EMERGENCY ROOM: AN UNRECOGNIZED SOURCE OF EXTENDED-SPECTRUM β-LACTAMASE PRODUCING *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE*

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Abstract. Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* are the leading causes of hospital-associated infections, but community-acquired cases are increasingly being reported. This study determined the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* carriers, their *bla* genes and risk factors of 452 patients admitted to the emergency room (ER) of Ramathibodi Hospital, Mahidol University, Bangkok, Thailand between April and August 2011. Prevalence of ESBL-producing *E. coli* and *K. pneumoniae* from rectal swabs was 16.5% and 1.0%, respectively. Factors associated with ESBL-producing carriers were a previous history of hospital admission ($p = 0.001$) and visits to health care facilities ($p = 0.002$) during the previous 3 months. All ESBL-producing isolates were susceptible to imipenem, meropenem and ertapenem. The majority (78%) of ESBL-producing *E. coli* isolates showed very high resistance to cefotaxime and ceftriaxone (MIC_{50} and $MIC_{90} > 256$ μg/ml). ESBL-producing *E. coli* harbored chromosomal bla_{TEM} (96%), bla_{CTX-M} (70%) and bla_{SHV} (1%), while 8%, 73% and 3%, respectively, were located on plasmid. The prevalence of these genes in ESBL-producing *K. pneumoniae* was 75%, 50% and 25%, respectively on chromosome; and 100%, 25% and 50%, respectively on plasmid. Nucleotide sequence analysis revealed that these *bla* genes were of the type bla_{TEM-1}, $bla_{TEM-116}$, $bla_{CTX-M-15}$, $bla_{CTX-M-161}$, bla_{SHV-12}, bla_{SHV-28} and $bla_{SHV-148}$. Detailed epidemiologic and clinical characteristics of ER patients with history of prior hospital visits should be carried out to identify the ESBL-producing organisms they have acquired in order to institute appropriate treatment for these patients as well as control measures against further dissemination of these life-threatening organisms.

Keywords: emergency room, ESBL, fecal carrier

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INTRODUCTION

Emergence of extended-spectrum β-lactamase (ESBL) in gram-negative bacilli (GNB) is a major challenge in health care-associated infections (HAIs) (Pitout and Laupland, 2008). *Escherichia coli* and

Klebsiella pneumoniae are the major ESBL-producing organisms isolated worldwide (Pitout and Laupland, 2008). Conditions in emergency rooms (ERs) are typically hectic, fast-paced and involve multidisciplinary medical personnel. ER is also the most upfront of the hospital with a high risk of being reservoir of ESBL-producing bacteria coming from the community or other hospitals.

The majority of ESBLs belong to 3 groups, namely, TEM, SHV and CTX-M. ESBLs arise from mutations of the genes encoding TEM-1, TEM-2 or SHV-1 β-lactamases (Paterson, 2006). TEM- and SHV-type ESBLs are less common, while CTX-M-type has become the most prevalent worldwide (Bonnet, 2004).

Ramathibodi Hospital, located in Bangkok, Thailand, is a university hospital providing tertiary care for some 100,000 patients per year, and approximately 5,500 patients attend ER every month. The hectic and crowded environment of ER could compromise infection control measures. If ESBL carriers are among the ER patients, then a possibility of the spread of ESBL bacteria might occur in this setting, which could later spread to other patients within the hospital.

This study determined the prevalence and risk factors of ESBL-producing *E. coli* and *K. pneumoniae* among patients attending Ramathibodi Hospital ER. We also identified the presence of bla_{CTX-M}, bla_{TEM}, and bla_{SHV} and their types among ESBL-producing *E. coli* and *K. pneumoniae* isolates.

