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RESEARCH ARTICLE

Occurrence and Characteristics of the Extended-spectrum Beta-lactamase-producing *Enterobacterale* in a Hospital Setting

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Abstract:

Objectives:

Infectious diseases caused by ESBL-producing *Enterobacterale* are an emerging problem worldwide, which increase hospital costs, empirical treatment failure, together with rates of morbidity and mortality. The aims of this study were to determine the antibiotic-resistant patterns and the frequency of *bla*TEM, *bla*CTX-M and *bla*SHV genes among *Enterobacterale*.

Methods:

A total of 239 non-repeated clinical isolates of the *Enterobacterale* family, including 202 (84.5%) *Escherichia coli* and *Klebsiella pneumoniae* 25 (12.3%) were collected. Identification and susceptibility tests were carried out on 60 (25.9%) ESBL phenotypes using biomérieux VITEK[®] 2 compact system. Isolates showing ESBL positivity by the phenotypic method were all screened for *bla*TEM, *bla*CTX-M and *bla*SHV genes by PCR.

Results:

The prevalence of ESBL-producing *Enterobacterale* isolates was found to be 25.9%. These ESBL-producing isolates displayed an increasing rate of resistance for aminopenicillins (ampicillin) (96.3%), followed by piperacillin-tazobactam (54.2%), amoxicillin/clavulanic acid (45.8), the cephalosporin groups, ceftriaxone (52.4%), ceftazidime (46.7%), and fluoroquinolone (ciprofloxacin) (42.7%). Both the organisms showed a higher susceptibility to the carbapenems (Imipenem) and aminoglycosides (Amikacin). Out of the 62 ESBL positive isolates, 11 (17.7%) carried the TEM gene, 22 (35.5%) carried the CTX-M gene alone, 8 (12.9%) carried both TEM and CTX-M genes, 1 (1.6%) carried both SHV and CTX-M genes, and 20 (32.3%) carried the TEM, SHV, and CTX-M genes. SHV gene alone was not reported in any of the isolates.

Conclusion:

The study indicated a moderate occurrence of ESBL-producing *Enterobacterale* with CTX-M being the most dominant gene. The co-existence of all three genes on many occasions suggested the carriage of multiple plasmids with three resistance genes that might pose a serious epidemiological, clinical and public health threat.

Keywords: *Enterobacterale*, *Bla*TEM gene, *Bla*CTX-M gene, *Bla*SHV gene, ESBL gene, Aminopenicillins (ampicillin).

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1. INTRODUCTION

Extended-Spectrum Beta-Lactamases (ESBLs) are rapidly evolving plasmid-mediated enzymes produced by certain bacteria that can hydrolyze extended-spectrum cephalosporin, thus making them effective against beta-lactam antibiotics [1, 2]. ESBLs are found in gram-negative bacteria, especially in

Enterobacterale species [1], the main bacterial family associated with ESBL production, of which *Escherichia coli* and *klebsiella pneumoniae* are the most important. These organisms are of medical importance, causing infections in the various body systems. The dissemination of ESBL-producing multi-drug resistant *Enterobacterale* has resulted in increased bacterial resistance to antibiotics in healthcare settings and in the community worldwide [3].

The plasmid-mediated lactamase-producing isolates of the family *Enterobacterale* mainly possess the *bla*_{TEM} gene which can hydrolyze penicillin and first-generation cephalosporins

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and the *bla*_{CTX-M} gene that preferentially hydrolyze cefotaxime [4]. Other types of ESBLs genes include SHV, which is responsible for plasmid-mediated ampicillin resistance. The majority of the ESBLs are derivatives of TEM or SHV enzymes, which are most often found in the enterobacteria *E. coli* and *K. pneumoniae* [5].

Because of the global spread of CTX-M enzyme, ESBL are the cause of increasing numbers of both nosocomial and community infections [6]. Rates of ESBL production among *Enterobacterale* range between 6-38.5% in the Kingdom of Saudi Arabia [7, 8]. ESBL producing *Enterobacterale* in Saudi Arabia is dominated by *E. coli* where both hospital and community-based studies have shown that more than 70% harbored the gene *bla*_{CTM-X} [9].

Detection of these genes in ESBL-producing bacteria by the most sensitive molecular methods can give valuable information about its epidemiology and help in the reasonable treatment of infections [10].

