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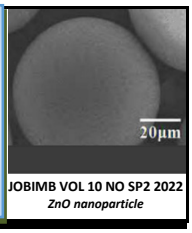

Correlation Between Patients with Wounds and Isolated *Pseudomonas aeruginosa* and *Staphylococcus aureus* at Barau Dikko Teaching Hospital, Kaduna, Nigeria

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ABSTRACT

This study was carried out to determine the correlation between patients with wounds and isolated *Pseudomonas aeruginosa* and *Staphylococcus aureus* in clinics at Barau Dikko Teaching Hospital, Kaduna, Nigeria. The Socio-demographic, medical and drug histories and characteristics of the wounds from each consented patient were taken using a questionnaire along with sixty samples of the patient's wound swab samples. Isolation of *Pseudomonas aeruginosa* and *Staphylococcus aureus* from the wound swab samples was carried out using standard phenotypic and genotypic procedures. Out of the 60 samples collected, 30 (50.0%) each were from general out-patient and inpatients. The higher percentage 12 (20.0%) and 39 (65.0%) were patients in the age group between 61 and above and male patients respectively. Regarding the patient's occupations, the higher percentage 20 (33.3%) of the patients were businessmen and women. The patient's wound location indicated that a higher percentage of 38 (63.3%) wounds were located on the leg. Also, only 13 (21.7%) patients had diabetes and 44 (73.3%) of the wound patients were receiving antimicrobials; the commonest being metronidazole 11 (18.33%), followed by mupirocin/Supirocin 9 (15.6%). A total of eleven isolates each of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were isolated from the sixty wound swab samples of the in-and out-patients. Association between antimicrobial agents use and the organisms showed significant difference ($P < 0.05$), while the association between sex, diabetes status, duration of the wound and the isolation of *Pseudomonas aeruginosa* and *Staphylococcus aureus* from the wound of patients showed no statistically significant difference ($p > 0.05$).

INTRODUCTION

Globally, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are important bacterial pathogens causing a wide range of community and hospital-acquired infections. Studies showed that major infectious pathogens in acute and chronic wounds are *Pseudomonas aeruginosa, Staphylococcus aureus* and beta-hemolytic Streptococci [1]. Acute and chronic wounds are susceptible to colonization and proliferation by these bacterial pathogens. *Pseudomonas aeruginosa* contributes substantially to wound-related morbidity and mortality worldwide and causes wound infection, skin infection (especially at burn sites), ulcers and pressure sores [2]. Similarly, Staphylococcus *aureus* has been reported as a wound infection with varying frequencies from 43% of infected leg ulcers [3] to 88% of non-infected leg ulcers [4]. It has been reported that *Staphylococcus aureus* is a pathogenic organism that causes human infection including boils, abscesses and impetigo. This organism has also been reported as

a common cause of secondary infections of ulcers, burns, wounds, skin disorders and insect bites [5], [6]. Factors which influence the colonisation and proliferation of microorganisms in wounds include the type of wound, its depth, location, the level of tissue perfusion and the antimicrobial efficacy of the host immune response [1].

Generally, the mechanism of action and class of antibiotics are important factors in antibiotic choice for the therapy of diagnosed and identified wound infectious pathogens. Quinolones are known to act against most Gram-negative bacteria from acute and chronic wounds. Similarly, cephalosporins B- Lactam antibiotics are known to act against Gram-negative bacteria in chronic wound infection. Hence, Benjamin and Christopher [7] recommended these antibiotics for effective therapy of wounds colonised by Gram-negative bacteria including *Pseudomonas aeruginosa*. On the other hand, the flucloxacillin antibiotic is considered a drug of choice for the treatment of wounds infected by *Staphylococcus aureus* [8].

