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Characterization of VIM, VEB and CTX-M Beta-lactamase Gene in *Escherichia coli* and *Pseudomonas aeruginosa* Isolated from Urine Samples of Patients Visiting a Tertiary Hospital in Abakaliki

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Abstract

The spread and convergence of multiple beta-lactamase genes across distinct resistant bacterial populations from various hosts and settings demonstrates increased risk of morbidity and mortality in humans. This study was undertaken to characterize bla_{VIM}, bla_{VEB} and bla_{CTX-M} beta-lactamase gene in *Escherichia coli* and *P. aeruginosa* isolates from patients visiting a tertiary hospital in Abakaliki. A total of three hundred (300) urine samples were collected from patients and were subjected to bacteriological examination using culture, Gram staining and biochemical technique, for routine microbiological identification and further confirmed using the VITEK-2 Automated System (Biomerieux, France). Antimicrobial susceptibility studies were determined using the Kirby–Bauer disk diffusion method. All isolate were further screen for various beta-lactamase resistant gene by PCR using specific primer. Of the 300 urine samples collected, prevalence rate of 187 (62.3%) and 91 (30.3 %) *E. coli* and *P. aeruginosa* were recorded. The isolates exhibited 50.0-100% percentage of resistance to Amoxycillin-Clavulanic acid, Azetronam, Cefoxitin, Ceftriaxone and Piperacillin/tazobactam. The proportion of beta-lactamase gene in *E. coli* were as follows (VEB 143/76.5 %; CTX-M 175/93.5 %; VIM 77/41.2 %) while beta-lactamase gene in *P. aeruginosa* were as follows (VEB 91/100 %; CTX-M 63/69.2%; VIM 48/52.7 %). The presence of these gene in our study indicates the possibility of therapeutic failure, serious consequences for infection control and increased risk of morbidity and mortality in patients. Hence, continuous effort in hospital surveillance, infection control, and clinical audits must be conducted to fight against the rapid development and spread of antibiotic-resistant bacteria pathogens.

Keywords: Beta-lactamase, *Escherichia coli*, *Pseudomonas aeruginosa,* VIM, VEB, CTX-M

INTRODUCTION

Escherichia coli and *Pseudomonas aeruginosa* are Gram-negative rods associated with health care, and are significant causes for infection with antibiotic resistance burden [1, 2, 3, 4, 5]. (Yusof *et al.,* 2022; Ogba *et al.,* 2022; Nomeh *et al.,* 2023; Egwu *et al.,* 2023; Mustafai *et al.,* 2023). The treatment of human infection associated with this pathogen are commonly with beta-lactam antimicrobials agent. The World Health Organization has classified beta-lactam antimicrobials such as extended-spectrum cephalosporins and carbapenems as 'last resort' and 'critically important antimicrobials' because antimicrobial alternatives for treating last resort antimicrobial resistant bacteria are limited [6]. (WHO, 2019).

Most *Escherichia coli* and *Pseudomonas aeruginosa* resistance to beta-lactams is mainly due to the production of beta-lactamases enzymes, which are often encoded either on the chromosome or plasmid [2, 3, 4,] (Ogba *et al.,* 2022; Nomeh *et al.,* 2023; Egwu *et al.,* 2023). Beta-lactamases, such as carbapenemase beta-lactamases and extended-spectrum beta-lactamases (ESBLs), are increasingly being identified in food and companion animals, wildlife, humans, and the environment around the world [3, 4, 7, 8]. (Egwu *et al.,* 2023; Nomeh *et al.,* 2023; Awosile *et al.,* 2022; Joseph *et al.,* 2023). The spread and convergence of multiple beta-lactamase genes across distinct resistant bacterial populations from various hosts and settings demonstrates that antimicrobial resistance (AMR) is a One Health issue [9]. (Rousham *et al.,* 2018). Beta-lactamase production in *Escherichia coli* and *Pseudomonas aeruginosa* is a public health concern due to the possibility of therapeutic failure, serious consequences for infection control and increased risk of morbidity and mortality in animals and humans [7, 10.] (Awosile *et al.,* 2022; EFSA, 2011). The predominant ESBL genes encountered are blaCTX-M, blaTEM, and blaSHV while for carbapenemases, blaOXA-48 and blaNDM-1 have been reported globally [7.] (Awosile *et al.,* 2022). Although beta-lactamase genes are globally disseminated, they are not equally prevalent among human bacteria. Also, the occurrence and prevalence of these resistance genes varies across different geographic regions. For instance while blaCTX-M is widely disseminated and has been reported in almost every region of the world, blaVIM and VEB has been mostly encountered in Asian and Arabic pennisula in both animal and human hosts [11, 12, 13, 14, 15]. (Hashemizadeh *et al*., 2020; Tian *et al*., 2018; Haghighi and Goli, 2022; Haghighifar *et al.,* 2021; Zafer *et al.,* 2014). For this reason, ongoing monitoring of beta-lactamase resistance genes is necessary in order to gain a

