— **Regular Article** —

Antimicrobial Resistance, Class 1 Integrons and Extended-Spectrum β-Lactamases in *Escherichia coli* Clinical Isolates from Patients in South Thailand

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Five hundred and ninety seven *Escherichia coli* (*E. coli*) isolates were obtained from clinical specimens at the Songklanagarind Hospital in Songkhla Province, Thailand during 2003–2005. Antimicrobial susceptibilities to ten antimicrobial agents were tested by a standard disk diffusion method. The presence of class 1 integrons was based on the detection of the integrase gene (*intI1*) by PCR. Extended-spectrum β-lactamases (ESBLs) were detected by a combination disk method. The highest percentage of resistance was found to ciprofloxacin (40.5%), norfloxacin (39.0%), and cefuroxime (33.2%). The *IntI1* was detected in 59.5% of the tested isolates. Resistance to gentamicin, cefazolin, cefuroxime, cefotaxime, ceftriaxone, norfloxacin, and ciprofloxacin was significantly higher in class 1 integron-positive isolates ($p < 0.05$). The most prominent resistance pattern was for norfloxacin-ciprofloxacin (17.7%). ESBLs were detected in 75 out of 597 (12.6%) isolates; 56/302 (18.5%) and 19/295 (6.4%) were from hospitalized and non-hospitalized patients, respectively. Seventy-five percent of ESBL-positive strains were integron-positive isolates. Imipenem and meropenem were still able to inhibit all ESBL-producing strains. The results indicated that class 1 integrons are widely prevalent among clinical isolates of resistant *E. coli* especially in ESBL-producers and are probably a reservoir for producing multidrug resistance and nosocomial infections in hospitals.

Key words —— class 1 integron, extended-spectrum β-lactamase, *Escherichia coli*, multidrug resistance, hospitalized patient, non-hospitalized patient

INTRODUCTION

Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) is an emerging pathogen.[1] Outbreaks due to ESBL-producing organisms have been reported from hospitals all over the world.[2–6] It has been recognized as an important cause of nosocomial infections.[7] ESBL-producing isolates are not only resistant to β-lactams (penicillins, first-, second-, and third-generation cephalosporins, and aztreonam) but also aminoglycosides, tetracyclines, chloramphenicol, trimethoprim, sulfonamides, and quinolones. In addition, many of the more recently described ESBL genes are frequently found within integron-like structures.[3,8,9] Integrons appear to play an important role in the transfer of antibiotic resistance in *E. coli* derived from animals and humans because they can capture, integrate, and express gene cassettes encoding antibiotic resistance.[10–19] However, there have been only a few reports on ESBL-producing bacteria isolated in Thailand.[5,20–23] Our objective for this study was to determine the prevalence of resistance factors, including the production of ESBL and the presence of class 1 integrons, in *E. coli* isolated from clinical specimens obtained at the Songklanagarind Hospital the major university hospital in southern Thailand.

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MATERIALS AND METHODS

Clinical Isolates —— A total of 597 *E. coli* iso-

lates each collected from a single patient as a clinical specimen: urine (446), blood (45), pus (40), body fluid (30), sputum (16), and others (20) from 295 non-hospitalized and 302 hospitalized (> 48 hr) patients from Songklanagarind Hospital, a modern tertiary care university hospital in southern Thailand during July 2003–August 2005. All isolates appearing as Gram-negative, nonspore-forming rods, fermenting lactose with gas production within 48 hr at 35°C, demonstrating Indole test, Methyl Red test, Voges Proskauer test and Citrate test (IMViC) patterns of positive-positive-negative-negative (++−−), urease negative, and hydrogen sulfide negative were considered to be *E. coli*.[24] All isolates were maintained in 15% glycerol at −80°C.

