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MULTI-DRUG RESISTANCE PATTERN OF *PSEUDOMONAS AERUGINOSA* AMONGST CLINICAL SAMPLES FROM SURGICAL UNIT IN EDO SPECIALIST HOSPITAL BENIN CITY, NIGERIA

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ABSTRACT

Pseudomonas aeruginosa is a leading cause of nosocomial infections in surgical units, presenting significant challenges in hospital settings worldwide. The study aims to determine the prevalence and antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* isolated from Surgical Unit in Edo specialist Hospital (ESH), Benin City, Edo State. This was a cross-sectional based study conducted at Edo Specialist Hospital, from November 2023 to July, 2024. A total of one hundred clinical wound swabs were obtained from the participants using pre-tested questionnaires. Samples were inoculated and bacteria were isolated using standard microbiological methods. Antimicrobial susceptibility testing was performed by modified Kirby Bauer's method. Multidrug resistant isolates were further subjected to molecular method of plasmid DNA analysis. Obtained data was analyzed using Statistical package for social science (SPSS). The prevalence of *Pseudomonas aeruginosa* was 21.3% irrespective of other bacteria isolates. Susceptibility profiles of *P. aeruginosa* isolates in our study were all 100% resistant to Cefuroxime, Ceftriaxone, Oxacillin, Augmentin and Erythromycin and 70% least resistant to Ciprofloxacin antibiotics used. The bacteria isolates exhibited a higher sensitivity pattern to the Imipenem antibiotic. The PCR techniques also revealed that all the *P. aeruginosa* that were resistant to Augmentin expressed the (SHV) resistance genes with bands at 100bp in the clinical isolates from surgical wound infections in ESH. In conclusion, there was a high prevalence of multi-drug-resistant *P. aeruginosa* in surgical wound infections at ESH. The genetic evidence of resistance mechanisms in this study indicates the need for rigorous infection control measures to prevent the spread of these resistant strains.

Keywords: Nosocomial, Antimicrobial, Inoculated, Susceptibility, Resistance.

INTRODUCTION

Surgical wound infections are among the most prevalent nosocomial infections and is defined as a wound infection that occurs within 30 days of an operative procedure or within a year if an implant is left in place. Surgical wound infections are classified into Superficial, Deep incisional and Organ/space

infections (Sharan *et al.,* 2015). Surgical wound infections pose significant risks to patients undergoing surgical procedures, leading to prolonged hospital stays, increased healthcare costs, and in severe cases, morbidity and mortality (Kaoutzanis *et al*., 2015).

Surgical wound infections occur when microorganisms, such as bacteria, fungi, or viruses, invade the surgical incision site. These pathogens can be introduced during the surgical procedure itself or through contamination from the patient's own flora, healthcare workers, or the environment. The common causative bacteria are *Staphylococcus aureus* (31.58%) followed by *Klebsiella pneumonia* (26.31%) and *Pseudomonas aeruginosa* (17.8%) (Motie *et al.*, 2014).

Pseudomonas aeruginosa is a Gram-negative, rod-shaped bacterium that is a significant opportunistic pathogen, particularly in healthcare settings. This organism is ubiquitous in the environment, found in soil, water, and on various surfaces, which contributes to its presence in hospitals (Panda *et al*., 2022). *Pseudomonas aeruginosa* is known for causing a wide range of infections, including respiratory tract infections, urinary tract infections, dermatitis, soft tissue infections, bacteremia, and particularly infections in burn patients and those with surgical wounds (Silva *et al*., 2020). Its pathogenicity is enhanced by its ability to form biofilms, which protect bacterial communities from both the host immune response and antibiotic treatment, making infections difficult to eradicate (Qin *et al*., 2022).

