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Molecular Detection of Extended Spectrum Beta-lactamases Among Clinical Isolates of *Pseudomonas aeruginosa* from Patients Attending Two Selected Hospitals in Niger State, Nigeria.

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ABSTRACT

Pseudomonas aeruginosa belongs to the ESKAPE group of superbugs and has been implicated in both healthcare-associated infections and as an opportunistic infection affecting patients with debilitating underlying conditions. The study aims to determine the prevalence of extended spectrum beta-lactamases (ESBL)- producing *P. aeruginosa*, antibiogram and associated risk factors for acquisition of *P. aeruginosa* infection whilst using conventional PCR to detect the molecular presence of ESBL genes (particularly *CTX-M*, *TEM* and *SHV*) among isolates of *P. aeruginosa* from patients attending Federal Medical Centre, Bida and General Hospital, Minna, Niger State, Nigeria. A total of 200 samples of wound swabs, pus, urine, ear and eye swabs were analyzed by standard bacteriological methods. Screening and confirmation of ESBL were done phenotypically by Kirby-Baur method of antibiotic susceptibility testing and Double Disk Synergy Testing (DDST), respectively. Molecular detection of ESBL genes: *CTX-M*, *TEM* and *SHV* was done using conventional PCR technique. Of a total of 200 samples collected, 27 (13.5%) were isolates of *P. aeruginosa*, 14 (51.9%) confirmed positive for ESBL using DDST and 12(44.4%) confirmed positive for the presence of one or more of the targeted ESBL gene using the conventional PCR technique. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (PPV) of DDST given as 58.3%, 53.3%, 50% and 61.5% respectively using the PCR technique as standard against DDST technique. Prevalence of *CTX-M* (499kbp), TEM (862kbp) and SHV (827kbp) genes were distributed as 9(40.9%), 8(36.4%) and 5(22.7%), respectively. Prevalence of ESBL producing *P. aeruginosa* is high thus endemic in this region. A high prevalence in occurrence of *CTX-M* reinstates the changing pattern of ESBL producing *P. aeruginosa* in this geographical region. Therefore, this calls for a public health concern and highlights the need to develop an active surveillance system to track the spread and activating a robust Infection prevention and control measure.

Keywords: Niger State; Extended Spectrum; Beta-Lactamases; Molecular Detection; *Pseudomonas aeruginosa*

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GET Journal of Biosecurity and One Health (2024) 3(1) 35-44.
DOI: 10.36108/GJOBOH/4202.30.0140

INTRODUCTION

The World Health Organization (WHO) has classified *P. aeruginosa* as tier one priority pathogen of urgent concern, and new drugs are urgently needed as a result of the emergence of multi-drug resistance [1]. The frequency of *P. aeruginosa* infections, its clinical challenges and poor outcomes associated with these infections, and in particular the proportion of *P. aeruginosa* isolates that are resistant to antibiotics have resulted in these bacteria being classified as one of a group of six pathogens (the ESKAPE pathogens) that are the most problematic to treat [2]. As typical of ESKAPE group of pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp.), drug-resistant nosocomial pathogens such as *Pseudomonas aeruginosa* poses unwavering and increasing global threats as they can be extensively drug resistant and pan drug-resistant [3]. *P. aeruginosa* that has become resistant to the carbapenem as well as the cephalosporins class of antibiotics as classified by the WHO is one of the three “Priority 1: Critical” groups of bacteria for which new treatment strategies are most critically needed [2]. Due to the recent COVID-19, there has been increased usage of antibiotics as well as hand sanitizers and disinfectants worldwide, thus accelerating the spread of cross resistance, this stems largely from the treatment of secondary underlying infections with the use of antibiotics [1,4]. Moreover, *P. aeruginosa* has been found to be the primary causative agent of nosocomial morbidity and mortality in people with Cystic Fibrosis (CF), Chronic Obstructive Pulmonary Disease (COPD) and non-CF bronchiectasis [3] a report showed that by 2050 if no action has been taken, mortality and morbidity from Antimicrobial Resistance (AMR) will surpass any acute or chronic illnesses, including heart diseases and cancer with an estimated annual mortality of 10 million cases and cost estimates as high as US $100 trillion worldwide [3,5].

In Nigeria, *P. aeruginosa* is one of the most common pathogens implicated in all hospital acquired infections and one of the most common causes of post-operative wound infection (burns, wounds etc.) [6]. It has also been found to be one of the most common pathogens associated with both ventilator-associated pneumonia and catheter-associated urinary tract infections [2,4,6]. *P. aeruginosa* is an effective opportunistic pathogen unlikely to cross healthy, intact anatomical barrier, thus its infectiousness results from invasive medical procedures or weakened host defences. These predisposing conditions include debilitating illness, immuno-suppressant medication or intravenous injections. Once in the tissues, these bacteria express virulent factors including exotoxins and also causes endotoxic shock [7].

