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JOBIMB VOL 12 NO 2 2024 BACTERIAL ACRYLAMIDASE

Occurrence of CTX-M and qnrA Genes Among ESBL-Producing *Pseudomonas aeruginosa* Isolated from Patients with Diabetic Foot Ulcers Attending ATBUTH Bauchi

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ABSTRACT

Diabetic foot ulcers (DFUs) pose a significant global health challenge due to its association with high morbidity and mortality rates. These chronic lesions, prevalent among individuals with diabetes mellitus (DM), result from a complex interplay of factors including peripheral neuropathy, peripheral artery disease, trauma, and impaired wound healing. *Pseudomonas aeruginosa*, a notorious pathogen linked to DFU infections, exhibits inherent resistance mechanisms and biofilm formation capabilities, complicating treatment strategies. This study aimed to assess the occurrence of CTX-M and qnrA genes among ESBL-producing *P. aeruginosa* isolates from DFUs patients at Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH) in Bauchi, Nigeria. From 150 collected samples, *P. aeruginosa* was the most frequently isolated organism (37 isolates). Antimicrobial susceptibility testing revealed significant resistance to cephalosporins and fluoroquinolones, with 46.7% of isolates exhibiting ESBL production. Molecular analysis identified the presence of CTX-M genes in three ESBL-producing isolates while qnrA genes were not detected, the absence of qnrA suggests a lack of plasmid-mediated quinolone resistance in the isolates. Clinically, this finding could imply that quinolone-based treatment may still effective against infection. These findings underscore the urgent need for antimicrobial stewardship and tailored treatment strategies to address multidrug-resistant *P. aeruginosa* infections in DFU patients, emphasizing the importance of molecular surveillance to inform targeted interventions and improve patient outcomes.

INTRODUCTION

Diabetic foot ulcers (DFUs) are long-term lesions that develop in people with diabetes mellitus (DM) and are linked to a high global rate of morbidity and death [1]. Diabetes mellitus (DM) patients can develop diabetic foot ulcers (DFUs), which are chronic lesions that pose a major global health and socioeconomic risk. Peripheral neuropathy, peripheral artery disease, trauma, and poor wound healing are some of the numerous interplaying variables that cause these ulcers, making effective management and treatment difficult [2]. DFUs have a high rate of morbidity and death, which increases the need for lower limb amputations, lengthier hospital stays, and more expensive medical care [1]. A number of factors, such as the length and severity of diabetes, glycemic management, peripheral neuropathy, peripheral vascular disease, foot deformities, and a history of prior foot ulcers, might affect the occurrence of DFUs, which differs among different populations. Foot ulcers are estimated to be 6.3% prevalent worldwide among diabetic individuals, with a lifetime risk of 10–25% for those with the disease [3]. The development of DFU is associated with an increased risk of age, prolonged diabetes, poor glycemic management, obesity, smoking, and concomitant conditions like dyslipidemia and hypertension [4].

The high prevalence, related morbidity and death, and socioeconomic burden of DFUs make them a major healthcare concern, DFUs to be fully managed, a multidisciplinary strategy that is adapted to each patient's needs is needed [5,6]. This strategy must address the underlying pathophysiology, optimize wound healing, avoid complications, and encourage patient education and self-care behaviors [5]. The ultimate goal of this crippling complication of diabetes mellitus is to improve outcomes and lessen its burden, which will require more study to better understand the etiology, risk factors, and best management practices for DFUs [7].

Pseudomonas aeruginosa is a particularly dangerous pathogen among the wide range of microorganisms linked to DFU infections because of its inherent resistance mechanisms and capacity to build biofilms, which make it extremely resistant to standard antibiotic therapy [8]. The management of DFU infections has become more difficult due to the advent and spread of *P. aeruginosa* strains that are resistant to drugs, which has resulted in higher rates of morbidity, death, and healthcare expenses [9,30]. *P. aeruginosa* produces extended-spectrum β-lactamase (ESBL), which confers resistance to a wide range of β-lactam antibiotics, such as monobactams, cephalosporins, and penicillin [10].

