

Incidence and molecular characterization of the extended spectrum beta lactamase-producing *Escherichia coli* isolated from urinary tract infections in Eastern Saudi Arabia

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ABSTRACT

الأهداف: للعثور على الاشريكية القولونية المنتشرة اللاصقة والمنتجة لطيف بيتا أكتاماز في مجمع الملك فهد الطبي العسكري، الظهران (KFMMC) وللكشف عن الجينات المسؤولة عن إنتاجه. وبالإضافة إلى ذلك، فإننا نحدد نمط مقاومة الأدوية المتعددة بين العزلات.

الطريقة: اشتملت الدراسة على مجموعة 117 اشريكية القولونية المنتشرة جمعت من KFMMC خلال فترة 4 أشهر من مارس 2014م إلى يونيو 2014م. وقد أرسلت لمختبر علم الأحياء الدقيقة في كلية الأمير سلطان العسكرية للعلوم الصحية (PSMCHS)، الظهران، المملكة العربية السعودية لتحليلها. تم فحص السلالات لـESBL باستخدام VITEK® 2 Compact واستخدم سلسلة تفاعل البلمرة (PCR) لتحديد جينات TEM, SHV and CTX-M.

النتائج: تشير النتائج التي توصلنا إليها إلى وجود نسبة عالية من ESBLs بين الاشريكية القولونية المعزولة عن UTI (23.1%). كما تشير أن جينات CTX-M هي الأكثر انتشاراً بين العزلات في KFMMC يليها الجين TEM (6%)، ولكن هناك نسبة عالية من الاشريكية القولونية (3.4%) تحمل جينات TEM و CTX-M. لا شيء من العزلات لدينا يحمل الجينات SHV.

الخاتمة: تسجل النتائج خطر ESBL على عزلات الاشريكية القولونية من UTI لاسيما مجموعة CTX-M في مستشفانا مع حدوث هذه السلالات كعوامل مسببة للعدوى في المستشفى والمجتمع.

Objectives: To find the prevalence of uropathogenic *Escherichia coli* (*E. coli*)-producing extended spectrum beta lactamase (ESBL) at King Fahd Military Medical Complex in Dhahran (KFMMC) and to detect the genes responsible for its production. In addition, we determined the pattern of multi-drug resistance among isolates.

Methods: A total of 117 uropathogenic *E. coli* isolates were collected from KFMMC over a period of 4 months from March 2014 to June 2014. These were received in the Microbiology Laboratory at Prince Sultan Military

College of Health Sciences (PSMCHS), Dhahran, Saudi Arabia for analysis. The isolates were screened for ESBL using VITEK® 2 Compact. Polymerase chain reaction (PCR) examination was used to determine TEM, SHV, and CTX-M genes.

Results: Our findings indicated that there is a high incidence of ESBLs among the *E. coli* isolated from UTI (23.1%). Our study also indicated that CTX-M genes are the most prevalent among the isolates at KFMMC followed by TEM class (6%), but there was also a higher percentage *E. coli* (3.4%) simultaneously harboring TEM and CTX-M genes. None of our isolates harbored the SHV genes.

Conclusion: The findings document the threat of ESBL among *E. coli* isolates from UTI especially the CTX-M class in our hospital with the occurrence of these strains as etiologic agents of infection in the hospital and community.

