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# Prevalence of Extended-Spectrum of Beta-Lactamase-producing class a genes in *Escherichia coli* and *Klebsiella pneumoniae* isolated from patients urine samples in United Arab Emirates

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***Abstract*---**The current study aimed to determine the prevalence of class A genes in the Extended Spectrum of β-Lactamase producing *E. coli* and *K. pneumoniae* isolated from Patients Urine Samples in United

Arab Emirates. A total of 3457 urine samples were collected from October 2018 to January 2019. ESBL screening and confirmatory test were done using MicroScan WalkAway automated bacterial identification system. The ESBL class A gene primers were used to detect the *bla*$_{CTX-M}$, *bla*$_{SHV}$ & *bla*$_{TEM}$. Out of the collected urine samples, 474 were reported positive for urinary tract infection. A total of 130 samples were identified as Gram negative ESBL producing species, *Escherichia coli* (n=107) and *Klebsiella pneumoniae* (n=23). Amid these 130 ESBL producers, the class A ESBL genes *bla*$_{TEM}$, *bla*$_{CTX-M}$ & *bla*$_{SHV}$ were detected in 78 isolates of which 59 were *E. coli* and 19 *K. pneumoniae*. In the identified 59 *E. coli* isolates, the most predominant gene was *bla*$_{TEM}$, followed by *bla*$_{CTX-M}$ and *bla*$_{SHV}$, and these three genes were detected together in 02 *E. coli*. In 10 *E. coli* and 11 *K. pneumoniae* two class A genes were detected in combination of *bla*$_{CTX-M}$ + *bla*$_{SHV}$, *bla*$_{SHV}$ + *bla*$_{TEM}$ and *bla*$_{TEM}$ + *bla*$_{CTX-M}$. The prevalence of ESBL producing *E. coli* and *K. pneumoniae was* 27.45%. The rate of ESBL producing *E. coli* were more compared to *K. pneumoniae* from the urine specimens. There should be a continuous surveillance towards appropriate antibiotic usage to control the infections in the future.

***Keywords*---**ESBL genes, *bla*$_{CTX-M}$, *bla*$_{SHV}$, *bla*$_{TEM}$, *E. coli, K. pneumonia.*

## 1. Introduction

Gram-negative bacilli belonging to family Enterobacteriaceae are found to be one of the major causative pathogens for urinary tract infections [1]. Antibiotic resistance is greatest concerns in today's modern medicine. The development of resistance to newer β-lactam antibiotics in Gram-negative bacteria is an epidemiological problem in recent years [2]. Extended spectrum β-lactamases are a group of enzymes produced by Gram-negative bacteria which leads to multi drug resistance to β-lactam antibiotics [3]. These enzymes hydrolyze the β-lactam ring inactivating the antimicrobial agents leading to ineffective treatment for urniary tract infections. Clinical outcome data indicate that ESBLs are clinically significant and, when detected, indicate the need for the use of appropriate antibacterial agents [4].

ESBL genes are often located on plasmids that are transferable from strain to strain and between bacterial species. Molecular classification divides β-lactamases into A, B, C, D enzymes based on the amino acid sequence among which class A, C and D utilize serine for β-lactam hydrolysis whereas class B metalloenzymes require divalent zinc ions for substrate hydrolysis. Class A is further divided into TEM, SHV and CTX-M genes [5].

The gene *bla*$_{TEM}$ was first reported in *E. coli* [6] and it is the most commonly encountered β-lactamase gene in Gram-negative bacteria [7, 8]. The *bla*$_{CTX-M}$ gene produces an enzyme called cefotaximase that inhibits the activity of the cephalosporin; cefotaxime. The first emergence of CTX-M β-lactamase was reported in 1991 from France [9,10]. As of March 2019, over 172 CTX-M types

have been identified and described [11]. The *bla*$_{SHV}$ is one of the most common ESBLs which is chromosomally encoded within the larger part of segregates of *K. pneumoniae* and generally plasmid mediated in *E. coli* [12]. The beta lactamase enzymes produced by SHV and TEM genes are structurally similar. These are ESBLs belonging to Ambler's class A / Bush's group 2be [13] and as of February 2018, over 223 TEM and 193 SHV types have been listed in public databases [14]. The prevalence of ESBLs among clinical isolates especially in the Gram-negative bacteria have increased significantly over the past two decades [15]. There are limited number of similar studies available in this region and there by lay down a foundation to work further to understand the current situation. Hence, the current study focused on the prevalence of ESBL genes *bla*$_{TEM}$, *bla*$_{CTX-M}$ and *bla*$_{SHV}$ in the ESBL producing gram negative bacterial isoates in urine samples collected from Thumbay Hospitals, United Arab Emirates.