Extended spectrum β-lactamases (ESBL) hydrolyzes oxyimino-aminothiazole cephalosporins (cefotaxime, cefuroxime, cefepime, ceftriaxone and ceftazidime) as well as penicillins and other cephalosporins, excluding cephamycins [6]. Extended spectrum beta-lactamases were first reported in Germany, and persistent exposure of bacterial strains to myriads of beta-lactams has caused a dynamic and continuous production and mutation of lactamase (into a new beta lactamase called ESBLs) in many bacteria, thus expanding their activity even against the third and fourth-generation cephalosporins [6]. The most common and predominant ESBL in *P. aeruginosa* are the Ambler class A SHV-, TEM, CTX, VEB-, PER- and GES-type enzymes and the OXA-type enzymes from Ambler class D [8–10]. It has been reported that the presence of ESBLs confers a higher level of resistance to extended spectrum cephalosporins than AmpC, impermeability and efflux pump hyper-expression combined [3,11]. However, the production of ESBLs confers resistance at various levels to expanded spectrum cephalosporins [12–14]. These enzymes are encoded by different genes located on either chromosomes or plasmids as such ESBL-producing bacteria may not be detectable by the routine disk diffusion susceptibility test, leading to inappropriate use of antibiotics and treatment failure [12–14]. Although studies abound in Nigeria on the prevalence of multi-drug-resistant *P. aeruginosa,* there is paucity of data on the molecular epidemiology of ESBL gene in Niger State. This study aims to detect the presence of Extended Spectrum Beta Lactamase (ESBL) among clinical isolates of *P. aeruginosa* of patients attending selected government hospitals in Niger State and also to determine the prevalence of ESBL genes *bla*$_{SHV}$, *bla*$_{TEM}$ and *bla*$_{CTX-M}$ using conventional PCR among clinical isolates of *P. aeruginosa.*

METHODOLOGY

Study Design and Sample Size

This was a descriptive cross-sectional study conducted between July 2023 to December 2023 among patients attending Federal Medical Centre Bida, Niger State and General Hospital Minna, Niger State, North Central Nigeria. The Sample size (200) was calculated by Leslie Fisher’s formular and the prevalence used was 13.5% from a similar study conducted in Minna, Nigeria. Participants were recruited by convenient or purposive sampling and inclusion criteria involving patients diagnosed with bacterial infection and those who consented to the research. Written informed consent was obtained from each

participant before questionnaire administration, while those unwilling were excluded. A semi-structured interviewer-administered questionnaire was used to obtain data on socio-demographics and associated factors predisposing individuals to ESBL infection. Ethical approval with ethical clearance number FMCB/HCS/HREC/APPR/VOL 2/39/23, HMB/GHM/136/VOL.III/653 was obtained from the Research Ethics Committee of both Federal Medical Centre Bida and General Hospital Minna, respectively.

Sample Collection

Approval was obtained in August and October for both General Hospital and FMC Bida respectively with sample collection beginning immediately. Specimens such as wound, pus, urine and ear swabs were collected from the participants and processed at the Medical Microbiology Laboratory of both hospitals following standard microbiology procedures.

Laboratory Methods and Analysis

Pseudomonas aeruginosa was identified as catalase positive, oxidase positive and produced greenish pigmentation on Nutrient agar and Cetrimide agar. The Kirby-Baur method was used in the antibiotic susceptibility test for *P. aeruginosa*. Double Disk Synergy Test (DDST) was done using CLSI guidelines for Co-amoxiclav (20/10 µg), Ceftazidime (30 µg) and ceftriaxone (30 µg) (Fidson & Co, Nigeria German Chemicals, Glaxo, Swipha, Abtex and Oxiod UK).

DNA extraction of *P. aeruginosa* was done by taking fresh colonies from the nutrient agar slant, suspended in 200 µl of TE buffer and turbidity was adjusted according to 0.5 McFarland's standard. The procedure for DNA extraction was performed according to RUN MEI DNA Extraction Kit Manual, China. PCR amplification was done using Transgen Biotech 2×EasyTaq PCR SuperMix, China. The bla_{SHV}, bla_{TEM} and bla_{CTX-M} primers (Table 1) and thermocycling protocols were used as previously described. The PCR conditions for all reactions involved an initial denaturation for 5minutes at 94°C followed by a final denaturation of 35 cycles for 30seconds (with specific annealing at 56°C for CTX-M and 53°C for TEM and SHV for 30seconds) followed by a final extension at 72°C for 35 cycles in 5 minutes. A total volume of 50 µl reaction mixture was used to amplify each of the bla_{SHV}, bla_{TEM} and bla_{CTX-M} genes separately. The Polymerase Chain Reaction (PCR) amplified products were detected by 1% agarose gel electrophoresis at 80V for 30 minutes. Primers were optimized to assess Tm and TA. DDST evaluation was done in comparison with PCR as gold standard. Performance evaluation was done using markers such as true positives, true negatives, false positives and false negatives to determine sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV). The study was analyzed using SPSS version 25 software.

Table 1: Sequences of primers that were used [27].

Primer	Sequence (5'-3')	Product Size (bp)	Annealing (˚C)
***bla*SHV (F)**	ATTTGTCGCTTCTTACTCGCC	827	53
***bla*SHV(R)**	TTCACCACCATCATTACCGACC		
***bla*TEM (F)**	GTGCGCGGAACCCCTATT	862	53
***bla*TEM (R)**	GGGATTTTGGTCATGAGATTATC		
***bla*CTX-M (F)**	CGATGTGCAGTACCAGTAA	499	56
***bla*CTX-M (R)**	TAAGTGACCAGAATCAGCGG		

RESULTS

Table 2 shows the distribution for age, gender and place of residence. Also, 16 out of 27 (59.3%) isolates of *P. aeruginosa* are male, while 11 out of 27 (40.7%) are female with a *P-value* of 0.978.