In Nigeria, the resistance of *Pseudomonas aeruginosa* and *Staphylococcus aureus* to locally available antibiotics is increasing but the basis of resistance and the population structure of these organisms particularly in chronic wounds are poorly described. Similarly, the correlation between factors such as sociodemographic, medical, antimicrobial usage, location and duration of the wound and the occurrence of bacterial pathogens in the wound of patients are rarely available in the biomedical scientist's domain, however, this information is essential. This study was carried out to determine the correlation between patients with wounds and isolated *Pseudomonas aeruginosa and Staphylococcus aureus* isolated in clinics at Barau Dikko Teaching Hospital, Kaduna, Nigeria.

MATERIALS AND METHODS

Study Area

Kaduna is a cosmopolitan city and the state capital of Kaduna State. The state has a population of 8, 252, 400 people according to the 2016 population projection, and it has a land mass of 45,061km^2. Barau Dikko Teaching Hospital is located at Longitude 7^{0}26'29''E and Latitude 10^{0}31'33" N. The hospital provides training to resident doctors and medical students of Kaduna State University, Kaduna. It consists of many departments and units that provide twenty-four hours' medical services to the populace 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 carried out between February and December 2020.

Study Population

This consisted of in and out-patients with various wounds attending clinics at Barau Dikko Teaching Hospital Kaduna State. Eligible patients with infected wound at female and male surgical wards, accident and emergency (A and E) ward, obstetrics and gynaecology (O and G) ward, Maternity Ward, and General out-patient (GOP) wound dressing unit within the period of the study were included. The inclusion criteria were patients with different categories of wounds showing secretion/pus or purulent exudates or discharge, and those with old wounds without signs of healing despite antibiotics treatment. Patients with fresh wounds, and/or receiving topical antibiotics/antiseptics (e.g., cream or tincture iodine) treatment or critically ill or unconscious were excluded.

Ethical Consideration

Permission to conduct the research was sought and obtained from the Health Research Ethics Committee of Barau Dikko Teaching Hospital, Kaduna State (Reference number: HREC - 20-0004). Permission was obtained from all the selected ward/ unit heads and written informed consent from the eligible participants. The participants were also informed of their freedom to consent or decline participation. Guardians or parents of children were requested to give assent for the children.

Sample collection and transportation

A total of sixty samples were collected between February to December 2020 from in - and out-patients in the clinics at Barau Dikko Teaching Hospital, Kaduna. In- and out-patients with infected wounds receiving medical care at the selected units or wards in the hospital were selected for collection of wound swabs. One wound swab sample was collected from each patient. In the case of patients with two or more wounds, one sample was collected from only one wound using balloting. All the collected samples were appropriately labelled for analysis.

With the help of nurses, exudate or purulent or pus discharge from the wounds was aseptically swabbed with a 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 labelling of the wound swab, the questionnaire was filled out to obtain 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

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

Isolation of *Pseudomonas aeruginosa* and *Staphylococcus aureus* from the wound

All clinical samples collected were cultured aerobically for isolation of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in the laboratory as described by Vallis and Nacente [9] and Cheesbrough [10]. The collected swabs were first cultured aerobically in an enrichment medium, BHI broth at 37 ^{o}C for 24 h. The broth cultures from the BHI broth was 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 subcultured aerobically on MacConkey agar plates at 37 ^{o}C for 24 h to confirm its characteristically non-lactose fermenters colonies. While the yellow colonies of presumptive *Staphylococcus aureus* on MSA plates were subcultured aerobically on another Chromogenic media (Baird Parker agar) plates at 37 ^{o}C for 24 h for the appearance of grey – 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 ^{o}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 as described [6,10].

Biochemical Characterisation of presumptive *Pseudomonas aeruginosa* and *Staphylococcus aureus* Isolates
Biochemical characterisation of the obtained pure isolates was carried out as described by Aneja , Ochai and Kolhatkar [6] and Cheesbrough [10]. 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 - Bioneer accuprep genomic DNA extraction kit (K-3032) as described by Bobai *et al.* [12].