In Saudi Arabia, few studies have been performed to determine the prevalence of ESBL-producing *Enterobacterale* and little is known about their molecular characteristics. However, the few studies conducted on ESBL phenotypes isolated from clinical samples previously indicated a predominance of *bla*_{CTX-M}-like genes [8, 9, 11, 12]. Therefore, the aims of this study were to determine the prevalence of ESBL-producing *Enterobacterale* in a hospital setting from various clinical specimens taken from patients diagnosed with various infections and to detect the frequency of TEM, SHV, and CTX-M genes in an ever-changing prevalence and resistant pattern of ESBL-producing enterobacteria.

2. MATERIALS AND METHODS

The ethical committee of Prince Sultan Military College of Health Science approved this study. This retrospective study included 239 isolates diagnosed with culture-positive *Enterobacterale* at the Medical Laboratory of King Fahad Military Medical Complex, Dhahran, from various clinical specimens from March 2014 to June 2014. Isolates were then received at the Microbiology Laboratory of Prince Sultan Military College of Health Sciences (PSMCHS) where identification and susceptibility tests were carried out using biomérieux VITEK® 2 compact system (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions (2009 FDA breakpoints).

Gram-negative (GN) cards and antibiotic susceptibility cards (AST) were used for the identification and the antibiotic sensitivity profile of the isolates, respectively. Antimicrobial susceptibility testing was performed using software version 06.01 and the AST-GN291 and AST-XN06 cards, according to the manufacturer's instructions. Susceptibility cards used included aminoglycosides (amikacin, gentamicin); aminopenicillins (ampicillin); cephalosporin (cefepime, ceftazidime, ceftriaxone, cefuroxime); carbapenems (imipenem); amoxicillin/clavulanate (augmentin); trimethoprim/sulfamethoxazole (Bactrim); fluoroquinolone (ciprofloxacin); and piperacillin-tazobactam. Identification and susceptibility cards were inoculated and interpreted according to the manufacturer's

instructions. The quality control strains tested with each run included the ESBL-producing strains *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 for the antimicrobial identification and susceptibility testing.

Out of 239 non-repeated isolates, 62 were ESBL producers, which were further tested for the detection of ESBL genes in the PSMCHS Molecular Biology Laboratory. DNA was extracted using the method previously described [13]. The PCR mixture was made using QIAGEN Fast Cycling PCR Kit and the primers, which were designed and procured by the Eurofins Company (Eurofins Advantur Inc, San Diego USA). PCR was performed using CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc. USA). SHV, TEM, and CTX-M primers were used on all of the 62 ESBL-producing *Enterobacterale* isolates (Table 1). The following PCR protocol was used for all primers as previously described [11]: initial denaturation (hot start) at 95°C for 5 minutes, followed by 30 cycles of denaturation, annealing, and extension at 92°C, 61°C, and 72°C, respectively, for 30 seconds each. The mixture was then incubated for final elongation at 72°C for 5 minutes.

PCR products were then separated on 2% agarose electrophoresis gel and visualized using Syngene U Genius Gel Imaging System (Cambridge Scientific, Watertown, Massachusetts) for the presence of 403 bp, 293 bp, and 569 bp bands representing the TEM, SHV, and CTX-M primers, respectively.

3. RESULTS

A total of 239 non-repeated multi-resistant clinical isolates of the *Enterobacterale* family, including *Escherichia coli* 202 (84.5%) and *Klebsiella pneumoniae* 25 (10.5%), while 12 (5%) were identified as *Proteus mirabilis* and *Citrobacter diversus*. Out of the total *Enterobacterale* isolates recovered, 62 or 25.9% were ESBL phenotypes. *E. coli*, and *K. pneumoniae* represented by 48 (77.4%), and 12 (19.4%), respectively. Most of the isolates were recovered from urine specimen 198 (70.3%), followed by wound swabs being 23 (9.6%), vaginal swab being 10 (4.2%), sputum being 4 (1.7%), and tissue being 4 (1.7%). (Table 2).