Table 3 and Figures 2, 3 and 4 show the prevalence of ESBL among clinical isolates of *P. aeruginosa* was 14(51.9%) and 12(44.4%) for both DDST and PCR respectively. Table 3 shows the sensitivity, specificity, PPV and NPV of DDST given as 58.3%, 53.3%, 50% and 61.5% respectively using the PCR technique as standard.

Figure 1 show a prevalence of 13.5% representing 27 *P. aeruginosa* isolates out of a total of 200 samples.

Table 2: Socio-demographic characteristics of clinical isolates *Pseudomonas aeruginosa* and respondents

Variables	*Pseudomonas aeruginosa* N=27 (n, %)	Participants N=200 (n, %)	*P-value*
Age Groups			0.998
Children (0-12)	3(11.1)	22(11.0)	
Adolescents (13-17)	1 (3.7)	7(3.5)	
Adults (>18)	23 (85.2)	171(85.5)	
Gender			0.978
Male	16 (59.3)	119(59.5)	
Female	11(40.7)	81(40.5)	
Place of residence			0.987
Urban	19 (70.4)	141(70.5)	
Rural	8(29.6)	59(29.5)	

Key: *=Statistically significant (i.e. < 0.05) n = number of isolates, N = Total number of respondents

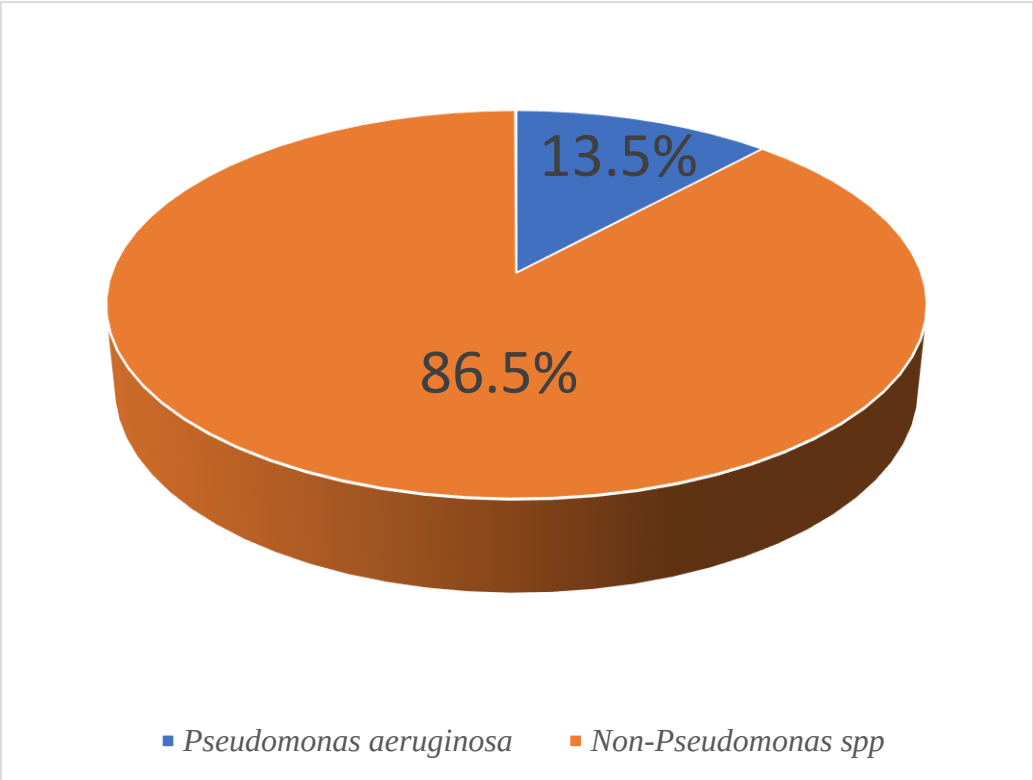

Figure 1: Percentage of confirmed *Pseudomonas aeruginosa* isolates

Table 3: Comparison of ESBL isolates using PCR and DDST

Variables	PCR		Total
	Positive (n, %)	Negative (n, %)	
DDST			
Positive	7(58)	7(47)	14
Negative	5(42)	8(53)	13
Total	12(100)	15(100)	27(100)