Polymerase Chain Reaction (PCR) - Accupower Hotstart PCR premix (Bioneer)
Twenty microlitres (20 µl) reaction PCR set-up was prepared by adding 16µl dH_2O, 1µl forward primer - GGACTACAGGGTATCTAAT 16S (RIBOSE-1), 1µl reverse primer- AGAGTTTGATCCTGG 16S (RIBOSE-2), and 2µl template DNA. PCR amplification reaction was performed using PTC 100 thermal cycler with Pre- denaturation at 95 ºC for 5 min, denaturation at 94 ºC for 1 min, primer annealing at 54 ºC for 1 min, extension at 72 ºC for 1 min for 25 cycles, and final extension at 72 ºC for 5 min. The PCR products were separated by electrophoresis in 1.5% agarose gel for 35 min 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.

Data Analysis
The data was analysed using SPSS version 23 and results were presented as charts, and the Chi-square test was used to test the association between categorical variables.

Ethical approval
Permission to collect patients' wound swab samples for isolation of Pseudomonas aeruginosa was obtained from the research ethic committee (Reference number: HREC - 20-0004) of Barau Dikko Teaching Hospital, Kaduna. Written informed consent was also obtained from the respondents. Prior to the collection of wound swabs from patients, the hospital ethical committee approval and appropriate intended research information were disseminated to the nurses of the selected hospital wards and units. A brief explanation of the aim and objectives of the research was done to enlighten the patients. Patients were also informed of their freedom to consent or decline participation. Guardians or parents of children with wound infections were requested to give assent to the children.

RESULTS

Sociodemographic characteristics and medical history of the respondents
One-fifth (20%) of the respondents were aged 61 years and above, and 65.0% were males. About 2/3 (66.7%) were married and the majority (33.3%) were businessmen and women and 45% had secondary education. Half (50.0%) of the respondents were in-patients (**Table 1**).

About 1/5 (21.7%) of the respondents were known diabetic and 73% of the respondents were on antimicrobial (s) during the study (**Table 2**). The most commonly used antimicrobials by the respondents were metronidazole (18.3%), followed by mupirocin/Supirocin (15.6%) (**Fig. 1**). **Fig. 2** showed the location of the wounds, with 63.3% located on the legs, 15% hands, on 5% on the buttocks, among others.

Table 1. Sociodemographic characteristics of the respondents (n=60).

Variables	Frequency	Percent
Age (in years)		
0-10	6	10.0
11-20	10	16.7
21-30	3	5.0
31-40	9	15.0
41-50	11	18.3
51-60	9	15.0
≥61	12	20.0
Sex		
Male	39	65.0
Female	21	35.0
Marital status		
Married	40	66.7
Single	19	31.6
Widow	1	1.7
Occupation		
Business	20	33.3
Civil servant/public servant	7	11.7
Retirees	6	10.0
Students	17	28.3
Farming	5	8.3
Artisan	4	6.7
Non-formal teaching	1	1.7
Educational Status		
Non-formal	6	10.0
Primary	18	30.0
Secondary	27	45.0
Tertiary	9	15.0
Patient category		
In-patient	30	50.0
Out-patient	30	50.0

Table 2. Medical and drugs history of the respondents (n=60).

Variables	Frequency	Percent
Diabetes status		
Yes	13	21.7
No	31	51.7
Don't know	16	26.6
Patient on antimicrobial(s) during the study		
Yes	44	73.3
No	16	26.7

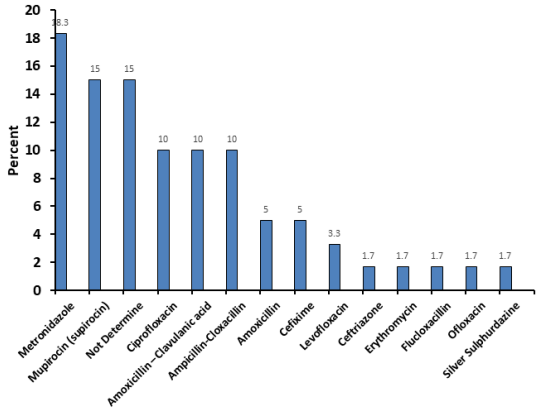

Fig. 1. Types of antimicrobial agents, patients were administered for the treatment of their wounds.

