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## Extended-Spectrum $\beta$ -Lactamases-Producing *Escherichia coli* from a Tertiary Hospital in Malaysia: Emergence of CTX-M-Type $\beta$ -Lactamases Variation

Zamberi Sekawi, Rusmah Yusof and Mariana Nor Shamsudin  
Department of Medical Microbiology and Parasitology,  
Faculty of Medicine and Health Sciences,  
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor DE, Malaysia

**Abstract:** A study was conducted to portray a preliminary characteristic of extended-spectrum  $\beta$ -lactamases (ESBLs)-producing *Escherichia coli* in a local tertiary hospital in Malaysia. Sixteen clinical isolates of ESBLs producing *E. coli* from different sources were examined for  $bla_{SHV/TEM/CTX-M}$  ESBL genes by PCR molecular assay. Each strain was found to carry at least one of the genes. This study demonstrated a high prevalence of  $bla_{CTX-M}$  (81.3%) and  $bla_{TEM}$  genes (75%). Only two strains (12.5%) carried the  $bla_{SHV}$  gene. Nucleotide and deduced protein sequences determination showed; 61% produced CTX-M-15, 31% produced CTX-M-14 and 8% produced CTX-M-3. The antimicrobial susceptibility testing data determined that almost all sixteen isolates were resistant to oxyimino-cephalosporins, 46% resistant to gentamicin, 69% resistant to trimethoprim-sulfamethoxazole and 46% resistant to ciprofloxacin. This study preliminary emphasizes the epidemiology of the ESBL-producing *E. coli* particularly SHV, TEM and CTX-M-type producing *E. coli* in Malaysia.

**Key words:** TEM, SHV, CTX-M, ESBL, *E. coli*, Malaysia

### INTRODUCTION

The increasing resistance of gram negative bacteria is of worldwide concern. Its rapid evolution to become resistant is an immense challenge to clinicians, scientists and researchers worldwide. Despite many new  $\beta$ -lactam antibiotics being developed over the last 20 years, new  $\beta$ -lactamases emerged, causing them to be resistant to these antibiotics (Bradford, 2001). This is presumably due to overuse of new antibiotics resulting in selective pressure to select new variants of  $\beta$ -lactamases. Its infection has now become even more difficult to treat as its resistance level has increased over the years. Now with the threat of the so-called ESBL-producing pathogens, it looks like we are fighting a losing battle.

Extended spectrum  $\beta$ -lactamase or ESBL is a  $\beta$ -lactamase enzyme with increased spectrum of activity especially against the oxyimino-cephalosporins such as cefotaxime and ceftazidime. Most ESBLs are derivatives of TEM or SHV enzymes (Bush *et al.*, 1995). There are now >150 TEM-type  $\beta$ -lactamases and >100 SHV-type enzymes ([www.lahey.org/studies/webt.htm](http://www.lahey.org/studies/webt.htm)). They are easily transmissible from one organism to another. Hence, SHV-and TEM-types which have been recognized since the 1980s are now a major cause of nosocomial infections all over the world.

In Malaysia, a national surveillance on antibiotic resistance was conducted in 2005 and found that ceftazidime and cefotaxime resistance in *E. coli* was 8.9% (0.9% from the community) and 10.1% (1.0% from the community) respectively (unpublished data). Tests for ESBL production was

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**Corresponding Author:** Zamberi Sekawi, Department of Medical Microbiology and Parasitology,  
Faculty of Medicine and Health Sciences, Universiti Putra Malaysia,  
43400 UPM Serdang, Selangor DE, Malaysia

unfortunately not performed routinely in this survey. Another study done in a university hospital in Malaysia reported *bla<sub>SHV-5</sub>* to be dominant in ESBL-producing *E. coli* and was postulated to have been acquired from ESBL-producing *Klebsiella pneumoniae* via plasmid transfer (Subramaniam *et al.*, 2006).

Another family of plasmid-mediated ESBLs which are non-TEM and non-SHV derivatives, called CTX-M has emerged. They preferentially hydrolyze cefotaxime and have mainly found in wide range of bacteria, especially members of the *Enterobacteriaceae* family (Bonnet, 2004). Strains expressing CTX-M-type  $\beta$ -lactamases have been isolated from many parts of the world. Based on communications with various experts in many local centres, CTX-M-type is also available in Malaysia and it poses a potential threat in healthcare management. Realizing the lack of published local research on this issue, the aim of this study is to demonstrate the emergence of CTX-M-type in ESBL-producing *E. coli* in a tertiary hospital in Malaysia.