Key: n = number of isolates

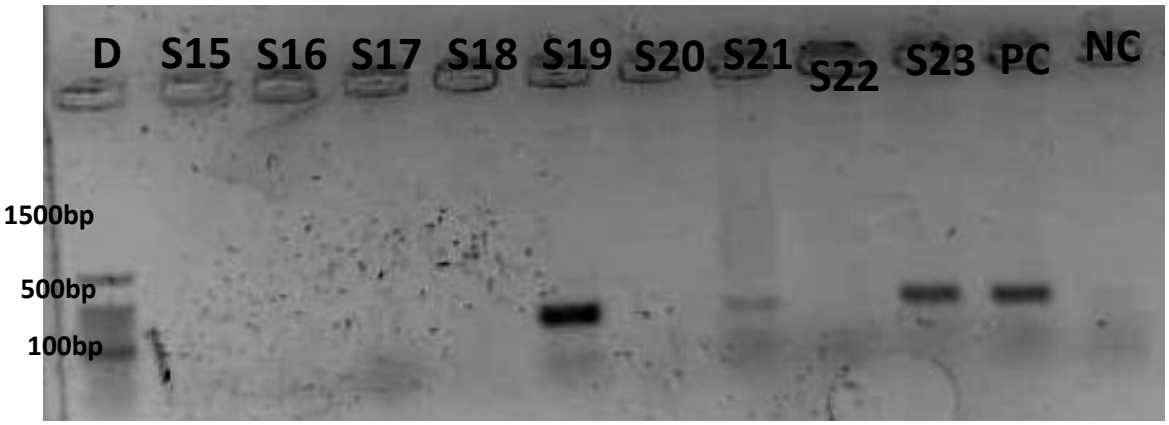

Figure 2: Photograph of CTX-M PCR product (Positives: S19, S21 and 23). PC-Positive control; NC-Negative control;D-DNA ladder; Target gene: *blaCTX-M* (499kbkbp)

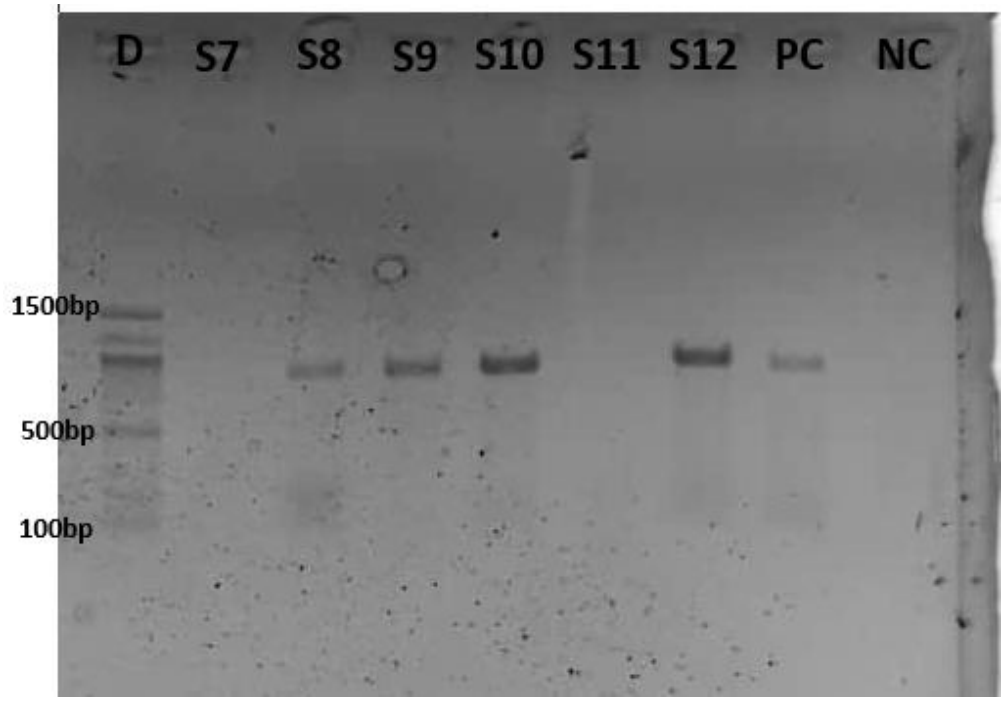

Figure 3: Photograph of TEM PCR product (Positives: S8, S9, S10, S12 and S13). PC-Positive control; NC-Negative control; D- DNA ladder; Target gene: *blaTEM* (862kbp)

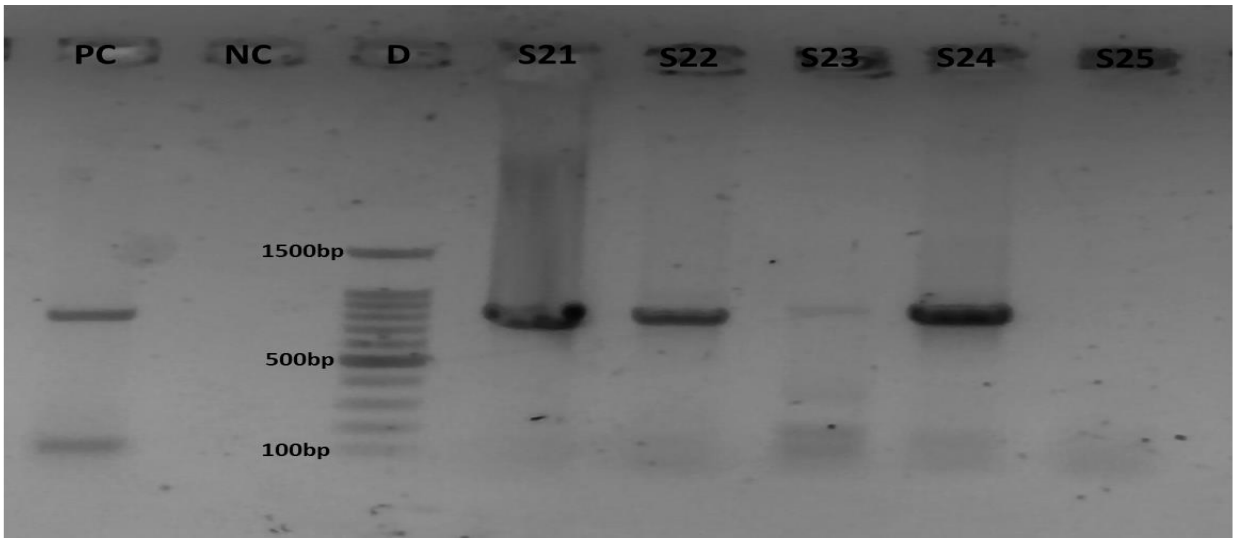

Figure 4: Photograph of SHV PCR product (Positives: S21, S22, S23, S24 and S25). PC-Positive control; NC-Negative control; D- DNA ladder; Target gene: *blaSHV* (827kbp).

