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Multidrug resistance *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated from wound patients in Alex Ekwueme Federal University Teaching Hospital Abakaliki.

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Abstract
The inappropriate use of antimicrobials, along with environmental conditions, leads to the emergence of multi-drug-resistant bacteria. The present study determined the Multidrug-resistance *Pseudomonas aeruginosa* and *Staphylococcus aureus* Isolated from Wound Patients in Alex Ekwueme Federal University Teaching Hospital Abakaliki. A total number of 229 wound samples were collected from wound patients and analyzed using standard microbiology techniques to determine the presence and distribution of *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolates. Antibiotic susceptibility testing and multidrug resistance of these isolates were performed using a modified Kirby-Bauer disk diffusion method. Out of the 229 wound samples analyzed, Gram positive and negative bacteria were isolated in 132 (57.6 %) samples, out of which, *Pseudomonas aeruginosa* was 39.4 % while 60.6 % was *S. aureus*. Analysis of antibiotic resistance rates indicated that most of the *S. aureus* isolates were resistant to, imipenem (95.0 %), oxacillin (85.0 %), cefotaxime (85.0 %), and erythromycin (55.0 %), while the *P. aeruginosa* isolates showed resistance to cefepime (86.5 %), trimethoprim/sulphamethaxazole (84.6 %), amoxicillin/clavulanic acid (80.8 %), cefotaxime (80.8 %), ceftazidime (78.8 %) and ceftriaxone (73.1 %). The multiple antibiotic resistance indexes showed an average of 0.70, which indicated that isolated *P. aeruginosa* and *S. aureus* were multi-drug-resistant. The identification of the most effective antibiotics against some microbial species could orient the clinicians towards the administration of some antimicrobials rather than others, resulting in a limitation in the use of less effective drugs for the treatment of wound infections.

Keywords: Multidrug-resistance, wound, susceptibility

Introduction

Multidrug-resistance (MDR) is an antimicrobial resistance exhibited by some microorganisms to multiple antimicrobial drugs (Magiorakos, 2012; WHO, 2018) [19]. The emergence of drug-resistant pathogens necessitates the development of novel antimicrobial agents with alternative modes of action to conventional antimicrobial agents (Kumar *et al.*, 2021) [17]. The rise in drug resistance has been caused partly by the incorrect use of treatments, and this has led to reduced efficacy against Gram-positive and Gram-negative bacteria (Lee Ventola *et al.*, 2015) [18].

Wound infection occurs when virulence factors expressed by one or more microorganisms in a wound outcompete the host natural immune system and subsequent invasion and dissemination of microorganisms in viable tissue provokes a series of local and systemic host responses (Pandith, 2013) [21].

The progression of a wound to an infected state is likely to involve a multitude of microbial and host factors, including the type, site, size, and depth of the wound, the extent of nonviable exogenous contamination, the level of blood perfusion to the wound, the general health and immune status of the host, the microbial load, and the combined level of virulence expressed by the types of microorganisms involved. This study aimed at to determine the Multidrug Resistance *Pseudomonas aeruginosa* and *Staphylococcus aureus* Isolated from Wound Patients in Alex Ekwueme Federal University Teaching Hospital Abakaliki.

Methodology

Study Area

This study was carried out at Alex Ekwueme Federal University Teaching Hospital Abakaliki, and Ebonyi State University Abakaliki. Both are in Abakaliki town, the capital city of Ebonyi State. Alex Ekwueme Federal University Teaching Hospital Abakaliki is located at 6.32ºN latitude and 8.12ºE longitude and is situated at an elevation of 117 meters above sea level, while Ebonyi State University Abakaliki is located at 6.20ºN latitude and 8.6ºE longitude. Abakaliki is populated and inhabited by indigenes and people from other parts of Nigeria (Ezegwui *et al.*, 2013) [9].