deeper understanding of the regional distribution of these genes.

MATERIAL AND METHODS

Study area and Duration

The study was conducted at Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFUTHA). The tertiary hospital is located at latitude 6.32629167 and longitude 8.11168167 in Abakaliki, Ebonyi State, South eastern Nigeria [16]. (Adibe-Nwafor *et al.,* 2023). The duration of the study was between January-August. 2023.

Ethical Approval

The ethical approval for the study was granted in 2022 by the AE-FUTHA Ethics and Research Committee with reference number: SMOH/ERC/043/22. Every fundamental study was done in accordance with the ARRIVE guidelines.

Sample collection and bacteriological examination

A total of three hundred (300) urine samples were collected from patients visiting AE-FUTHA and the collected samples were subjected to bacteriological examination using culture, Gram staining and biochemical techniques for routine microbiological identification. A loopful of the urine sample were enriched in Brain-heart infusion broth (Merck Co., Germany) and incubated at 35◦ C for 18–24 h. After overnight incubation, the turbid broth culture were aseptically streak on solidified plate of CM1046 brilliance™ *E. coli* Agar (Thermo-fisher Scientific, U. S.A) and *Pseudomonas* Isolation Agar (PIA) (Merck Co., Germany) and incubated at 35◦ C for 18–24 h. After incubation, *E. coli* and *Pseudomonas aeruginosa* was identified by standard microbiology techniques such as colonial morphology, Gram staining, motility, and biochemical tests as previously described and further confirmed using the VITEK-2 Automated System (Biomerieux, France)

Antimicrobial susceptibility Testing

This was done by disc diffusion technique on MHA according to the guidelines of the Clinical and Laboratory Standards Institute [17]. (CLSI, 2019). The following antibiotics were tested against isolated bacteria: ceftriaxone (30 µg), aztreonam (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefoxitin (30 µg), cefepime (10 µg), aztreonam (30 µg), amoxycillinclavulanic acid (20/10 µg), Piperacillin/tazobactam (30 µg), (Oxoid, UK). Zones of inhibition diameters were measured, recorded, and interpreted as resistant or susceptible according to established criteria [18, 19]. (Oke *et al.,* 2020; Uzoije *et al.,* 2021).

Molecular analysis and Amplification of beta-lactamase genes

The bacteria DNA were extracted according to the manufacturer's instructions using advanced BioRobot EZ1 XL instrument (QIAGEN, Germany). The Master Mix QuantiTect Probe PCR Kit (QIAGEN, Hilden, Germany) and PCR specific primers were used for amplification of extended spectrum β-lactamase genes (bla_{VIM}, bla_{VEB}, and bla_{CTX-M}) according to previous studies [2, 3, 4, 13]. (Ogba *et al.,* 2022; Nomeh *et al.,* 2023; Egwu *et al.*, 2023; Haghighi and Goli, 2022). The Amplified gene were detected by electrophoresis on agarose gels containing SYBR-safe (Invitrogen, USA), along with a DNA molecular weight marker (BenchTop *p*GEM®DNA Marker, Promega, Madison, WI, USA). Visualization of gels was displayed using The BenchTop pGEM®DNA Marker (Promega, Madison, WI, USA) under ultraviolet illumination.