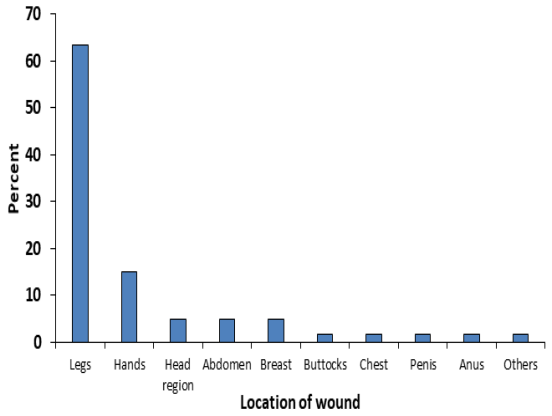

Fig. 2. Characteristics of the patient's wounds (n=60).

Phenotypic and Genotypic Characteristics of *Pseudomonas aeruginosa* and *Staphylococcus aureus* Isolates

Table 3 showed isolates of a presumptive *Pseudomonas aeruginosa* with characteristic colonial morphology blue–green and yellow–green in colour with circular, smooth edges, and flat and moderate shape on Cetrimide agar. Also, on MacConkey agar, the organism showed colourless, flat and circular colonies with smooth edges indicating typical characteristics. **Table 4** showed isolates of a presumptive *Staphylococcus aureus* with characteristic colonial morphology appearing completely yellowish in colour with raised, circular and smooth edges on Mannitol Salt Agar (MSA). On Baird Parker agar, the colonies of this organism 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. **Figs. 3** and **4** showed the Gel electrophoresis of amplified PCR 16SrRNA gene bands of *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolates respectively at 789 bp of the 100 bp plus DNA marker.

Table 3. morphological and biochemical characteristics of presumptive *Pseudomonas aeruginosa* isolates.

Morphological Characteristics		*Biochemical characteristics*													
										TSI					Probable organism
Colonial morphology on cetrimide and MacConkey agar	Cellular/ microscopic morphology	*Gram reaction*	*Motility*	*oxidase*	*Catalase*	*Indole*	*Methyl red*	*Voge-proskaver*	*Citrate utilization*	*Ureas utilization*	*Butt*	*slat*	*H_2S*	*GAS*	
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 aeruginosa*
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 aeruginosa*

Note:
Key: + = present, - = absent, TST = triple sugar iron, H_2S = Hydrogen sulphide, A = Acid and Al = Alkaline A = acid, AL = alkaline

Table 4. Morphological and biochemical characteristics of presumptive *Staphylococcus aureus* isolates.

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	Motility	Catalase	coagulase	Indole	Methyl red	Vogas-proskaver	Cirate utilization Hemolysis	lactose	mannitol	Sucrose	
Complete yellow, raised, circular and smooth edges, and moderate colonies on Black shining colonies with lysis at their edge	Cocci appeared in cluster (gape- like) or bundge with few singles and pairs	Gram positive	-	+	+	-	-	+	+ +	-	+	-	*Staphylococcus aureus*

Note
Keys: + = present, - = absent

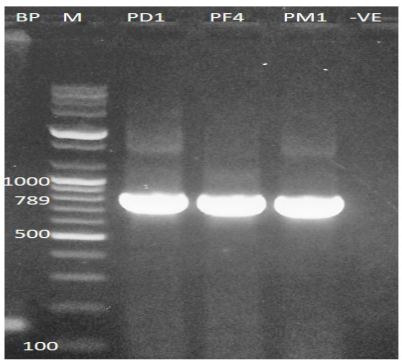

Fig. 3. Gel electrophoresis of amplified PCR 16SrRNA genes bands of *Pseudomonas aeruginosa* isolates at 789bp of the 100 bp plus DNA marker. Key: M = 100bp Marker, P = *Pseudomonas aeruginosa*, D= dressing room unit, F = female surgical ward, M = male medical ward, bp = base pair, - Ve = Negative Control.