## 2. Material And Methods

This cross-sectional study was conducted at Thumbay Laboratory, Gulf Medical University, Ajman, United Arab Emirates from October 2018 to January 2019. A total of 3457 urine samples were collected from the patients who had been admitted for urinary tract infection in the Gulf Medical Center, Ajman, U.A.E.

### *2.1 Bacterial Isolation*

The urine samples were cultured on Cystine-Lactose-Electrolyte-Deficient (CLED) agar & MacConkey agar plates for 24-48 hours at 37˚C. Bacterial pathogens were isolated from only 474 urine samples and were reported positive for urinary tract infection. The isolated bacterial pathogens were employed for Gram staining, and culture characteristic identification on Eosin Methylene blue agar & MacConkey agar.

### *2.2 Bacterial identification and determination of ESBL producer*

MicroScan WalkAway automated detection system was used to identify the bacteria as well to determine the ESBL producers. The inoculum was prepared from single colonies of isolated bacteria by using the Prompt™ Inoculation System-D (Beckman Coulter, Brea, CA, USA), consisting of a wand designed to hold a specific quantity of bacterial material and a 30 ml Prompt™ Inoculation Water bottle (Beckman Coulter, Brea, CA, USA). The inoculum was suspended thoroughly, poured in to a RENOK inoculator tray and the suspension was transferred in the MicroScan panel for Gram-negative organism (MicroScan Neg Breakpoint Combo 50, *BECKMAN COULTER)* using RENOK inoculator (*BECKMAN COULTER*, Brea, CA, USA). The plate was incubated in the automated MicroScan WalkAway system DxM 1096 (*BECKMAN COULTER*) for 16 – 18˚C. Table 1 & 1a demonstrates the different tests performed on the MicroScan Neg Breakpoint Combo 50 panel. The Beckman Coulter MicroScan LabPro software WalkAway 9631049 used for this evaluation.

Table 1: Test wells on the MicroScan Gram-negative panels (Neg Breakpoint Combo 50). Underlined substrates are wells for which coverage with mineral oil

| Abbreviation | Substrate | Abbreviation | Substrate | Abbreviation | Substrate |
|---|---|---|---|---|---|
| GLU | Glucose | LYS | Lysine | OF/B | Oxidation Base control |
| SUC | Sucrose | ARG | Arginine | OF/G | Oxidation of Glucose |
| SOR | Sorbitol | ORN | Ornithine | DCB | Decarboxylase Base control |
| RAF | Raffinose | TDA | Tryptophan diaminase | NIT | Nitrate |
| RHA | Rhamnose | ESC | Esculine | K4 | Kanamycin 4µg/ml |
| ARA | ARabinose | VP | Voges-Praskauer | Cl4 | Colistin 4µg/ml |
| INO | Inocitol | CIT | Citrate | P4 | Penicillin G4µg/ml |
| ADO | Adonitol | MAL | Malonate | Fd64 | Nitrofurantoin 64g/ml |
| MEL | Melibiose | ONPG | O-nitrophenyl -D-Galacto-pyranoside | Cf8 | Cephalotin 8 µg/ml |
| URE | Urea | TAR | Tartrate | To4 | Tobramycin 4 g/ml |
| $H_2S$ | Hydrogen Sulfide | ACE | Acetamide | | |
| IND | Indole | CET | Cetrimide | | |

