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PHENOTYPIC AND MOLECULAR CHARACTERISATION OF *PSEUDOMONAS AERUGINOSA* AND *STAPHYLOCOCCUS AUREUS* ISOLATED FROM PATIENTS' WOUNDS IN BARAU DIKKO TEACHING HOSPITAL, KADUNA, NIGERIA

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ABSTRACT

This study was carried out to determine the phenotypic and molecular characteristics of *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated from patients' wounds in Barau Dikko Teaching Hospital, Kaduna. Sixty wound swabs were collected from in –and out - patients in the hospital. Each of the collected wound swab was inoculated in Brain Heart Infusion (BHI) broth at 37^0 C for 24 hrs, then sub-cultured on cetrimide and mannitol salt agar (MSA) at 37^0 C for 24 hrs for isolation of *Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively. The presumptive organisms were sub-cultured on MacConkey and Baird Parker agar respectively for identification based on cultural characteristics, followed by biochemical characterization using standard procedures. Three representative isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were confirmed using molecular methods. The *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolates were tested against conventional antibiotics using Kirby-Bauer disc diffusion method. Biofilm formation and extended spectrum beta-lactamase production by the isolates were determined using standard procedures. Out of the 11 *Pseudomonas aeruginosa* isolates screened using 11 antibiotics; 11 (100.0 %) were resistant to chloramphenicol, ampicillin, meropenem, and nalidixic acid; 8 (72.7 %) resistant to kanamycin and tetracycline, 7 (63.6 %) resistant to ceftriazone and amoxicillin-clavulanic acid, 4 (36.4 %) resistant to gentamycin, 2 (8.2 %) resistant to ciprofloxacin, and 11 (100.0 %) were sensitive to imipenem. Similarly, out of the 11 *Staphylococcus aureus* isolates screened using 12 antibiotics, 2(18.2 %) were resistant to gentamycin, 3(27.3 %) resistant to kanamycin, 5(45.5 %) resistant to ciprofloxacin, 7(63.6 %) resistant to chloramphenicol and vancomycin, 10(90.9 %) resistant to amoxicillin-clavulanic acid and sulphamethoxazole, and 11(100.0 %) resistant to ceftazidime, ampicillin, oxacillin and cefoxitin. Eleven (100.0 %) isolates were sensitive to imipenem. All the 11 *Pseudomonas aeruginosa* strains were non-biofilm formers and 3 were extended spectrum beta-lactamase producers. The 11 *Staphylococcus aureus* strains were biofilm formers. These findings are of public health importance, therefore, there is need for public education on rational drug prescription and use.

KEYWORDS: **Multidrug Resistance; *Pseudomonas aeruginosa*; *Staphylococcus aureus*; Wound; Barau Dikko Teaching Hospital.**

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INTRODUCTION

Resistance to antimicrobials and spread of drug resistant pathogens have become increasing threats to public health in the 21st century [1]. Antimicrobial resistance (AMR) is the ability of microorganisms to grow despite exposure to antimicrobial agent meant to inhibit their growth [2] and it is currently one of the major threats faced by mankind. Globally, infections caused by resistant bacteria are now alarming and thereby resulting in the availability of fewer treatment options.

The economic and health impact of AMR on a global scale is enormous and has health security challenges. Globally, it has been estimated that about 700,000 lives are lost annually due to antimicrobial-resistant infections [3-5]. The impact of AMR is worse in developing nations, including Nigeria, with unaccounted cost of treatment and associated deaths.

Multidrug-resistant (MDR) *Pseudomonas aeruginosa* has proved difficult to eradicate due to elevated intrinsic resistance that is chromosomally encoded, and its ability to acquire resistance to multiple antibiotics [1]. Likewise, *Staphylococcus aureus* is known to acquire resistance to new drugs and continues to defy attempts to control it. Globally, all infections caused by the antibiotic- resistant strains of *Staphylococcus aureus* have reached epidemic proportions, resulting in fewer treatment options available [6].

Major infectious pathogens in acute and chronic wounds include multidrug-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa.* These antibiotic-resistant bacterial infections narrow therapeutic options for patients with wounds, delay in the healing of wounds, prolonged hospital stay, poor cosmetic outcomes, increase in treatment costs, morbidity, poor patients' outcomes and mortality [7]. Globally, bacteria from wounds continually develop antimicrobial resistance and render even novel antibiotic ineffective on daily basis with no timely knowledge on the causes of the AMR. Hence, there is need for research to establish the phenotypic and genotypic bacterial characteristics responsible for the changes and development of AMR for prompt effective therapy, prevention and control. This study was carried out to determine the phenotypic and molecular characteristics of *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated from wounds of patients in Barau Dikko Teaching Hospital (BDTH), Kaduna State.

METHODS

Study Area

Kaduna State has an area size of 45,061 km^2 with a 2016 projected population of 8,252,400. Barau Dikko Teaching hospital is geographically located on Longitude 7^{0}26'29''E and Latitude 10^{0}31'33" N. It is the teaching hospital of Kaduna State University, where students and resident doctors are trained. It consists of many departments and units which provide twenty-four hrs medical services to people within and outside Kaduna State. The departments include community medicine, obstetrics and gynaecology, surgery, paediatrics, radiology, anaesthesia, ophthalmology and laboratory medicine, among others.

Study Design

This was a cross-sectional descriptive study of in- and out- patients with wounds attending clinics at BDTH between February and December, 2020.

Study Population

This consisted of in and out-patients with various wounds attending clinics at BDTH and various units of the hospital. Patients with different category of wound infection showing secretion/pus or purulent exudates or discharge, and those with long time (old) wound infection without signs of healings despite antibiotics treatment were included in the study. Patients with fresh wound, and/or topical antibiotics or tincture iodine treatment or those critically ill or unconscious were excluded.

Sample Size

A total of sixty samples were collected. In- and out-patients with infected wounds receiving medical care in clinics, units and wards in the hospital were selected for wound swabs. One wound swab sample was collected from each patient. In case of patients with two wounds, one swab sample was collected from only one wound using balloting. All the collected wound swab samples were appropriately labeled and transported to the laboratory for analysis.

The socio-demographic information and medical histories were also collected from the patients that wound swabs were collected.

Ethical Consideration

Permission to conduct the research was obtained from the Health Research Ethics Committee of BDTH (Reference number: HREC - 20-0004). Permission was obtained from all heads of the clinics, units and wards. Written informed consents were obtained from the eligible patients. Patients

were also informed of their freedom to consent or decline participation. Guardian or parents of eligible children gave assent for the children to participate in the study. The researchers paid for all the cost of the laboratory analysis for the collected samples.

Collection and Transportation of Clinical Wound Swabs

A total of sixty wound swabs were collected from the patients between February and December, 2020. With the help of nurses, exudate or purulent or pus discharge from the wounds were aseptically swabbed with sterile swab cotton tip and the cotton tip broke immediately into a sterile Brain Heart Infusion (BHI) broth in a universal bottle. After the collection and labeling of the wound swab, the questionnaire was filled to obtain the socio-demographic information about the patient. The samples collected were then transported in ice packed thermo flasks to the Department of Microbiology, Faculty of Science, Kaduna State University Postgraduate Medical Microbiology Laboratory for isolation and identification of *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Media Preparation

All media were prepared according to the manufacturer's instructions. Cetrimide Agar and MacConkey Agar were used for isolation of *Pseudomonas aeruginosa.* Mannitol Salt Agar (MSA) and Baird Parker Agar were used for isolation of *Staphylococcus aureus.* Nutrient Agar was used for culture preservation.

Isolation of *Pseudomonas aeruginosa* and *Staphylococcus aureus* from Wound Swabs

All clinical samples collected were cultured aerobically for isolation of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in the laboratory [8,9] The collected swabs were first cultured aerobically in an enrichment medium (BHI broth) at 37^0 C for 24 hrs. The broth cultures from the BHI broth were then inoculated on Cetrimide agar and Mannitol Salt agar (MSA plates) for selective isolation of *Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively. Pure culture colonies of *Pseudomonas aeruginosa* identified were further sub-cultured aerobically on MacConkey agar plates at 37^0 C for 24 hrs to confirm its characteristically non-lactose fermenters colonies. While the yellow colonies of presumptive *Staphylococcus aureus* on MSA plates were sub-cultured aerobically on another chromogenic media (Baird Parker agar) plates at 37^0 C for 24 hrs for appearance of grey to black shining with lytic edges morphological characteristics colonies isolates. Pure single colonies from these selective and differential media were subculture on nutrient agar slant and kept at 4^0 C for further identification and research purposes.

Morphological Characterisation of presumptive *Pseudomonas aeruginosa and Staphylococcus aureus* isolates

The cellular morphological characterization of the pure isolates obtained was carried out [9-10] A culture smear was prepared on a clean grease-free glass slide and allowed to dry, then fixed with gentle heat by passing the back side of the slide three times over a Bunsen burner flame. It was flooded with crystal violet stain for 1 min, then washed with tap water. Lugol's iodine was applied and left for 1 min, followed by washing with tap water. Ethanol (95 %) was applied for 1min then washed with tap water. A Safranin counterstain was applied for 1 min and then washed with tap water. The slide was then air dried and examined under an oil-immersion objective lens (×100) of a binocular bright field microscope. This was done for all the isolates. Gram-negative bacteria appeared red under view, while gram-positive appears blueish in colour.

Biochemical Characterisation of presumptive *Pseudomonas aeruginosa* and *Staphylococcus aureus* Isolates

Biochemical characterisation of the pure isolates obtained was carried out as earlier described [9-11] Motility, oxidase, motility, indole, triple sugar iron (TSI), citrate utilization, urease, methyl red and Voges-Proskauer test were carried out for identification of *Pseudomonas aeruginosa*; while motility, catalase, coagulase, and hemolysis, citrate utilization, methyl red, Voges-Proskauer, indole and sugars (lactose, mannitol and sucrose) fermentation test were carried out for identification of *Staphylococcus aureus* isolates.