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A urinary tract infection (UTI) is a situation in which one or more sites of the urinary system (kidneys, ureters, bladder, and urethra) become infected. Urinary tract infections are a universal health issue in both outpatient and inpatient locations, and urine cultures bear most of the workload in practically all clinical microbiology laboratories. Approximately 95% of cases of UTIs are produced by bacteria that consistently proliferate at the orifice of the urethra and navigate up to the bladder. Occasionally, bacteria disseminate to the kidney from the bloodstream. Urinary tract infections are a severe public health problem and are caused by a diverse collection of organisms, but frequently by *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus saprophyticus*.¹ High recurrence rates and expanding antimicrobial resistance amidst uropathogens result in several management and therapeutic issues that are expensive.² Although a wide range of pathogens can cause UTI, *E. coli* continues to be the most common cause due its ubiquitous presence in the perianal area. Production of beta-lactamase is one of the most critical mechanisms in Gram-negative bacteria against beta-lactam antibiotics.³ Due to the treatment of bacterial infections with the broad-spectrum antibiotics, it leads to broad spectrum enzymes called beta-lactamase.⁴ This hydrolyses penicillins, broad-spectrum cephalosporin, and monobactams. Beta-lactamase species are ordinarily derived from TEM and SHV-type enzymes.⁵ extended spectrum beta lactamase (ESBL)-producing *Enterobacteriaceae* have been responsible for various outbreaks of infection around the globe and pose challenging infection prevention problems.⁶ The incidence of point mutations in the sequence of the primary beta-lactamase gene leads to the production of different enzymes.⁷ Their inhibitory mechanism is regulated by the type of substrate and physical characteristics such as molecular weight. Here, beta-lactamase enzymes are classified into 4 fundamental groups: A, B, C, and D.⁸ Broad spectrum beta-lactamases are in group A.⁹ In the previous 20 years, Gram-negative bacteria have elaborated their resistance to broad spectrum beta-lactam antibiotics.¹⁰ Scientists have discovered more than 400 types of ESBLs, and majority of them reside in the *Enterobacteriaceae* family.¹¹ *Escherichia coli* is one bacteria that can induce ESBL enzymes and can distinguish between phenotypic or genotypic methods.¹² The genotypic methods are more specific at identifying such resistant strains. *Escherichia coli* -producing ESBL have been described in Saudi Arabia, but limited data are available on the genotypic characteristics and susceptibility patterns.^{13,14}

The objective of this study was to phenotypically and genotypically enumerate ESBL-producing *E. coli* isolated from hospitalized patient with UTI at KFMMC, Dhahran. The study also evaluated the resistance profile of these bacteria to carbapenems and quinolones that are therapeutic options for ESBL producers.

Methods. Data collection. This cross-sectional study included a total of 117 *E. coli* isolates from urine collected from KFMMC over a period of 4 months from March 2014 to June 2014 and was received in the Microbiology Laboratory at Prince Sultan Military College of Health Sciences (PSMCHS), Dhahran, Saudi Arabia for analysis. Repeated positive culture of the same patient was eliminated. The isolates were all from different wards. There were isolates from the primary care (n=41), specialty clinic (n=12), female medical ward (n=7), male surgical (n=5), and other wards (n=52). *Escherichia coli* ATCC 25922 was used as a positive control in each assay.

In this study, the identification and susceptibility tests were carried out using the automated biomérieux VITEK® 2 compact system (bioMérieux, Marcy l'Etoile, France). Gram-negative GN cards containing different substrates were utilized for isolate identification, and antibiotics susceptibility card AST-N291 was utilized to estimate antimicrobial susceptibility containing ESBL detection antibiotics cefotaxime, cefotaxime with clavulanic acid, ceftazidime, and ceftazidime with clavulanic acid. The Vitek system reported the tested isolate as positive or negative according to the susceptibility.

Out of 117 isolates, 27 were found to be ESBL positive that were further tested in PSMCHS molecular biology laboratory using CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc. USA). Deoxyribonucleic acid extraction used a heavy suspension of fresh bacterial colony in 0.5 ml sterile saline followed by boiling the suspension for 10 minutes. The suspension was then centrifuged at 10,000 RPM, and the supernatant containing the DNA was used to prepare the PCR mixture. The PCR mixture was made using QIAGEN Fast Cycling PCR Kit comprising deoxynucleosides triphosphate, dNTPs, Taq (Thermo-stable) polymerase enzymes, buffer, and Magnesium Chloride that acted as the cofactor and catalyzer to increase the productivity of Taq polymerase. Other ingredients were DNase-free water and the primer. For this study, the primers were exclusively designed and procured from the Eurofins Company (Eurofins Advantur Inc, San Diego USA).