## MATERIALS AND METHODS

### Bacterial Strains

Sixteen non-duplicate isolates of validated ESBL-producing *E. coli* from multiple types of specimens collected from a tertiary hospital in Malaysia in 2006 were analyzed in this study.

### $\beta$ -Lactamase Gene Identification

Chromosomal DNA was prepared from an overnight bacterial culture (18-24 h) on a MacConkey agar plate. Approximately ten colonies were re-suspended into 50  $\mu$ L sterile distilled water and the cells were lysed by boiling for 15 min, then immediately cooled on ice for another 2 min. Cellular debris were removed by centrifugation at 10,000 g for 1 min and the supernatant was used as a source of template DNA for amplification of *bla<sub>TEM</sub>* and *bla<sub>SHV</sub>* genes.

Plasmid DNA was prepared by using a commercial kit (Invitrogen, BioDiagnostic, USA). The plasmid DNA was used as a source of template DNA for amplification of *bla<sub>CTX-M</sub>* gene.

PCR primers used for amplification of CTX-M types were universal primers of CTX-MA1 (5'-SCS ATG TGC AGY ACC AGT AA-3') and CTX-MA2 (5'-CCG CRA TAT GRT TGG TG-3') described by Saladin *et al.* (2002) yielding a 544 bp amplicon (S = G or C; Y = C or T; R = A or G). The primers used for PCR amplification of *bla<sub>TEM</sub>* and *bla<sub>SHV</sub>* were mentioned previously by Perilli *et al.* (2002) yielding amplicons of 1080 and 1050 bp in size, respectively.

### PCR Amplification

The cycling condition for the amplification of *bla<sub>TEM</sub>* and *bla<sub>SHV</sub>* were described previously by Perilli *et al.* (2002) and the DNA amplification steps for *bla<sub>CTX-M</sub>* were as follows: initial denaturation at 94°C for 4 min followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 57.6°C for 1 min and elongation at 72°C for 1 min with final extension at 72°C for 4 min. All reactions were performed in a 25  $\mu$ L volume using 6 units of Taq DNA polymerase (Bioron, Germany), 2.5  $\mu$ L of 10 $\times$  PCR buffer, 2 mM MgCl<sub>2</sub>, 0.2 mM deoxynucleoside triphosphates, 0.3  $\mu$ L of 10 pmol mL<sup>-1</sup> primers each and 1  $\mu$ L DNA template. The PCR reactions were performed using PCR Thermal Cycler (Biometra-TPersonal, Germany). All negative reactions were repeated twice.

### DNA Sequencing

All purified PCR product were sent for commercial sequencing (1st Base Laboratories Sdn. Bhd., Malaysia). The sequences were edited and analyzed by using DNAsis for Windows 2.6 (Hitachi Software Engineering, USA). The nucleotide and the deduced protein sequences were analyzed by using the software available online at the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov>).

### Control Strains

The quality control strains used were *E. coli* ATCC 25922 (sensitive strain), *K. pneumoniae* ATCC 700603 (*bla*<sub>SHV-12</sub>), *E. coli* 13-00 (*bla*<sub>CTX-M-14</sub>), *E. coli* 78-01 (*bla*<sub>CTX-M-15</sub>) and *E. coli* 188-04 (*bla*<sub>CTX-M-2</sub>).

## RESULTS AND DISCUSSION

Sixteen clinical isolates of ESBLs-producing *E. coli* carried at least one of the *bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub>, or *bla*<sub>SHV</sub> ESBL genes (Table 1). A vast majority of the strains carried *bla*<sub>CTX-M</sub> (81.3%) and *bla*<sub>TEM</sub> genes (75%). Only two strains (12.5%) carried the *bla*<sub>SHV</sub> gene. There were at least three circulating strains of which CTX-M-15 being the most dominant (61%), followed by CTX-M-14 (31%) and CTX-M-3 (8%).

Almost all strains demonstrated resistance to the second, third and fourth generation of cephalosporins (Table 2). There were more resistance to cefotaxime than ceftazidime among CTX-M-types, phenotypically supporting the presence of CTX-M enzymes.