RESULTS

Of the 300 urine samples samples, the prevalence rate of 187 (62.3%) and 91 (30.3 %) *E. coli* and *P. aeruginosa* were recorded through standard microbiology techniques as presented in figure 1. The proportion of 2.5 %, 27.3 % and 45.6 % of *E. coli* were susceptible to Azetronam, ceftriaxone, cefepime while resistance rate of 100 % to amoxycillin/clavulanic acid and cefoxitin, Piperacillin/tazobactam 89.7 % as shown in figure 2.

P. aeruginosa exhibit resistance to aztreonam 100 %, cefoxitin 100 %, cefotaxime 91.5 %, amoxycillin/clavulanic acid 89.6 %, ceftriaxone 78.4 % and Piperacillin/tazobactam 50 % but displayed low susceptibility to cefotaxime 8.5 % and amoxycillin/clavulanic acid 10.4 % as presented in figure 3.

The proportion of beta-lactamase genes; blaVEB 143 (76.5 %), blaCTX-M 175(93.5 %), blaVIM 77 (41.2 %) were identified in *E. coli* while *P. aeruginosa* harbored blaVEB 91 (100 %), blaCTX-M 63 (69.2%) and blaVIM 48 (52.7 %) as shown in figure 4.

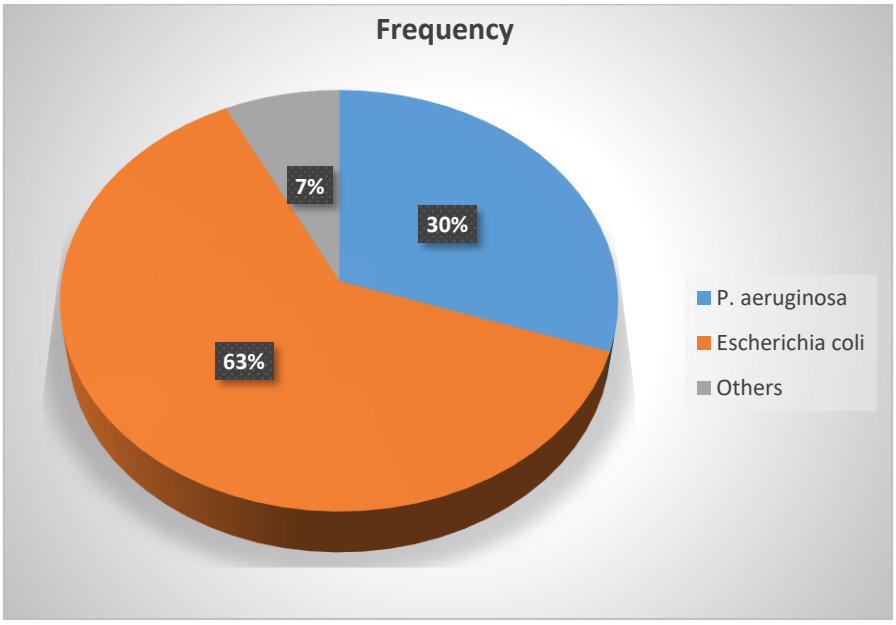

Figure 1: Percentage prevalence of *E. coli* and *P. aeruginosa*

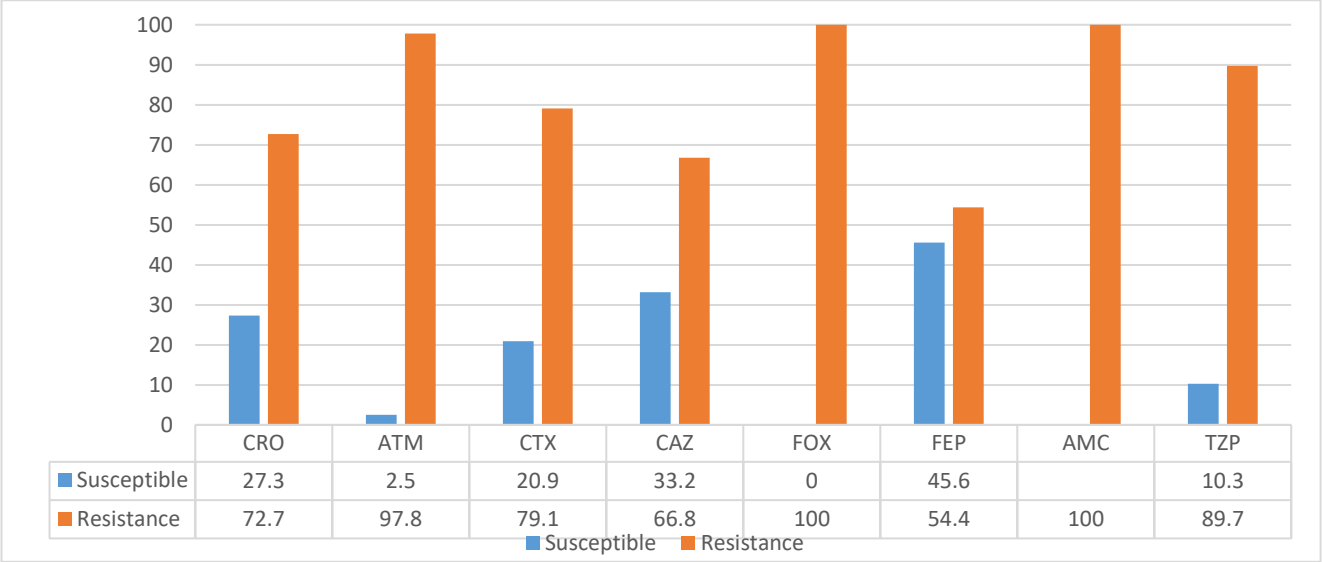

Figure 2: Percentage susceptibility profile of *E. coli* from urine samples

Key: CRO: ceftriaxone (30 µg), ATM: Aztreonam (30 µg), CTX: cefotaxime (30 µg), CAZ: ceftazidime (30 µg), FOX: cefoxitin (30 µg), FEP: cefepime (10 µg), AMC: amoxycillinclavulanic acid (20/10 µg), TZP: Piperacillin/tazobactam (30 µg),

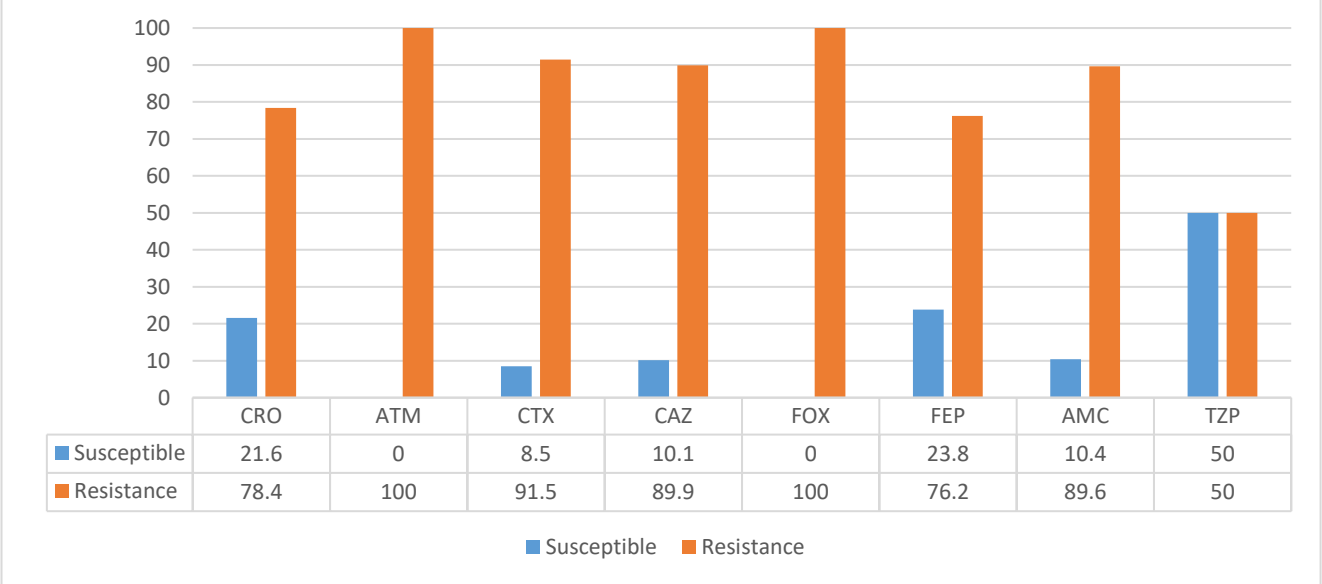

Figure 3: Percentage susceptibility profile of *P. aeruginosa* from urine samples