MATERIALS AND METHODS

Subjects

Criteria for eligible patients attending ER of Ramathibodi Hospital, Mahidol University were as follows: age > 15 years, conscious, stayed in ER within 24 hours of data collection, and provided written informed consent. The participants were asked to provide information regarding their health status. Rectal swab samples were collected prior to medical investigation or admission process. Exclusion criteria included unconsciousness, psychological disorders and life threatening conditions. In addition, patients' information was obtained from medical records, including demographic and clinical characteristics. The study was approved by the Ethics Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University (MURA2011/146).

Detection of bacterial ESBL producers

Rectal swab samples were streaked on MacConkey agar plates and isolates were identified using standard biochemical and microbiological methods. ESBL production was detected using a double-disk synergy test with cefpodoxime (10 μg), ceftriaxone (30 μg), ceftazidime (30 μg), and ceftazidime/ clavulanic acid (30/10 μg) (CLSI, 2010).

Minimum inhibitory concentration (MIC) determination

MICs of all ESBL-producing *E. coli* and *K. pneumoniae* isolates were determined using the E-test against amikacin, cefepime, ciprofloxacin, cefotaxime, ceftazidime, ceftriaxone, gentamicin, ertapenem, imipenem and meropenem (BioMerieux, Hazelwood, MO). Six strips were placed per 150-mm plate. Plates were incubated at 35°C for 16 to 20 hours. MIC was recorded, compared with the standard MIC and classified as susceptible (S), intermediate (I), and resistant (R) according to CLSI 2010 recommendations (CLSI,

Table 1
Primers used in PCR amplification of *bla* genes.

Primer	Sequence (5' - 3')	Amplicon size (bp)	Reference
bla-SHV.SE	ATGCGTTATATTCGCCTGTG	747	Paterson *et al*, 2003
bla-SHV.AS	TGCTTTGTTATTCGGGCCAA		
TEM-164.SE	TCGCCGCATACACTATTCTCAGAATGA	445	Monstein *et al*, 2007
TEM-165.AS	ACGCTCACCGGCTCCAGATTTAT		
CTX-M-U1	ATGTGCAGYACCAGTAARGTKATGGC	593	Boyd *et al*, 2004
CTX-M-U2	TGGGTRAARTARGTSACCAGAAYCAGCGG		

Y=C or T; R=A or G; K=G or T; S=C or G

Table 2
General characteristics of the 452 ER patients enrolled.

Characteristics	Number	%
Sex		
Male	133	29.5
Female	319	70.5
Age (years)		
15-25	25	5.5
26-35	23	5.0
36-45	36	8.0
46-55	77	17.0
56-65	83	18.5
66-75	95	21.0
>75	113	25.0
Range	15-96	
Median	64	
Residence		
Bangkok	266	59.0
Other provinces	186	41.0
Had underlying medical conditions	371	82.0
Prior use of antibiotics during previous 3 months	50	11.0
β-lactams	38	8.5
Macrolides	7	1.5
Fluoroquinolones	4	1.0
Others	9	2.0
Hospital admission during previous 3 months	106	23.5
Visited health care facilities during previous 3 months	227	49.0
Retained medical device upon ER visit[a]	22	5.0
Pervious history of MDR infection	10	2.0

[a]Nasogastric tube, permanent catheter, percutaneous transhepatic biliary drainage, Tenkoft catheter, Arteriovenous bridge graft (AVBG), Foley catheter, Biphasic positive airway pressure (Bipap). ER, emergency room; MDR, multidrug resistance.

2010). Quality control was performed using *Escherichia coli* ATCC 25922 on each day of testing.