ESBL producers demonstrated the highest resistance rates to ampicillin (96.3%) followed by piperacillin/tazobactam (54.2%), ceftriaxone (52.4%), ceftazidime (46.7%), amoxicillin/clavulanic acid (45.8), and ciprofloxacin (42.7%). *E. coli* was the most resistance to ampicillin (96.5%), cefepime (56.4%), ceftriaxone (50.0%), ceftazidime (48.5%), piperacillin/tazobactam (46.0%), amoxicillin/Clavulanate (43.6%), and ciprofloxacin (41.6%). *K. pneumoniae* was resistant to ampicillin (92.0%), amoxicillin/clavulanate (64.0%), ciprofloxacin (52%), cefepime (52.0%), and piperacillin/tazobactam (44.0%). ESBL producers showed a higher susceptibility to imipenem and amikacin as the resistance rate was 2.2% and 7.9%, respectively. The resistance pattern is almost similar in both organisms. However, *E. coli* was more resistant to ceftriaxone and ceftazidime than *K. pneumoniae*. On the other hand, *K. pneumoniae* showed more resistance to amoxicillin/clavulanic than *E. coli* (Table 3, Fig. 1).

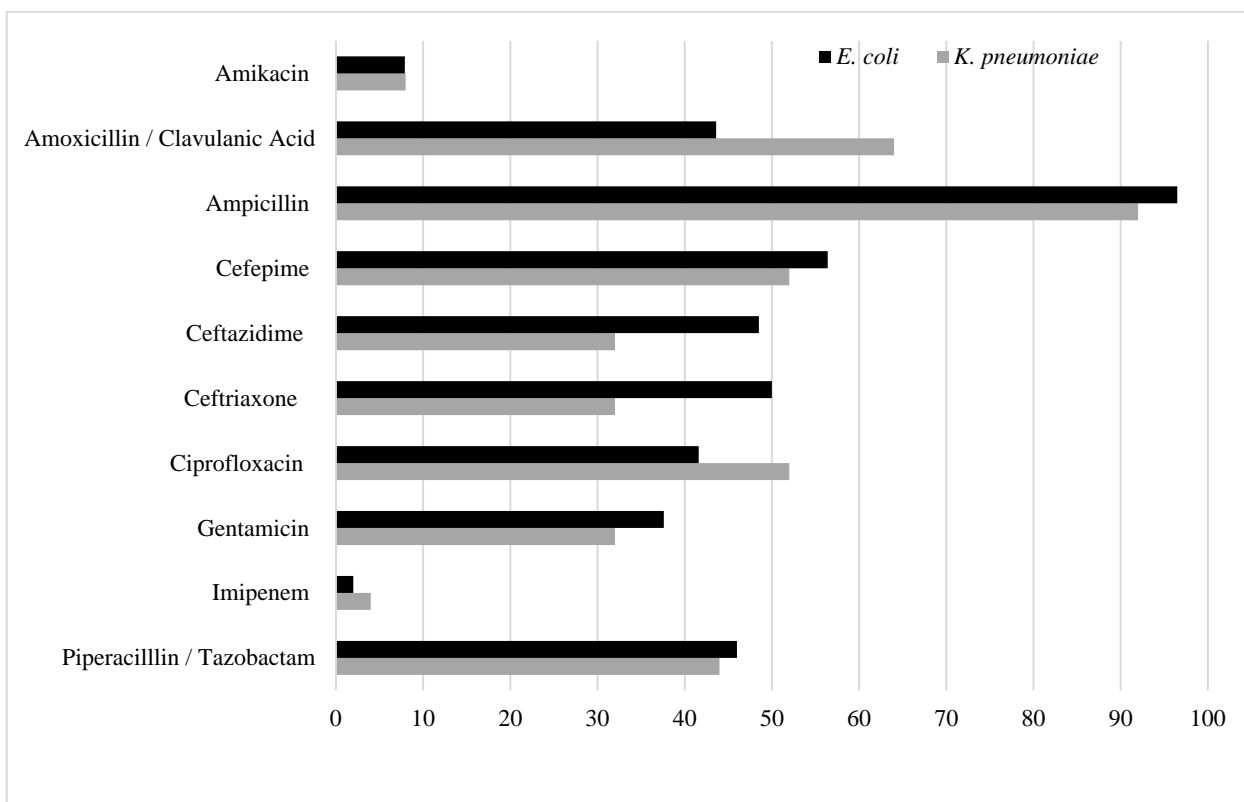


Fig. (1). Antibiotic sensitivity profile (resistance %) of *E. coli* and *K. pneumoniae*.