Three different sets of primers were used on each of the 27 *E. coli* producing ESBL isolates: SHV, TEM, and CTX-M primers (Table 1). The process was amplified under the following conditions: initial denaturation (hot start) at 95°C for 5 minutes once, followed by 30 cycles of denaturation at 92°C for 30 seconds, annealing at 61°C for TEM, SHV, and CTX-M for 30 seconds, extension at 72°C for 30 seconds, and a final elongation at 72°C for 5 minutes. Specific bands were then visualized by gel electrophoresis using Syngene U Genius Gel Imaging System (Cambridge Scientific, Watertown, Massachusetts).

Data analysis (Chi-square test) was carried out using the Statistical Package for Social Sciences version 22 (Armonk, NY: IBM Corp.). A *p*-value of >0.05 was considered statistically significant.

Results. Out of the 117 *E. coli* isolates obtained from KFMMC, ESBL were detected in 23.1% (27/117). We conclude that the CTX-M gene was the most prevalent among the isolates at KFMMC. Out of 27 ESBL-producing *E. coli* strains, 8.5% (10/27) harbored the CTX-M gene, 2.6% (3/27) harbored the TEM

gene, 3.4% (4/27) carried both CTX-M and TEM genes, and 8.5% (10/27) carried other type of genes (Figure 1). The antibiotic susceptibility pattern of the ESBL-producing *E. coli* to carbapenams and quinolone was recorded. Out of 27 ESBL-producing *E. coli*, 24 (88.9%) were susceptible to carbapenams and only 2 (7.4%) were resistant (*p*=0.02) (Figure 2). In contrast, of the 27 ESBL-producing *E. coli*, 5 (18.5%) were only susceptible to quinolones whereas 22 (81.5%) were resistant (*p*=0.01) (Figure 3).

Discussion. Urinary tract infections are a common health problem in both outpatient and inpatient settings. *Escherichia coli* is the most common organism causing UTIs both in the community and hospital settings.¹⁵ Antimicrobial resistance is increasing at an alarming rate in these pathogens due to genetic recombination.¹⁶ Extended spectrum beta lactamase production is a pivotal mechanism used by these pathogens to jeopardize UTI treatment options.¹⁷ Dissemination of various ESBLs has appeared globally with a remarkable surge of CTX-M enzymes over TEM and SHV variants.¹⁸ In this ever changing scenario, our study was launched to detail ESBLs among urinary isolates of *E. coli* in the KFMMC, a major hospital of the Saudi Arabian armed forces, catering to the medical needs of a significant number of people in the eastern province. We enumerated the molecular aspect of resistance, which shed light on the exact genes carried by these resistant isolates. Our findings conclude that there is high incidence of ESBLs (23.1%) from *E. coli* isolated from urine. Our study also concludes that the CTX-M genes are the most common (8.5%) amid isolates from KFMMC, which agrees with other studies carried out in the Middle East and in many parts of the

Table 1 - Three different sets of primers were used on each of the 27 *Escherichia coli* producing ESBL isolates: SHV, TEM and CTX-M primers.

Primers	Primer sequence 5-3	Gene product length (bp)
TEM Forward	TTTCGTGTCGCCCTTATTCC	403
TEM Reverse	ATCGTTGTCAGAAGTAAGTTGG	
SHV Forward	CGCCTGTGTATTATCTCCCT	293
SHV Reverse	CGAGTAGTCCACCAGATCCT	
CTX-M Forward	CGCTGTTGTTAGGAAGTGTG	569
CTX-M Reverse	GGCTGGGTGAAGTAAGTGAC	