Specimen collection

Wound swabs were conveniently collected from 229 patients with wound infection from March 2023 to May 2024. The Swabs were collected using commercially available sterile cotton swabs and following existing guidelines of the outpatient department. Only one swab per patient was collected after carefully cleaning the wound with physiological sterile normal saline solution in order to prevent surface contamination. After sample collection, wound specimens were transported to microbiology laboratory unit of Ebonyi State University, Abakaliki, within 30 minutes by placing the swabs into the sterile test

tubes having 0.5 mL of sterile normal saline for bacteriological analysis.

Laboratory Analysis

The media used were: (Nutrient Agar (NA), MacConkey Agar (MA), Cysteine Lactose Electrolyte Deficiency (CLED), Peptone Water (PW), Mueller-Hinton Agar (MHA), Preparation of Simmon's Citrate Agar, Preparation of Peptone Water). All media were prepared following strict adherence to the manufacturer's instructions.

Identification of Isolates

The organisms were identified based on gram staining and their reactions to standard microbiology biochemical tests which include: (Motility test, Indole test, Oxidase test, Catalase test, Methyl red test, Voges Proskauer test, Simmon's Citrate Agar, Coagulase test, Deoxyribonuclease (DNA-ase test) test, Nitrate reduction test (NR), Triple Sugar – Iron test).

Antibiotics Susceptibility Testing

A standard inoculum equivalent to 0.5 MacFarland standard of the isolate was inoculated aseptically on the surface of a prepared Mueller-Hinton agar plates. The inoculated plates were allowed for 10-15 minutes. The antibiotics disc was placed on the surface of the plate and the disk was aseptically placed on the plates using forceps and incubated at 37º C for 18-24 h. After 24 h, the Muller-Hinton agar plates were observed and the diameter of the zone of inhibition was measured in millimeter using a meter rule. The resistance (R), intermediate (1), susceptible (S) of the isolates to the antibiotic discs was determined according to the Clinical Laboratory Standard Institute guidelines (CLSI, 2007), standard and the results were recorded.

Determination of multiple antibiotic resistance index (MARI)

Multiple antibiotic resistance (MAR) index was determined for each isolate by using the formula MAR = a/b, where 'a' represents the number of antibiotics to which the test isolate depicted resistance and 'b' represents the total number of antibiotics to which the test isolate has been evaluated for susceptibility (Akinjogunle *et al*., 2010).

Results

Morphology and biochemical characteristics of *Pseudomonas aeruginosa and Staphylococcus aureus* from wound samples of patient attending Alex Ekwueme Federal University Teaching Hospital (AE-FUTHA).

Table 1 showed the result of morphological and biochemical characteristics of the bacteria isolated from wound swab samples. The test result revealed Gram-negative rod bacteria, motile, catalase negative, oxidase positive, indole negative, methyl red and Voges-Proskauer negative and the isolate was identified as *Pseudomonas aeruginosa*, on the other hand, the characteristics of Gram-positive organisms in cocci shape bacteria, non-motile, coagulase positive, catalase positive, oxidase negative, indole negative, methyl red and Voges-Proskauer positive were observed and identified as *Staphylococcus aureus*.

Percentage distribution of Bacterial Isolates from Wound Swabs Infected Patients at Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA).

Table 2 showed that out of the 229 wound swabs analyzed, Gram positive and Gram-negative bacteria were isolated in 132 (57.6 %) samples, indicating distribution of 39.4 % (52/132) *Pseudomonas aeruginosa* and 60.6 % (80/132) *S. aureus*. This indicates that *S. aureus* is most prevalent and may pose a serious threat to the patients and the hospital.

Table 1: Morphology and biochemical characteristics of *Pseudomonas aeruginosa and Staphylococcus aureus* from wound samples of patient attending Alex Ekwueme Federal University Teaching Hospital (AE-FUTHA)

staining	Shape	Coagulase	Catalase	TSI	Oxidase	Motility	VP	red	Indole	Citrate	Glucose	Lactose	Galactose	Arabinose	Sorbitol	Xylose	Fructose	Bacterial isolates
-	Rod	-	-	A	+	+	-	-	-	+	+	-	+	-	-	+	+	*P. aeruginosa*
+	Coci	+	+	+	-	-	+	+	-	+	+	+	+	-	-	-	+	*S. aureus*

KEYS: A/A- Acid/Alkaline, A-Acid, A/G-Acid/Gas, VP-Voges-Proskauer, TSI-Triple Sugar Iron, (+)- positive, (-)- negative

Table 2: Percentage Distribution of Bacterial Isolates from Wound Swabs of Infected Patients at Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA)

Bacterial isolates	Frequency	Percentage (%)
Staphylococcus aureus	80	60.6
Pseudomonas aeruginosa	52	39.4
Total	132	100

Antibiotics Profiles of *S. aureus* and *P. aeruginosa* Isolated from Wound Infected Patients at Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA).