Table 4 shows the antibiogram profile of *P. aeruginosa* isolates to various antibiotics and that Imipenem had the highest sensitivity. The highest sensitivity of 15(55.6%) was observed against Imipenem followed closely by Ciprofloxacin and Gentamycin with both having 8(29.6%) respectively. However, the highest resistance was observed against Cefotaxime and Cefoxitin with both sharing 21(77.8%) followed by Ceftriaxone, Tazobactam/Piperacillin and Ceftazidime with 18(66.7%), 14(51.9%) and 13(48.1%) respectively. Susceptibility pattern is shown in Table 4, with maximum resistance occurring against Cefoxitin (71.4%), Cefotaxime (64.3%), and Ceftriaxone (50%) of isolates. Table 5 and Figures 2, 3 and 4, depict the PCR result which showed the highest percentage in the co-occurrence of CTX-M+TEM followed by CTX-M+SHV with 5(18.5%) and 3(11.1%) respectively.

Table 4: Antibiogram profile of *Pseudomonas aeruginosa* isolates to various antibiotics

S/No.	Antibiotics	Sensitive (n, %)	Intermediate (n, %)	Resistant (n, %)
1.	Imipenem (10µg)	15(55.6)	4(14.8)	8(29.6)
2.	Ciprofloxacin (5µg)	8(29.6)	6(22.2)	13(48.1)
3.	Cefoxitin (30µg)	1(3.7)	5(18.5)	21(77.8)
4.	Ceftazidime (30µg)	8(29.6)	6(22.2)	13(48.1)
5.	Gentamycin (10µg)	4(14.8)	10(37.0)	13(48.1)
6.	Cefotaxime (30µg)	1(3.7)	5(18.5)	21(77.8)
7.	Ceftriaxone (30µg)	2(7.4)	7(25.9)	18(66.7)
8.	Tazobactam/Piperacillin (100/10µg)	4(14.8)	9(33.3)	14(51.9)
9.	Co-Amoxiclav (20/10µg)	8(29.6)	9(33.3)	10(37.0)

Key: n = number of isolates

Table 5: Distribution of CTX-M, TEM and SHV among *Pseudomonas aeruginosa* isolates

Distribution of ESBL among isolates

S/No	ESBL genes (n=27)	Frequency (n, %)
1	*bla*TEM	2(7.4)
2	*bla*SHV	1(3.7)
3	*bla*CTX-M + *bla*TEM	5(18.5)
4	*bla*CTX-M + *bla*SHV	3(11.1)
5	*bla*CTX-M + *bla*SHV + *bla*TEM	1(3.7)
	Total	**12(44.4)**

Distribution of Single ESBL genes

S/No	ESBL genes (n=27)	Frequency (n, %)
1	*bla*CTX-M	9(40.9)
2	*bla*TEM	8(36.4)
3	*bla*SHV	5(22.7)

Key: n = number of isolates

DISCUSSION

Although a significant portion of those found positive for *P. aeruginosa* were male, the relationship was not statistically significant. However, it was previously reported in Maiduguri and Minna that a significant relationship exists between *P. aeruginosa* acquisition and the male gender [6,7]. The high prevalence of *P. aeruginosa* shows an increasing endemicity in this part of the country buttressing the research carried out by previous authors [7,15,16].

ESBL producing *P. aeruginosa* were screened and confirmed using both DDST and conventional PCR technique respectively, as such, a prevalence of 51.9% for DDST and 44.4% for PCR was reported ESBL among clinical isolates of *P. aeruginosa* in this current study. While it is arguably true that variations in study designs may affect the result and outcome especially for genomic surveillance of ESBL and its types, the prevalence of the studied isolates are not in agreement with the study in Jos by Ejaz [17] who identified the prevalence of 7.2% for ESBL-producing *P. aeruginosa* in North-Central Nigeria. These findings are not in agreement with the work of Umar et al. [15] in Sokoto who identified *P. aeruginosa* in 36.7% of wound isolates and identified multi-drug resistance based on the antibiogram data. The study however did not go further to determine the phenotypic and molecular presence of ESBL. These findings are in agreement with the work of Olowo-Okere and colleagues [18] as well as Oli et al. [19] who reported a prevalence of 65% and 55.7% respectively. However, it is important to note a prevailing endemicity of ESBL in North-Central part of the country. Also, it is important to note that the present study is hospital-based research as such community-wide research might reveal an even higher and daunting prevalence. Both studies also used both DDST and PCR technique to obtain their findings. Similarly, the findings of sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of DDST were 58.3%, 53.3%, 50% and 61.5% respectively using the PCR technique as standard. This study is not in agreement with the reports of two studies in Ibadan [20,21] where the sensitivity, specificity, PPV and NPV were determined as 100%, 98%, 96% and 100% respectively. The difference could be attributed to the prevalence of co-occurrence of non-Class A ESBL genes not covered by this study. However, in the current study, the result indicates a low sensitivity for the detection of the 3 target genes thus buttressing the prevalence of false positives and false negatives associated with the phenotypic technique [22]. While it is true that the PCR technique is highly specific and sensitive, it targets only the genes probed, whereas ESBL is not only encoded by these 3 genes aforementioned earlier and DDST does not differentiate between the classes of ESBL. Due to the inexpensive nature of the DDST compared to PCR it is however, recommended for use in local settings or low-cost areas.