It is clearly shown that at least in this study, CTX-M-types have been the dominant strains. This is in parallel to the worldwide trend where CTX-M-types have overtaken dominance of TEM- and SHV-types globally. Though prior data on the prevalence of TEM-types and SHV-types are not known in Malaysia to demonstrate this fact, we believe similar trend is valid. It is difficult to determine the proportion of our strains which originated from the community as these patients were admitted to the hospital where medical records access is restricted owing to confidentiality and security issues.

In our study, we found at least three circulating strains of which CTX-M-15 being the most dominant (61%), followed by CTX-M-14 (31%) and CTX-M-3 (8%). CTX-M-15 and CTX-M-3 belong to the CTX-M-1 group while CTX-M-14 to the CTX-M-9 group (Bush *et al.*, 1995). Based on this finding, we agree to the hypothesis that states the current spread of CTX-M enzymes, is allodemic in nature, rather than an epidemic pattern, reflecting that the increase of CTX-M enzymes is a result of both multiple specific clones and/ or mobile genetic elements and not dissemination of a particular clone (Baquero *et al.*, 2002). Owing to the dynamic nature of these clones, it is not surprising if the dominant strains would change over time (Bonnet, 2004).

Table 1: The  $\beta$ -lactamase types of ESBL-producing *Escherichia coli*

ESBL <i>E. coli</i> strain	Source	Bla genes		
		TEM	SHV	CTX-M (Sequenced $\beta$ -lactamase)
E001	Pus	+	-	+(CTX-M-15)
E005	Blood	+	-	+(CTX-M-3)
E008	Body fluid	+	+	-
E011	Urine	-	-	+(CTX-M-15)
E014	Blood	+	-	-
E020	Urine	-	-	+(CTX-M-15)
E023	Urine	-	-	+(CTX-M-15)
E027	Pus	+	-	+(CTX-M-14)
E029	Pus	+	-	+(CTX-M-15)
E031	Urine	+	-	+(CTX-M-15)
E043	Blood	+	-	+(CTX-M-14)
E051	Pus	+	-	+(CTX-M-15)
E052	PD fluid	-	-	+(CTX-M-15)
E066	Body fluid	+	-	+(CTX-M-14)
E067	Body fluid	+	-	+(CTX-M-14)
E068	Body fluid	+	+	-
Total		12	2	13

+: Present; -: Absent

Table 2: Antibiotic resistant pattern of sixteen ESBL-producing *Escherichia coli*

ESBL-producing <i>E. coli</i> strain	Interpretation of antibiotic (Disc diffusion method by CLSI guideline)*									
	CXM	CFP	CAZ	CTX	FEP	GN	SXT	CIP	IMP	MEN
E001	R	R	R	R	R	S	R	R	S	S
E005	R	R	R	R	R	S	R	S	S	S
E008	R	R	R	R	R	S	R	R	S	S
E011	R	-	R	R	-	R	S	R	S	S
E014	R	R	R	R	R	S	R	R	S	S
E020	R	-	S	R	-	R	R	R	S	S
E023	R	-	R	R	R	R	R	R	S	S
E027	R	R	S	I	-	R	R	R	S	-
E029	R	R	S	R	-	S	S	S	S	-
E031	R	-	R	R	R	S	R	S	S	S
E043	R	R	R	R	R	S	R	S	S	S
E051	R	R	R	R	R	S	S	S	-	S
E052	R	R	R	R	R	R	S	S	S	S
E066	R	R	I	R	R	R	R	R	R	S
E067	R	R	R	R	R	S	R	S	S	S
E068	R	R	R	R	R	S	R	R	S	S

\*CXM: Cefuroxime; CFP: Cefoperazone; CAZ: Ceftazidime; CTX: Cefotaxime; FEP: Cefepime; GN: Gentamicin; SXT: Trimethoprim-sulfamethoxazole; CIP: Ciprofloxacin; IMP: Imipenem; MEM: Meropenem; S: Susceptible; R: Resistance; I: Intermediate; -: Absent

Several studies have shown an increasing association with resistance to other classes of antimicrobial agents among ESBL-producing organisms isolated from the community (Pitout *et al.*, 2005). This includes co-resistant with trimethoprim-sulfamethoxazole, tetracycline, gentamicin and ciprofloxacin. This association may be evident in this study as 46% of the strain was resistant to gentamicin, 69% resistant to trimethoprim-sulfamethoxazole and 46% resistant to ciprofloxacin.