A study examined the prevalence and patterns of antibiotic susceptibility of *P. aeruginosa* isolates from DFUs [30]. A notable percentage of isolates were discovered in the study to be resistant to several antibiotics, such as fluoroquinolones and β-lactams [8,11]. A subgroup of isolates had ESBL genes, including CTX-M, identified by molecular analysis, indicating the role that ESBL synthesis plays in promoting antibiotic resistance in *P. aeruginosa* from DFU infections [12]. Plasmid-mediated quinolone resistance (PMQR) genes have been linked to decreased sensitivity to fluoroquinolone treatments in *P. aeruginosa* strains isolated from diabetic foot ulcers, in addition to ESBL production. qnrA-positive isolates were highly prevalent in *P. aeruginosa* isolates from DFU infections, according to a retrospective study assessing the prevalence of PMQR genes in these isolates [13,14].

Despite the growing body of literature on ESBL-producing *P. aeruginosa* and antibiotic resistance in diabetic foot ulcer infections, there remains a paucity of data on the prevalence and molecular epidemiology of these strains in healthcare settings in Nigeria, particularly in the context of Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH) in Bauchi. Therefore, our study aims to fill this knowledge gap by investigating the occurrence of ESBL-producing *P. aeruginosa* isolates from DFU patients attending ATBUTH and characterizing the genetic determinants of ESBL production, with a specific focus on the presence of CTX-M and qnrA genes.

MATERIALS AND METHODS

Study Design

This study employed a cross-sectional design to investigate the occurrence of CTX-M and qnrA genes among extended-spectrum β-lactamase (ESBL)-producing *Pseudomonas aeruginosa* isolates from diabetic foot ulcer (DFU) patients attending Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH) in Bauchi, Nigeria.

Isolation and Identification of *Pseudomonas aeruginosa*

DFU specimens were collected using sterile swabs and immediately transported to the medical microbiology laboratory for processing. Swabs were streaked onto MacConkey agar, Chocolate Agar and Blood Agar plates and incubated aerobically at 37°C for 18-24 hours. Suspected *Pseudomonas aeruginosa* colonies were identified based on characteristic colony morphology, Gram staining, and biochemical tests, including oxidase, catalase, indole, urease, citrate and triple sugar iron (TSI) tests.

Antimicrobial Susceptibility Testing

Following the criteria of the Clinical and Laboratory Standards Institute (CLSI), *Pseudomonas aeruginosa* isolates were subjected to antimicrobial susceptibility testing on Mueller Hinton agar plates inoculated with 0.5 McFarland standard inoculums using the modified Kirby-Bauer disk diffusion method. The antibacterial drugs listed below were examined: Gentamicin (10µg), Amoxicillin-clavulanic Acid (30µg), Ofloxacin (5ug), Levofloxacin (5ug), Cefotaxime (25ug), Ceftriaxone Sulbactam (45ug), Cefexime (5ug), and Nalidixic Acid (10ug). The plates were incubated at 37°C for 24 hr and the inhibition zone diameter were measured and the results was interpreted based on the CLSI guidelines [2020].

Detection of ESBL Production

Using the Double Disk Synergy Test (DDST), ESBL production was assessed phenotypically in *P. aeruginosa* isolates resistant to two or more third-generation cephalosporins. Using sterile swab sticks, an aseptic inoculation was performed on Muller-Hinton agar (Oxoid, UK) plates with a suspension of the ESBL-producing *P. aeruginosa* isolate that had been adjusted to 0.5 McFarland turbidity standards. A combination disk containing amoxycillin-clavulanic acid, AMC (20/10 µg), was placed in the center of the plate using a template. Two disks, one each containing 30 µg of cefotaxime and 30 µg of ceftazidime, were placed 15 mm apart on either side of the central disk containing AMC 20/10 µg. The inhibitory zone diameter was determined after the plates were incubated for 24 hours at 37°C, and the results were interpreted in accordance with the CLSI standards [2020].