Table 1a: Cont. Antibiotic sensitivity test wells on the MicroScan Gram-negative panels (Neg Breakpoint Combo 50)

| Abbreviation | Substrate | Concentration (µg/ml) | Abbreviation | Substrate | Concentration (µg/ml) |
|---|---|---|---|---|---|
| **Ak** | Amikacin | 16 -32 | **Crm** | Cefuroxime | 4-16 |
| **Aug** | Augmentin | 8/4 – 16/8 | **Cp** | Ciprofloxacin | 1-2 |
| **Am** | AmiKacin | 8-16 | **Cl** | Colistin | 2 -4 |
| **A/S** | Ampicillin /Sulbactum | 8/4-16/8 | **Etp** | Ertapenem | 0.5-1 |
| **Azt** | Aztreonam | 4-16 | **Gm** | Gentamicin | 4-8 |
| **Cfz** | Cefazolin | 2-4 | **Imp** | Imipenem | 1-8 |
| **Cpe** | Cefepime | 8-16 | **Lvx** | Levofloxacin | 2-4 |
| **Cft** | Cefotaxime | 1-2, 16 | **Mer** | Meropenem | 1-8 |
| **Cft/CA** | Cefotaxim | 0.5/4, 4/4 | **Mxf** | Moxifloxac | 0.5-1 |

| | | | | | |
|---|---|---|---|---|---|
| | e/K Clavulanate | | | in | |
| **Cfx** | Cefoxitin | 8-16 | **Fd** | Nitrofurantoin | 32-64 |

### *2.3 ESBL gene analysis*

After the identification of ESBL strains, the bacterial strains were subcultured in sterile double strength Muller Hinton broth at 37°C for 24 hours for bacterial DNA extraction with the G-spin™ for Genomic DNA extraction kit (iNtRON). The DNA extracts positive for the 16SrRNA gene in PCR assays were processed for the presence of the ESBL genes *bla*$_{SHV}$, *bla*$_{CTX-M}$, and *bla*$_{TEM}$ with consensus primers in a Veriti™ 96-well Thermal cycler (Applied Bio-systems™). The PCR assays were performed with Thermoscientific PCR Master mix kit (ThermoFisher). The PCR primers and their specifications are provided in the Table 2 [16]. All the ESBL gene's primers were synthesized by e-oligos, Gene Link™, NY, USA. The PCR products underwent agarose gel electrophoresis to separate the fragments of DNA and visualized the target genes.

Table 2: PCR primers and their specifications

| **Amplicon** | **Primer sequence (5' – 3')** | **Size of amplicon (bp)** | **Annealing temperature˚C** |
|---|---|---|---|
| *bla*$_{SHV}$ | F'ATG CGT TAT ATT CGC CTG TG<br>R'AGC GTT GCC AGT GCT CGA TC | 1018 | 58 |
| *bla*$_{TEM}$ | F'ATG AGT ATT CAA CAT TTC CG<br>R'CCA ATG CTT AAT CAG TGA GG | 921 | 50 |
| *bla*$_{CTX-M}$ | F'SCS ATG TGC AGY ACC AGT AA<br>R'ACC AGA AYV AGC GGB GC | 544 | 58 |
| 16S rRNA gene | F'AGA GTT TGA TCM TGG CTC AG<br>R'ACG GHT ACC TTG TTA CGA CTT | 1,500 | 55 |

## 3. Results

### *3.1 Isolation of ESBL producers & their antibiotic susceptibility*

Of the 3457 urine samples, 474 (13.7%) urine samples were reported positive for urinary tract infection (UTI). Of which 130 samples (27.4%) were identified as ESBL producing isolates. Among the remaining 344 (73%) samples, the non-ESBL producers included both Gram positive and Gram negative bacteria. Among 130 ESBL producers, 107 (82.31%) were *E. coli* and 23 (17.69%) were *K pneumoniae*.
All the ESBL *E. coli* (n=107) and *K. pneumoniae* (n=23) were resistant to Ampicillin, Aztreonam, cefazolin, cefepime, cefotaxime, ceftazidime and, cefuroxime and susceptible to Ertapenem and Meropenem. In addition, all the *K. pneumoniae* (n=23) were also susceptible to Amikacin and Imipenem. Whereas in the case of *E. coli,* more than 90% strains were susceptibility to imipenem, amikacin, piperacillin/Tazobactam, Tigecycline, Nitrofurantoin and cefoxitin.