Antimicrobial Susceptibility Test and ESBL Detection —— Susceptibility to 10 antimicrobial agents commonly used in the treatment of Gram-negative bacterial infections [amikacin (AMK) 30 μg, gentamicin (GEN) 10 μg, cephazolin (FAM) 30 μg, cefuroxime (CXM) 30 μg, cefotaxime (CTX) 30 μg, ceftriaxone (CRO) 30 μg, imipenem (IMP) 10 μg, meropenem (MEM) 10 μg, norfloxacin (NOR) 10 μg, and ciprofloxacin (CIP) 5 μg] was determined by the standard disk diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines.[25] All intermediate susceptible strains were considered to be resistant strains. The presence of the ESBL phenotype was initially screened using four antimicrobial disks containing CTX (30 μg), CRO (30 μg), ceftazidime (30 μg) and cefpodoxime (10 μg). The phenotypic confirmatory test used the clavulanate double-disk synergy method, was carried out with disks containing ceftazidime (30 μg) and CTX (30 μg) either alone or in combination with clavulanic acid (30/10 μg, oxoid). The results were interpreted according to CLSI.[25] Quality control was performed using *E. coli* ATCC 25922 and *Klebsiella pneumoniae* (*K. pneumoniae*) ATCC 700603.

Detection of Class 1 Integrons —— Integrons were detected using a multiplex PCR that targetted three conserved sequences of class 1 integrons including *qacEΔ1* (gene encoding resistance to quaternary ammonium compounds), *intI1* (integrase gene), and *sul1* (sulphonamide resistance gene), as previously described.[19] Primer pairs were purchased from a commercial source (QIAGEN Operon GmbH, Cologne, Germany). Primers, (reported from 5′ to 3′) included, GGTTCGAATGTCGTAACCGC and ACGCCCTTGAGCGGAAGTATC for amplification of the *intI1* gene, ATCAGACGTCGTGGATGTCG and CGAAGAACCGCACAATCTCG for amplification of the *sul1* gene and GAGGGCTTTACTAAGCTTGC and ATACCTACAAAGCCCCACGC for amplification of the *qacEΔ1* gene. Template DNA was prepared by boiling overnight cultures in 0.2% Triton-X for 10 min. Boiled cultures were cooled on ice for 5 min and 1 μl volumes were used immediately for PCR using a PTC-100™ Peltier thermocyler, MJ Research, Inc. (Waltham, Massachusetts, U.S.A.) with the following cycle: one cycle of 94°C for 4 min; 10 "touchdown" cycles of 94°C for 1 min, 65°C for 30 s (decreasing 1°C/cycle), 70°C for 2 min; 24 cycles of 94°C for 1 min, 55°C for 30 s, 70°C for 2 min; and one final cycle of 70°C for 5 min. *Salmonella enterica* serovar Typhimurium DT104, a known carrier of a class 1 integron, was used as a positive control. The PCR products were visualized by ethidium bromide staining after agarose gel electrophoresis. The prevalence of class 1 integrons was based on the presence of the *intI1* gene.

Statistical Analysis —— The χ^2 test was used to determine any significant differences in resistance or prevalence where appropriate. Differences were considered significant at $p < 0.05$.

RESULTS

Prevalence of Class 1 Integrons and ESBL in *E. coli*

Class 1 integrons, as indicated by the presence of *intI1* was detected in 355 (59.5%) *E. coli* clinical isolates. One hundred and sixty-six isolates (27.8%) contained all three conserved genes associated with class 1 integrons (*intI1*, *qacEΔ1*, *sul1*) and 214 isolates (35.8%) contained only *sul1*. The occurrence of a class 1 integron was similar among the isolates from hospitalized and non-hospitalized patients (60.3 and 58.6%, respectively) as shown in Table 1.

Of the 597 isolates tested, ESBL production was demonstrated in 75 isolates (12.6%). ESBL-positive isolates were recovered from the following specimens: urine 62.7%, pus 12.0%, body fluid 9.3%, blood 8.0%, sputum 5.3%, and other tissues 2.7%. ESBL production was more prevalent in isolates from hospitalized (18.5%) compared with non-hospitalized patients (6.4%). Class 1 integrons were more frequently found among ESBL-positive (56/75, 74.7%) than among ESBL-negative (19/75, 57.3%) *E. coli* strains ($p < 0.05$).

Table 1. Prevalence of Integrase Genes and Integron Component Genes in *E. coli* from Hospitalized and Non-hospitalized Patients

	Hospitalized patients (n = 302)	Non-hospitalized patients (n = 295)	Total (n = 597)
IntI1 (i)	182 (60.3)[a)]	173 (58.6)[a)]	355 (59.5)
SulI (s)	109 (36.1)[a)]	105 (35.6)[a)]	214 (35.8)
All three conserved genes (i, s, q)	80 (26.5)[a)]	86 (29.2)[a)]	166 (27.8)
None	91 (30.1)[a)]	103 (34.9)[a)]	194 (32.5)

Data shows numbers and percentages of clinical *E. coli* isolates carrying class 1 integrons and genes associated with integrons, as determined by multiplex PCR. q, *qacEΔ;* i *intI1*; s, *sul1*. *a*) Values within the same row are not significantly different ($p > 0.05$).