Out of the 62 ESBL positive isolates, 11 (17.7%) carried the TEM gene, 22 (35.5%) carried the CTX-M gene alone, 8 (12.9%) carried both TEM and CTX-M genes, 1(1.6%) carried

both SHV and CTX-M genes, and 20 (32.3%) carried the TEM, SHV, and CTX-M genes. SHV gene alone was not reported in any of the isolates. The results indicated a Predominance of CTX-M gene among ESBL isolates (Table 4).

Table 1. Oligonucleotide primers used for detection of beta-lactamase genes.

Primers	Forward Primers 5'-3'	Reverse Primers 3' - 5'	Reference
SHV	AGGATTGACTGCCTTTTGG	CGAGTAGTCCACCAGATCCT	EF125011
TEM	TTTCGTGTCGCCCTTATCC	ATCGTTGTCAGAAGTAAGTTGG	AB282997
CTX-M	CGCTGTTGTTAGGAAGTGTG	GGCTGGGTGAAGTAAGTGAC	DQ303459

Table 2. Total multi-drug resistant *Enterobacterale* and the number ESBL-producing isolates and the percentage (in parenthesis) according to the site of isolation.

ESBL genotype	Number of isolates (%)							
	<i>E. coli</i>		<i>k. pneumoniae</i>		Others		Total	
	MRD	ESBL	MRD	ESBL	MRD	ESBL	MRD	ESBL
Urine	168	40 (23.8)	18	9 (50.0)	12	2 (16.7)	198	51 (25.8)
Wound swab	18	4 (22.2)	5	2 (40.0)	0	0 (0.0)	23	6 (26.1)
Vaginal swab	10	2 (20.0)	0	0 (0.0)	0	0 (0.0)	10	2 (20.0)
Sputum	4	1 (25.0)	0	0 (0.0)	0	0 (0.0)	4	1 (25.0)
Tissue	2	1 (50.0)	2	1 (50.0)	0	0 (0.0)	4	2 (50.0)
Total	202	48 (23.8)	25	12 (48.0)	12	2 (16.7)	239	62 (25.9)

MDR = Total multi-drug resistant isolates.
 ESBL = Extended spectrum beta-lactamases isolates.

Table 3. Number of resistant ESBL-producing *Enterobacterale* isolates and the percentage (in parenthesis).

Antibiotic (Class)	MIC range mg/L	<i>E. coli</i> n =202	<i>K. pneumoniae</i> n =25	Total n =227
Amikacin	8 – 64	16 (7.9)	2 (8.0)	18 (7.9)
Amoxicillin/Clavulanic acid	4/2 – 32/16	88 (43.6)	16 (64.0)	104 (45.8)
Ampicillin	4 – 32	195 (96.5)	23 (92.0)	206 (96.3)
Cefepime	2 – 32	114 (56.4)	13 (52.0)	58 (27.1)
Ceftazidime	1 – 32	98 (48.5)	8 (32.0)	106 (46.7)
Ceftriaxone	1 – 32	101 (50.0)	8 (32.0)	119 (52.4)
Ciprofloxacin	0.5 – 4	84 (41.6)	13 (52.0)	97 (42.7)
Gentamicin	4 – 32	76 (37.6)	8 (32.0)	84 (37.0)
Imipenem	1 – 12	4 (2.0)	1 (4.0)	5 (2.2)
Piperacillin/Tazobactam	2/4 – 48/8	93 (46.0)	11 (44.0)	104 (54.2)

Table 4. Distribution of TEM, SHV, and CTX-M genes in *Enterobacterale* isolates (n = 62).

ESBL genotype	Number of isolates (%)		Total
	<i>E. coli</i>	<i>K. pneumoniae</i>	
TEM	7 (13.0)	4 (57.1)	11(17.7)
CTX-M	20 (37.0)	2 (28.6)	22 (35.5)
TEM and CTX-M	8 (14.8)	0 (00)	8 (12.9)
SHV and CTX-M	0 (00)	1 (14.3)	1 (1.6)
TEM, SHV, and CTX-M	19 (35.2)	1 (14.3)	20 (32.3)
Total	54 (100)	7 (100)	62 (100)

4. DISCUSSION

Enterobacterale are among the most important causes of nosocomial and community-acquired infections. Infections caused by these microorganisms are mainly treated with beta-lactams and fluoroquinolones [6]. However, resistance to these compounds is being reported more frequently [14].