Molecular Detection of CTX-M and qnrA Genes

Using the procedures and guidelines included with the promega wizard TM genomic DNA purification kits for bacterial isolation, the genomic DNA of bacteria isolates was extracted for molecular diagnostics [15]. Using primers CTX-M F:(5' -TTT GCG ATG TGC AGT ACC AGT AA-3') R:(5' -CGA TAT CGT TGG TGG TGC CAT A-3') with 480bp [16] and qnrA F:(5' -ATT TCT CAC GCC AGG ATT TG-3') R:(5' -GAT CGG CAA AGG TTA GGT CA-3') with 516bp [17]. DNA amplification for CTX-M and qnrA genes was carried out in a total volume of 25 µl. PCR ampliication was achieved as follows: 5 µl of genomic DNA sample was added to 20µl of PCR mixture (20 mmol/l Tris-HCl, pH 8.4; 50 mmol/l KCl, 10 mmol/l MgCl2, and 200 µmol/l each of deoxynucleoside triphosphates (dNTPs), 0.6 µmol/l each primer and 1 U Taq DNA polymerase.

The ampliication process were started with an initial denaturation step (94°C, 3 min). Each cycle consisted of three steps (denaturation, annealing, and extension). Each PCR reaction consisted of 30 cycles of ampliication (denaturation at 94°C for 1 min, annealing at 55.5°C for 1 min, and extension at 72°C for 1 min). A final extension cycle was performed at 72°C for 10 min. The agarasoe gel electrophoresis was used to evaluate the PCR product of the CTX-M and qnrA genes. A 25 ul PCR product was loaded with 5 ul of 6X loading dye. To compare the bands, 5ul and a 1.5 kb plus DNA ladder were employed. The gel (1.5 percent agarose) was prepared using 1X tris-acetate-EDTA (TAE) buffer. After loading the PCR products with 5 ul, 1 kb, and a DNA ladder, the gel was run for 25 minutes at 80 volts. The

DNA band ladder was viewed using a UV transilluminator [18,19].

Data Analysis
Statistical data analysis was done using Statistical Package for Social Science program (SPSS) version 24.

Ethical Approval
The ethical approval was obtained from the committee of Abubakar Tafawa Balewa University Teaching hospital Bauchi before collecting the swab specimens from diabetic foot ulcers patients and clinical details, with their consent.

RESULTS AND DISCUSSION

The results of the microbial analysis from 132 out of 150 collected specimens revealed a diverse range of bacterial pathogens isolated from diabetic foot ulcer patients, with *Pseudomonas aeruginosa* being the most commonly identified organism (37 isolates), followed by *Staphylococcus aureus* (25 isolates), *Proteus vulgaris* (23 isolates), *Escherichia coli* (15 isolates), *Klebsiella pneumoniae* (13 isolates), *Enterobacter agglomerans* (10 isolates), *Klebsiella oxytoca* (6 isolates) and *Streptococcus pyogenes* (3 isolates).These findings underscore the polymicrobial nature of diabetic foot ulcer infections and highlight the importance of considering both Gram-negative and Gram-positive pathogens in clinical management.

Table 1. Distribution of isolates according to antimicrobial susceptibility pattern.

Antimicrobial agent (ug)	Number (%) of Antimicrobial Susceptibility Pattern (n=37)	
	Sensitive	Resistant
Cefotaxime (25)	19(51.3)	18(48.7)
Ceftriaxone Sulbactam (45)	20(54.1)	17(45.9)
Cefexime (5)	19(51.3)	18(48.7)
Gentamycin (10)	26(70.3)	11(29.7)
Levofloxacin (5)	22(59.5)	15(40.5)
Nalidixic Acid (10)	28(75.7)	09(24.3)
Ofloxacin (5)	15(40.5)	22(59.5)
Amoxicillin Clavulanate (30)	25(67.6)	12(32.4)

The antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* isolates from diabetic foot ulcer (DFU) infections, as revealed in the provided data, demonstrate varying levels of resistance to commonly used antimicrobial agents. Notably, approximately half of the isolates displayed resistance to cephalosporins like cefotaxime, ceftriaxone sulbactam, and cefexime, while 40-60% showed resistance to fluoroquinolones such as levofloxacin and ofloxacin. Resistance levels also varied for gentamicin and amoxicillin clavulanate, with around one-third of isolates resistant to each. The findings underscore the challenges in effectively managing *Pseudomonas aeruginosa* infections in DFUs and emphasize the importance of judicious antimicrobial prescribing and exploring alternative treatment options to address multidrug-resistant strains

Frequency of ESBL MDR among *P. aeruginosa* Isolates During the Study
Among the 37 *Pseudomonas aeruginosa* isolates 15 which show MDR to two to three cephalosporins antibiotics were screen for Extended Spectrum of Beta Lactamase Production in which 7(46.5%) show positive to ESBL producer (**Fig. 1**).