Key: CRO: ceftriaxone (30 µg), ATM: Aztreonam (30 µg), CTX: cefotaxime (30 µg), CAZ: ceftazidime (30 µg), FOX: cefoxitin (30 µg), FEP: cefepime (10 µg), AMC: amoxycillinclavulanic acid (20/10 µg), TZP: Piperacillin/tazobactam (30 µg),

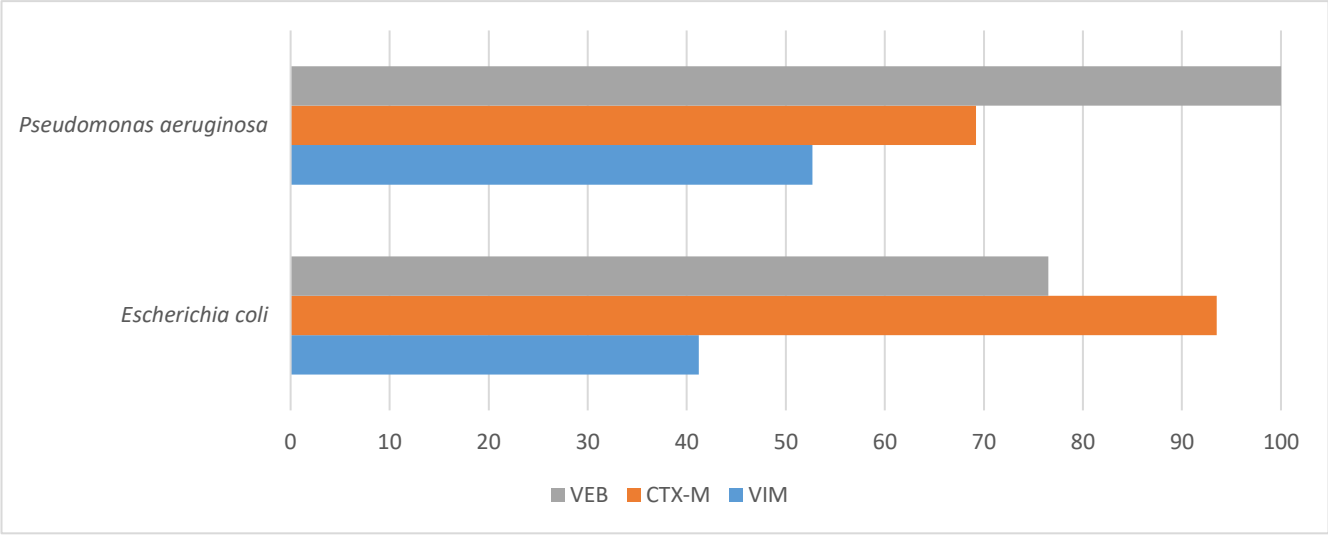

Figure 4: Percentage distribution of beta-lactamase gene.