In the present study, a low resistance was recorded against Imipenem, Ciprofloxacin and Ceftazidime for all isolates of *P. aeruginosa.* Resistance was highest in Cefoxitin, Cefotaxime and Gentamycin with 71.4%, 64.3% and 42.9% respectively. Similarly, a high resistance was recorded for co-amoxiclav with 42.9% which further emphasizes the possibility of the *P. aeruginosa* having more than the three dominant genes detected. However, the high frequency of

resistance among the aforementioned cephalosporins may not be unconnected to the indiscriminate use and misuse of cephalosporin antibiotics and in addition to the fact that these antibiotics have existed much longer in circulation. These findings are in agreement with a study in Bangladesh [23] and another study in Anambra [19] as well as in Minna [7], with the identification of the various antibiotic profiles of ESBL producing *P. aeruginosa,* thus ensuring the right drugs is given for the right treatment ultimately preventing therapeutic failure. Moreover, the low resistance and high sensitivity observed against Imipenem might be due to the fact that this antibiotic is of the 4th generation cephalosporin and expensive, as such abuse and misuse are usually minimal. Although ESBL production was observed in slightly above 50% of the clinical isolates in this present study, resistance to 3rd generation cephalosporin was indicative of more non-class A ESBL gene carried by the clinical isolates. It is evident that the varied resistance mechanisms of this organisms has increased its adaptability to varying environmental conditions thus yielding varying antibiotic pattern [3,24].

The present study highlights a significant increase in the prevalence of CTX-M gene (40.9%), thus taking the lead with TEM (36.4%) and SHV (22.7%) following behind accordingly. This study is in agreement with another hospital based research on the prevalence of ESBL *P. aeruginosa* in paediatric patients focusing on only this three dominant genes which was carried out in Saudi Arabia [17]. However, this study does not agree with the findings of Rahimi et al. [25], where TEM (48.2%), SHV (28.2%) and CTX-M (18.8%) gene occurred frequently in that order among isolates of *P. aeruginosa* in Iran. Similarly, a study in south eastern part of Nigeria revealed a frequency of TEM(55%), CTX-M(45%) and SHV(35%) among clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* [26]. The differences in climatic conditions, geography and lifestyle pattern might have been associated with the differences.

The variation in the prevalence of ESBL-producing *P. aeruginosa* upon confirmation by PCR technique in this present study is evidence of the possible existence of non-class A ESBL and carbapenemases genes not sought by this study. The high occurrence of CTX-M can be explained by the changing patterns previously observed in Europe and Asia that may now have caught up with this part of Nigeria. The detection of multiple carriage of two or more beta-lactam genes within the same organism was reported in this study as well as numerous other studies [16,20,26] with CTX-M leading at 40.9% with SHV (36.4%) and TEM (22.7%) following closely. This study is in agreement with the work of Ejaz, in Saudi Arabia [17]. However, this present study does not agree with the findings of Rahimi and colleagues in Iran [25] as well as that of Ugbo and colleagues in Ebonyi [26]. The prevalence of CTX-M and co-occurrence amongst other genes such as in CTX-M+TEM followed by CTX-M+SHV with 5(18.5%) and 3(11.1%) respectively can be attributed to an existing relationship between antibiotic resistance and multiple carriage of resistant genes in addition to differences in climatic conditions, geography and lifestyle pattern earlier reported in other continents that might now have caught up with this part of Nigeria.

CONCLUSION

Our study highlights a high prevalence of ESBL among clinical isolates of *P. aeruginosa* thereby affecting the susceptibility pattern (antibiogram) of the isolates. Imipenem was found to be the most effective in its activity against *P. aeruginosa* isolates despite being the last drug of choice for treating multidrug resistance. The high cost of imipenem and the fact that AMR is gradually eroding the gains in the antibiotic production industry is worrisome. The relatively high prevalence of ESBL recorded following screening and confirmation by both phenotypic and molecular techniques showed the predominance of the target genes with CTX-M leading while TEM and SHV follows closely. Co-occurrences of CTX-M/TEM/SHV was recorded frequently and buttresses the relationship of antibiotic resistance with multiple resistant gene carriage. The assessment of DDST and PCR reveals the need for a robust diagnostic system for the detection of ESBL among *P. aeruginosa*. While PCR remains a gold standard for gene identification, it is relatively expensive and does not account for genes not included in the probe.

Therefore, it is pertinent to curb the spread of this organism by ensuring adequate adherence to standard operating procedures in the routine testing of ESBL in not just *P. aeruginosa* but other Gram-negative organisms. The economic burden of antimicrobial resistance (AMR) is gradually impoverishing Nigeria and the continent of Africa at large. As such the roles of infection prevention control measures and antibiotic stewardship will go a long way in surveillance, genomic mapping, by providing information for policies and improving treatment outcomes.