Identification of *bla* genes from ESBL-producing isolates

Chromosomal and plasmid DNA were prepared using the NucleoSpin® Tissue kit (Macherey-Nagel, Bethlehem, PA) and NucleoSpin® Plasmid kit in accordance with the manufacturer's recommendations. Purified DNA concentrations were determined by agarose gel-electrophoresis in comparison with standard markers. The primer sets used and expected PCR amplicon sizes are listed in Table 1. Three single PCR amplification assays were carried out targeting bla_{SHV}, bla_{TEM} and bla_{CTX-M}. Each reaction contained 200 ng of purified DNA, 0.2 mM each dNTP, 1x ThermoPol buffer, 1 U *Taq* (New England Biolabs, Beverly, MA) and 200 nM each gene-specific primer pair in a final reaction volume of 20 μl. Amplification reaction (conducted in Px2 Thermal Cycler; Thermo Hybaid Scientific, Waltham, MA) was carried out as follows: 95°C for 2 minutes; 35 cycles of 95°C for 15 seconds, 56°C for bla_{SHV} or 50°C for bla_{TEM} or 58°C for bla_{CTX-M} for 30 seconds, and 68°C for 45 seconds; with a final step at 68°C for 5 minutes. Amplicons were submitted for DNA sequencing (Pacific Science, Bangkok, Thailand) and sequences compared to previously published sequences using BLAST program (www.NCBI.nlm.Gov/BLAST).

Data analysis

Multivariate logistic regression analysis was performed to identify risk factors and outcome between ESBL-producing and non-ESBL-producing carriers. Difference is considered statistically significant when 2-tailed *p*-value is ≤ 0.05. Statistical analysis was conducted using PASW statistics package 18.

RESULTS

Characteristics of enrolled ER patients

The majority (71%) of 452 patients enrolled in study were female, 25% were more than 75 years old, over half (59%) resided in Bangkok, and the majority (82%) had underlying health problems (Table 2).

ESBL-producing *E. coli* and *K. pneumoniae*

Overall, 78/452 (17%) of the patients carried ESBL-producing isolates: 74 (16%) were *E. coli* and 4 (1%) were *K. pneumoniae* samples. ESBL-producing *K. oxytoca* also were found in 2 patients, with one case carrying both ESBL-producing *K. oxytoca* and *E. coli*.

Factors associated with ESBL-producing *E. coli* and *K. pneumoniae* carriers

Multivariate analysis revealed that the history of hospital admission and healthcare visits in the previous 3 months are significantly different between carriers and non-carriers of ESBL-producers ($p = 0.001$, 95%CI 2.21-6.39 and $p = 0.002$, 95% CI 1.38-3.77, respectively) (Table 3). Follow-up of the outcome of each case after receiving health care service at ER showed that about 70% of ESBL carriers returned home after the treatment, 28% were admitted to the hospital for further treatment and another 3% were referred to other health care settings.

Antimicrobial susceptibility of ESBL-producing *E. coli* and *K. pneumoniae* isolates

In general, MIC_{50} values of ESBL-producing *K. pneumoniae* isolates were lower than those of ESBL-producing *E. coli* (Table 4). All isolates were susceptible to carbapenems, with MIC_{50} and MIC_{90} ranging from 0.008-1 μg/ml for ertapenem and 0.125-1.5 μg/ml for imipenem (Table 4). About 3% and 62% of the 74 ESBL-producing *E. coli* isolates were non-susceptible (intermediate and resistant)

Table 3
Factors associated with ESBL-producing *E. coli* and *K. pneumoniae* carriers.

Factor	ESBL carrier *n* (%)	Non-ESBL carrier *n* (%)	*p*-value
Sex			0.833
Male	24 (31)	103 (29.5)	
Female	54 (69.2)	246 (70.5)	
Age (years)			0.098
<35	7 (9)	37 (10.5)	
>35	71 (91)	312 (89.5)	
Residence			0.237
Bangkok	39 (50)	209 (60.0)	
Other provinces	39 (50)	140 (40.0)	
Underlying disease			0.215
Yes	68 (87)	283 (81.0)	
None	10 (13)	66 (19.0)	
Use of antibiotics during previous 3 months			
Yes	17 (22)	29 (8.0)	0.231
None	61 (78)	320 (92.0)	
Admitted to hospital during previous 3 months			
Yes	33 (42)	52 (15.0)	**0.001**
No	45 (58)	297 (85.0)	
Visited health care facilities during previous 3 months			
Yes	48 (62)	144 (41.0)	**0.002**
No	30 (38)	205 (59.0)	
Retained medical device upon ER visit[a]			
Yes	5 (6)	16 (4.5)	0.697
No	73 (94)	333 (95.5)	
Previous history of ER visit			
Yes	38 (49)	112 (32.0)	0.058
No	40 (51)	237 (68.0)	
History of past treatment[b]			
Yes	19 (24)	53 (15.0)	0.071
No	59 (76)	296 (85.0)	
Pervious history of MDR infection			
Yes	4 (5)	5 (1.5)	0.615
No	74 (95)	344 (98.5)	