DISCUSSION

In this study, bla*CTX-M* gene was the most predominant gene in *E. coli* and the second most predominant gene in *P. aeruginosa* but such observed prevalence does not undermine it global spread and predominant in different continent of the world. In practice, earlier study, in Kano, Nigeria, *bla*CTX-M 73.3% were the most predominant resistance genes in the ESBLs positive *E. coli* [20]. (Saka *et al.,* 2020) while in another study, among the 27 patients with previous hospitalizations, bla*CTX-M* were found in 18 (67%) of patients [21]. (Rodríguez-Baño *et al*., 2004). In yet another study detection of β-lactamase genes in 59 ESBL-producing *E. coli* isolates showed high prevalence of *bla*$_{CTX-M}$ 69.5%, over other genes, respectively, using specific PCR primers [22]. (Mirkalantari *et al.,* 2020) while the frequency of bla*CTX-M* in *P. aeruginosa* has been reported in Jordan 68.9% [23]. (Al-dawodeyahin., 2018), India 7.0% [24]. (Murugan *et al.,* 2017), Saudi Arabia 11%, Brazil 2.8% [25]. (de Almeida Silva *et al.,* 2017). CTX-M gene are known to contained a plasmid mediated cefotaxime hydrolyzing bla*CTX-M* enzyme encoded on a 42 kb plasmid which may be associated with transferable resistance to ceftriaxone, ceftazidime and other advanced generation cephalosporins observed in this study and have been reported as a vital mechanism of resistance [26, 27]. (Ugbo *et al*., 2020; Hussain *et al*., 2011).

Our study reports carbapenemase bla*VIM* 41.2 % and 52.7 % in *E. coli* and *P. aeruginosa* respectively, earlier in the same country this gene was first reported in Kano [28] (Aminu, *et al*., 2022) and Abakaliki, Nigeria [3].(Nomeh *et al.,* 2023). Elsewhere isolate positive for the bla*VIM* gene has been found by other researchers [11, 12, 29]. (Hashemizadeh *et al*., 2020; Tian *et al*., 2018; Murugan *et al.,* 2019). The dissemination of bla*VIM* among Gram-negative rod should not be underrate and in recent time, bla*VIM, IMP, NDM* have become the two most prevalent class B carbapenemases in worldwide *P. aeruginosa* isolates [30]. (Yoon and Jeong, 2021).

The rate of bla*VEB* gene was 76.5 % and 100 % in *E. coli* and *P. aeruginosa* respectively. The *bla*VEB was first detected in *Escherichia coli* isolates in Vietnam in 1996 and then detected in many members of Enterobacteriaceae and *Pseudomonas* [14]. (Haghighifar *et al.,* 2021). In an Egyptian study conducted by [15]. Zafer *et al.* in 2014, 7.4% of the *P. aeruginosa* isolates were ESBL positive, while 10.4% of them carried the *bla*$_{VEB-1}$ gene, and 60.6%, 45.1%, and 25.4% of their isolates were resistant to ceftazidime, aztreonam, and piperacillin-tazobactam, respectively [15]. (Zafer *et al.,* 2014) while 93.02% *P. aeruginosa* carried *bla*$_{VEB-1}$ gene in Iran [13]. (Haghighi and Goli, 2022). This genotype are highly prevalent and significant in clinical isolates of *P. aeruginosa* [13]. (Haghighi and Goli, 2022). The bla*VEB* enzyme also promotes resistance to ceftazidime, aztreonam, and cefepime. In the present study, 50.0%-100% of the isolates resistant to aztreonam, ceftazidime, ceftriaxone, cefuroxime, indicating the important role of the bla*VEB* enzyme in resistance to these antibiotics. The genes encoding these enzymes are usually carried by transposons and can move between Gram-negative bacteria [31] (John-Onwe *et al.,* 2023) and may cause the creation of MDR strains that make it difficult to choose the appropriate treatment for any GNB disease condition.

CONCLUSION

Our study provides an in-depth characterization of bla*VIM,* bla*VEB* and bla*CTX-M* beta-lactamase gene and the proportion of the amplified gene reported in our study may apparently revealed the endemic convergence of this gene in our study area. Thus, it is of paramount importance for each hospital to have an antibiotic guidance or stewardship program for all pharmacists and the physicians based on the most accurate microbiological data. In conjunction with this guidance, a continuous effort in hospital surveillance, infection control, and clinical audits must be conducted to fight against the rapid development of antibiotic-resistant pathogens.

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Author's Contribution

All authors investigated the study, did literature searches and did data Validation and Visualization. All the authors reviewed and approved the final draft, and are responsible for all aspects of the work

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Conflict of Interest: None

Ethical consideration: Ethical approval with reference No: AEFUTHA/ERC/179/R22 was obtained from the Research and Ethics Committee of AEFUTHA.

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