LIST OF ABREVIATIONS

µg/ml - microgram/millilitre
Amp C - Ampicillin Resistant Gene
Kbp – Kilobase Pairs
CLSI - Clinical and Laboratory Standard Institute
CTX-M - Cefotaxime Resistance Gene
DNA - Deoxyribonucleic Acid
Esbls - Extended Spectrum Beta Lactamases

GES - Guyana Extended Spectrum Beta Lactamases
IPAC – Infection Prevention And Control
OXA- Oxacillinase Gene
PCR - Polymerase Chain Reaction
PER - *Pseudomonas* Extended Resistance Gene
SHV - Sulphahydryl Variable
SPSS - Statistical Package for Social Science
TEM - Temoniera (Greek Patient) Gene
UK - United Kingdom
VEB - Vietnam Extended-Spectrum-Beta Lactamases
WHO - World Health Organization

AUTHOR CONTRIBUTIONS

HIC being the lead author participated in the research design, collation of data, manuscript preparation and overseeing most of the experiments including sample collection and statistical analysis. KM participated in the review of study design and overall manuscript review. MY contributed towards review of research design and provision of some materials used in the research experiments. MKG contributed largely in the laboratory workflow design and supported in the preparation of laboratory reagents used during the experiments.

CONFLICTS OF INTEREST

There authors have no conflict of interest to declare.

FUNDING

There was no financial support or funding for this research.

ETHICAL APPROVAL

Ethical approval with ethical clearance number FMCB/HCS/HREC/APPR/VOL 2/39/23, HMB/GHM/136/VOL.III/653 was obtained from the Research Ethics Committee of both Federal Medical Centre Bida and General Hospital Minna, respectively. Informed consent was obtained from all participants and guardians of participants below the age of 16 years.

REFERENCES

[1] Langendonk RF, Neill DR, Fothergill JL. The Building Blocks of Antimicrobial Resistance in *Pseudomonas aeruginosa*: Implications for Current Resistance-Breaking Therapies. Front Cell Infect Microbiol. 2021;11:665759. doi:10.3389/fcimb.2021.665759

[2] Glen KA, Lamont IL. β-lactam Resistance in *Pseudomonas aeruginosa*: Current Status, Future Prospects. Pathogens. 2021; 10(12):1638. doi: 10.3390/pathogens10121638.

[3] Panda SK, Buroni S, Swain SS, Bonacorsi A, da Fonseca Amorim EA, Kulshrestha M, et al. Recent advances to combat ESKAPE pathogens with special reference to essential oils. Front Microbiol. 2022;13:1029098. doi: 10.3389/fmicb.2022.1029098

[4] Timbrook TT, Prinzi AM, Spivak ES. Diagnostic and antimicrobial stewardship workforce challenges: A crisis in combating antimicrobial resistance. Antimicrob Steward Healthc Epidemiol. 2023;3(1):e60. doi:10.1017/ash.2023.147

[5] Al-Orphaly M, Hadi HA, Eltayeb FK, Al-Hail H, Samuel BG, Sultan AA, et al. Epidemiology of Multidrug-Resistant *Pseudomonas aeruginosa* in the Middle East and North Africa Region. mSphere. 2021; 6(3):e00202-21. doi: 10.1128/mSphere.00202-21

[6] Mohammed Y, Ibrahim BS, Umar KA, Adamu H, Mahmoud DM, Mohammed I, et al. Evaluation of Two Phenotypic Methods for the Confirmatory Testing of Extended Spectrum Beta Lactamases Producing. Int J Health Sci Res 2017;7(4):113–20.

[7] Ndukwe A, Omosigho O, Udeani T, Onoja A, Ogedengbe S, Ndochi MS, et al. Prevalence and Antibiotic Susceptibility Patterns of *Pseudomonas aeruginosa* in Minna, North Central Nigeria. Int J Epidemiol Infect. 2014;2(2):25-31.

[8] Ortiz De La Rosa JM, Nordmann P, Poirel L. ESBLs and resistance to ceftazidime/avibactam and ceftolozane/tazobactam combinations in *Escherichia coli* and *Pseudomonas aeruginosa*. J Antimicrob Chemother. 2019;74(7):1934-1939.

[9] Hassan H, Abdalhamid B. Molecular Characterization of Extended-Spectrum Beta-Lactamase Producing Enterobacteriaceae in a Saudi Arabian Tertiary Hospital. J Infect Dev Ctries. 2014;8(3):282-188.

[10] Tanko N, Bolaji RO, Olayinka AT, Olayinka BO. A Systematic Review On The Prevalence Of Extended-Spectrum Beta Lactamase-Producing Gram-Negative Bacteria In Nigeria. J Glob Antimicrob Resist. 2020;22:488-496. doi: 10.1016/j.jgar.2020.04.010.

[11] Adejobi A, Ojo O, Alaka O, Odetoyin B, Onipede A. Antibiotic Resistance Pattern of *Pseudomonas* spp. From Patients in a Tertiary Hospital in South-West Nigeria. GERMS. 2021;11(2):238-245.

[12] Begum S, Salam MA, Alam KF, Begum N, Hassan P, Haq JA. Detection of Extended Spectrum B-Lactamase in *Pseudomonas* spp. Isolated From Two Tertiary Care Hospitals in Bangladesh. BMC Res Notes. 2013;6:7 doi: 10.1186/1756-0500-6-7.