Molecular Identification of *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Chromosomal DNA Extraction

The DNA extraction was carried out using Bioneer bacterial extraction kits (Genomic DNA extraction kits) protocols - Bioneeraccuprep genomic DNA extraction kit (K-3032).

Standard inoculum (a density of $1x10^8$ cells/ml) of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were prepared from 24 hrs broth culture.

Two millilitres (2 ml) of the prepared standard inoculum was transferred to 5 ml sterile eppendorf tube and centrifuged for 5 mins at 10,000 rpm. The

supernatant was carefully discarded without disturbing the pellet. Another 2 ml of the standard inoculum added and centrifuged at 10,000 rpm for 5 mins, followed by carefully discarding the supernatant, and repeated once again to obtained more quantity of DNA.

The pellets obtained was re-suspended in 200 µl of phosphate buffer saline (PBS) in the eppendorf tube. Twenty microlitres (20 µl) of Proteinase k was added to the tube containing the pellet in PBS, followed by addition of 10 µl of RNase, then mixed thoroughly by vortexing and incubated at room temperature.

Two hundred microlitres (200 µl) of GB buffer (lysis buffer) was added to the sample and mixed by vortexing, followed by incubation at 60^{o} C for 10 mins using heating block.

Four hundred microlitres (400 µl) of absolute ethanol (Biological grade) was added and mixed well by pipetting, followed by careful transferred of the lysate into the upper reservoir of the binding or absorption column (fitted in the collection tube) without wetting the rim. The tube was closed and centrifuged at 8,000 rpm for 1 min. followed by discarding the solution from the collection tube and then reused the collection tube.

Five hundred microlitres (500 µl) of W_2 buffer was added without wetting the rim, followed by closing the tube and then centrifuged at 8,000 rpm for 1 min. The solution from the collection tube was discarded and then reused the collection tube.

The sample was centrifuged once more at 13,000 rpm for 1 min to completely removed ethanol, followed by checking to ensured that there were no droplets clinging to the bottom of the binding column tube. The binding column tube was transferred to new 1.5ml tube for elution and 100 µl of elution buffer (EA buffer) was added on to the binding column tube and then kept at room temperature (15-25^{o} c) for 1 min.

Polymerase Chain Reaction (PCR) - AccupowerHotstart PCR premix (Bioneer)

Twenty microlitres (20µl) reaction PCR set - up was prepared by adding; 16ul dH_2O, 1 µl forward primer – GGACTACAGGGTATCTAAT 16S (RIBOSE-1), 1 µl reverse primer- AGAGTTTGATCCTGG 16S (RIBOSE-2), and 2ul template DNA. PCR amplification reaction was performed using PTC 100 thermal cycler with Pre- denaturation at 95^{o} C for 5 mins, denaturation at 94^{o} C for 1 min, primer annealing at 54^{o} C 1 min, extension at 72^{o} C 1 min for 25 cycles, and final extension at 72^{o} C 5 mins. The PCR products were separated by electrophoresis in 1.5 % agarose gel for 35 mins at 125 volt and then visualized the gel DNA bands using UV lightbox/ gel imaging system (Biorad). Amplified PCR products were sequenced and the nucleotides sequences of the 16SrRNA genes were searched for sequences similarities using online BLASTn.

Antimicrobial Susceptibility Tests Using Selected Conventional Antimicrobial Agents used for Treatments of Wound Infections

Antimicrobial susceptibility test against *Pseudomonas aeruginosa and Staphylococcus aureus isolates* was carried out using Kirby-Bauer disc diffusion techniques [12]. A loopful of 24 hrs' growth culture of each isolate in nutrient broth was suspended in 10 ml sterile distilled water and then diluted in steps of 1:10 to give turbidity equivalent to the 0.5 McFarland standards (a density of 1x10^{8} cells/ml) before inoculation. Sterile cotton wool swabs were dipped in the suspensions adjusted to 1x10^{8} cells/ml, the excess fluid was removed by pressing and rotating the swabs against the wall of the tubes, and then streaked on the surface of MHA plates. The inoculated plates were allowed to dry for about 5 mins. Using disc dispenser, single disc Gram positive antibiotics made by Oxoid namely; Gentamycin (10 µg), Amoxicillin- Clavulanic acid (30 µg), Nalidixic acid (30 µg), Kanamycin (30 µg), Ciprofloxacin (5 µg), Vancomycin (30 µg), Ampicillin (10 µg), Oxacillin (1 µg), Chloramphenicol (30 µg), Imipenem (10 µg), Cefoxitin (30 µg), and Sulphamethaxole (25 µg) were dispensed on inoculated plates of *Staphylococcus aureus.* Gram-negative antibiotics made by Oxoid namely; Ceftriazone (30 µg), chloramphenicol (30 µg), Gentamycin (10 µg), Imipenem (10 µg), Ampicillin (10 µg), Ciprofloxacin (5 µg), Kanamycin (30 µg), Meropenem (10 µg), Nalidixic acid (30 µg), Amoxicillin-Clavulanic acid (30 µg), and Tetracycline (30 µg) were dispensed onto the surface of the inoculated agar plates of *Pseudomonas aeruginosa*. After 30 mins of applying the discs, the plates were then incubated aerobically at 37^{0}C for 24 hrs in an inverted position. Diameter of zone of growth inhibition were measured using a transparent metric ruler and the results interpreted as either susceptible, intermediate, or resistant according to Clinical and Laboratory Standard Institute (CLSI) guidelines [13].

Phenotypic Biofilm Assay of Antibiotics Resistant *Pseudomonas aeruginosa* and *Staphylococcus aureus* Isolates

Determination of Biofilm Formation

The test organisms were grown for 18 hrs in Luria Bertani medium (LB) [14]. The 18 hrs culture was

diluted to 1:100 with a fresh LB medium for *Staphylococcus aureus* and *E. coli.* M63 minimal medium supplemented with 0.1% (w/v) arginine as the sole carbon and energy source was used for the 1:100 dilutions of *Pseudomonas aeruginosa*. One hundred microliter (100 µL) of the 1:100 dilutions were added per well in a 96-well microtitre plate.
For quantitative assays, 4 replicate wells were used for each treatment. The microtiter plate was incubated for 24 hrs at 37° C. The 96-well microtitre plate was stained after incubation. Cells were dumped out of the 96-well microtitre plate by turning the plate over and shaking out the liquid. The plate was gently submerged in a small tube of water. The water was drained off by shaking the plate. This process was repeated a second time. The step helped to removed unattached cells and media components that could be stained with crystal violet and significantly lowers background staining. A 125 µL aliquot of a 0.1% (w\v) solution of crystal violet in water was added to each well of the microtitre plate. The microtitre plate was incubated at room temperature for 15 mins and was rinsed three times by submerging in a tube of water. The rinsed plates were drained by shaking gently and then blotted vigorously on a stack of paper towels to rid the plate of all excess cells and dye. The microtiter plate was turned upside down and dried for 7 hrs, then photographed for qualitative assays.

Quantification of Biofilm Formation

One hundred and twenty-five microlitres of 30% acetic acid was dispensed into each well of the 96-well microtiter plate to solubilize the Crystal Violet (CV) and incubated at room temperature for 15 mins. 125 µL of the solubilized CV was transferred to a new flat bottomed 96-well microtitre plate. Absorbance was then quantified using ELISA plate reader at 630 nm using 30 % acetic acid in water as the blank. To categorise the biofilm forming capacity of these bacteria isolates used in this study, the following criteria were used;
OD_{cut} = (Optical density cut-off, is the unit of activity in a serodiagnostic test above which a test is classified as positive and below which they are considered negative) $OD_{average}$ of negative control + 3 x standard deviation (SD) of ODs of negative control [15].

1. $OD \leq OD_{cut}$ = Non-biofilm former
2. $OD_{cut} < OD \leq 2 \times OD_{cut}$ = Weak biofilm former
3. $2 \times OD_{cut} < OD \leq 4 \times OD_{cut}$ = Moderate biofilm former
4. $OD > 4 \times OD_{cut}$ = Strong biofilm former.

Phenotypic Detection of Extended Spectrum Beta-Lactamase (ESBL) Production in *Pseudomonas aeruginosa*

A double- disk synergy test was employed. This was carried out as described by Drieux *et al*. [16]. Overnight broth culture of the pure isolates was standardized using 0.5 McFarland standards. Mueller Hinton agar plate was inoculated with the test organisms. Cefpodoxine, Ceftazidime, Cefixime were used for screening of the isolate while Amoxicillin-Clavulanic acid, Cefpodoxime and Cefixime were positioned at a distance of 30 mm centre to centre. This was incubated aerobically at 35^0 C for 18-24 hrs. A greater than 5 mm increase in zone diameter for either antimicrobial agent tested in combination with Clavulanic acid versus it zone when tested alone confirmed as positive for phenotypic ESBL production. In other words, the test was considered as positive when a decreased susceptibility to Cefpodoxime and Cefixime was combined with a clear-cut enhancement of the inhibition zone of ceftazidime in front of the clavulanate-containing disk, often resulting in a characteristic shape-zone referred to as 'champagne-cork' or 'keyhole'.

Statistical Analysis

Data analysis was carried out using descriptive statistical method and results presented in tables and charts. SPSS version 23.0 was used for the analysis.