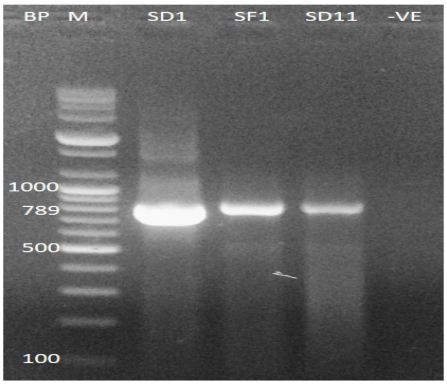

Fig. 4. Gel electrophoresis of amplified PCR 16SrRNA genes bands of *Staphylococcus aureus* isolates at 789 bp of the 100 bp plus DNA marker. Key: M = 100 bp DNA marker, S = *Staphylococcus aureus*, D = dressing room unit, F = female medical ward, M = male medical ward, bp = base pair, - Ve = Negative Control.

Distribution of *Pseudomonas aeruginosa* and *Staphylococcus aureus* Isolated from the Wounds of In - and Out - Patients

From the 60 samples collected, 11 isolates were *Pseudomonas aeruginosa (*72.7% from out-patients) *and 11* were *Staphylococcus aureus* (54.5% from out-patients) (**Table 5**). The test of association between the respondents' antimicrobial agents use and the organisms showed a significant difference (P > 0.05), while the test of association between respondents' sex, diabetes status, duration of the wound and the isolation of *Pseudomonas aeruginosa* and *Staphylococcus aureus* from the wound of patients showed no statistically significant differences (P > 0.05) (**Table 6**).

Table 5. Distribution of *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated from the wounds of in - and out–patients.

Category patients	of *Pseudomonas aeruginosa* Frequency (%)	*Staphylococcus aureus* Frequency (%)	Total Frequency (%)
In-Patient	3 (27.3)	5 (45.5)	8 (36.4)
Out-Patient	8 (72.7)	6 (54.5)	14 (63.6)
Total *(%)*	11(100.0)	11(100.0)	22(100.0)

Table 6. Associations between sex, diabetes status, antimicrobial use, duration of the wound and the isolation of *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

Variables	Organism			P value
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Staphylococcus aureus and Pseudomonas aeruginosa*	
Sex				
Male	6 (10.0%)	5 (8.3%)	2 (3.3%)	0.696
Female	3 (5.0%)	4(6.7%)	0 (0.0%)	
Diabetes status				
Diabetic	2 (3.3%)	2(3.3%)	0 (0.0%)	
Non-diabetic	6 (10.0%)	5 (8.3%)	0 (0.0%)	0.336
Don't know	1 (1.7%)	2(3.3%)	2(3.3%)	
Antimicrobial status				
Those on antimicrobial	6 (10.0%)	8 (13.3%)	2 (3.3%)	0.004
Those not on antimicrobial	3 (5.0%)	1(1.7%)	0 (0.0%)	
Duration of wound				
1 day to 3 months	2(3.3%)	1 (1.7%)	1(1.7%)	0.386
>3 months to 6 months	5(8.3%)	4 (6.7%)	1 (1.7%)	
>6 months	2 (3.3%)	4 (6.7%)	0 (0.0%)	