Table 3 reveals that most of *P. aeruginosa* isolates showed the resistance to FEP (86.5 %), SXT (84.6 %), AMC (80.8 %), CTX (80.8 %), CTZ (78.8 %) and CRO (73.1 %). On the other hand, *P. aeruginosa* isolates were susceptible to MEM, CN and TPZ with resistance rates of 0.0 %, 0.0 %, and 7.7 % while most of the *S. aureus* isolates were resistant to, imipenem (95.0 %), oxacillin (85.0 %), cefotaxime (85.0 %), and erythromycin (55.0 %). On the other hand, *S aureus* was susceptible to vancomycin, ciprofloxacin and lincomycin with resistance rates of 7.5 %, 18.0 %, and 35.0 %.

MARI of *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated from wound infection Patients at Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA).

Table 4 reveals that both *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from the wound samples had equal average MARI value of 0.70.

Table 3: Antibiotics Profiles of *P. aeruginosa* and *S. aureus* Isolated from Wound Infected Patients at Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA).

Antibiotics	***Pseudomonas aeruginosa* N = 52**		**Antibiotics**		***Staphylococcus aureus* N = 80**
Conc. (µg)	**Resistance (%)**	**Susceptibility (%)**	**Conc. (µg)**	**Resistance (%)**	**Susceptibility (%)**
MEM	0.0	100	DA	40.0	60.0
ATM	32.7	67.3	E	55.0	45.0
TPZ	7.7	92.3	CIP	18.0	82.0
AMC	80.8	19.2	L	35.0	65.0
CRO	73.1	26.9	OX	85.0	15.0
CN	0.0	100	FOX	45.0	55.0
OFX	48.1	51.9	VA	7.5	92.5
SXT	84.6	15.4	CTX	85.0	15.0
TE	32.7	67.3	IPM	95.0	5.0
CTX	80.8	19.2			
FEP	86.5	13.5			
CTZ	78.8	21.2			

MEM-Meropenem, ATM-Aztreonam, TPZ-Piperacillin/tazobactam, AMC- Amoxicillin/clavulanic acid, CRO-Ceftriaxone, CN-Gentamycin, OFX- Ofloxacin, SXT-Trimethoprim/sulphamethaxazole, TE-Tetracycline, CTX-Cefotaxime, FEP-Cefepime, CTZ-Ceftazidime. DA-Clindamycin, L-Lincomycin, CIP-Ciprofloxacin, OX-Oxacillin, FOX-Cefoxitin, E-Erythromycin, VA-Vancomycin, CTX-Cefotaxime, IPM-Imipenem

Table 4: MARI of *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from wound infection Patients at Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA).

Organism	**MARI Value**
Staphylococcus aureus	0.70
Pseudomonas aeruginosa	0.70
Total	1.4 Average= 0.70

Discussion of Results

In this study, the most common isolate was *S. aureus* 88 (60.6 %) as shown in Table 2, which was also reported in many other studies to be the predominant bacteria (40–60 % of the total bacteria) isolated from different types of wounds (Davies *et al.*, 2004; Gjødsbøl *et al.*, 2006; Körber *et al.*, 2010; Urbancic-Rovan and Gubina, 2000) [6, 11, 16, 27]. *P. aeruginosa* 52 (39.4 %) was the Gram-negative bacterium detected out of 132 isolates, which is also in agreement with other reports of (Burmølle *et al.*, 2010; Davies *et al.*, 2004; Serra *et al.*, 2015; Gjødsbøl *et al.*, 2006) [4, 6, 11, 24], who reported that *S. aureus* and *P. aeruginosa* play a central role, colonizing about 93.5 % and 52.2 % of patients with chronic leg ulcers, respectively. This finding is also in line with the work of Guan *et al.*, 2021 [12], who reported that the most common bacteria isolates among patients with chronic wounds were *S. aureus* (29.2 %), *E. coli* (11.4 %), *P. aeruginosa* 52 (11.0 %), *Proteus mirabilis* (8.0 %) and *Klebsiella pneumoniae* (5.8 %).