RESULTS

Morphological and Biochemical Characteristics of Presumptive *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Table 1 revealed eleven isolates identified which appeared blue – green and yellow – green in colour with circular, smooth edges, flat and moderate shape on Cetrimide agar, while on MacConkey agar, the colonies appeared colourless, flat and circular with smooth edge indicating typical characteristics of *Pseudomonas aeruginosa.* Table 2 revealed eleven isolates identified *Staphylococcus aureus* which appeared completely yellowish in colour with raised, circular and smooth edges on MSA. On Baird Parker agar, the colonies appeared grey - black with shining characteristics and lytic edges, while on blood agar, the colonies showed complete lysis of blood cells surrounding the colonies indicating typical characteristics of beta-hemolysis. The isolates revealed typical characteristics of *Staphylococcus aureus.*

Molecular Characteristics of *Pseudomonas aeruginosa* and *Staphylococcus aureus* Isolates

Plates 1 and 2 showed the Gel electrophoresis of amplified PCR 16SrRNA genes bands of *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolates respectively at 789 bp of the 100 bp plus DNA marker. The sequences BLAST results (Table 3) of the 16SrRNA genes of the presumptive *Pseudomonas aeruginosa* isolates; PD1, PF4 and PM1 revealed the percent identity or similarity of these isolates from GenBank database as 92.7 %, 97.7 % and 91.8 % respectively, confirming the identity of these isolates as *Pseudomonas aeruginosa* strains. Similarly, the sequences BLAST results of the presumptive *Staphylococcus aureus* isolates; SD1, SF1 and SD11 16SrRNA genes revealed the percent identity or similarity of these isolates from the GenBank database as 76.9 %, 91.6 % and 86.9 % respectively, confirming the identity of these isolates as *Staphylococcus aureus* strains.

Antimicrobial Activity of Selected Conventional Antibiotics Against *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains

Figures 1a and b, and figures 2a and b showed that all *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains were multi-drug resistant isolates. Figure 1a showed out of eleven *Pseudomonas aeruginosa* isolates screened using eleven selected conventional antibiotics, 11 (100 %) were resistant to chloramphenicol, ampicillin, meropenem, and nalidixic acid, 8 (72.7 %) resistant to kanamycin and tetracycline, 7 (63.6 %) resistant to ceftriazone and amoxicillin-clavulanic acid, 4 (36.4 %) resistant to Gentamycin, 2 (8.18 %) resistant to Ciprofloxacin; and all the isolates 11 (100.0 %) were sensitive to imipenem**.** The resistant pattern of *Pseudomonas aeruginosa* isolates showed in figure 1b indicated that three isolates (DR_{14}, $DR_{17,}$ and DR_{19}) were resistant each to 6 (54.6 %) antibiotics used, two isolates (DR_{12} and DR_{24}) resistant each to 7 (63.6 %) antibiotics used, three isolates (DR_1, DR_{23}, and DR_{30}) resistant each to 8 (72.7 %) antibiotics, two isolates (FSW_3 and FSW_4) resistant each to 9 (81.2 %) antibiotics, and only one isolate (MSW_1) was resistant to 10 (81.8 %) antibiotics used. According to the results imipenem, ciprofloxacin and gentamycin were the most effective antibiotics against all the *Pseudomonas aeruginosa* strains. Similarly, figure 2a revealed that out of eleven *Staphylococcus aureus* isolates screened using twelve selected conventional antibiotics, 2 (18.2 %) resistant to gentamycin, 3 (27.3 %) resistant to kanamycin, 5 (45.5 %) resistant to ciprofloxacin, 7 (63.6 %) resistant to chloramphenicol and vancomycin, 10 (90.9 %) resistant to amoxicillin-clavulanic acid and sulphamethoxazole, and 11 (100.0 %) resistant to ceftazidime, ampicillin, oxacillin and cefoxitin. All the 11 (100.0 %) isolates were sensitive to imipenem. The resistant pattern of *Staphylococcus aureus* isolates showed in figure 2b indicated that four isolates (DR_{19}, $DR_{21,}$ $FSW_1$and FSW_6) were resistant each to 7 (58.3 %) antibiotics used, five isolates (DR_3, DR_5, $DR_{11,}$ MSW_3 and MSW_4) resistant each to 8 (66.6 %) antibiotics used, and two isolates (DR_{12} and MSW_2) resistant each to 9 (75.0 %) antibiotics. According to the results; imipenem, gentamycin and kanamycin were the most effective antibiotics against all the *Staphylococcus aureus* strains.

Biofilm Formation Characteristics of *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Table 4 revealed that all *Pseudomonas aeruginosa* isolates had lower Optical Density (OD) than their respective Optical Density cut-off (OD_{cut}) indicating that they are non-biofilm formers. The biofilm characteristics of *Staphylococcus aureus* (Table 5) showed 8 isolates; DR_3, DR_5, DR_{11}, DR_{19}, DR_{21}, FSW_6, MSW_3 and MSW_4 are strong biofilm formers, while three (3); DR_{12}, FSW_1 and MSW_2 are moderate biofilm formers.

Extended Spectrum Beta- Lactamase (ESBL) Characteristics of Multidrug Resistant *Pseudomonas aeruginosa*

Multidrug-resistant *Pseudomonas aeruginosa* isolates were screened for ESBL using third generation cephalosporin namely: Cefpodoxime (10 µg), Ceftazidime (30 µg) and Cefixime (30 µg) with confirmatory test using Amoxicillin-Clavulanic acid (30 µg) (table 6). The results showed that out of 11 *Pseudomonas aeruginosa* isolates, three – DR_{14}, FSW_3 and FSW_4 were ESBL producers.

DISCUSSION

This study isolated and identified *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains from wounds of patients seeking medical care at Barau Dikko Teaching Hospital, Kaduna using both phenotypic and molecular methods. Cultural morphology of *Pseudomonas aeruginosa* on cetrimide agar revealed a characteristic blue-green

Table 1: Morphological and Biochemical Characteristics of Presumptive *Pseudomomas aeruginosa* Isolates

Isolate Identification Code	**Morphological Characteristics**			***Biochemical characteristics***									***TSI***				**Probable organism**
	Colonial morphology on cetrimide and MacConkey agar	Cellular/microscopic morphology	*Gram reaction*	*Matility*	*oxidase*	*Catalase*	*Indole*	*Methyl red*	*Voge-proskaver*	*Citrale-utilization*	*Ureas utilization*		*Butt*	*slat*	*H_2S*	*GAS*	
DR1	Blue-green, smooth circular, flats & moderate colonies on cetrimide agar. Colourless, flat, circular and smooth edge colonies on MacConkey agar	Shot rod appeared mostly single formed	*Gram negative*	+	+	+	–	–	–	–	–		*AL*	*A*	–	–	*Pseudomonas aeroginosa*
DR12, DR14, DR17 DR19, DR23, DR24 DR30, MSW1 FSW3, FSW4	Yellow-green, circular and smooth edges, flat and moderate colonies on cetrimide agar	Shot rod appeared mostly in single formed	*Gram negative*	+	+	+	–	–	–	–	–		*AL*	*A*	–	–	*Pseudomonas aeroginosa*

Key: + = positive, - = negative, TST = triple sugar iron, H_2S = Hydrogen sulphide, A = Acid and Al = Alkaline
A = acid, AL = alkaline, DR = dressing room, MSW = male surgical ward, FSW = female surgical ward

Table 2: Morphological and Biochemical Characteristics of Presumptive *Staphylococcus aureus* Isolates

Isolate Identification Code	Morphological Characteristics			Biochemical Characteristics											Probable Organism
	Colonial morphology on mannitol salt agar (MSA) and Baird Parker agar and blood agar	Cellular/ microscopic morphology	Gram reaction	Molility	Catalase	coagulase	Indole	Methyl red	Vogas-proskaver	Cirat utilization	Hemolysis	lactose	mannitol	Surcrose	
DR3, DR5, DR11, DR12, DR19, DR21, FSW1, FSW6, MSW2, MSW3, MSW4.	Complete yellow, raised, circular and smooth edges, and moderate colonies on MSA Black shining colonies with lysis at their edge	Cocci appeared in cluster (gape-like) or bundge with few singles and pairs	Gram positive	-	+	+	-	-	+	+	+	-	+	-	*Staphylocccus aureus*

Keys: + = positive, - = negative, DR = dressing room unit, MSW = male surgical ward, FSW = female surgical ward.

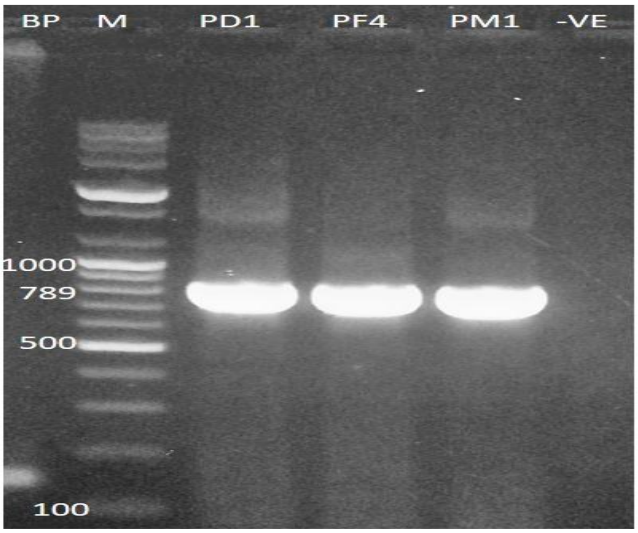

Plate 1: Gel electrophoresis of amplified PCR 16SrRNA genes bands of *Pseudomonas aeruginosa* isolates at 789bp of the 100 bp plus DNA marker.