A major concern with *Pseudomonas aeruginosa* is its high level of antibiotic resistance. This resistance arises from multiple mechanisms, including the production of beta-lactamases, efflux pumps that expel antibiotics out of the cell, and mutations that alter antibiotic targets (Pachori *et al*., 2019). These mechanisms collectively contribute to its classification as a multi-drug resistant (MDR) pathogen. The bacterium's resistance to many commonly used antibiotics complicates treatment regimens, often requiring the use of more potent and sometimes more toxic drugs (Rocha *et al*., 2019). The adaptability and resilience of *P. aeruginosa* make it a critical target for infection control practices and antibiotic stewardship programs, as controlling its spread and mitigating its impact on patient health are crucial for improving clinical outcomes (Coque *et al*., 2023).

In Nigeria, the burden of infections caused by multi-drug resistant *Pseudomonas aeruginosa* (especially in surgical units) is particularly concerning due to limited healthcare resources and the high prevalence of hospital-acquired infections (HAIs). Particularly, there is lack of documented information on the prevalence rate, Antimicrobial Susceptibility and genetic determination of multi-drug resistant *Pseudomonas aeruginosa* in Edo Specialist Hospital Benin City, Edo State. Therefore, this study was aimed at determining the prevalence and antibiotics susceptibility patterns of *Pseudomonas aeruginosa* isolated from Surgical Unit in Edo Sspecialist hospital, Benin city, Edo state.

MATERIALS AND METHODS

Study Area

This study was carried out at Edo Specialist Hospital, Benin City, Edo State. Edo State is located at latitude 6.6342°N and longitude 5.9304°E of the equator and prime meridian, respectively. The state covers an area of 19,794 km² and had a provisional population of 2,159,484, with Benin City estimated at 1,147,188 (National Population Census, 2006).

Study Design

A cross- sectional based clinical samples of hospitalized post operative surgical patients and other samples were used in the study. Ethical approval was obtained from the ethics committees of Military of health before the commencement of this study.

Study Population

The study subjects included a total of 50 male and 50 female hospitalized Patients having post operative surgical wounds from the department of general surgery, obstetrics/gynaecology and orthopaedic wards of Specialist Hospital in Benin city within the ages 17 and 80. A total of 100 clinical wound swabs samples were collected.

Sample Collection

Random samplings of patients with post operative surgical wounds were used in this study. Each day samples were collected from postoperative patient by using even number or odd number such that patients with postoperative infections will have equal chances of been sample randomly. An informed consent form was signed before sample collection. Samples were collected in duplicate before surgical wound dressing. All collections were done under strict aseptic conditions. Specimens were transported immediately to the Medical Microbiology Laboratories at BIU. Benin City, Edo state for analysis within 3-4 hours of sample collection. Structured questionnaires were used to gather information from the patients.

Laboratory Procedures

Isolation of Organisms

Samples were transported to the Medical Microbiology Laboratory at Benson Idahosa University and inoculated into Blood agar, MacConkey, and CLED agar. Phenotypic and biochemical characterization of bacterial isolates was performed using standard procedures, including Gram staining, colony isolation, and biochemical tests such as catalase, coagulase, and oxidase. *Pseudomonas aeruginosa* was identified based on its pale-colored colonies on MAC and greenish colonies on nutrient agar, with additional morphological and biochemical observations.

Antimicrobial Susceptibility

Antimicrobial susceptibility testing was performed on each isolate using the Kirby-Bauer disc diffusion method following the National Committee for Clinical Laboratory Standards (NCCL, 2003) guidelines to assess the sensitivity of the test organisms to various antibiotics. The test isolates were cultured on Nutrient agar and incubated at 37°C for 24 hours. The resulting colonies were then suspended in sterile peptone water, and the inoculum density was adjusted to match the 0.5 McFarland turbidity standard. This inoculated broth suspension was used to evenly coat the surface of freshly prepared, dried Mueller-Hinton agar plates. The antimicrobial discs used against *Pseudomonas aeruginosa* isolates included Erythromycin (15 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Imipenem (10 µg), Cefuroxime (30 µg), Ceftriaxone (30 µg), Cefepime (30 µg), Augmentin (30 µg and Oxacillin (1 µg). The discs were placed on the surface of the inoculated Mueller-Hinton agar plates, which were then incubated at 37°C for 24 hours. Following incubation, the diameters of the inhibition zones were measured to the nearest millimeters using a transparent ruler. The results were interpreted according to the standard zone sizes outlined in the Clinical and Laboratory Standards Institute (CLSI) guidelines (2017).