64.5% to 76.6% of the *E. coli* were susceptibility to Amoxicillin/k clavulanic acid, Gentamycin & Tobramycin. Nearly 60% of the *E. coli* were resistant to Norfloxacin (63.5%), Ciprofloxacin (62.6%), Levofloxacin (60.7%) and Trimethoprim/Sulfamethoxazole (52.3%). Similarly, 47.8% of the bacteria were resistant to Ampicillin/Sulbactam (47.8%). But 91.3% of the strains were susceptible to Gentamicin, Levofloxacin and Norfloxacin, 82.6% were susceptible to Ciprofloxacin, Piperacillin/Tazobactam, Tobramycin, and Tigecycline. Only 60.86 – 52.17% strains were susceptible to Amoxicillin/K Clavulanate & Nitrofurantoin and in the drug Trimethoprim/Sulfamethoxazole and Ampicillin/Sulbactam only 17.4% - 21.73% of the strains were susceptible (Fig. 1).

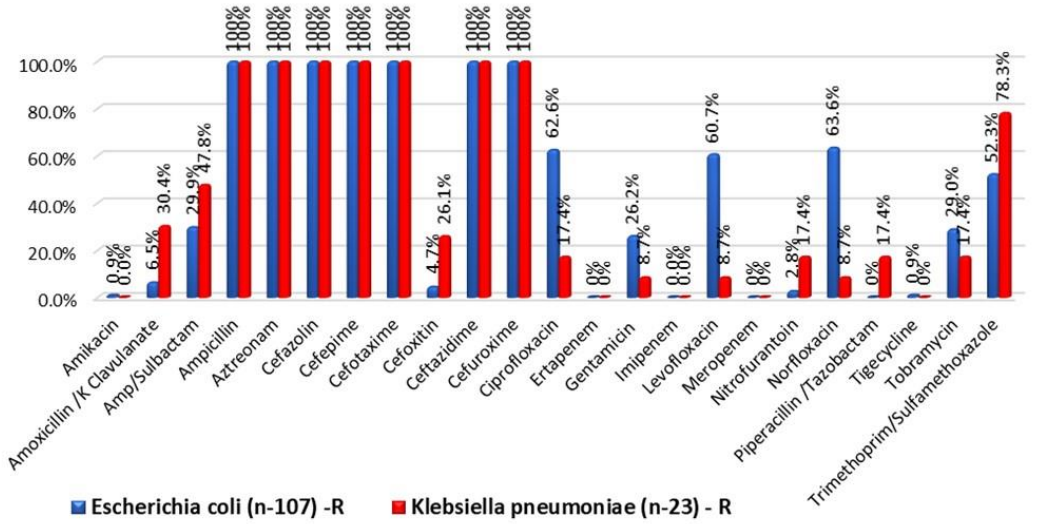


Figure 1: Rate of Resistance to various antibiotics by *E. coli* (n=107) & *K. pneumoniae* (n=23)

### *3.2 Detection of bla genes*

Of the 130 ESBL positive phenotypes, 78 (60%) bacteria including both *E. coli* (n=59) & *K. pneumoniae* (n=19) showed positive amplification to ESBL class A genes (*bla*$_{TEM}$, *bla*$_{CTX\text{-}M}$ and *bla*$_{SHV}$) (Fig. 2).

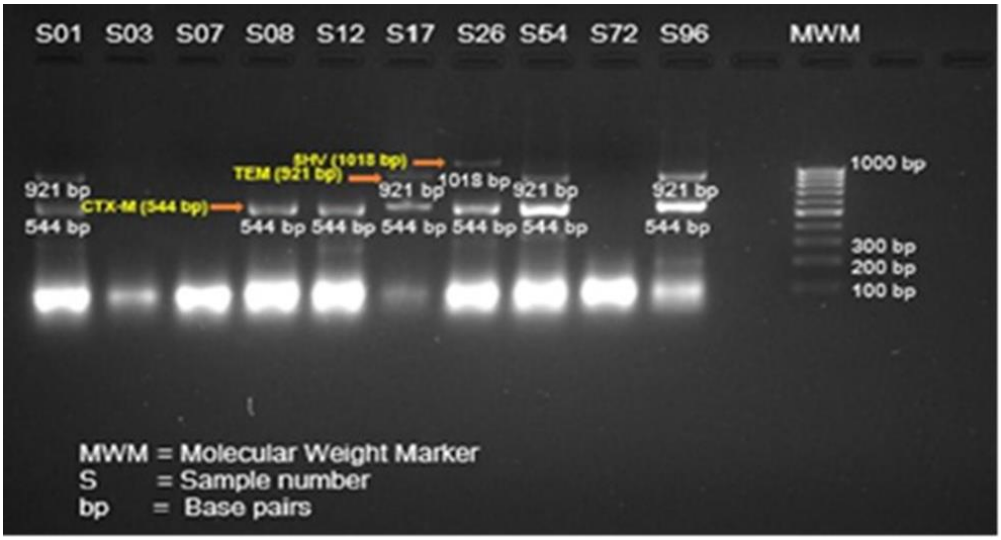


Figure 2: Detection of *bla*$_{TEM}$, *bla*$_{CTX-M}$ and *bla*$_{SHV}$ gene bands in 2% Agarose gel.