Key: M = 100bp Marker, P = *Pseudomonas aeruginosa*, D= dressing room unit, F = female surgical ward, M = male medical ward, bp = base pair, - Ve = Negative Control
PD1 = DR_1, PF4 = FSW_4, PM1 = MSW_1

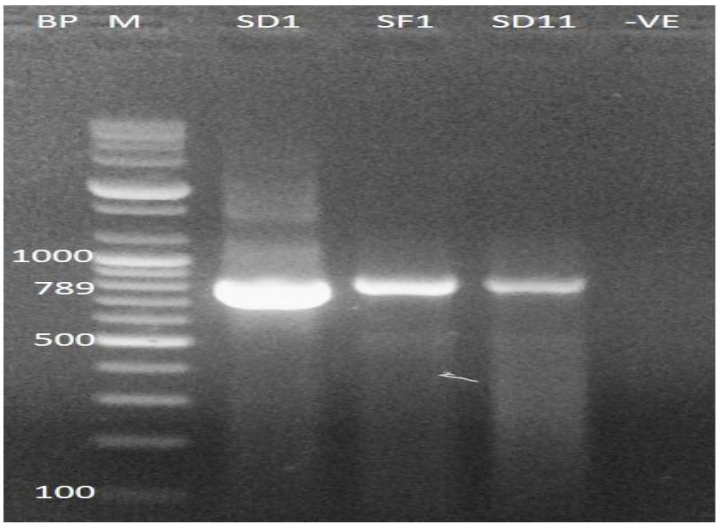

Plate 2: Gel electrophoresis of amplified PCR 16SrRNA genes bands of *Staphylococcus aureus* isolates at 789 bp of the 100 bp plus DNA marker.

Key: M = 100 bp DNA marker, S = *Staphylococcus aureus*, D = dressing room unit,
F = female medical ward, M = male medical ward, bp = base pair, - Ve = Negative Control, SD1 = DR_{12}, SF1 = FSW_1, SD11 = DR_{11}

Table 3: BLAST Characteristics of *Pseudomonas* aeruginosa and *Staphylococcus aureus*

S/N	Sample Code	Organism	Searched Gene	Total Scores	Identity and Similarity (%)	E-Value	Query cover (%)
1.	SD1	*Staphylococcus aureus*	16SrRNA	134	76.87	8e-29	44
2.	SF1	*Staphylococcus aureus*	16SrRNA	878	91.64	0.0	99
3.	SD11	*Staphylococcus aureus*	16SrRNA	360	86.94	9e-94	43
4.	PD1	*Pseudomonas aeruginosa*	16SrRNA	756	92.71	0.0	100
5.	PF4	*Pseudomonas aeruginosa*	16SrRNA	73.1	97.67	2e-10	5
6.	PM1	*Pseudomonas aeruginosa*	16SrRNA	815	91.81	0.0	93

Key: SD1= *Staphylococcus aureus* strain from dressing room unit
SF1= *Staphylococcus aureus* strain from female surgical ward.
SD11 = *Staphylococcus aureus* strain from dressing room unit
PD1 = *Pseudomonas aeruginosa* strain from dressing room
PF4 = *Pseudomonas aeruginosa* strain from female surgical ward
PM1 = *Pseudomonas aeruginosa* strain from male surgical ward

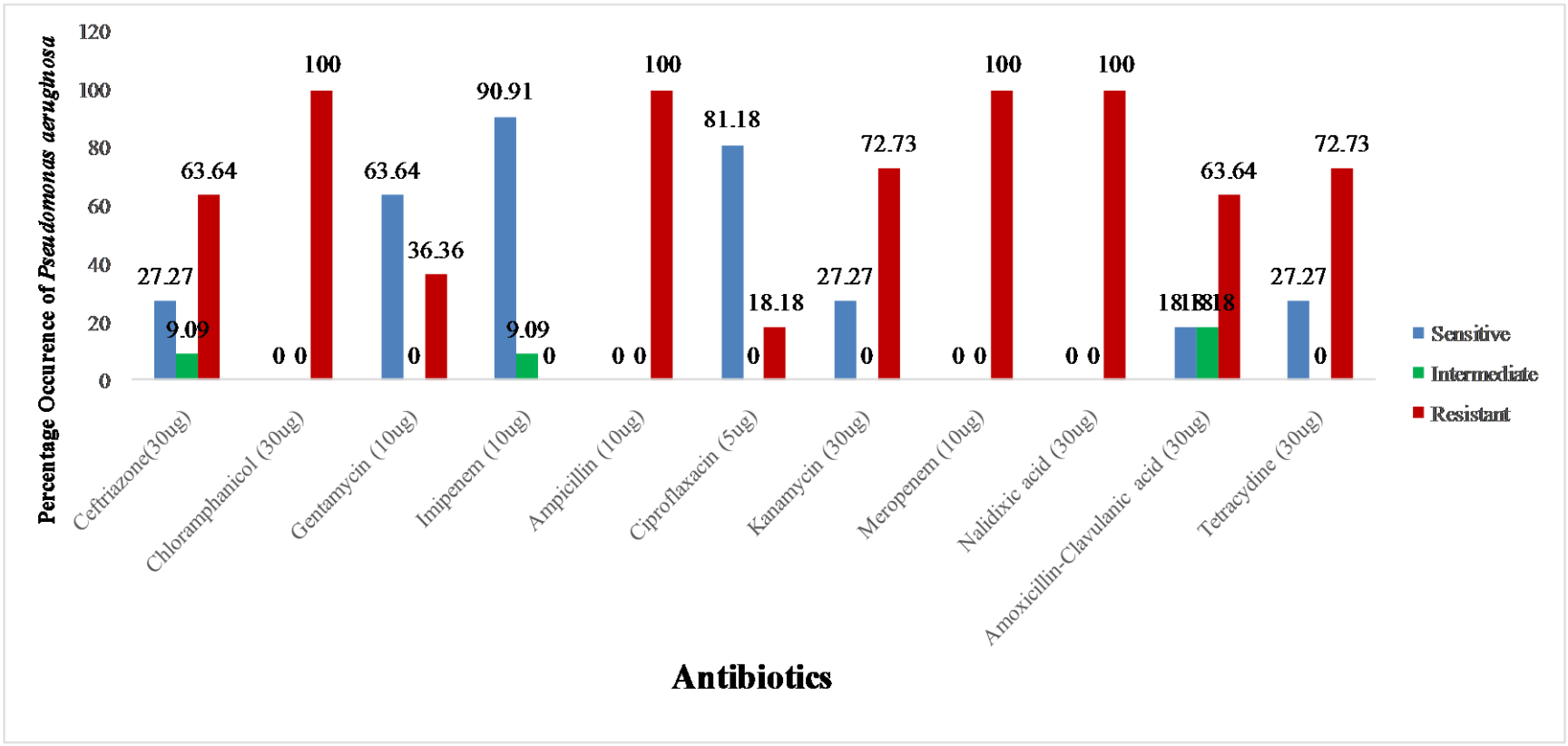

Figure 1a: Susceptibility Profile of *Pseudomonas aeruginosa* Strains against selected antibiotics.

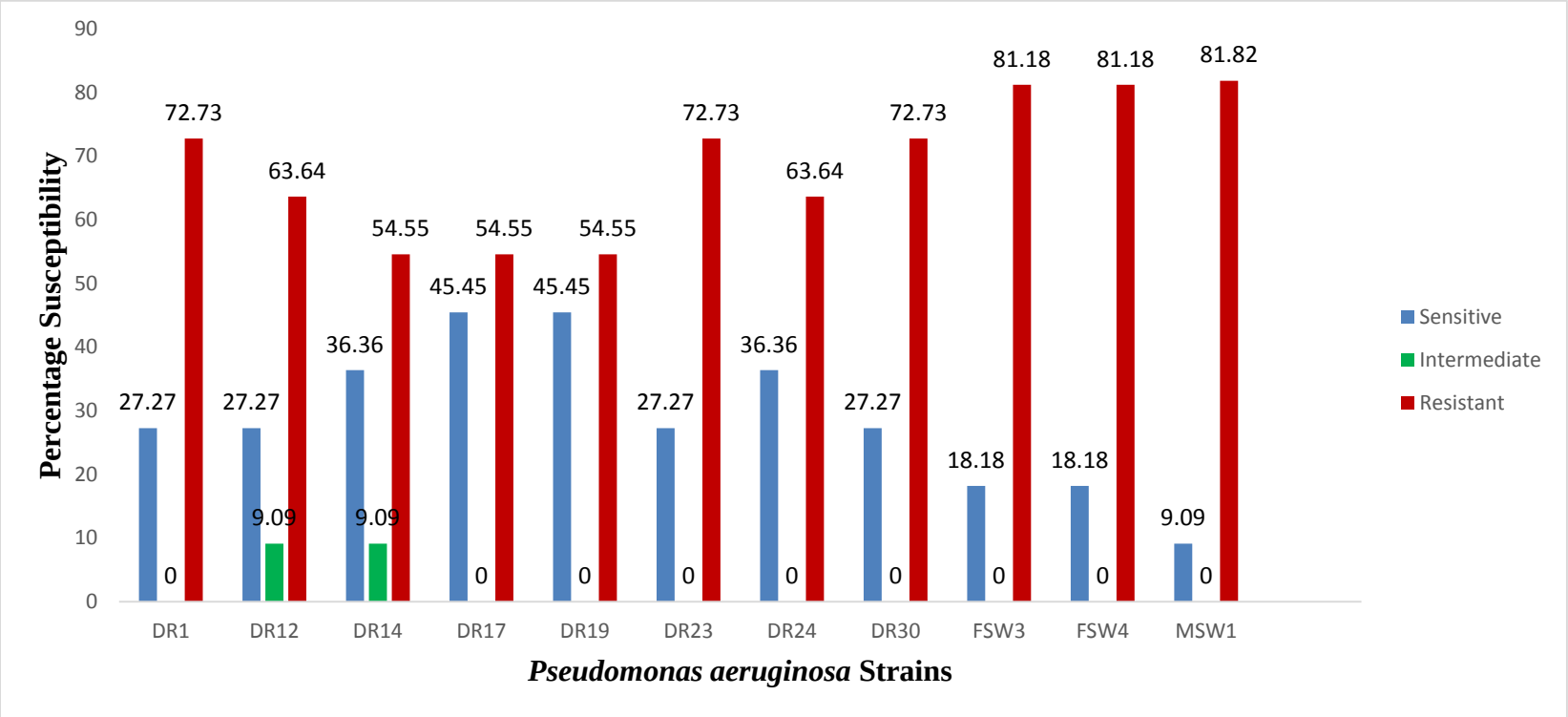

Figure 1b: Susceptibility Pattern of Selected Antibiotics tested against *Pseudomonas aeruginosa* Strains.