Table 2. Antimicrobial Resistance in *E. coli* with Respect to ESBL Production and Presence of Class 1 Integrons

Antimicrobial agents	Resistance (%)		p	Resistance (%)		p	Total
	ESBL-positive (n = 75)	ESBL-negative (n = 522)		Integron-positive (n = 355)	Integron-negative (n = 242)		(n = 597)
Amikacin	35 (46.7)	29 (5.6)	< .001	37(10.4)	27(11.2)	.776	64(10.7)
Gentamicin	61 (81.3)	59(11.3)	< .001	88(24.8)	32(13.2)	< .001	120(20.1)
Cephazolin	74 (98.7)	86(16.5)	< .001	113(31.8)	47(19.4)	< .001	160(26.8)
Cefuroxime	75(100.0)	123(23.6)	< .001	134(37.7)	64(26.4)	.004	198(33.2)
Cefotaxime	73 (97.3)	24 (4.6)	< .001	67(18.9)	30(12.4)	.035	97(16.2)
Ceftriaxone	74 (98.7)	26 (5.0)	< .001	69(19.4)	31(12.8)	.033	100(16.8)
Imipenem	0 (0.0)	2 (0.4)	NA	2 (0.6)	0 (0.0)	NA	2 (0.3)
Meropenem	0 (0.0)	3 (0.6)	NA	2 (0.6)	1 (0.4)	NA	3 (0.5)
Norfloxacin	56 (74.7)	177(33.9)	< .001	158(44.5)	75(31.0)	< .001	233(39.0)
Ciprofloxacin	58 (77.3)	184(35.2)	< .001	166(46.8)	76(31.4)	< .001	242(40.5)

Antimicrobial Susceptibility

The antimicrobial susceptibility of *E. coli* isolates is shown in Table 2. Overall, the highest percentage of resistance was found to CIP (40.5%), NOR (39.0%), CXM (33.2%), FAM (26.8%), GEN (20.1%), CRO (16.8%), CTX (16.2%), AMK (10.7%), MEM (0.5%), and IMP (0.3%). Resistance to all antimicrobials tested except for IMP and MEM, was significantly more common ($p < 0.05$) in ESBL-positive isolates than in ESBL-negative isolates.

All ESBL-producing isolates were multidrug resistant (MDR) from 3 to 8 antimicrobials, whilst only 52.5% of ESBL-negative isolates were resistant to at least one antimicrobial, and 75.5% of those resistant isolates were MDR strains (Table 3).

Table 3 provides a comparison of the resistance patterns for ESBL-positive and ESBL-negative *E. coli* isolates. A total of 12 different resistance patterns were observed among the 75 ESBL-producing isolates. The most frequent pattern was GEN-FAM-CXM-CTX-CRO-NOR-CIP (33.3%), followed by AMK-GEN-FAM-CXM-CTX-CRO-NOR-CIP (28.0%) and AMK-GEN-FAM-CXM-CTX-CRO (12.0%). Forty-three resistance patterns were found among the 276 drug resistant ESBL-negative isolates. The top three resistance patterns were NOR-CIP (22.5%), CXM-NOR-CIP (11.6%), and GEN-NOR-CIP and FAM (7.6%). Only five resistance patterns were common to both groups. In addition, among the ESBL-negative isolates the MDR pattern was more prevalent in the integron-positive (45.8%) compared with the integron-negative strains (31.4%, $p < 0.05$).

DISCUSSION

In Asia, the prevalence of ESBL-positive isolates among clinical isolates of *E. coli* has been shown to vary among countries. National survey data have indicated the presence of ESBLs in 5 to 8% of *E. coli* isolates from Korea, Japan, Malaysia and Singapore but in 12–24% in Thailand, Taiwan, the Philippines, Indonesia, Hong Kong and China.[2,6,7)] In the present study, the prevalence of ESBL-positive isolates from various sources in a major university hospital in the south of Thailand was 12.6%. A study by Jitsurong and Yodsawat[5)] documented only a 5.1% prevalence of