Fig. 1. ESBL Phenotypic Confirmation Test.

The results indicate a significant frequency of ESBL (Extended-Spectrum Beta-Lactamase) producers among *Pseudomonas aeruginosa* isolates during the study, with ESBL producers accounting for 46.7% of the isolates. This proportion is consistent with findings reported in similar studies conducted in Nigeria, where ESBL-mediated resistance mechanisms are increasingly recognized as major contributors to antibiotic resistance among gram-negative bacteria [20,21]. Studies conducted in different countries such as India, Pakistan, and China have reported varying but substantial proportions of ESBL producers among *Pseudomonas aeruginosa* isolates, ranging from 30% to 60% [22–24].

Molecular Detection of CTX_M and qnrA Gene Among ESBL Producer *P. aeruginosa* Isolates During the Study
The figure shows the present of CTX-M gene in agarose gel electrophorese. The molecular detection of the CTX-M gene among ESBL (Extended-Spectrum Beta-Lactamase) producer *Pseudomonas aeruginosa* isolates, with three out of seven isolates testing positive for the CTX-M gene (**Fig. 2**), aligns with previous studies documenting the presence of CTX-M genes in ESBL-producing *Pseudomonas aeruginosa* strains [25–27]. However, the absence of the qnrA gene in the tested isolates suggests alternative mechanisms of quinolone resistance, consistent with previous research indicating diverse mechanisms of quinolone resistance in *Pseudomonas aeruginosa*, including target site mutations and efflux pump overexpression [28,29]. This finding underscores the importance of molecular surveillance to monitor the prevalence and dissemination of ESBL genes in clinical settings. Understanding the genetic mechanisms underlying antibiotic resistance in *Pseudomonas aeruginosa* is crucial for informing antibiotic stewardship practices and developing effective treatment strategies to combat multidrug-resistant infections caused by this opportunistic pathogen.

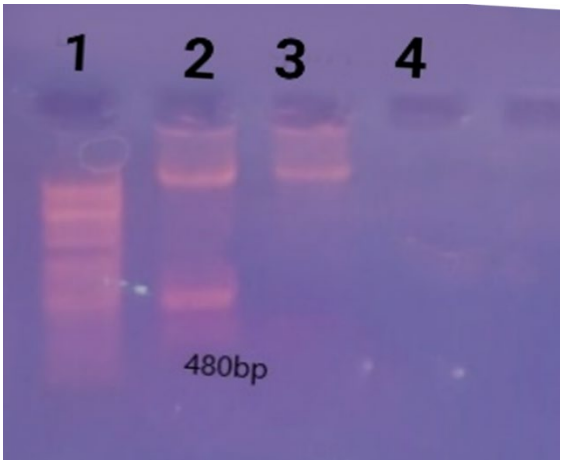

Fig. 2. Agarose gel electrophoresis. PCR-amplified CTX_M (480bp), and qnrA (516bp) gene; Lane 1: DNA ladder (Thermo Scientific), Lane 2: Positive (for CTX_M gene), Lane 3: Negative (for both gene).

CONCLUSION

The study was conducted at ATBUTH Bauchi provided comprehensive insights into various aspects of diabetic foot ulcer infections, focusing on *C* isolates, antimicrobial sensitivity testing, and the frequency of ESBL producers among *Pseudomonas aeruginosa* isolates, along with molecular identification of specific resistance genes. The finding of CTX-m gene presence in ESBL-producing *Pseudomonas aeruginosa* align with global antibiotics resistance trends, while the absence of qnrA gene suggests limited quinolone resistance locally. To combat rising multidrug resistance, local practices should priotize routine susceptibility testing, strict infection control, and antibiotic stewardship programs in hospital settings.

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