DISCUSSION

Findings from this study showed the majority of the respondents were elderly persons. The predominance of wounds in this age group could be due to their poor immune status as a result of old age or immunosuppression, other diseases like diabetes and cancers, among others. Sharmina *et al.* [13] reported that advancing in age, obesity, smoking, poor nutrition and immunosuppression due to diseases like Human Immunodeficiency Virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS), diabetes, cancer, chemotherapy or radiotherapy may lead to chronic ulceration. Bowler *et al.* [1] also reported that in old age, patients may have low antimicrobial efficacy of host immune response, a factor that influences the colonization and proliferation of microbial pathogens in wounds. However, it remains controversial whether ageing delays wound healing as some studies reported that there is no difference in the wound healing rate of old and young patients [14], while some studies reported a slow healing rate of the wound with advanced age [14].

Furthermore, about 2/3 of the swab samples were obtained from male patients indicating that a higher percentage of the patients with the wound that showed signs of infections were male compared to their female counterparts. This could be attributed to different wound healing rates due to sex, and age, among others. The study has shown that the ability of the human wound to heal is modulated by the host sex since sex hormones play a vital role in skin maintenance of homeostasis [15]. A number of studies clearly showed faster healing of wounds among female patients [15], and sex steroid hormones in particular, estrogens have been attributed to that [15]. Compared with aged females, aged males have been shown to have delayed healing to acuteness wounds [16].

Though this study's findings revealed infection of wounds of the patients predominantly by *Staphylococcus aureus* than *Pseudomonas aeruginosa* and a combination of the two organisms, it generally indicated bacterial pathogens infection predominantly in male than female patients, with no statistically significant difference (P > 0.05). This agreed with the findings recorded by Guo and Dipietro in 2010.

Findings from this study revealed a lower percentage of patients with wounds and diabetes compared to non-diabetic patients with wounds and those who don't know their status. Hence, the findings showed wound infection predominantly by *Staphylococcus aureus* and *Pseudomonas aeruginosa* in non-diabetes than diabetes patients. The statistical correlation between these wound pathogens and diabetic patients' status showed no significant difference (P > 0.05). Generally, people with diabetes are known to develop poor wound healing rates, consequently increasing the tendency of the proliferation of bacterial pathogens. However, studies have also reported that good management of blood glucose levels, thorough care, and prompt and good treatment of wounds as freshmen as they occur to improve their rate of healing. There are studies that showed that patients with wounds and chronic diabetes are more likely to have their wounds heal faster and fully if their blood glucose is well controlled [15,17].

The high prevalence of use of antimicrobial agents of different classes by the respondents during this study is of public health significance because it could serve as a driver for the development of antimicrobial resistance. The most prevalent antibiotic was mupirocin (Bacitracin + Neomycin), followed by ciprofloxacin, ampicillin-cloxacillin and amoxicillin, among others. Studies by Shittu *et al*. [18], Bowler *et al*. [1], Benjamin and Christopher [7], Sharmina *et al*. [18], Wendy *et al*. [19] and Twilley *et al*. [20] also reported the use of these antimicrobial agents by patients. Statistical analysis correlating the isolation of these wound pathogens from patients administered or those not administered antimicrobial agents showed a significant difference (P<0.05), with the study findings showing that a higher number of *Pseudomonas aeruginosa* were isolated from both patients who were administered or not administered antimicrobial agents compared to *Staphylococcus aureus* and a combination of the two organisms isolated from the patients.

In addition, this showed a higher percentage of the patients' wounds were located on their legs, and a lower percentage on the buttock, chest, penis and anus. Bowler *et al.* [1] reported similar findings and stated that the location of the wound and the level of tissue perfusion influence wound microbial pathogen proliferation. More so, that together with other factors, the antimicrobial efficacy of the host immune responses plays a vital role in microbial colonization of wounds. The patients' duration of the wound in this study showed both short and long periods ranging from ≤ 7 days to ≥1≤15 years, with a higher percentage of patients' wound duration being ≥1≤4 weeks. Kozar *et al.,* [21] reported that bacterial infection of wounds can be a major cause of complications and delay in wound healing.