In order to further understand the role of ESBL to healthcare, more extensive studies will be conducted. These include linking CTX-M-types with *E. coli* phylogenetic group. We certainly would like to know this association with our strains, especially the dominant strains. CTX-M-types have been reported to be associated with groups D and B2, both groups of which are mainly associated with extraintestinal infections (Cantun and Conque, 2006). Virulence markers in our local CTX-M-producing *E. coli* would be investigated. It was reported that there were significant differences from individual virulence factors among CTX-M producers and non-producers; however, aggregate virulence factor scores were similar (Pitout *et al.*, 2007).

A comprehensive surveillance study is in the pipeline which, in our opinion will provide a good overview and more accurate picture of the problem of ESBL in our country.

The increasing evidence of CTX-M-producing bacteria in food-producing bacteria such as cattle and poultry and pets, namely, dogs and cats is worrying (Pitout *et al.*, 2005). This may act as reservoir for the acquisition of resistant organism which will make the quest for winning this 'battle' an uphill task. It is therefore, necessary for us to study this situation in our region.

In conclusion, ESBL is indeed a significant problem in our clinical settings in this tertiary hospital and therefore, it is not incorrect to postulate this problem as also a national issue. We have succeeded in determining the group type of the enzymes and in this preliminary study, the epidemiology of the enzymes seem to give a slightly clearer picture of the impact of ESBL in Malaysia. However, it is still premature to draw conclusions based on the existing data.

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## REFERENCES

- Baquero, F., T.M. Coque and R. Cantón, 2002. Allodemics. *Lancet Infect.*, 2 (10): 591-592.
- Bonnet, R., 2004. Growing group of extended-spectrum  $\beta$ -lactamases: The CTX-M enzymes. *Antimicrob. Agents Chemother.*, 48 (1): 1-14.
- Bradford, P.A., 2001. Extended-spectrum  $\beta$ -Lactamases in the 21st Century: Characterization, epidemiology and detection of this important resistance. *Threat. Clin. Microbiol. Rev.*, 14 (4): 933-951.
- Bush, K., G.A. Jacoby and Medeiros, 1995. A functional classification scheme for  $\beta$ -lactamases and its correlation with molecular structure. *Antimicrob. Agents Chemother.*, 39 (6): 1211-1233.
- Cantün, R. and T.M. Conque, 2006. The CTX-M  $\beta$ -Lactamase pandemic. *Curr. Opin. Microbiol.*, 9 (5): 466-475.
- Perilli, M., E. Dell'Amico, B. Segatore, M.R. De Massis, C. Bianchi, F. Luzzaro, G.M. Rossolini, A. Toniolo, G. Nicoletti and G. Amicosante, 2002. Molecular characterization of extended-spectrum  $\beta$ -lactamases produced by nosocomial isolates of enterobacteriaceae from an Italian nationwide survey. *J. Clin. Microbiol.*, 40 (2): 611-614.
- Pitout, J.D., K.B. Laupland, D.L. Church, M.L. Menard and J.R. Johnson, 2005. Virulence factors of *Escherichia coli* isolates that produce CTX-M-type extended-spectrum  $\beta$ -lactamases. *Antimicrob. Agents Chemother.*, 49 (11): 4667-4670.
- Pitout, J.D., D.L. Church, D.B. Gregson, B.L. Chow, M. McCracken, M.R. Mulvey and K.B. Laupland, 2007. Molecular epidemiology of CTX-M-producing *Escherichia coli* in the calgary health region: Emergence of CTX-M-15-producing isolates. *Antimicrob. Agents Chemother.*, 51 (4): 1281-1286.
- Saladin, M., V.T. Bao Cao, T. Lambert, J.L. Donay, J.L. Herrmann, Z.O. Hocine, C. Verdet, F. Delisle, A. Philippon and G. Arlet, 2002. Diversity of CTX-M  $\beta$ -lactamases and their promoter regions from *Enterobacteriaceae* isolated in the three Parisian hospitals. *FEMS. Microbiol. Lett.*, 209 (2): 161-168.
- Subramaniam, G., S. Palasubramaniam and P. Navaratnam, 2006. SHV-5 Extended-Spectrum  $\beta$ -Lactamases in clinical isolates of *Escherichia coli* in Malaysia. *Indian J. Med. Microbiol.*, 24 (3): 205-207.