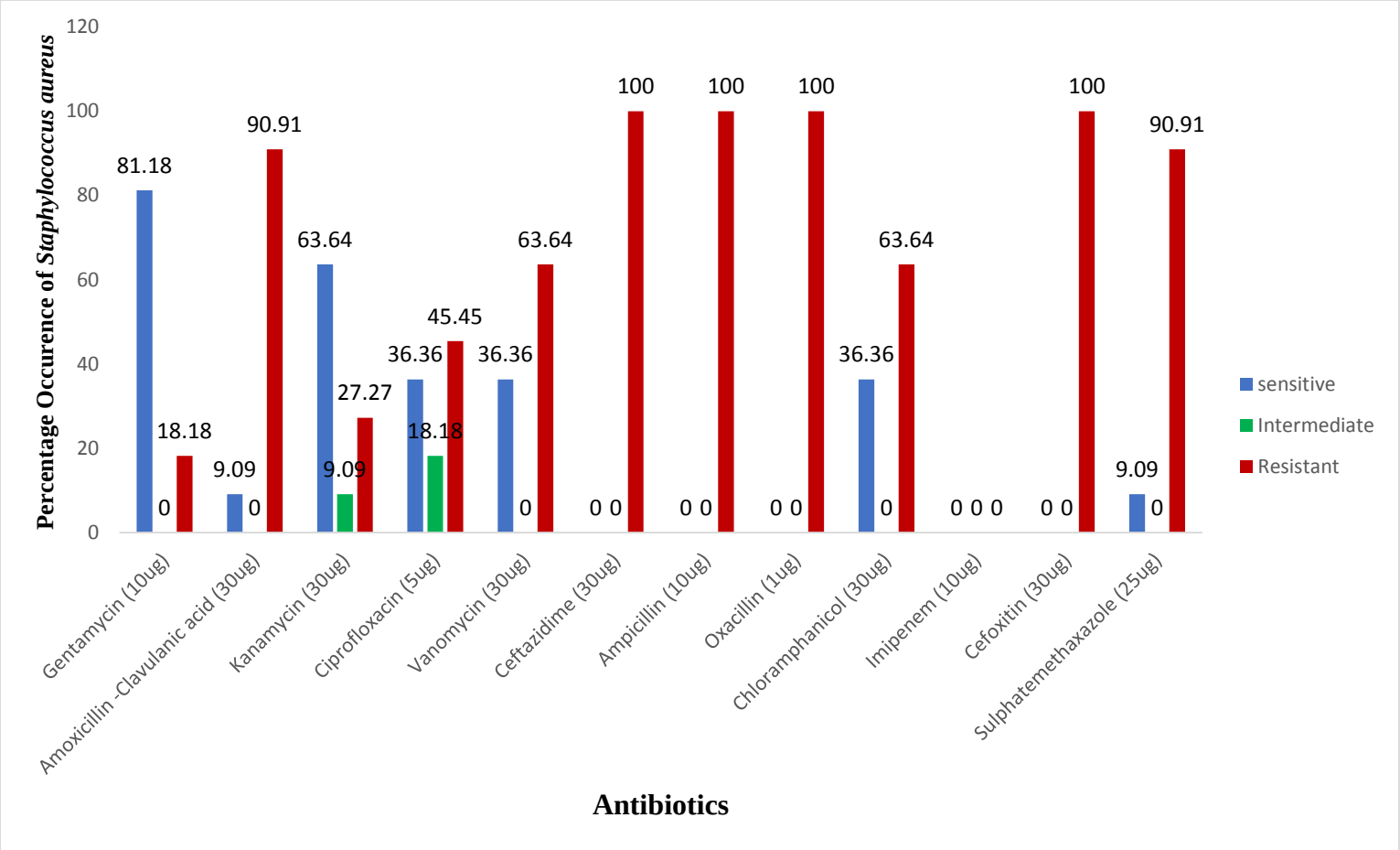

Figure 2a: Susceptibility Profile of *Staphylococcus aureus* Strains against selected antibiotics.

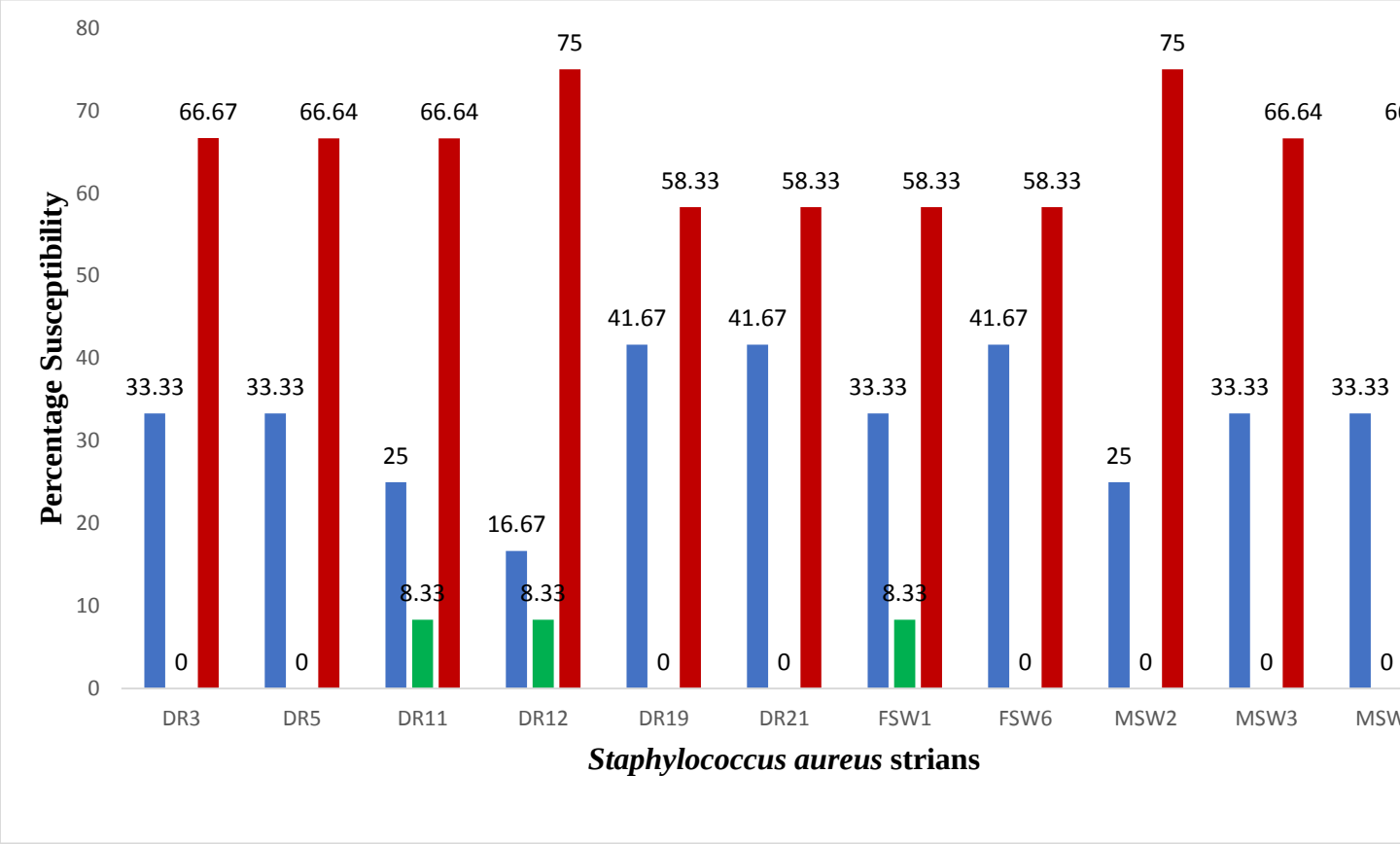

Figure 2b: Susceptibility Pattern of Selected Antibiotics tested against *Staphylococcus aureus* Strains.

Table 4: Biofilm Formation Characteristics of *Pseudomonas aeruginosa* Strains (OD_{cut} = 0.47910)

S/No	Isolate Identity Code	Biofilm Formation		
		Qualitative	Quantitative	
			OD	Category	Interpretation
1	DR1	-	0.3920	$OD \leq OD_{cut}$	Non-Biofilm former
2	DR12	-	0.17025	$OD \leq OD_{cut}$	Non-Biofilm former
3	DR14	-	0.14575	$OD \leq OD_{cut}$	Non-Biofilm former
4	DR17	-	0.22525	$OD \leq OD_{cut}$	Non-Biofilm former
5	DR19	-	0.3440	$OD \leq OD_{cut}$	Non-Biofilm former
6	DR23	-	0.16425	$OD \leq OD_{cut}$	Non-Biofilm former
7	DR24	-	0.2562	$OD \leq OD_{cut}$	Non-Biofilm former
8	DR30	-	0.2065	$OD \leq OD_{cut}$	Non-Biofilm former
9	FSW3	-	0.42525	$OD \leq OD_{cut}$	Non-Biofilm former
10	FSW5	-	0.16575	$OD \leq OD_{cut}$	Non-Biofilm former
11	MSW1	-	0.15425	$OD \leq OD_{cut}$	Non-Biofilm former

Key: OD = Optical Density of Isolate at 630nm
OD_{cut} = Optical Density Cut-off of negative control
(i) = Not Detected
\+ = Present/Detected
DR = dressing room,
FSW = female surgical ward
MSW = male surgical ward

Table 5: Biofilm Formation Characteristics of *Staphylococcus aureus* Strains
(OD_{cut} = 0.05907)