[13] Nuhu T, Beng E, Bolaji R, Olayinka T, Olayinka B, Yahaya M. Antimicrobial Resistant and Virulence Genes Profiles of

Some Gram-Negative Bacteria From Clinical Isolates at Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. J Pre-Clin Clin Res. 2020;14(2):52-57.

[14] Afunwa RA, Odimegwu DC, Iroha RI, Esimone CO. Antibiotic Combinatorial Approach Utilized Against Extended Spectrum Beta-Lactamase (ESBL) Bacteria Isolates From Enugu , South Eastern Nigeria. J Health Sci. 2014;4(1):19–25.

[15] Umar AI, Garba I, Jidda ML, Ganau AM, Fana AS, Umar AI, et al. Multidrug-resistant *Pseudomonas aeruginosa* Isolated From Ear and Wound Swabs in Some Selected Hospital Laboratories in Sokoto Metropolis, Nigeria. Calabar. J Health Sci. 2020;3(1):31-35.

[16] Daam KC, Samuel DA, Nwokoro U, et al. Detection of CTX-M and SHV Genes in Extended Spectrum Beta-Lactamase Producing *Klebsiella pneumoniae* and *Pseudomonas* aeruginosa in a Tertiary Hospital in North-central Nigeria. Niger Med J. 2023;64(2):196-204.

[17] Ejaz H. Dissemination of SHV, TEM and CTX-M Genotypes in *Pseudomonas aeruginosa*: A Pre-eminent Reason for Therapeutic Failure in Pediatrics. Ann Clin Lab Sci. 2020;50(6):797–805.

[18] Olowo-Okere A, Ibrahim YKE, Olayinka BO. Molecular Characterisation of Extended-Spectrum B-Lactamase-Producing Gram-Negative Bacterial Isolates From Surgical Wounds of Patients at A Hospital in North Central Nigeria. J Glob Antimicrob Resist. 2018;14:85-89.

[19] Oli AN, Eze DE, Gugu TH, Ezeobi I, Maduagwu UN, Ihekwereme CP. Multi-Antibiotic Resistant Extended-Spectrum Beta-Lactamase Producing Bacteria Pose a Challenge To The Effective Treatment of Wound and Skin Infections. Pan Afr Med J.2017; 27:66. . doi:10.11604/pamj.2017.27.66.10226

[20] Ajani TA, Elikwu CJ, Anaedobe CG, Onwuzo CN, Tayo B, Okangba CC, et al. Evaluation of Phenotypic and Molecular Technique in The Detection of Extended Spectrum Beta-Lactamase (ESBL) - Producing Gram Negative Bacilli in Ogun State, Nigeria. Ann Ib Postgrad Med. 2022;20:160-168.

[21] Hasan SM, Ibrahim KS. Molecular Characterization of Extended Spectrum β -Lactamase (ESBL) and Virulence Gene-Factors in Uropathogenic *Escherichia coli* (UPEC) in Children in Duhok City, Kurdistan Region, Iraq. Antibiotics (Basel). 2022;11(9): 1246. doi:10.3390/antibiotics11091246

[22] Mohammed AB, Anwar KA. Phenotypic and Genotypic Detection of Extended Spectrum Beta lactamase Enzyme in *Klebsiella pneumoniae*. PLoS One. 2022;17(9):e0267221. doi:10.1371/journal.pone.0267221

[23] Begum S, Salam A, Alam KF, Begum N, Hassan P. Detection of Extended Spectrum B -Lactamase in *Pseudomonas* spp. Isolated From Two Tertiary Care Hospitals in Bangladesh. BMC Res Notes. 2013;6:7 doi.org/10.1186/1756-0500-6-7.

[24] Singh-Moodley A, Duse A, Naicker P, Kularatne R, Nana T, Mbelle N, et al. Laboratory Based Antimicrobial Resistance Surveillance For *Pseudomonas aeruginosa* Blood Isolates from South Africa. J Infect Dev Ctries. 2018;12(8):616-624.

[25] Rahimi E, Asgari A, Azimi T, Soleiman-Meigooni S. Molecular Detection Of Carbapenemases And Extended-Spectrum B-Lactamases-Encoding Genes In Clinical Isolates of *Pseudomonas aeruginosa* in Iran. Jundishapur J Microbiol. 2021;14(7):14(7):e115977. https://doi.org/10.5812/jjm.115977.

[26] Ugbo EN, Anyamene CO, Moses IB, Iroha IR, Babalola OO, Ukpai EG, et al. Prevalence of blaTEM, blaSHV, and blaCTX-M Genes Among Extended Spectrum Beta-Lactamase-Producing *Escherichia coli* and *Klebsiella pneumoniae* of Clinical Origin. Gene Rep. 2021;21(5):100109. https://doi.org/10.1016/j.genrep.2020.100909

[27] Eguale T, Birungi J, Asrat D, Njahira MN, Njuguna J, Gebreyes WA, et al. Genetic Markers Associated With Resistance To Beta-Lactam and Quinolone Antimicrobials in Non-Typhoidal *Salmonella* Isolates From Humans and Animals in Central Ethiopia. Antimicrob Resist Infect Control. 2017;6:13. doi:10.1186/s13756-017-0171-6.