli*, observed in this study, has been previously reported (Baraniak *et al*, 2002; Cao *et al*, 2002; Dansuputra, 2002), as well as the low prevalence of bla_{SHV} gene (Naas *et al*, 1999; Rasheed *et al*, 1997; Dansuputra, 2002). The mobile genetic elements involved in the spread of certain gene families should be further explored.

Gene bla_{VEB-1} was first found in a single isolate of *E. coli* in a patient from Vietnam, but was subsequently also found in *Pseudomonas aeruginosa* isolate from a patient in Thailand (Naas *et al*, 1999). There was a report that bla_{VEB-1} ESBL seems to be highly prevalent in Thai isolates since they account for 60% of the ESBL-possessing *Enterobacteriaceae* isolates (Girlich *et al*, 2001). High prevalence of bla_{VEB} in *E. coli* was reported, but not bla_{SHV} (Dansuputra, 2002). In our study, 8% of isolates carried bla_{SHV} and 8% bla_{VEB}. The difference in bla_{VEB} prevalence may depend on isolates collected and may not be significant between these two studies. However, Girlich *et al* (2001) showed, for ceftazidime resistant *E. coli*, that 60% of these *E. coli* carried bla_{VEB} genes.

The ESBL producers in this study were much less susceptible to ampicillin, amoxicillin/ clavulanic acid, cefazolin, gentamicin, trimethoprim/ sulfamethoxazole, and norfloxacin (range 4-32%). Among the third generation cephalosporins, these isolates showed much less susceptibility to cefotaxime (8%) moderate susceptibility to ceftazidime (46%), and high susceptibility to cefoxitin (68%). This may be explained by the fact the enzymes of this class can not hydrolyze cephamycin (Jacoby *et al*,

1988). The recommended antibiotic susceptible test report by NCCLS would indicate resistance to all cephalosporins for these ESBL producers. It is noticeable that the use of cephamycin for clinical treatment of infections by ESBL producers is still debatable and awaiting for more clinical studies (Paterson, 2000 Lee *et al*, 2006). Many investigators (Nathisuwan *et al*, 2001; Ingviya *et al*, 2003; Babypadmini and Appalaraju, 2004) have reported a low percent susceptibility of ESBL-producing *E. coli* to non beta-lactam antibiotics. In this study, only 13% and 27% of *E. coli* isolates were susceptible to gentamicin and trimethoprim/sulfamethoxazole respectively. However, previous studies (Dansuputra, 2002; Ingviya *et al*, 2003; Goosen and Grabein, 2005) showed that the susceptibility of ESBL-producing *E. coli* against imipenem (carbapenem) is high (96.9-100%), consistent with results in this study. Therefore, there is a strong rationale for the use of carbapenem against the ESBL- producing *E. coli* during such nosocomial infections.

In summary, this study has shown a high recovery rate of integrase gene in both clinical and non clinical *E. coli* isolates. This will create more multidrug-resistant bacteria in the future due to the probable high rate of gene transfer. Sources of the integrase gene should be investigated and prevention of transfer of the resistant gene among nosocomial and community agents should be seriously attempted.

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