S/No	Isolate Identity Code	Biofilm Formation			
		Qualitative	Quantitative		
			OD	Category	Interpretation
1	DR_3	-	0.22225	$OD > 4 \times OD_{cut}$	Strong Biofilm former
2	DR_5	-	0.2620	$OD > 4 \times OD_{cut}$	Strong Biofilm Former
3	DR_{11}	+	0.39850	$OD > 4 \times OD_{cut}$	Strong Biofilm Former
4	DR_{12}	+	0.23225	$2 \times OD_{cut} < OD \leq 4 \times OD_{cut}$	Moderate Biofilm Former
5	DR_{19}	+	0.35400	$OD > 4 \times OD_{cut}$	Strong Biofilm Former
6	DR_{21}	+	0.67200	$OD > 4 \times OD_{cut}$	Strong Biofilm Former
7	FSW_1	-	0.15000	$2 \times OD_{cut} < OD \leq 4 \times OD_{cut}$	Moderate Biofilm former
8	FSW_6	+	0.3365	$OD > 4 \times OD_{cut}$	Strong Biofilm Former
9	MSW_2	+	0.21675	$2 \times OD_{cut} < OD \leq 4 \times OD_{cut}$	Moderate Biofilm former
10	MSW_3	+	0.83875	$OD > 4 \times OD_{cut}$	Strong Biofilm Former
11	$MSW_{4(6)}$	-	0.28875	$OD > 4 \times OD_{cut}$	Strong Biofilm Former

Key: OD = Optical Density of Isolate at 630nm
OD_{cut} = Optical Density Cut-off negative control
(ii) = Not Detected
\+ =Present/Detected
DR = dressing room,
FSW = female surgical ward
MSW = male surgical ward.

Table 6: Extended Spectrum Beta-lactamase (ESBL) Characteristics of Multidrug Resistant of *Pseudomonas aeruginosa* Isolates

S/N	Isolates Identify Code	Zone of Growth Inhibition (mm)							
		ESBL Screening			ESBL Confirmatory				
		CPD (10 μg)	CAZ (30 μg)	CFM (30 μg)	CPD (10 μg)	AMC	CFM	Difference (mm)	ESBL
1.	DR1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-
2.	DR12	0.00	0.00	23.00	0.00	0.00	23.00	0.00	-
3.	DR14	15.0	15.0	35.0	21.00	17.0	22.00	6.50	+
4	DR17	0.00	0.00	0.00	0.00	3.00	0.00	0.00	-
5	DR19	0.00	0.00	0.00	0.00	4.00	0.00	0.00	-
6	DR23	0.00	0.00	15.00	0.00	5.00	15.00	0.00	-
7	DR24	35.00	30.00	30.00	40.00	30.00	43.00	3.00	-
8	DR30	0.00	0.00	14.00	0.00	10.00	15.00	1.00	-
9	FSW3	14.00	0.00	35.00	20.00	15.00	41.00	5.50	+
10	FSW4	14.00	15.00	35.00	22.00	17.00	41.00	7.00	+
11	MSW1	16.00	15.00	30.00	17.00	18.00	32.00	1.50	-

Key: DR = dressing room wound isolates, FSW = female surgical ward wound isolate, MSW = male medical ward wound isolates. ESBL = Extended Spectrum Beta-Lactamase, CPD = cefpodoxime, CAZ = ceftazidime, CFM = cifixime, AMC = amoxicillin-clavulanic acid, mm = millimeter, μg = microgram**.**

colour for some colonies and yellow-green for other colonies. These colonies all appeared circular with smooth edges, flat and moderate shape. There is a reportof similar findings about the cultural characteristics of this organism on cetrimide agar [17]. The production of distinctive blue-green and yellow-green colour by *Pseudomonas aeruginosa* strains on Cetrimide agar is due to its water-soluble pigments, pyocyanin and pyoverdine which diffuses through the medium; and that *Pseudomonas aeruginosa* is the only species of Pseudomonas or gram-negative rod known to excrete pyocyanin [11]. The combination of this characteristic colour and grape-like smell of growth colonies on Cetrimide agar due to production of 2-aminoacepophenone distinguishes *Pseudomonas aeruginosa* from other Pseudomonas species. Aside from identification, pyocyanin and pyoverdine are also useful in the chelation of ions to support the metabolic process and control of the expression of *Pseudomonas aeruginosa* virulence factors such as exotoxin A, enoprotease and pyoverdine [18, 19]. In this study, *Pseudomonas aeruginosa* showed colourless, flat and smooth, and non-lactose fermenters colonies on MacConkey agar. Generally, the colonies showed regular margins and alligator skin-like appearance from top view. The colourless nature of the colonies is attributed to the fact that this organism does not ferment lactose. This agreed with Prasanna *et al.* report about cultural characteristics of this organism on MacConkey agar [17].

Cellular morphology and biochemical characteristics showed P*seudomonas aeruginosa* to be oxidase positive - an important biochemical characteristic which indicates that Pseudomonas species produces indophenol oxidase (an enzyme). This is a unique characteristic that distinguishes it from other Gram-negative bacteria [11]. It was showed by biochemical test in this study that this organism is motile, indole negative, methyl red negative, hydrogen sulphide production negative, Voges-Proskauer negative, citrate utilization positive, urea utilization negative, and non-lactose, glucose, mannitol and sucrose fermenter. The Gram stain and microscopy showed the organism to be Gram negative rod. This is in agreement with the findings reported by Walthiq and Mohammed [20].

Phenotypic identification revealed the colonies of *Staphylococcus aureus* on MSA as yellow with flat and moderate shape. The production of yellow colonies on MSA is due to fermentation of mannitol salt with consequent production of acid [21]. On Baird Parker medium, *Staphylococcus aureus* showed grey-black shining colonies with opaque halo surrounded by zone of clearing. A study reported similar characteristics of *Staphylococcus aureus* on Baird parker medium, where it was reported that the formation of grey black shining colonies is due to reduction of potassium tellurite and the proteolytic activity through breaking down of egg yolk by Lecithinase causing clear zone around respective colonies, while the opaque halo surrounding zone of clearing is as a result of Lipase activity [22]. The gram stain cell showed characteristic gram-positive cocci, that appeared as grape-like (cluster) under microscopic examination using x100 objective lens. Tong *et al*. reported similar cellular appearance of *Staphylococcus aureus* [23]. The biochemical characteristics showed that this organism is catalase and coagulase positive with characteristic production of beta-hemolysis on blood agar – a unique characteristic for phenotypic identification of pathogenic *Staphylococcus aureus* strains. Studies have reported that *Staphylococcus aureus* isolated from human have bound and free form of coagulase, with characteristic formation of beta-haemolysis on blood agar. The presence of the enzyme coagulase is phenotypically employed to differentiate between the strain of virulent and less virulent *Staphylococcus aureus.*

The phenotypic identification method in this study generally revealed cultural and biochemical characteristics related to *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolates. However, due to the need for identification of the pathogen-specific wound infection organisms to species level in this study, it became imperative to characterize the *Pseudomonas aeruginosa* and *Staphylococcus aureus* using molecular identification methods [24]. The molecular identification was employed to compare the genetic similarities of the studied bacteria (*Pseudomonas aeruginosa* and *Staphylococcus aureus)* isolated from wound with GenBank database [25]. The results of the molecular analysis in this study showed the gel electrophoresis of amplified PCR 16SrRNA genes bands of *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolates at 789 bp of the 100 bp plus DNA marker. The sequences BLAST results of the 16SrRNA genes of the presumptive *Pseudomonas aeruginosa* isolates; PD1, PF4 and PM1 revealed the percentage identity and similarity of these isolates to those from GenBank database as 92.7 %, 97.7 % and 91.8 % respectively, confirming the identity of these isolates as *Pseudomonas aeruginosa* strains. Similarly, the sequences BLAST results of the presumptive *Staphylococcus aureus* isolates; SD1, SF1 and SD11 16SrRNA genes showed the percentage identity and similarity of these isolates to those from the GenBank database

as 76.9, 91.6 and 86.9 % respectively, confirming the identity of these isolates as *Staphylococcus aureus* trains.

Generally, the percentage identity and similarity revealed by the sequences BLAST results for all the *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains ranged from 76.9 % to 97.7 %. It has been widely accepted that Prokaryotes whose genomes are at least 70 % homologous belongs to the same species [25]. This supports the confirmation of identity of these isolates as *Pseudomonas aeruginosa* and *Staphylococcus aureus* in this study.

The findings in this study showed all the *Pseudomonas aeruginosa* strains to be multidrug resistant to the selected conventional antibiotics - chloramphenicol, ampicillin, amoxicillin-clavulanic acid, kanamycin, tetracycline, ceftriazone, gentamycin, ciprofloxacin, meropenem and nalidixic acid; and all the isolates were sensitive to imipenem (figures 1a and b). According to the findings in this study, imipenem, ciprofloxacin and gentamycin were the most effective antibiotics against all the *Pseudomonas aeruginosa* isolates. The multidrug resistance exhibited by *Pseudomonas aeruginosa* might be attributed to some factors which include; possession of unique outer membrane that excludes certain drugs and antibiotics from penetrating the cell [7], elevated intrinsic resistance i.e. chromosomally encoded resistance and ability to acquire multidrug resistance to antibiotics [26, 27], ability of resistant strain in clinical settings to spread in high density antibiotic environment [28] and rapid spreading of high risk "clone" of *Pseudomonas aeruginosa* ST235 carrying extremely drug resistant phenotypes thereby causing difficulty to treat infections caused by the organism [29].