The current findings have detected a high occurrence of ESBLs (25.9%) among the *Enterobacterale* members isolated from various specimens. Previous studies have reported various ranges of ESBL prevalence of 10% to 36% [8, 15 - 18]. ESBL-producing *Enterobacterale* prevalence varies greatly and has increased over time worldwide. The pooled estimated prevalence was the highest in the West Pacific (46%), followed by southeast Asia and Africa (22%), the Eastern Mediterranean (15%) and Northern Europe (4%) [19]. Factors associated with a higher risk of colonization were antibiotic use and international travel in the previous year [19].

In the present study, ESBL-producing *Enterobacterale* displayed an increasing rate of resistance for aminopenicillins (ampicillin), followed by piperacillin-tazobactam, cephalosporin groups (ceftriaxone, ceftazidime, cefepime, ciprofloxacin), and fluoroquinolone (ciprofloxacin). The study has shown that both *E. coli* and *K. pneumoniae* have developed a high rate of resistance to ampicillin, piperacillin-tazobactam, amoxicillin/clavulanic acid, the cephalosporin groups, ceftriaxone, ceftazidime, and fluoroquinolone (ciprofloxacin) (42.7%). Both the organisms showed a higher susceptibility to the carbapenems (imipenem) and the aminoglycosides (Amikacin). This resistance pattern has been observed in many other studies [8, 9, 12, 15, 17, 18] and might probably be attributed

to the extensive use of these antibiotics.

Our study also included the molecular aspect of resistance through the identification of genes carried by these resistant isolates. The ESBL types detected in these isolates belonged to the CTX-M and TEM, while the SHV type was not detected alone in any of the tested isolates.

The CTX-M genes are the most prevalent (35.5%) among the isolates of this study. This finding agrees with other studies conducted elsewhere in the Middle East and many parts of the world [7, 20]. Among the different ESBL families, CTX-M has become the most prevalent worldwide, both in nosocomial and community-acquired infections [20].

The TEM class alone was the next frequent class of ESBLs (11.3%), but a higher percentage of harboring TEM also had CTX genes along with it (12.9%). Isolates were not found to harbor the SHV genes alone, whereas 12.9% had CTX- M also.

Many different studies show that the distribution of ESBL genes is diverse. Some studies have shown a predominance of the TEM gene with less SHV and CTX-M [21]. In one study in Saudi Arabia, characterization of the resistance genes revealed the dominant presence of SHV, followed by TEM and CTX-M [22]. Other findings agree with ours, which indicated a predominance of CTX-M [9, 23].

Earlier reports mentioned that the most prevalent type of ESBL genes is SHV, TEM, and CTX-M. During the past decade, TEM and SHV types were reported to be the most common types of b-lactamase genes, but recently, the CTX-M type has spread worldwide compared to TEM and SHV genotypes [24].

CTX-M is predominant in many regions; several reports were made from Iran (74%) [25], Morocco, North Africa (70%) [26] and India (93.7%) [27]. However, TEM-type β -lactamase gene was predominant in Italy (45.4%) [28], Portugal (40.9%) [29], and Turkey (72.7%) [30].

The most important finding in our study was the co-existence of different ESBLs. A number of the isolates had multiple genes, namely TEM and CTX-M; TEM, SHV, and CTX; and SHV and CTX-M in their genome suggesting the carriage of multiple plasmids. The co-existence of all three *bla* genes was observed in 32.5% of the ESBL isolates. Several studies reported the co-existence of various ESBL genes within the same isolates [5, 24, 31]. The co-existence of all the three *bla* genes observed in our study was similar to that previously described in Iran [21].

CONCLUSION

In the present study, ESBL-producing *Enterobacterale* displayed an increasing rate of resistance for aminopenicillins (ampicillin), followed by piperacillin-tazobactam, cephalosporin groups (ceftriaxone, ceftazidime, cefepime, ciprofloxacin), and fluoroquinolone (ciprofloxacin).

The findings in this study document the threat of ESBL among *Enterobacterale* especially the CTX-M class in our geographical region with the occurrence of these strains as etiological agents of infection in the hospital setting. The frequency of antibiotic usage and even the dosages and period of administration vary greatly from one country to another. This has led to large differentials in the emergence of resistant patterns. Therefore, it is essential to study and report trends in antimicrobial resistance on a regular basis. Our findings represent a preliminary study for follow-up work, establishing novel features concerning the potential of multiple plasmids.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The ethical committee of Prince Sultan Military College of Health Science approved this study.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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