Among the ESBL positive *E. coli*, the *bla*$_{TEM}$ (28; 47.4%) was the most predominant gene, followed by *bla*$_{CTX-M}$ (24; 40.6%) and *bla*$_{SHV}$ (7; 11.8%) (Fig. 3). Out of 59 *E. coli*, only in 12 *E. coli,* the class A

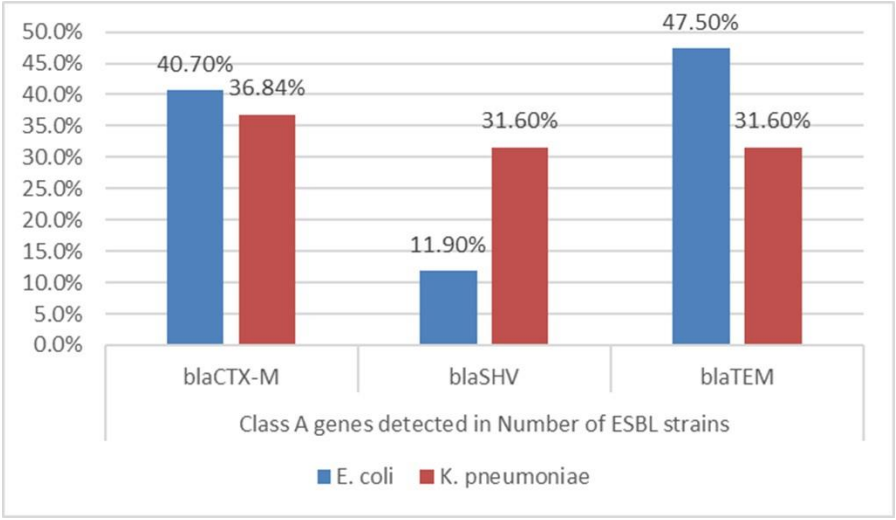


Figure 3: Prevalence of class A genes

genes were combined in their plasmid DNA as in the combination of *bla*$_{CTX-M}$ + *bla*$_{SHV}$ (n= 5), *bla*$_{TEM}$ + *bla*$_{CTX-M}$ (n= 5) and *bla*$_{TEM}$ + *bla*$_{CTX-M}$ + *bla*$_{SHV}$ (n= 2). In *K. pneumoniae*, the predominant ESBL gene was *bla*$_{CTX-M}$ gene (7; 36.8%) followed by *bla*$_{TEM}$ (6; 31.6%) and *bla*$_{SHV}$ (6; 31.6%). The class A gene combinations were also identified in 11/19 *K. pneumoniae*. The gene combinations were in the pattern of *bla*$_{CTX-M}$ + *bla*$_{SHV}$ (n= 4), *bla*$_{SHV}$ + *bla*$_{TEM}$ (n= 1) and *bla*$_{TEM}$ + *bla*$_{CTX-M}$ (n= 6) (Fig. 4).