Table 3. Antibiotic Patterns among ESBL-positive and ESBL-negative *E. coli*

Resistance pattern			
ESBL-positive *E. coli* (*n* = 75)	No. of isolates (%)	ESBL-negative *E. coli* (*n* = 276)	No. of isolates (%)
Resistance to 8 antimicrobials		Resistance to 9 antimicrobials	
AMK-GEN-FAM-CXM-CTX-CRO-NOR-CIP	21 (28.0)	AMK-GEN-FAM-CXM-CTX-CRO-IPM-NOR-CIP	1 (0.4)
Resistance to 7 antimicrobials		Resistance to 7 antimicrobials	
GEN-FAM-CXM-CTX-CRO-NOR-CIP	25 (33.3)	GEN-FAM-CXM-CTX-CRO-NOR-CIP	2 (0.7)
AMK-FAM-CXM-CTX-CRO-NOR-CIP	2 (2.7)	AMK-FAM-CXM-CTX-CRO-NOR-CIP	1 (0.4)
AMK-GEN-FAM-CXM-CTX-CRO-CIP	1 (1.3)	FAM-CXM-CTX-CRO-IMP-MEM-CIP	1 (0.4)
Resistance to 6 antimicrobials		FAM-CXM-CTX-CRO-MEM-NOR-CIP	1 (0.4)
AMK-GEN-FAM-CXM-CTX-CRO	9 (12.0)	Resistance to 6 antimicrobials	
FAM-CXM-CTX-CRO-NOR-CIP	6 (8.0)	FAM-CXM-CTX-CRO-NOR-CIP	5 (1.8)
GEN-FAM-CXM-CTX CRO-CIP	1 (1.3)	FAM-CXM-CTX-CRO-MEM-CIP	1 (0.4)
GEN-FAM-CXM-CRO-NOR-CIP	1 (1.3)	Resistance to 5 antimicrobials	
Resistance to 5 antimicrobials		GEN-FAM-CXM-NOR-CIP	3 (1.1)
GEN-FAM-CXM-CTX-CRO	3 (4.0)	AMK-FAM-CXM-NOR-CIP	2 (0.7)
AMK-FAM-CXM-CTX-CRO	1 (1.3)	GEN-FAM-CXM-CTX-CRO	2 (0.7)
Resistance to 4 antimicrobials		AMK-GEN-FAM-NOR-CIP	1 (0.4)
FAM-CXM-CTX-CRO	4 (5.3)	Resistance to 4 antimicrobials	
Resistance to 3 antimicrobials		FAM-CXM-NOR-CIP	12 (4.3)
CXM-NOR-CIP	1 (1.3)	AMK-CXM-NOR-CIP	11 (4.0)
		GEN-CXM-NOR-CIP	7 (2.5)
		FAM-CXM-CTX-CRO	5 (1.8)
		GEN-FAM-NOR-CIP	5 (1.8)
		AMK-FAM-NOR-CIP	1 (0.4)
		FAM-CTX-CRO-CIP	1 (0.4)
		Resistance to 3 antimicrobials	
		CXM-NOR-CIP	32 (11.6)
		GEN-NOR-CIP	21 (7.6)
		AMK-NOR-CIP	4 (1.4)
		FAM-NOR-CIP	4 (1.4)
		AMK-FAM-CXM	1 (0.4)
		FAM-CXM-CRO	1 (0.4)
		GEN-CTX-CRO	1 (0.4)
		Resistance to 2 antimicrobials	
		NOR-CIP	62 (22.5)
		FAM-CXM	11 (4.0)
		CXM-CIP	2 (0.7)
		GEN-FAM	2 (0.7)
		AMK-CXM	1 (0.4)
		AMK-FAM	1 (0.4)
		CTX-CRO	1 (0.4)
		CXM-NOR	1 (0.4)
		FAM-CRO	1 (0.4)
		GEN-CIP	1 (0.4)
		Resistance to 1 antimicrobial	
		FAM	21 (7.6)
		CXM	20 (7.2)
		GEN	13 (4.7)
		AMK	5 (1.8)
		CIP	3 (1.1)
		CRO	2 (0.7)
		CTX	2 (0.7)
		NOR	1 (0.4)

ESBL-positive isolates from blood in the same hospital. The ESBL-positive *E. coli* we isolated were mainly from urine. Moor *et al.*[26] also found that urine was the commonest source (97%) of ESBL-producing *E. coli* in their study. In the major teaching hospitals in Bangkok, 30.1% of Gram-negative bacteria and 31% of *E. coli* were ESBL producers.[21,23] Patients at high risk for developing colonization or infections with ESBL-producing organisms are often seriously ill patients with prolonged hospital stays and in whom invasive medical devices are present such as urinary catheters, and endotracheal tubes of central venous lines for prolonged duration.[7] We found that 74.7% of ESBL-producing *E. coli* was from hospitalized patients and 62.7% of these were from urine (data not shown).