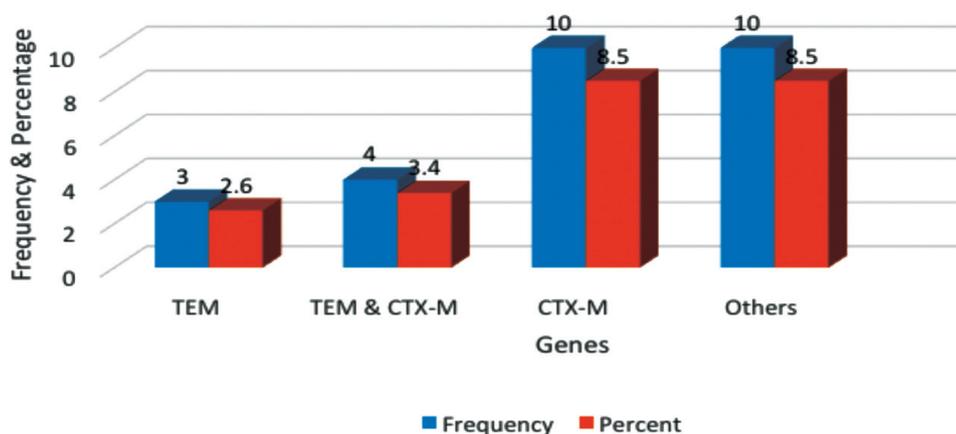


Figure 1 - Molecular analysis of the genes

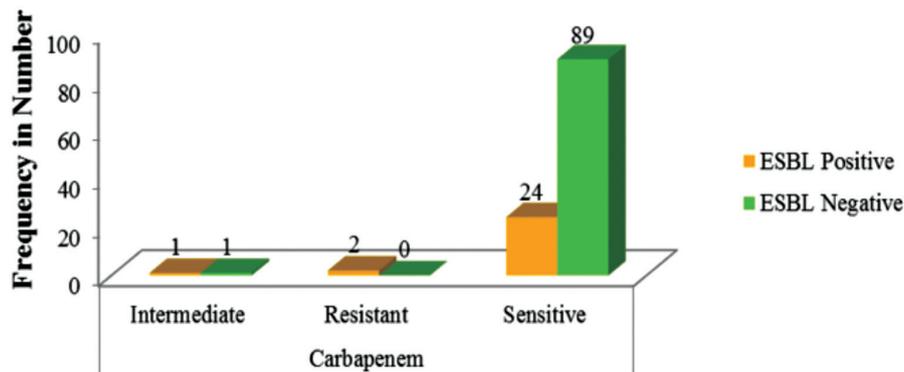


Figure 2 - Comparison between Carbapenem and extended spectrum beta lactamase (ESBL).

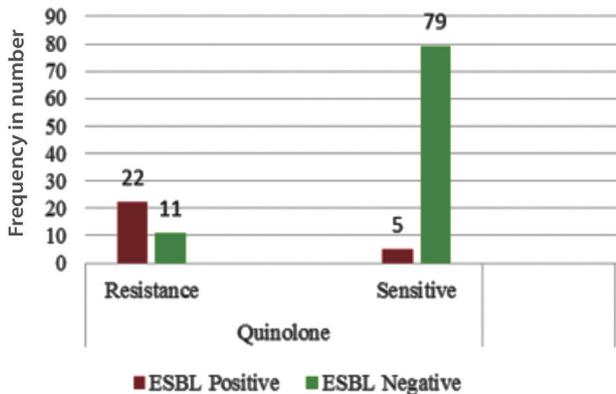


Figure 3 - Comparison between Quinolone and extended spectrum beta lactamase (ESBL).

world. A number of our isolates (11.9%) had multiple genes (TEM, SHV, and CTX-M) in their genome suggesting the carriage of multiple plasmids.

Many of the isolates producing these enzymes were also resistant to trimethoprim, quinolones, and aminoglycosides, often due to plasmid co-expression of other resistance mechanisms. Our study also showed that carbapenems still remain the best treatment option for these highly resistant pathogens.

In conclusion, the findings in this study document the threat of ESBL from *E. coli* in our hospital, especially the CTX-M class. These strains are etiological agents of infection in the hospital and community. Our study was relatively brief; the full extent of the spread could not be established. There was also a lack of primers for the various variants of ESBL genes, and we recommend further work on evaluating the ESBL types in these

isolates. It is critical to create faster, economical, and accurate diagnostic methodologies along with advanced potent antimicrobials.

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 - Methods (setting, date of study, design, subjects, intervention and analysis)
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