[a]Nasogastric tube, permanent catheter, percutaneous transhepatic biliary drainage, Tenkoft catheter, Arteriovenous bridge graft (AVBG), Foley catheter, Biphasic positive airway pressure (Bipap).
[b]Hemodialysis, chemotherapy, radiation therapy, immunosuppressive drugs, post-operation within 1 week. ER, emergency room; MDR, multidrug resistance.

to amikacin and gentamicin, respectively. Non-susceptibility to 3rd generation cephalosporins in ESBL-producing *E. coli* isolates (55%-79%) was higher than that of *K. pneumoniae* (25%). Non-susceptibility to ceftazidime was 55% in *E. coli* isolates but no ESBL-producing *K. pneumoniae* isolates were resistant. However, 43% and 25%

Table 4
MICs of 10 antibiotics against ESBL-producing *E. coli* and *K. pneumoniae* determined using agar gradient diffusion.

Antibiotic	*E. coli* (n = 74)				*K. pneumoniae* (n = 4)			
	Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% non-susceptible[a]	Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% non-susceptible[a]
Cefepime	0.016 - > 256	8	> 256	43.2	0.023 - 24	0.032	24	25
Cefotaxime	0.023 - > 256	> 256	> 256	78.4	0.032 - > 256	0.047	> 256	25
Ceftriaxone	0.032 - > 256	> 256	> 256	79.8	0.047 - > 256	0.047	> 256	25
Ceftazidime	0.064 - > 256	6	32	55.4	0.094 - 1.5	0.125	1.5	0
Ciprofloxacin	0.003 - > 32	> 32	> 32	70.3	0.012 - > 32	0.016	> 32	50
Gentamicin	0.19 - > 256	48	> 256	62.2	0.19 - 0.75	0.38	0.75	0
Amikacin	1.5 - > 256	3	12	2.8	1.5 - 6	2	6	0
Imipenem	0.125 - 1.5	0.19	0.25	0	0.125 - 0.25	0.19	0.25	0
Meropenem	0.016 - 0.25	0.032	0.064	0	0.016 - 0.047	0.016	0.047	0
Ertapenem	0.008 - 1	0.064	0.38	0	0.012 - 0.047	0.016	0.047	0

[a]Intermediate and resistance.

of ESBL-producing *E. coli* and *K. pneumoniae* isolates, respectively were non-susceptible to cefepime, with MICs in the range of 0.016 - > 256 μg/ml. Non-susceptibilty to ciprofloxacin (a fluoroquinolone) was also high in both ESBL-producing *E. coli* (66%) and *K. pneumoniae* (50%) isolates. More than 78% of ESBL-producing *E. coli* isolates were highly resistant to cefotaxime and ceftriaxone (MIC_{50} and MIC_{90} > 256 μg/ml), and their MIC_{50} were much higher than those of ESBL-producing *K. pneumoniae* (MIC_{50} of 0.047 μg/ml).