Molecular Assay

Genomic DNA was extracted using the THERMO SCIENTIFIC GENE JET (Thermo Fisher Scientific Inc. Waltham, Massachusetts, U.S) kit by culturing isolates, centrifuging, and processing with digestion and lysis solutions, followed by ethanol precipitation and purification via a genomic DNA column. PCR detection targeted MDR genes using specific primers, including SHV

F: ATTTGTCGCTTCTTTACTCGC and SHV R: TTTATGGCGTTACCTTTGACC (Monstein, *et al.*, 2007). Quick load One Taq One Step PCR was performed with a 50µl reaction mix, following a protocol of denaturation, annealing, and extension cycles. A 1.5% agarose gel was prepared with Tris EDTA buffer and ethidium bromide for electrophoresis, where amplified PCR products were analyzed, visualized using a UV transilluminator, and photographed for molecular weight calculation.

Statistical Analysis

Data obtained from this research were presented and analyzed using the Statistical Package for Social Sciences (SPSS) version 21.0 (IBM Inc., USA). Results were expressed in tables and charts as appropriate.

RESULTS

Prevalence of *Pseudomonas aeruginosa* among surgical wound infection in ESH

A total of Hundred (100) post-operative wound swab specimens from hospitalized patients at ESH were analysed. Samples were collected from patients aged between 17-80 years. Overall, 5 samples had no bacterial growth after 48 hours, while 95 had bacteria growth. Here, 20% of the samples were positive for *P. aeruginosa* (Figure 1). The prevalence of *P. aeruginosa* was 21.05%.

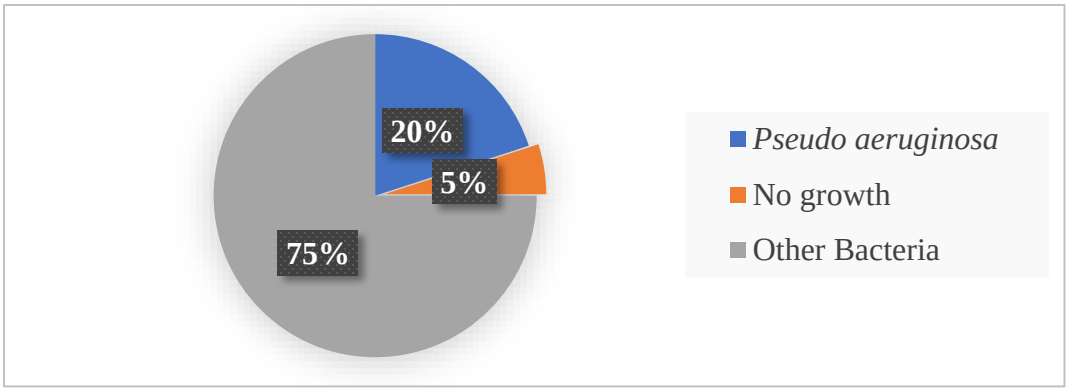

Figure 1: Bacterial growth from one hundred (100) post-operative wound swab specimens collected from hospitalized patients at ESH

Table 1: Phenotypic characterization of *Pseudomonas aeruginosa* from surgical unit.

Test	Result
Gram Reaction	Gram Positive Bacilli
Motility	Motile
Oxidase	Positive
Indole	Negative
Catalase	Positive
Urea	Negative
Gas	Negative

P. aeruginosa had some susceptibility to ciprofloxacin (30%), imipenem (40%) and cefepime (5%), but not to Augmentin, oxacillin, cefuroxime, ceftriaxone and erythromycin (Figure 2 and table 2).