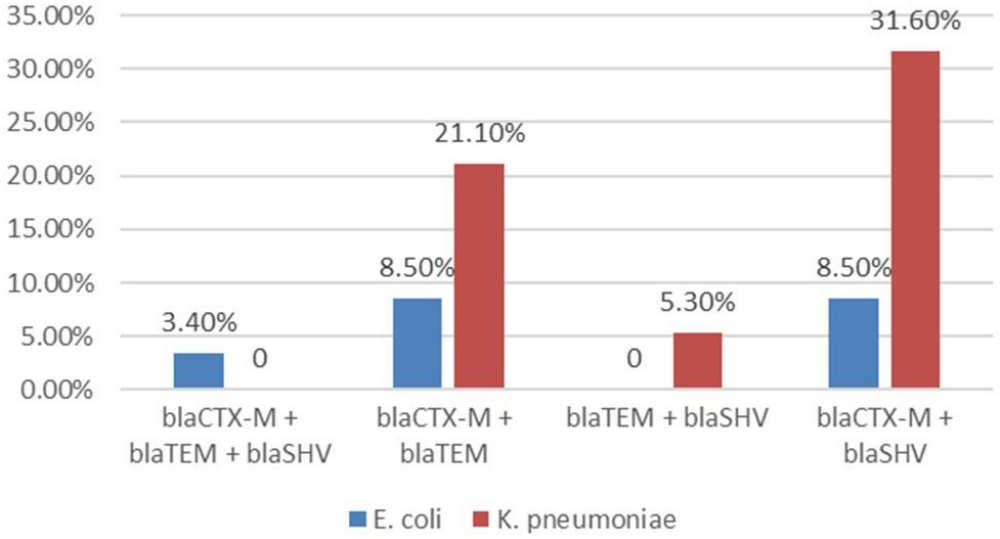


Figure 4: Prevalence of class A gene combination

Among the 130 ESBL positive phenotypes, 52 (40%) of them were showed negative amplification to these 03 genes. Furthermore, according to the molecular detection results, it was found that $bla_{TEM}$ gene is the most predominant among the three genes with a prevalence rate of 43.5% followed by the $bla_{CTX\text{-}M}$ gene with a prevalence of 39.7% among the multinational population of U.A.E.

## 4. Discussion

Multidrug resistance in ESBL producers have emerged as public health concern and difficult to treat infections with empirical treatment protocols [17]. Although the mechanism of resistance among ESBL producing Enterobacteriaceae may be varied, the most common resistance mechanism is β-lactamase [18, 19]. The current study was conducted on 130 ESBL producing *E. coli* (82.31%) and *K. pneumoniae* (17.69%) isolates. Shakya et al., 2017; isolated ESBL producing *E. coli* and *Klebsiella* spp. in their study were 91.7% & 8.3% [20]. In 2011 a similar study was conducted in UAE by Alfaresi et al., reporting 62% & 38% of ESBL producing *E. coli* and *K. pneumoniae* have isolated in their study in UAE [2] and consistent with previous similar studies conducted in Canada & Lebanon [21, 22]. All the isolates of *E. coli* & *K. pneumoniae* were resistant to ceftriaxone, cefazoline, cefixime, cefpodoxime, and cefuroxime and intermediate to cefotaxime. They were commonly susceptible to amikacin, ertapenem, meropenem, piperacillin/tazobactam, nitrofurantoin and ampicillin+sulbactam. *K. pneumoniae* became intermediate to gentamicin but *E. coli* was not. This is an alarm for clinicians that the prescription unregulated antibiotics against such kinds of resistant bacterial species must be replaced with safer antibiotics and those that does not promote resistance. Our finding is in accordance with recent data highlights towards the prevalence of ESBL producing Gram negative bacilli especially *E. coli* and *K. pneumoniae*, which are the predominant species that cause UTIs in patients. Molecular characterization in this study showed that the

predominant ESBL gene was *bla*$_{TEM}$ (43.5%), followed by *bla*$_{CTX-M}$ (39.7%) and *bla*$_{SHV}$ (16.6%) in both *E. coli* and *K. pneumoniae*.

Similar studies were conducted in Nepal and Qatar. In Nepal out of 2209 urine samples, 451 (20.4%) samples showed the Gram-negative bacteria, and the predominant species was *E. coli* followed by *K. pneumoniae* and *K. oxytoca* [20]. In Qatar, Sid Ahmed et al., 2016; stated that out of 629 urine samples, 109 (17.3%) samples were positive for ESBL producers mainly *E. coli* and *K. pneumonia* [23].
In 2008 a study was conducted in India, in which 31 isolates (48.4%) had only *bla*$_{TEM}$ gene and 13 (20.3%) had only *bla*$_{SHV}$ [24]. But in our study, the gene combinations such as *bla* $_{CTX-M\ \&\ SHV}$, *bla*$_{SHV\ \&\ TEM}$ and *bla*$_{TEM\ \&\ CTX-M}$ were detected in 10 *E. coli* and 11 *K. pneumoniae* and all the 3 genes were identified in 02 *E. coli* isolates. No previous studies have been reported in UAE with all 3 genes identified in single ESBL strains.