Prevalence of β-lactamase genes

The majority of ESBL-producing *E. coli* isolates carried chromosomal (96%) and plasmid (88%) bla_{TEM}, as well as chromosomal (70%) and plasmid (73%) bla_{CTX-M}, but the prevalence of bla_{SHV} was lower (1% and 3% in chromosome and plasmid, respectively) (Table 5). Similar results were observed among the 4 isolates of ESBL-producing *K. pneumoniae.*

Amplicons of *bla* genes from 9 ESBL-producing *E. coli* isolates and 4 ESBL-producing *K. pneumoniae* isolates were sequenced. The bla_{TEM} belonged to TEM-1 (non-ESBL) and TEM-116 (ESBL) type (Table 6). Four isolates of ESBL-producing *E. coli* and 2 isolates of ESBL-producing *K. pneumoniae* carried bla_{TEM-1} on both chromosome and plasmid, whereas only one isolate of ESBL-producing *E. coli* harbored $bla_{TEM-116}$ on both chromosome and plasmid. Interestingly, one each of ESBL-producing *E. coli* and

Table 5
Chromosomal and plasmid bla_{SHV}, bla_{TEM} and bla_{CTX-M} of ESBL-producing *E. coli* and *K. pneumoniae*.

ESBL producer	*n*	Chromosome		Plasmid	
		n	%	*n*	%
E. coli	74				
bla_{SHV}		1	1	2	3
bla_{TEM}		71	96	65	89
bla_{CTX-M}		52	70	54	73
K. pneumoniae	4				
bla_{SHV}		1	25	2	50
bla_{TEM}		3	75	4	100
bla_{CTX-M}		2	50	1	25

K. pneumoniae isolates carried $bla_{TEM-116}$ on their chromosome and bla_{TEM-1} on their plasmids. On the other hand, 2 isolates of ESBL-producing *E. coli* and 1 isolate of *K. pneumoniae* possessed only bla_{TEM-1} on their plasmid.

Among the bla_{CTX-M} carrying *K. pneumoniae* and *E. coli*, 1 and 4 isolates, respectively harbored $bla_{CTX-M15}$ on both chromosome and plasmid. Surprisingly, 2 isolates of ESBL-producing *E. coli* carried a new type of bla_{CTX-M}, designated as $bla_{CTX-M161}$, on both chromosome and plasmid.

The less common bla_{SHV} was found in only one ESBL-producing *E. coli* isolate. The strain possessed $bla_{SHV-148}$ on both chromosome and plasmid. On the other hand, one ESBL-producing *K. pneumoniae* carried bla_{SHV-12} on both chromosome and plasmid, and one isolate carried bla_{SHV-28} only on plasmid. The sequences were deposited in GenBank (Table 6).

DISCUSSION

ESBL-producing *E. coli* is an increasing cause of community-acquired infection, especially of the urinary tract (UTI) (Pitout *et al*, 2005; Rodríguez-Baño *et al*, 2008). Hence, empirical treatment of community-acquired UTI with 3rd generation cephalosporins may not be as effective as it has been in certain geographic areas (Lin *et al*, 2011). Fecal carrier rate of CTX-M β-lactamase-producing Enterobacteriaceae (85% being *E. coli*) in Kanchanaburi Province, Thailand is as high as 58% of 141 healthy volunteers (age > 20 years) (Sasaki *et al*, 2010). In addition, Kanchanaburi, being the province with highest prevalence (50.6%) of CTX-M type ESBL-producing Enterobacteriaceae, also had the highest rate of antibiotic use (67.5%) and purchase without prescriptions (40.3%) (Luvsansharav *et al*, 2011). In this study, the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* from rectal swabs of 452 ER patients was much lower (16% and 1%, respectively), even though a significant portion of our patient population had a history of health care attendance, either as outpatient or had been hospitalized. The lower prevalence could be due to low recovery rate of bacteria from rectal swabs as compared to that obtained from stool samples. However, we do not have

Table 6
Distribution of the tested β-lactamase genes (accession number) among the selected ESBL- producing *E. coli* and four *K. pneumoniae* isolates.