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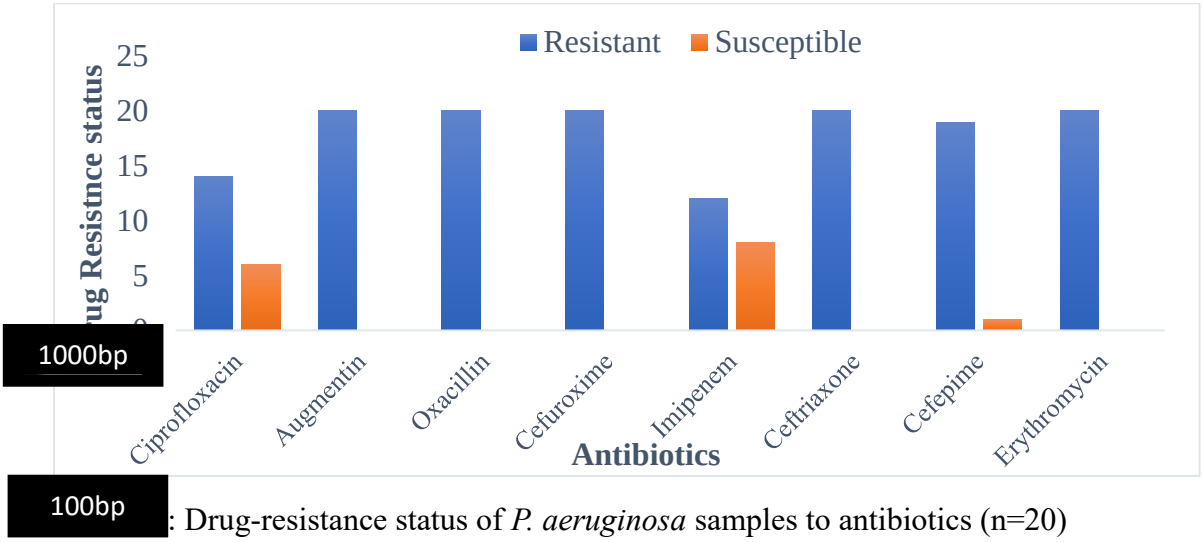
Table 2: Susceptibility profile of *Pseudomonas aeruginosa* to tested antibiotics

Class of Antibiotics	Type of antibiotics	No. tested =20 (%)	
		R	S
Penicillin	Augmentin (30µg)	20(100%)	0(0.0%)
	Oxacillin (4µg)	20(100%)	0(0.0%)
Macrolides	Erythromycin (10µg)	20(100%)	0(0.0%)
Cephalosporin	Ceftriaxone (30µg)	20(100%)	0(0.0%)
	Cefuroxime (30µg)	20(100%)	0(0.0%)
	Cefepime	19 (95%)	1 (5%)
Carbapenem	Imipenem (30µg)	12(60%)	8(40%)
Quinolones	Ciprofloxacin(5µg)	14(70%)	6(30%)

Keys: No. = Number of *Pseudomonas aeruginosa* tested, **R**= Number of bacteria resistant (%), **S**= Number of bacteria sensitive (%)



1000bp

100bp

: Drug-resistance status of *P. aeruginosa* samples to antibiotics (n=20)

Figure 2 above shows the drug resistance status of *P. aeruginosa* isolates against the various classes of antibiotics used. Where the *P. aeruginosa* expressed a high rate of resistivity.