Most of the studies from various countries alarming towards the prevalence of ESBLs in clinical isolates are continuously increasing over time [25-29]. All the three class A genes were identified together only in the *E. coli* and two class A genes were seen in combination of *bla*$_{SHV}$+*bla*$_{CTX-M}$ and *bla*$_{TEM}$+*bla*$_{CTX-M}$ in both *E. coli* and *K. pneumoniae.* The gene *bla*$_{SHV}$ and *bla*$_{TEM}$ were detected together in the combination of *bla*$_{SHV}$+*bla*$_{TEM}$ *K. pneumoniae*. About 40% of the ESBL positive phenotypes, the class A genes were not detected.

## 5. Conclusion

The current study highlights the emergence and prevalence of the ESBL class A genes *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$ in *E. coli* and *K. pneumoniae* isolates from the patients' urine samples in the United Arab Emirates. This is the recent report of all the 03 class A genes were identified together only in *E. coli* and 02 class A genes were identified in combination of *bla*$_{CTX-M}$+ *bla*$_{SHV}$, *bla*$_{SHV}$+*bla*$_{TEM}$ and *bla*$_{TEM}$+*bla*$_{CTX-M}$ in both *E. coli* and *K. pneumoniae*. Further investigations are required for the other variants of ESBL genes and finding of any mutations in those genes that could be helpful for the further surveillance by the clinical microbiology laboratories to guide towards better choice of antibiotics as well as implementation of infection control measures in the UAE.

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## References

1. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature Reviews Microbiology. 2015;13(5):269–84.

2. Alfaresi MS, Elkoush AA, Alshehhi HM, Abdulsalam AI. Molecular Characterization and Epidemiology of Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolates in the United Arab Emirates. Medical Principles and Practice. 2011; 20:177-180.
3. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for *β*-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother. 1995; 39 (6): 1211–1233.
4. Doi Y, Iovleva A, Bonomo RA. The ecology of extended-spectrum β-lactamases (ESBLs) in the developed world. J Travel Med. 2017;24, Suppl 1, S44–S51.
5. Bush K, Jacoby GA. Updated Functional Classification of β-Lactamases. Antimicrob Agents Chemother. 2010;54 (3): 969-976.
6. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev. 2005 Oct;18(4):657-86.
7. Kong KF, Schneper L, Mathee K. Beta-lactam Antibiotics: From Antibiosis to Resistance and Bacteriology. APMIS: acta pathologica, microbiologica, ET immunologica Scandinavica. 2010; 118 (1):1-36.
8. Kapoor G, Saigal S, Elongavan A. Action and resistance mechanisms of antibiotics: A guide for clinicians. J Anaesthesiol Clin Pharmacol. 2017 Jul-Sep;33(3):300-305.
9. Zhao WH, Hu ZQ. Epidemiology and genetics of CTX-M extended-spectrum β-lactamases in Gram-negative bacteria. Critical Reviews in Microbiology. 2013; 39 (1): 79-101.
10. Bernard H, Tancrede C, Livrelli V, Morand A, Barthelemy M, Labia R. A novel plasmid-mediated extended-spectrum b-lactamase not derived from TEM- or SHV-type enzymes. J. Antimicrob. Chemother. 1992; 29, 590–592.
11. Ahmed AR, Neveen AA, Magdy AA, Ramy KA. Novel *bla*CTX-M variants and genotype-phenotype correlations among clinical isolates of extended spectrum beta-lactamase-producing *Escherichia coli*. Nature SCIENTIFIC REPROTS.2019;9:4224.
12. Fernandes R, Amador P, Oliveira C, Prudêncio C. Molecular characterization of ESBL-Producing Enterobacteriaceae in Northern Portugal. The Scientific World Journal. Vol. 2014; 782897: 1- 6.
13. Shaikh S, Fatima J, Shakil S, Rizvi SM and Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi. J. Biol. Sci. 2015; 22: 90–101.
14. Antonopoulos DA, Rida A, Ramy KA, Thomas B, Christopher B, Neal C, et al. PATRIC as a unique resource for studying antimicrobial resistance. Briefings in Bioinformatics. 2019; 20(4):1094–1102.
15. Pitout JD, Laupland KB. Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis 2008, 8(3):159–166
16. Tofteland S, Haldorsen B, Dahl KH, Simonsen GS, Steinbakk M, Walsh TR, et al. Norwegian ESBL Study Group: Effects of phenotype and genotype on methods for detection of extended-spectrum-beta-lactamase-producing clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* in Norway. J Clin Microbiol 2007;45:199–205.
17. Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, et al. Antibiotic resistance: a rundown of a global crisis. Infect Drug Res. 2018;11:1645–8.