The possible reason for the high frequency of *S. aureus* and *P. aeruginosa* may be that these bacteria are commonly found in the hospital environment. In addition, *S. aureus* is a normal flora of healthy person (especially on skin), so whenever there will be breaks and cuts on skins and soft tissue, it can easily disseminate.

It is well documented that bacteria such as *S. aureus* and *P. aeruginosa* produce very destructive virulence factors, responsible for maintaining infection and delay healing in chronic wounds. *S. aureus* causes clinically relevant infections mostly because of its virulence factors such as coagulase, catalase, clumping-factor A and leucocidines (Dissemond, 2009) [7]. Similarly, the production of an elastase by *P. aeruginosa* has been associated to its pathogenicity in the wound environment (Schmidtchen *et al.*, 2003) [23].

Globally, antibiotic resistance has become a growing concern in the public health sector. This is because resistance often results in treatment failure, which can have serious consequences especially in critically ill patients (WHO, 2022). Resistant bacteria may spread and create broader infection control problems, both within healthcare institutions and in the community. Multiple antibiotic resistant *Staphylococcus aureus and P. aeruginosa* are major threats to patients care, owing to their stubborn intransigence to chemotherapy (Nwobodo *et al.*, 2022) [20].

Analysis of species-specific resistance rates from our study indicated that most of the *S. aureus* isolates were resistant to, imipenem (95.0 %), oxacillin (85.0 %), cefotaxime (85.0 %), and erythromycin (55.0 %). On the other hand, *S. aureus* was susceptible to vancomycin, ciprofloxacin and lincomycin with resistance rates of 7.5 %, 18.0 %, and 35.0 %, respectively.

The resistance rate of cefotaxime (85.0 %) and erythromycin (55.0 %) presented in this study is in agreement with the work of Ibrahim *et al.*, 2013 [13], where antibiotic susceptibility results of *S. aureus* showed high resistance against cefotaxime (81 %), and erythromycin is 59 %. The resistance rates of erythromycin (55.0 %) and vancomycin (7.5 %) is in line with the work of (Guan *et al.*, 2021) [12], who reported erythromycin (58.3 %) and vancomycin (0.0 %). However, ciprofloxacin resistance rates of 18.0 %, presented in this study is not in agreement with the work of (Ibrahim *et al.*, 2013) [13], where antibiotic susceptibility results of *S. aureus* showed high resistance against ciprofloxacin (41 %).

More-over, analysis of species-specific resistance rates indicated that *Pseudomonas aeruginosa* isolates demonstrated high resistance to various antibiotics, with cefepime showing the highest resistance at 86.5 %. Other significant resistance levels were observed against timethoprim/sulphamethaxazole (84.6 %), amoxicillin/clavulanic acid (80.8 %), cefotaxime (80.8 %), ceftazidine (78.8 %), and ceftriaxone (73.1 %). On the other hand, *P. aeruginosa* isolates were susceptible to meropenem (100 %), gentamycin (100 %) and piperacillin/tazobactam (92.3 %).

This finding is in line with the work of (Egwuatu *et al.*, 2022) [8], who reported high level of antibiotics resistance in third generation cephalosporins (cefotaxime, 61.3 % and ceftriaxone, 58.1 %), and penicillin + β-lactamase inhibitor (amoxicillin clavulanic acid, 58.1 %). This indicates a concerning trend of increasing antibiotic resistance in *P. aeruginosa*, potentially impacting treatment options. This data highlights the need for alternative antimicrobial strategies and emphasizes the importance of antibiotic stewardship to combat the growing challenge of antibiotic resistance in *P. aeruginosa* infections.