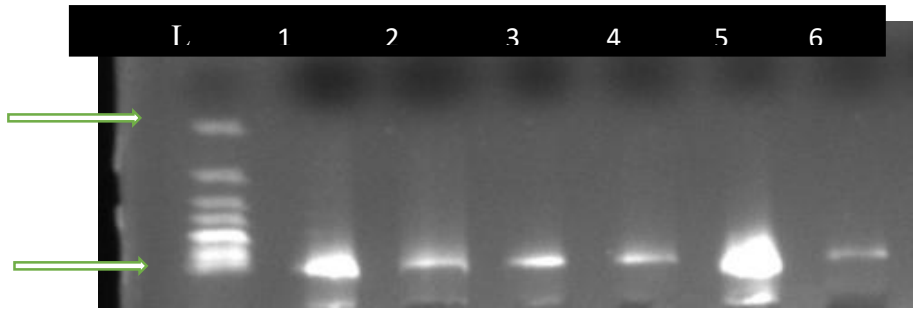


Figure 3: Polymerase chain reaction results of SHV resistant genes detected in clinical isolate analyzed on a 1.5% agarose gel electrophoresis stained with ethidium bromide. L is 100bp-1kb

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DNA ladder (molecular marker). Samples 1, 2, 3, 4, 5 and 6 were resistant to Augmentin and were also positive for SHV resistant genes with bands at 100 bp.

Figure 4: shows the comparison of phenotypic and genotypic detection of multidrug resistant *pseudomonas aeruginosa* that was resistant to Augmentin antibiotic phenotypically also were all positive to SHV resistant genes genotypically. This high resistance rate indicates that Augmentin may be less effective for treating surgical wound infections in this population, necessitating alternative or combination therapies.

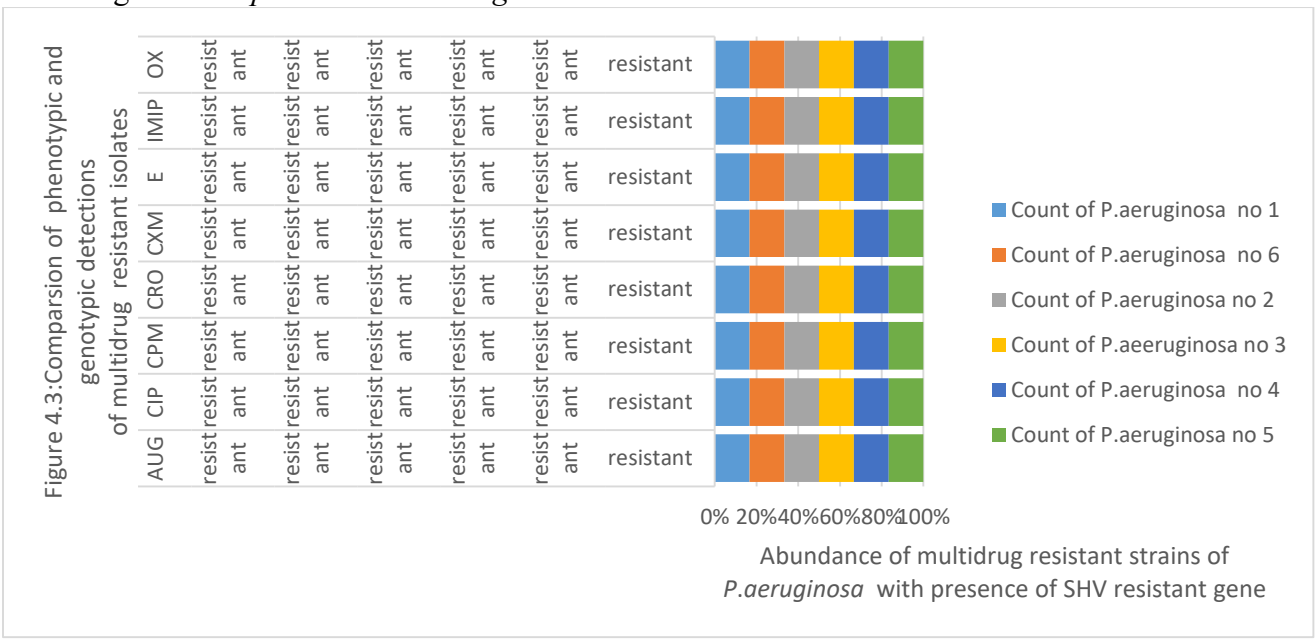

Figure 4: A comparison of phenotypic and genotypic multidrug resistant strains of *Pseudomonas aeruginosa* to Augmentin with presence of SHV resistant gene.