18. Akova M. Epidemiology of antimicrobial resistance in bloodstream infections. Virulence. 2016;7(3):252-266.
19. Al-Agamy MH, Shibl AM, Hafez MM, Al-Ahdal MN, Memish ZA, Khubnani H. Molecular characteristics of extended-spectrum β-lactamase-producing Escherichia coli in Riyadh: emergence of CTX-M-15-producing E. coli ST131. *Annals of Clinical Microbiology and Antimicrobials* 2014, 13:4.
20. Shakya P, Shrestha D, Maharjan E, Sharma VK, Paudyal R. ESBL Production Among *E. coli* and *Klebsiella* spp. Causing Urinary Tract Infection: A Hospital Based Study. Open Microbiol J. 2017 Apr 28;11:23-30.
21. Mulvey MR, Bryce E, Boyd D, Ofner AM, Christianson S, Simor AE, et al. Ambler class A extended-spectrum β -lactamase producing *Escherichia coli* and *Klebsiella* spp. in Canadian hospitals. Antimicrob Agents Chemother 2004;48:1204–1214.
22. Moubareck C, Daoud Z, Hakimé NI, Hamzé M, Mangeney N, Matta H, et al. Countrywide spread of community- and hospital-acquired extended-spectrum beta-lactamase (CTX-M-15)-producing Enterobacteriaceae in Lebanon. J Clin Microbiol 2005;43:3309–3313.
23. Sid-Ahmed MA, Bansal D, Acharya A, Elmi AA, Hamid MJ, Sid Ahmed AM, et al. Antimicrobial susceptibility and molecular epidemiology of extended-spectrum beta-lactamase-producing Enterobacteriaceae from intensive care units at Hamad Medical Corporation, Qatar. *Antimicrobial Resistance and Infection Control* (2016) 5:4.
24. Jain A, Mondal R. TEM & SHV genes in extended spectrum beta-lactamase producing *Klebsiella* species beta their antimicrobial resistance pattern. Indian J Med Res. 2008;128(6):759–64.
25. Babypadmini S, Appalaraju B. Extended spectrum-lactamases in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae*-prevalence and susceptibility pattern in a tertiary care hospital. Indian J Med Microbiol. 2004;22(3):172-4.
26. Teawtrakul N, Jetsrisuparb A, Sirijerachai C, Chansung K, Wanitpongpun C. Severe bacterial infections in patients with non-transfusion-dependent thalassemia: prevalence and clinical risk factors. International Journal of Infectious Diseases. 2015;39:53-56.
27. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2022). Post-pandemic health and its sustainability: Educational situation. International Journal of Health Sciences, 6(1), i-v. https://doi.org/10.53730/ijhs.v6n1.5949
28. Ricciardi W, Giubbini G, Laurenti P. Surveillance and control of antibiotic resistance in the mediterranean region. Mediterr J Hematol Infect Dis. 2016;8:2016036.
29. Girmenia C, Canichella M, Serrao A. Epidemiology of carbapenem resistant *klebsiella pneumoniae* infections in Mediterranean countries. Mediterr J Hematol Infect Dis. 2016;8:2016032.
30. Rakhmonov, O. M., Shadmanov, A. K., & Juraev, F. M. (2021). Results of endoscopic treatment of benign prostatic hyperplasia in patients with metabolic syndrome. International Journal of Health & Medical Sciences, 5(1), 21-25. https://doi.org/10.21744/ijhms.v5n1.1811
31. Devrim F, Serdaroğlu E, Çağlar İ, Oruç Y, Demiray N, Bayram N et al. The emerging resistance in nosocomial urinary tract infections: from the pediatrics perspective. Mediterr J Hematol Infect Dis. 2018;10(1):e2018055.