	Chromosome			Plasmid		
	bla_{TEM}	bla_{SHV}	bla_{CTX-M}	bla_{TEM}	bla_{SHV}	bla_{CTX-M}
E. coli (9)						
E101	$bla_{TEM-116}$ (KM077047)	$bla_{SHV-148}$ (KJ815122)		$bla_{TEM-116}$ (KM077048)	$bla_{SHV-148}$ (KJ815123)	
E571	bla_{TEM-1} (KM077049)		$bla_{CTX-M-15}$ (KJ815105)	bla_{TEM-1} (KM077050)		$bla_{CTX-M-15}$ (KJ815110)
E420	bla_{TEM-1} (KM077051)		$bla_{CTX-M-15}$ (KJ815106)	bla_{TEM-1} (KM077052)		
E504	$bla_{TEM-116}$ (KM077053)		$bla_{CTX-M-15}$ (KJ815107)	bla_{TEM-1} (KM077054)		$bla_{CTX-M-15}$ (KJ815111)
E480	$bla_{TEM-116}$ (KM077055)		$bla_{CTX-M-15}$ (KJ815108)			$bla_{CTX-M-15}$ (KJ815112)
E536			$bla_{CTX-M-15}$ (KJ815109)	bla_{TEM-1} (KM077056)		$bla_{CTX-M-15}$ (KJ815113)
E462	bla_{TEM-1} (KM077057)		$bla_{CTX-M-161}$ (KJ815115)	bla_{TEM-1} (KM077058)		$bla_{CTX-M-161}$ (KJ815117)
E383	bla_{TEM-1} (KM077059)		$bla_{CTX-M-161}$ (KJ815116)	bla_{TEM-1} (KM077060)		$bla_{CTX-M-161}$ (KJ815118)
E455				bla_{TEM-1} (KM077061)		$bla_{CTX-M-15}$ (KJ815114)
K. pneumoniae (4)						
KP107	bla_{TEM-1} (KM077062)			bla_{TEM-1} (KM077063)		
KP296	$bla_{TEM-116}$ (KM077064)			bla_{TEM-1} (KM077065)	bla_{SHV-28} (KJ815124)	
KP339			$bla_{CTX-M-15}$ (KJ815119)	bla_{TEM-1} (KM077066)		
KP565	bla_{TEM-1} (KM077067)	bla_{SHV-12} (KJ815125)	$bla_{CTX-M-15}$ (KJ815120)	bla_{TEM-1} (KM077068)	bla_{SHV-12} (KJ815126)	$bla_{CTX-M-15}$ (KJ815121)

data of self-prescribed antibiotic in the Bangkok Metropolitan area, which may affect the baseline prevalence of resistant organisms in the community, although it is expected to be high.

More than 75% of ESBL-producing *E. coli* isolates were non-susceptible to cefotaxime and ceftriaxone, and about 50% were non-susceptible to ceftazidime and cefepime. This is a worrying trend as ceftriaxone and cefotaxime are two of the most commonly prescribed antibiotics in ER (Phuphuakrat *et al*, 2013).

We found that 20.5% of *E. coli* isolates harboring *bla* genes were still susceptible to all tested cephalosporins (cefotaxime, ceftazidime, ceftriaxone and cefepime). Similar observations have been reported in a previous study of fecal carriage of ESBL-producing Enterobacteriaceae in

healthy Thais (Sasaki *et al*, 2010). However, in the latter report 5% of non-ESBL-producing bacteria, as determined by double-disk synergy test, harbor bla_{CTX-M}, which could possibly produce more ESBLs upon subsequent exposure to the antibiotics. These false-positive antibiotic-susceptible results could mislead clinicians to treat such patients with drugs that may result in treatment failure (Paterson *et al*, 2001). However, more clinical data are needed to clarify this issue, which has important clinical implications.

A high proportion of ESBL-producing *E. coli* that were multidrug resistant (MDR) (to cefotaxime, ceftriaxone, and cefepime) and co-resistant to gentamicin and ciprofloxacin. MDR phenotypes are common among bacteria ESBL-producers because the genes encoding ESBL are found frequently on the same plasmids with resistance genes to aminoglycosides, sulfonamides and quinolones (Jacoby, 1994; Pitout and Laupland, 2008). Colonization of these MDR ESBL-producing bacterial strains in the gut of patients could potentially cause unrecognized dissemination in the communities and hospitals.