DISCUSSION

The present study successfully isolated and characterized *Pseudomonas aeruginosa* from surgical wound infections in the Edo Specialist Hospital (ESH), Benin City. Our findings revealed that *P. aeruginosa* is a significant pathogen in surgical site infections, accounting for 21.3% of the isolates, as the prevalence rate and the third most common pathogen in surgical wound infections. The prevalence of *P. aeruginosa* observed in this study aligns with previous research conducted in similar settings. For instance, a study by Izadi *et al*. (2021) reported that surgical wound infections accounted for 19.73% of healthcare-associated infections, which is consistent with our findings. The results are also comparable to those reported by Lakoh *et al*. (2022), who found that the prevalence of surgical wound infections in Africa ranges from 6.8% to 26%. The 21.3% prevalence of *P. aeruginosa* in our study falls within this range, suggesting that our findings are representative of the broader regional context.

The characterization of *P. aeruginosa* isolates revealed high levels of antibiotic resistance, which is consistent with the known adaptability and resilience of this pathogen. This observation aligns with the findings of (Pachori *et al*., 2019), who highlighted *P. aeruginosa* ability to resist conventional antibiotics due to complex drug-resistance

genes in plasmids or integrated into its genome.

This research third objective determine the antimicrobial susceptibility profiles of *Pseudomonas aeruginosa* isolates and prevalence rate of multi-drug resistance isolates from surgical wound infections in Edo Specialist Hospital, Benin City. This study revealed alarming rates of antibiotic resistance among *Pseudomonas aeruginosa* isolates from surgical wound infections in Edo Specialist Hospital (ESH). The observed resistance patterns are consistent with, and in some cases exceed, those reported in previous studies. These results align with the growing global concern over antibiotic resistance in *P. aeruginosa*. For instance, (Recio *et al*., 2020) and (Sader *et al*., 2018) reported prevalence rates ranging from 11.5% to 24.7% for MDR *P. aeruginosa* in the INFORM database. The finding suggests an even more critical situation in ESH, with resistance rates exceeding 90% for most antibiotics tested. The high resistance to ciprofloxacin (70%) observed in this study is alarming and the report by (Olowo *et al*., 2020) from Southwest Nigeria, also highlighted high rates of resistance to ciprofloxacin among *P. aeruginosa* isolates from chronic wounds. The 100% resistance observed against cephalosporins (cefuroxime, ceftriaxone) and near-complete resistance to cefepime (95%) in our study is alarming. These findings surpass the resistance rates reported by (Berrazeg *et al*., 2015), who noted increased resistance to cephalosporins due to overproduction of β-lactamases. Our results suggest a potentially more severe situation in ESH, possibly due to the overuse of these antibiotics or the spread of highly resistant strains. The 60% resistance to imipenem, while lower than for other antibiotics, is still concerning. This aligns with the World Health Organization's classification of carbapenem-resistant *P. aeruginosa* as a critical priority pathogen (Tacconelli *et al*., 2018). Our findings suggest that even last-resort antibiotics like carbapenems are losing effectiveness against *P. aeruginosa* in this setting. The high prevalence of MDR isolates in our study is consistent with the observations of Exner *et al*. (2017) who noted that MDR gram-negative bacterial infections, including those caused by *P. aeruginosa,* are major global health issues. Our findings show the severity of this problem in the surgical unit of ESH. Infections with MDR organisms are associated with poor clinical outcomes, increased morbidity and mortality, prolonged hospitalization, and high healthcare costs. The high prevalence of MDR *P. aeruginosa* in surgical wound infections at ESH suggests that these negative outcomes may be frequent occurrences (Huang 2018). Furthermore, our findings support the observations of Santajit *et al*. (2016), who reported that *P. aeruginosa* commonly possesses inherent resistance to antimicrobial agents through various mechanisms. The broad resistance profile observed in our study suggests the presence of multiple resistance mechanisms, potentially including reduced membrane permeability, efflux pump systems, enzymatic inactivation, and biofilm formation.