Also, it has been reported that *Pseudomonas aeruginosa* characteristic multidrug resistance could be due to numerous multidrug resistant efflux pump mechanism, acquisition of antibiotic resistance gene readily from bacteria by transformation, conjugation and transduction [30], biofilm formation-preventing host defense which prevent the antibiotic from reaching the bacteria, use of broad-spectrum beta-lactamases and metallo-B-lactamases (MBL) through alteration of penicillin binding proteins (PBP), Porin mutabion/porin impermeability (OPrD), plasmid enzymatic modification and DNA gyrase mutations [26, 27]. The major cause of *Pseudomonas aeruginosa* resistance to beta-lactam antibiotics used in this study could be due to beta-lactamases production. Both chromosomally-mediated and plasmid-mediated beta-lactamase characteristically produced AmPC-type which mediated resistance to the third generation cephoalosporin and the monobacterm [31]. Metalo-beta-lactamase (MBL) production is also an important mechanism in development of resistance to antibiotics [26].

Similarly, the antibacterial susceptibility profile of *Staphylococcus aureus* showed multidrug resistance pattern against all antibiotics used. According to the results from this study (figures 2a and b), imipenem was shown to be the most potent antibiotic against the *Staphylococcus aureus* isolates followed by gentamycin. This means that imipenem must be carefully prescribed by clinicians to avoid development of resistance by organism. Also, sensitivity result should always be used as the basis for rational prescription of these drugs to patients. There is also need to educate clinicians on this finding and the public health importance. More so, there is need to educate clinicians to prescribe these drugs for the correct duration and for the patients to ensure the prescribed drugs at correct dose, dosing and duration in order to prevent the development of resistance and ultimate poor clinical outcomes. Findings from this study are similar to that of susceptibility profile of *Staphylococcus aureus* study that reported imipenem as the most potential antibiotic with 90 % sensitivity, and 75 % of isolates also showing resistance to Oxacillin, Methicillin, Ciprofloxacin and Tetracycline [32]. Studies [33, 34] reported that *Staphylococcus aureus* is capable of producing many antibiotic resistant strains and that the organism has the ability to acquire resistance to many antibiotics, which is a worldwide problem.

This study reported *Staphylococcus aureus* isolates to be resistant to chloramphenicol similar to that in Bangladesh [32]. In addition, this study reported multidrug resistance exhibited by *Staphylococcus aureus* to ceftazidime as earlier reported [35]. Moreso, earlier study [32] reported that only 4 (36.6%) *Staphylococcus aureus* showed sensitivity to vancomycin which is a serious threat to patient management. Notably, this study reported vancomycin resistance by *Staphylococcus aureus*, and this is of public health significance. Researchers have reported vancomycin resistant *Staphylococcus aureus* (VRSA) to be currently one of the greatest threats mankind faces because the antibiotic is the last resort for the treatment of Staphylococcal infections [36, 37].

Previous study recommended tetracycline, chloramphenicol and gentamycin for the treatment of wound infection caused by *Staphylococcus aureus* [38]. Similarly, vancomycin, cefoxitin and imipenem have been recommended for effective treatment of wound infection [39]. Findings from this study

showed imipenem, gentamycin and ciprofloxacin to be the most potent antibiotics indicating that they are still effective as recommended.

The results from qualitative and quantitative bacteria biofilm assay of this study showed that all the *Pseudomonas aeruginosa* isolates were non-biofilm formers. The non-detection of biofilm by the qualitative and quantitative assay methods implies that these bacterial isolates do not have exopolymers (extracellular matrix) - one of which is the exopolysacharides called alginate (a polymer consisting of B-D-mannuranic acid and α-L-gluronic acid). The exopolysacharides of biofilm matrix is composed of a repeating pentasacharides consisting of D-mannose, D-glucose and L-rhamnose, a glucose rich polysaccharide and extracellular DNA and proteins [26, 27, 40, 41]. For biofilm formation by *Pseudomonas aeruginosa,* the organism requires high level of total and reducing sugars which are structural element for biofilm formation, therefore biofilm is a matrix that can be formed mainly by these sugars [26].

Contrary to the report that *Pseudomonas aeruginosa* exists as biofilm rather than single cells in wounds and has been observed existing as biofilm when colonizes human chronic wounds [42, 43]; in this study, biofilm formation was not detected among all the *Pseudomonas aeruginosa* isolates analyzed. The non-detection of biofilm formation from the *Pseudomonas aeruginosa* isolates in this study can be attributed to many factors. Study have reported that environmental factors such as nutrients level, temperature, PH, and ionic strength can influence both bacterial properties and biofilm production. This is mediated by changes in gene regulation and / or cell surface physiochemical and surface properties (44). Studies reported *Staphylococcus aureus* biofilm formation to be lower at highly acidic (pH 3) and alkaline (pH 12) pHs compared to neutral pH of 7. This indicated that most bacterial biofilm formation are effective at neutral pH. Similarly, optimum temperature enhances biofilm formation and non - optimum temperature cultures condition negatively affects biofilm formation. Oxygen availability also influences bacterial biofilm formation, and it has been reported by Azezo *et al* that *Pseudomonas aeruginosa* easily form biofilm when grown mostly under anaerobic condition (45). Studies have also addressed the effect of nutrients level on the formation of bacterial biofilm stating that glucose and sodium chloride level always exert great effect on biofilm formation. In respect to this, study has reported that high sodium chloride (NaCl) concentration inhibited biofilm formation by *Pseudomonas aeruginosa* and other bacteria such as *Salmonella* species and *Enterococcus faecalis (45)*. Quorum-sensing in *Pseudomonas aeruginosa* biofilm formation has also been said to influence hydrodynamic conditions such as higher shear forces or stress by inducing cell detachment through reduction of extracellular polysaccharides. This condition is known to also affect biofilm formation by changing nutrients and oxygen supply. Formation of bacterial biofilm occurs naturally as a result of balancing between variety of chemical, physical and biological processes, and hence, bacterial biofilm forming abilities depends on these numerous factors - the inherent biological characteristics and environmental factors [43]. The lack of biofilm formation by the *Pseudomonas aeruginosa* isolates in this study might be due to the influenced of one or the combination of these scientifically established factors during the course of the study.

The qualitative biofilm assay of *Staphylococcus aureus* isolates in this study showed that 2/3 of the screened *Staphylococcus aureus* isolates were biofilm formers. The quantitative biofilm detection assay also indicated that all the *Staphylococcus aureus* isolates were biofilm formers with 8 (72.7 %) isolates classified as strong biofilm formers having optical density (OD) greater than four times their optical density cutt (OD >4 X ODcutt), while 3 (27.3 %) isolates as moderated biofilm former with 2 X ODcutt<OD ≤4 X ODcutt. The detection of biofilm from the *Staphylococcus aureus* in this study implied that these bacterial isolates have cellular matrix of exopolysaccharides. This agreed with the Study by Ankit *et al.* [46] and Manandhar *et al.* [47] who reported *Staphylococcus aureus* to be biofilm producers and multidrug resistant. The study also reported that higher rate of multidrug resistance is found among the biofilm producing strains.

CONCLUSION

Pseudomonas aeruginosa and *Staphylococcus aureus* strains isolated from wounds of patients receiving treatment in clinics at Barau Dikko Teaching Hospital Kaduna, Nigeria were multidrug-resistant strains. Of all the antimicrobial agents used, imipenem, ciprofloxacin, kanamycin and gentamycin were the most effective drugs against the bacteria. Biofilm formation was not detected from all the *Pseudomonas aeruginosa* isolates analyzed. However, all the multidrug resistant *Staphylococcus aureus* were biofilm formers. Three *Pseudomonas aeruginosa* isolates were also found to be extended spectrum beta-lactamase producers. From the findings in this study, there is a need for awareness and education of clinicians to prescribe antibiotics

rationally, especially based on the local antibiogram. Similarly, there is the need for patient education on rational use of prescribed antibiotics too. Exhaustive molecular studies involving detection of specific resistant genes responsible for the multidrug resistant *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains will be helpful for development of new therapeutic agent for effective treatment of wounds.