Multivariate analysis of risk factors associated with ESBL fecal carriage in this study indicated that patients with a previous history of hospital admission and ER visits in the previous three months were at greater risk for ESBL fecal carriage ($p < 0.001$). These findings are similar to other studies (Kusum *et al*, 2004; Udomsantisuk *et al*, 2011; Lonchel *et al*, 2012; Ko *et al*, 2013), and it is postulated that frequent hospital admissions lead to increase exposure to ESBL-producers, which colonized and infected the patients admitted in the hospital especially in ICUs. Another study on the risk of ESBL-producing *E. coli* septicemia at a university hospital in northeastern Thailand showed that hospital acquisition, use of central venous line and previous use of fluoroquinolone are independent risk factors for acquisition of such organisms (Anunnatsiri *et al*, 2012). Other factors reported to contribute to the risk of transmission in ER included crowded conditions and pediatric beds shared with ESBL carriers (Isendahl *et al*, 2012). However, there is no significant difference in antibiotic exposure between patients with and without ESBL-producers in our study.

As expected chromosomal bla_{TEM} was found in nearly all (95%) ESBL-producing isolates. Among 13 randomly selected samples, the majority possessed bla_{TEM-1}, except 4 isolates that produced TEM-116, which is similar to other studies (Jeong et al, 2004; Miró *et al*, 2005; Lin *et al*, 2006; Bell *et al*, 2007; Dropa *et al*, 2010; Sasaki *et al*, 2010; Castanheira *et al*, 2013). TEM-1 β-lactamase has a limited spectrum to penicillins and early cephalosporins, whereas its variant TEM-116 confers resistance to penicillins, cephalosporins and monobactams (Du Bois *et al*, 1995).

Among the bla_{CTX-M} types, $bla_{CTX-M-15}$ was identified with the highest proportion (56%) in both chromosome and plasmid. This suggested that there might be a wider dissemination of this gene compared to other types in the future. We also found a new derivative of $bla_{CTX-M-27}$ among the patients' specimens, designated $bla_{CTX-M-161}$. The $bla_{CTX-M-161}$ showed high identity (99%) with that of $bla_{CTX-M-27}$ (AY196523) except for two nucleotide mutations (G781A, G789C). The mutation of nucleotide 781 (according to the numbering system of AY196523) led to the substitution of valine to isoleucine, and the mutation at position 789 was a silent mutation. Changes to functional properties need to be studied further.

Commensal microorganisms can play a role in dissemination of resistance genes to pathogenic bacteria. It is accepted that most plasmids encoding β-lactamase are derived from chromosomally located genes of commensal or environmental microorganisms (Vignoli *et al*, 2005). Therefore, persistence of ESBL genes among commensal *E. coli* has greatly affected public health policy with regards to the evolution of antibiotic resistance.

Fecal carriage of ESBL-producing *K. pneumoniae* is associated with ESBL-producing *K. pneumoniae* infection (odds ratio of 3.4) among patients admitted in ICU (Pena *et al,* 1998). Only four isolates of ESBL-producing *K. pneumoniae* were detected from rectal swabs in the present study. In contrast to *E. coli*, ESBL-producing *K. pneumoniae*, especially those harboring bla_{SHV-12}, was prevalent in the clinical isolates as a cause of nosocomial infection. Improvement of infection control measures has decreased the incidence of ESBL-producing *K. pneumoniae* infection (Paterson and Bonomo, 2005).

In conclusion, *bla* genes were detected in ESBL-producing *E. coli* and *K. pneumoniae* obtained from rectal swabs of ER patients. Thus, ESBL-fecal carriers in an ER setting should be the target of preventive measures to minimize further spread of these life-threatening Enterobacteriaceae.

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