The fourth objective of this research was to determine the genetic determinants of multi-drug-resistant *Pseudomonas aeruginosa* from surgical unit in ESH Benin City, Edo State. This study identified the presence of SHV resistant genes in clinical isolates of *Pseudomonas aeruginosa* from surgical wound infections in the Edo Specialist Hospital (ESH), Benin City. This finding provides crucial genetic evidence for the observed phenotypic multi-drug resistance in these isolates. The detection of SHV resistant genes through PCR analysis, as shown in figure 3, aligns with previous research on the genetic basis of antibiotic resistance in *P. aeruginosa*. Our results corroborate the

findings of Potron *et al*. (2015), who identified various metallo-beta-lactamase (MBL) genes in *P. aeruginosa*, including IMP, VIM, SPM, GIM, NDM, and FIM. The presence of SHV genes in our isolates adds to this list of known resistance genes and underscores the genetic complexity underlying multi-drug resistance in *P. aeruginosa.* The identification of SHV genes in our study is particularly significant when considered alongside the high levels of phenotypic resistance observed in the antibiogram tests. This genetic evidence provides a mechanistic explanation for the broad-spectrum resistance seen in our isolates, which showed high resistance rates to multiple classes of antibiotics, including beta-lactams, fluoroquinolones, and aminoglycosides. These findings are consistent with those reported by Bhat *et al*. (2023), who emphasized the role of integron as reservoirs for numerous antibiotic resistance genes in *P. aeruginosa*.

The presence of SHV genes in our isolates suggests that these genetic elements may be part of larger mobile genetic structures, potentially facilitating the spread of resistance within the bacterial population in the surgical unit. The detection of SHV genes also aligns with the observations of Michaelis and Grohmann (2023), who highlighted the importance of horizontal gene transfer in the acquisition of antibiotic resistance genes by *P. aeruginosa*. The presence of these genes in our isolates suggests that horizontal gene transfer may be occurring within the hospital environment, contributing to the spread of multi-drug resistance. Furthermore, these findings support the concept of the "resistome" in *P. aeruginosa* (Murray *et al*., 2015).

The presence of SHV genes in our isolates is particularly concerning when considered in the context of the clinical impact of multi-drug-resistant *P. aeruginosa.* As noted by Kang *et al*. (2003), In Nigerian, these findings provide new insights into the genetic determinants of antibiotic resistance in *P. aeruginosa* isolates from surgical wound infections. While previous studies by Olowo *et al*. (2020) and Umeokonkwo *et al*. (2021) reported on the phenotypic resistance patterns of *P. aeruginosa in* Nigerian healthcare settings, the study adds valuable information about the genetic underpinnings of this resistance. The identification of SHV genes in our isolates also has implications for infection control and antibiotic stewardship in ESH and similar healthcare settings. As highlighted by Exner *et al*. (2017), multi-drug-resistant organisms pose significant challenges in hospital environments. The genetic evidence of resistance mechanisms in our study underscores the need for rigorous infection control measures to prevent the spread of these resistant strains.

CONCLUSION

This study revealed the prevalence rate of *Pseudomonas aeruginosa* in surgical wound infections at ESH to be 21.3%. This prevalence positions of *P. aeruginosa* as the third most common pathogen in surgical wound infections thrive in healthcare environments and cause infections indicate its importance as a nosocomial pathogen. The findings emphasize the critical need for enhanced infection control practices and targeted antimicrobial strategies to combat the growing threat of MDR *P. aeruginosa* in healthcare settings. Further research is recommended to explore alternative treatment options and develop effective interventions to manage and prevent *P. aeruginosa* infections in surgical units.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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