REFERENCES

1. Muraj CJK, Ikuba KS, Sahara FS, Luclen RA, Gisela G, Authia H, Chich B, Catherine RPW. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet, 2022, 399(10325):629-655. .https://doi.grg/10.1016/so140-6736(21)02724-0.
2. World Health Organisation (WHO). Antimicrobial resistance, 2021. Available at: www.who.int/newsroom. Accessed on 31/06/2022.
3. WHO. International organizations unite on critical recommendations to combat drug-resistant infections and prevent staggering number of deaths each year. 2019. Available at www.who.int/newsroom. Accessed on 31/06/2022.
4. Tadesse TB, Ashley AE, Ongarello warden M, Gonzalez JI, Dittrich S, Antimicrobial in Africa; a systematic review. *BMC Infections Disease*. 616(2017), 2017. https://doi.org/10.1/86/s12879-017-2713-1
5. Cheng G, Dai M, Ahmed S, Hao H, Wang X, Yuan Z. Antimicrobial Drugs in Fighting against Antimicrobial Resistance. *Frontier in Microbiology*, 7.2016:470. doi.10.3388/fmicb.2016.00470
6. Iileka AEK, Mukesi M, Engelbrecht F, Moyo SR. Antimicrobial Susceptibility Patterns of *Staphylococcus aureus* strains Isolated at the Namibia Institute of Pathology from 2012 to 2014. *Journal of Medical Microbiology*, 6. 2016:116-124. http://dx.doi.org/10.4236/ojmm.2016.63016
7. Xiatong D, Qinghan T, Zeyu X, Ye X, Hao Z, Dongfeng Z, Shugin W, *et al*. Challenges and innovations in treating chronic and acute wound infections: from basic science to chemical practice. Oxford University Press, 2002. Doi:10.1093/burns/tkac014
8. Vallis SJ, Nacente BJ. Hand book of Microbiological culture media, 9 edition, ScherlauChemie SA, export@scharlau.com, 2006; Pp 68.
9. Cheesbrough M. District Laboratory practice in tropical countries, part 2, low price edition, Cambridge University Press, 2010; Pp 63-197.
10. Aneja KR. Experiment in Microbiology plant pathology biotechnology, 4th edition, new age international (p) Ltd, new Delhi New York. www.new age publisher.com. 2007; Pp390.
11. Ochai JO. Kolhatkar A. Medical Laboratory Science and practice, Tata McGrew Hill publishing limited new Delhi, New York. 2008; Pp535, 539, 632-635.
12. Arora DR, Arora BA. Text book of Microbiology; 3rd edition, CBS Publisher, New Delhi. 2011; Pp 75-80, 213 and 418.
13. CLSI. Performance Standard for antimicrobial susceptibility testing; twenty-nineth edition. 2020.
14. Puja N, Hari PN, Rojeet S, Osamu U, Yoshihiro A. (2018). In vitro biofilm formation by *Staphylococcus aureus* isolated from wounds of hospital admitted patients and their association with antimicrobial resistance. *International journal of general medicine*. Dove medical press limited. Doi:10.2/47/IJGM. S153268.
15. Singh DR, Morinda CL. (Noni): A review of the scientific validation for its nutritional and therapeutic properties. *Journal of Diabetes Endocrinology*. 3, 2012: 77-99. Doi.10.5897/JD810.006.
16. Drieux L, Brossier F, Sougakoff W, Jarlier V. Phenotypic detection of extended-spectrum b-lactamase production in Enterobacteriaceae: review and bench guide. *Clin Microbiol Infect.*14 (1), 2008: 90–103.
17. Anupama S. *Pseudomonas aeruginosa*: an overview. Microbe note, 2021. Available at microbenote.com>bacteriology. Accessed on 04/07/2022.
18. Saga A. Centrimide agar composition, principle, uses, preparation, and colony morphology. Microbiologyinfo.com. microbiologyinfo.com centrimide agar.com (2022). Accessed on 15/07/2022.
19. Lamont IL, Beare PA, Ochsner U, Vasil AI, Vasil ML. Siderophore-mediated signaling regulates virulence factor production in *Pseudomonas aeruginosa*. *Proc Natl Acad Sci* U S A. 99(10), 2002:7072-7.
20. Walthiq AHA, Mohammed SAA. Molecular detection f or nosocomial *Pseudomonas aeruginosa* and its Relationship with multidrug resistance, Isolated from Hospitals government. Medico-Legal Update. 20(1), 2020: 633-6335.

21. Fitzgerald N, Ogunjobi AA, Ogunjobi TE. Comparative of antibacterial Activities of ethanol extracts of the Band seeds *Garania kola* and *Carica papaya. African Journal of Biomedicine.* 14, 2014: 14152.
22. Silva WP, Destra MT, Landgraf M, Franco DGM. Biochemical Characteristics of typical and atypical *Staphylococcus aureus* in mastitis milk and environmental samples. *Brazillian Journal of Microbiology.* 31, 2000:103-106.
23. Tong D, Njume C, Afolayan AJ, Clarke AM, Ndip RN. Crude ethanol extracts of *Garcinia Kola* Seeds Heckel prolong the lag phase of *Helicobacter pylori* Inhibitory and bactericidal potential, *Journal of medical food.*14 (7-6), 2015: 822-827.
24. VanVuuren SF. Antimicrobial activity of South African medicinal plants. *Journal of Ethnopharmacology.* 119, 2008:462–72.
25. Prescott LM, Harley JP, Klein AD. Microbiology, 10th edition McGraw-Hill, New York. Available at https://www.worldcat.org/title/prescots-microbiology//oclc/1934479924, 2017. Accessed on 20/07/2022.
26. Sarah AO, Ariadnna C, Gerardo ER, Vicenta CD, Gerardo E, Jose A, Rigoberto H, Castro AR, Juan X. Phenotypic characterization of multidrug-resistant *Pseudomonas aeruginosa* strains isolated from pediatric patients associated to biofilm formation. Microbiological Research. 172, 2015:68-78. https://doi.org/10.1016/j.micres.2014.11.005
27. Mohammad E, Maryam B, Mhboubeh S, Nafiseh A, Reza B, Willem BL, Fereshteh J. Evaluation of Mannosidase and Trypsin Enzymes Effects on Biofilm Production of *Pseudomonas aeruginosa* Isolated from Burn Wound Infections. *PLoS ONE.* 11(10), 2016: e0164622. doi:10.1371/journal.pone.0164622.
28. Larsson JDG, FlachC. Antibiotic resistance in the environment nature. Review Microbiology. 20(5), 2021: 257-259. doi:10.1038/S41579-021-00649-4.
29. Friedman DN, Temkin E,Carmeli Y. The negative impact of antibiotic resistance. *Clinical Microbiology and Infection*. 22 (5), 2016: 416-422. https://doi.org/10.1016/j.cmi.2015.12.002
30. Sadikot RT, Blackwell TS, Christman JW, Prince AS. Pathogen-host interactions in *Pseudomonas aeruginosa* pneumonia. *American Journal of Respiratory and Critical Care Medicine.* 171(11), 2005:1209-23.
31. Shigeki F, Kathryn SM, Victor LY. *Pseudomonas aeruginosa*: Antimicrobe: Infectious disease antimicrobial agent. 2017. Available at: (http://www.antimicrobe.org/final/inde.asp. Accessed on 15/10/2017
32. Rashedul H. Mrityunjoy A, Rashed N. Prevalence of vancomycin resistant *Staphylococcus aureus* (VRSA) in methicillin resistant *S. aureus* (MRSA) strains isolated from burn wound infections. *Tzu Chi Medical Journal.* 28(2),2016:49-53. https://doi.org/10.1016/j.tcmj.2016.03.002
33. Kitara LD, Anywar AD, Acullu D., Odongo-Aginya E, Aloyo J. Fendu M. Antibiotic Susceptibility of *Staphylococcus aureus* in Suppurative Lesions in Lacor Hospital, Uganda. African Health Sciences. 11, 2011: S34-S39. http://dx.doi.org/10.4314/ahs.v11i3.70068
34. Brown PD, Ngeno C. Antimicrobial Resistance in Clinical Isolates of *Staphylococcus aureus* from hospital and community sources in Southern Jamaica. *International Journal of Infectious Diseases.* 11(3), 2007: 220-225.
35. Aisha N, Abdul R, Sadia I, Aisha N, Arfan A, Afshan K. Characterization of antibiotic resistant gene in *Staphylococcus aureus* isolated from surgical wounds. *Advances life sciences.* 3(3), 2016:83-88.
36. Selim S, Faried AO, Almhayawi S.M, Saleh MF, Sharaf M, Nahhas EW, Warrad M. Incidence of vancomyai resistant *Staphylococcus aureus* strains among patients with urinary tracks infections. Antibiotics (Basel). 11(3), 2022:408-412. Doi:10.3390/antibiotics.11030408.
37. Cong Y, Yang S, Rao X. Vancomycin resistant *Staphylococcus aureus* infections. A review of case update and clinical features. *Journal of Advanced research.* 2020, 2019:169-176. Doi:10.1016/j.jae 2019.10.005.
38. Benjamin AL, Christopher H. Topical Antimicrobial therapy for treating chronic wounds. *Clinical infectious diseases.* 49, 2009:1541-9. Doi:10.1086/644732.
39. Bowler PG, Duerden BI, Armstrong D. Wound Microbiology and Associated approach to wound management. *Clinical Microbiology Review*, 2001, 14, 2001: 244-269.https:/doi.org/10.1128/CMR14.2.244-269 2001.
40. Kamali E, Jamali A, Ardebili A, Zadi F, Mohebbi A. Evaluation of antimicrobial resistant biofilm forming potential, and the presence of biofilm related genes among clinical isolates of *Pseudomonas aeruginosa*. BMC research notes. 13, 2020:27. https://doi.org/10/1186/s13104-020-48.90-2.

41. Secor RP, Michaels AL, Bubit CD, Jenning SKL, Singh KP. The depletion Mechanisms actuates bacterial aggregation by exopolysaccharide and determines species distribution and composition in bacteria aggregates. *Frontier in cellular and infection microbiology.* 2022. https://doi/10.3389/fcimb/2022.869736.
42. Andy JS, AH, Nancy LC, Simon CW, Jane A. Colmer- H., Ben H. B. Diversity of biofilms produced by quorum-sensing-deficient clinical isolates of *Pseudomonas aeruginosa.* 2017. Available from: www.microbiologyresearch.org Accessed on 15/07/2022.
43. Ansari AF, Fafri H, Ahmad I, Abdulresh H.H. Factors affecting biofilm formation in in vitro and in the rhizophere. Biofilm in plant and soil health. 2018: Pp 275 – 290. doi:10.1002/9781119246329.ch15.
44. Alotaibi FG, Bukhari AM. Factor influencing bacteria biofilm formation and dcilopnut. *American journal of biomedical science journal of biomedical science and research.* 12(6), 2021. doi:10.34297/AJBSR.2021.12.001820.
45. Azezo F, Haluk B, Thomas EB, Anders O, Douglas RC *et al*.. Osmotic compounds enhance antibiotics efficiency against *Acinetobacter baumanii* biofilm communities; *Applied and Environmental Microbiology.* 83(19), 2017:e01297-e01317.
46. Ankit B, Narayan DP, Krishus N, Bibhusan N, Rikesh B, Reena B. Binod L. (2017). Antibiotic resistance and biofilm production among the strains of *Staphylococcus aureus* isolated from pus/wound swab samples in a tertiary care hospital in Nepal. *Annals of Clinical Microbiology and Antimicrobials.* 16, 2017:15;https://doi.org/10.1186/s12941-017-0194-0
47. Manandhar S, Singh A, Varmar A, Randey S. Shirvastavai M. Biofilm producing chemical *Staphylococcus aureus* isolates augmented Prevalence of antibiotic resistant cases in tertiary care hospitals of Nepal. *Frontiers in Microbiology.* 9, 2018: 2749. doi:10.3389/fmicb.2018.02749.