It has been well documented that class 1 integrons are most commonly found in clinical isolates of Gram-negative bacteria.[27] Su *et al.*[28] reported the detection of three classes of integrons in 111 clinical strains of *E. coli* and found that 85.6 and 3.6% of the isolates carried class 1 and class 2 integrons respectively, whereas no class 3 integron was detected. Yan *et al.*[29] also found a high rate of class 1 integrons (76.3%) in both Gram-positive and Gram-negative bacteria while only 0.8% of class 2 integrons was detected. Integrons have been identified as a primary source of resistance genes and are claimed to be reservoirs of antimicrobial resistance genes within microbial populations.[27,30] In this study, the class 1 integron integrase gene (*intI1*) was detected in 59.5% of *E. coli* clinical isolates which is comparable to the study in *E. coli* isolated from fecal specimens of non-hospitalized patients (63%) by Phongpaichit *et al.*[31] but much lower than that reported by Pongpech *et al.*[23] who detected *IntI1* in 99% of the clinical and 87% of the non-clinical *E. coli* isolates. The result indicated that there was less selective pressure for integron-positive isolates in the southern part of Thailand than in the central part (Bangkok *etc.*).

In this study, 74.7% of ESBL-positive isolates carried a class 1 integron compared to 57.3% of the ESBL-negative isolates. ESBL enzymes including TEM, SHV and CTX-M are mostly found in clinical isolates.[32] Pongpech *et al.*[23] found that bla_{TEM} and $bla_{\text{CTX-M}}$ were predominant among ESBL producing *E. coli*. In Argentina, CTX-M-2 is the most frequently found ESBL among clinical isolates in Argentinean hospitals. Almost all open reading frame (orf) 513-bearing class 1 integrons are associated with $bla_{\text{CTX-M-2}}$ in the Gram-negative bacterial population under study and the sequences adjacent to the $bla_{\text{CTX-M-2}}$ gene are conserved in all the studied isolates.[33] Eckert *et al.*[34] examined the $bla_{\text{CTX-M}}$ genes in *E. coli* and *K. pneumoniae* by cloning, sequencing, and PCR analysis and detected a complex *sul1*-type integron including an orf513, that carried the *bla* gene together with its surrounding DNA. We found that 60% of *intI1*-positive isolates also contained the *sul1* gene (data not shown). The ESBL-encoding genes have been reported to be located on large plasmids which may also contain other resistance genes. Resistance genes can occur within related species. Novais *et al.*[35] reported that the spread of the CTX-M gene in enterobacterial clinical isolates in Spain was associated with Incompatibility group (Inc)N broad-host-range and IncFII narrow-host-range plasmids. Mshana *et al.*[36] also found the conjugative IncF1 plasmids carrying CTX-M-5 among *E. coli* ESBL producing isolates at a University hospital in Germany also had a high rate of transferable antibiotic resistances for sulphamethoxazole (61%), tetracycline (61%) and GEN (33%).

Evaluation of the antimicrobial resistance patterns of ESBL-positive isolates showed that antimicrobial agents with a high level of effectiveness were MEM and IMP, followed by AMK. Furthermore, an association between the production of ESBL and resistance to CIP and NOR was demonstrated. This association has been observed in ESBL-producing *K. pneumoniae*.[37,38] All our ESBL producers were MDR from 3 to 8 antimicrobial agents. The high resistance profile of ESBL-producing *E. coli* to the antimicrobial agents readily available for treatment reflects the severe consequences of ESBL infection. Lee *et al.*[39] reported that the cost of infection caused by ESBL-producing *E. coli* and *Klebsiella* species was 1.7 times the cost of infection caused by non-ESBL producers. This study has demonstrated that class 1 integrons are widely prevalent among *E. coli* clinical isolates; especially among ESBL-producers and that they could be a reservoir for producing multidrug resistance and nosocomial infections in hospitals.

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