The aminoglycoside gentamicin, and carbapenem meropenem were however found to be effective against *P. aeruginosa* with 100 % and 100 % susceptibility respectively. This finding is slightly different from the work of (Egwuatu *et al.*, 2022) [8], who reported that aminoglycoside gentamicin (61.3 %), and carbapenem meropenem (83.9 %) were however found to be effective against *P. aeruginosa*.

Although gentamicin seems effective in this study, it had a resistance rate of 0.0 %, then the 35.4 % resistance reported in the study by (Adejobi *et al*., 2021) [1], in which gentamicin had the highest resistance rate. A lower susceptibility to meropenem was also observed in this study, with (Adejobi *et al*., 2021) [1], reporting 89 % susceptibility.

Antibiotics surveillance is expedient to monitor the effectiveness of commonly used antibiotics in order to mitigate selection of antibiotics resistant strains. Antibiotics prescription, particularly empiric therapy should be well guided, and prolonged antibiotics treatment of infections caused by antibiotic resistant bacteria discouraged to prevent further selection of resistant strains (Tamma *et al*., 2021) [26].

The result of gentamycin (0.0 %) from this study is not in agreement with the work of (Sanusi *et al.*, 2019) [22], who reported that the highest resistance rates among isolates were observed towards gentamicin (35.4 %), however, the result of Piperacillin/tazobactam with resistance rates (7.7 %) from this study is in agreement with (Sanusi *et al.*, 2019) [22] who reported that piperacillin/tazobactam was the most active antibiotic with a low resistance rate (6 %). Our observation is slightly different from what (Shrestha *et al.*, 2019) [25] reported from Kathmandu, Nepal. In their study, *P. aeruginosa* exhibited high rates of resistance to piperacillin (57.1 %) among others, while only 6.5 % of the isolates were resistant to meropenem which is in agreement with our reported resistance rate of 0.0 % for imipenem. Also, our finding is not in agreement with (Gad *et al.*, 2007) [10] reported from Egypt a higher rate for gentamicin (59 %) and meropenem (22 %) in an earlier study.

P. aeruginosa has also been implicated as a prominent cause of post-operative wound infection in Nigeria (Iduh *et al.*, 2015) [14]. *Pseudomonas aeruginosa* is one of the most important opportunistic pathogens responsible for 10-15 % of nosocomial infections worldwide. From the foregoing, it can be unequivocally stated that *P. aeruginosa* has now emerged as a highly multidrug-resistant pathogen with concomitant high multiple antibiotic resistance index in this environment. This is also in addition to the intrinsic nature of *Pseudomonas* being inertly impervious to most antibiotics due to the cell wall structure as well as its ease of spread in nosocomial settings.

All the MDR strains had a high MAR index suggesting a high-risk source where antibiotics are regularly and inappropriately used leading to high selective pressure. We can, therefore, safely speculate that the widespread, easy access, non-hygienic measures in hospitals, the ability of some bacteria to grow in hospital materials or indiscriminate use of antibiotics, fake drugs, self-prescription among patients and unrestrained antibiotic use have accelerated the incidence of antibiotic resistance and MDR strains in this environment.

Conclusion

Wound infections are underestimated problems that result into a chronic disease and the detection of microbial species, pathogens distribution and antimicrobial susceptibility patterns are important aspects, often underestimated, in order to limit the spread of antibiotic-resistant isolates. Multiple antibiotic resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* are major threats to patients care, owing to their stubborn intransigence to chemothrapy. In particular, the detection of the different microbial species colonizing a wound, as well as their susceptibility to the antimicrobials, can provide an indication for a more appropriate therapy to be administered to patients, significantly reducing the health care costs. Resistant bacteria may spread and create broader infection control problems, both within healthcare institutions and in the community. The identification of the most effective antibiotics against some microbial species could orient the clinicians towards the administration of some antimicrobials rather than others, resulting in a limitation in the use of less effective drugs for the treatment of wound infections.

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