Since microbial proliferation in wounds delays healing, this might be strongly attributed to the reason why this study's findings clearly revealed that some of the wound patients have been managing their wounds for a period of one to fifteen years, some for a period of one to twelve months, some for a period of one to four weeks, and others managing for a period of one to less than seven days. This finding agreed with that by Bowler *et al*., [1] who carried out a study and demonstrated an increase in the average hospitalisation period of wound patients from fourteen days when the wound healed to twenty-four days when the wound has a complication and is infected by bacterial pathogens. More so, Emma and Warren [22] reported that antibiotic-resistant bacterial wound infections narrow therapeutic options for patients and consequently result to poorer patient outcomes. *Pseudomonas aeruginosa* contributes substantially to wound-related morbidity and mortality worldwide and causes wound infection, skin infection (especially at burn sites), ulcers and pressure sores (Khan *et al.,* [2] thereby increasing treatment cost and delaying wound healing. Also, *Staphylococcus aureus* has been reported in wound infection frequencies varying from 43% of infected leg ulcers [3] to 88% of non-infected leg ulcers [[4] influencing wound healing duration. The findings showed that a higher number of *Pseudomonas aeruginosa* were isolated from both patients with different duration of a wound than *Staphylococcus aureus* and a combination of these two organisms. This was not statistically significant (P>0.05).

The application of appropriate therapeutic interactions requires differentiation of the types of wound-colonizing pathogens. Thus, would allow the application of a specific treatment that will result in faster and more efficient healing of the wound with further impact in slowing down the spread of antimicrobial resistance bacteria [21]. This study isolated and identified *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains from wound-infected patients using both phenotypic and genotypic approaches. As indicated in this study, Prasanna *et al.* [23] reported similar findings about the cultural and biochemical characteristics of *Pseudomonas aeruginosa* on cetrimide and MacConkey agar. Also, the Gram stain and microscopy showed the organism to be a Gram-negative rod. This is in agreement with the findings reported by Walthiq and Mohammed [24]. Similarly, phenotypic identification revealed the colonies of *Staphylococcus aureus* on MSA as yellow with flat and moderate shape. The production of yellow colonies on MSA has been reported by Fitzgerald [25].

As shown in this study, Silva *et al.* [26] reported similar characteristics of *Staphylococcus aureus* on the Baird parker medium. The biochemical characteristics showed that this organism is catalase and coagulase positive with characteristic production of beta-hemolysis on blood agar – a unique characteristic of pathogenic *Staphylococcus aureus* strains. Tong *et al*. [27] reported similar cellular and biochemical characteristics of *Staphylococcus aureus.* Molecular identification was employed to compare the genetic similarities of *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated from the wound with the GenBank database according to Prescott *et al.* [28]. 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 789bp of the 100 bp plus DNA marker.

Findings in this study showed infection of wounds of in-and out-patients by *Pseudomonas aeruginosa and Staphylococcus aureous* out-patients. One of the major complications associated with the wound is infected. The type of bacteria pathogens and the number of pathogens cells number in the wound is related to the wound healing outcome [15]. White blood cells are essential to the function of the immune system and when the white blood cells are able to function well, then the body has the ability to fight bacteria pathogens and close wounds. Though this study's findings showed infection of the wound with *Pseudomonas aeruginosa and Staphylococcus aureus*, there was no statistically significant difference (P > 0.05) between the presence of these bacterial wound pathogens.

CONCLUSION

In this study, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were isolated from wounds of in - and out-patients. The percentage of *Pseudomonas aeruginosa* isolates was higher compared *to Staphylococcus aureus* and a combination of these organisms. It is evidenced from this study that both acute and chronic wounds are susceptible to colonization and proliferation by bacteria pathogens. However, a correlation between patients with wounds and the isolation of *Pseudomonas aeruginosa and Staphylococcus aureus* showed no statistically significant difference.

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