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Full Length Research Article

ISOLATION, IDENTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF BACTERIA ASSOCIATED WITH WOUND OF PATIENTS ATTENDING AHMADU BELLO UNIVERSITY TEACHING HOSPITAL, SHIKA, ZARIA-NIGERIA

*Abdullahi B. and Lawal F.B.

Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria

*Corresponding Author Email Address: albishir13@gmail.com Phone: +2348054527359

ABSTRACT
An infected wound affects the quality of life, and compromises the wound's healing rate. This study was aimed at isolating bacteria from wounds and determining their antibiotics susceptibility patterns. A total of 100 wound swab samples were collected from wound patients attending Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. The samples were cultured and the resulting isolates identified using biochemical tests. The identified bacteria were subjected to antibiotic susceptibility test using agar disc diffusion method. Out of the 100 wound samples collected, 43 were infected with bacteria; given rise to overall prevalence of 43% bacterial wound infection. Out of the 43 isolated bacteria, 58.1% were *Staphylococcus aureus*, 18.6% were *Klebsiella* spp while 23.3% were identified to be *Pseudomonas* spp. Prevalence of bacterial wound infection was higher in female patients (47.3%) than their male counterpart (37.8%). Age group 21-40 years had the highest prevalence of the infections (48.3%) and the least was observed among 10-20 years (33.3%). None of the risk factors is significantly associated with the infection *(p>0.05).* All the isolates were 100% susceptible to ofloxacin. All the isolates showed 100% resistance to penicillin. All the *Pseudomonas* species were 100% MDR. *Staphylococcus aureus* was the most frequently encountered bacteria and ofloxacin was the first drug of choice in the treatment of wound infections.

Keywords: Wounds; Bacterial Infection; Antibiotics Susceptibility; MDR

INTRODUCTION

Wound is a breach in the skin and the exposure of subcutaneous tissues following loss of skin integrity which provides moist, warm, and conducive environment that is conducive to microbial colonization and proliferation (Esebelahie *et al.,* 2013). Skin is exposed to injuries, scratches and it is in contact with the external environment, thus it is more susceptible to colonization by pathogens (Simões *et al*., 2018). Since wound colonization is most frequently poly-microbial, involving different microorganisms that could be potentially pathogenic, any wound is at some risk of getting infected (Simões *et al.,* 2014). Wounds presented by patients vary from one setting to another, ranging from acute surgical wounds, traumatic wounds such as those that occur following an accident, burn wounds or chronic wounds such as diabetic foot, leg and pressure ulcers. All wounds are contaminated with microorganisms that are part of the saprophytic microflora of the skin and the type and quantity of these microorganisms vary from one wound to another (Cooper and Lawrence, 1996).

An infected wound affects the quality of life, and compromises the wound's healing rate. Wound infections represent one third of nosocomial infections among surgical patients and are responsible of 70–80% of mortality (Pallavali *et al.,* 2017). Differently, wound infection depends on the pathogenicity and virulence of the microorganisms and on the immune competency of the host and it is determined by the presence of clinical signs of infection such as erythema, pain, tenderness, heat, oedema, cellulites and abscess/pus (EWMA, 2005). Reports showed that, *Staphylococcus aureus*, *Pseudomonas* spp., *Klebsiella* spp, and *E. coli* are the leading bacterial pathogens in wound infection (Yasidi *et al.,* 2015). Similar reports have been observed in Nigeria (Okesola and Kehinde, 2008).

The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and to a greater incidence of infections caused by methicillin-resistant *S. aureus* polymicrobic flora and by fungi (Sibor *et al*., 2008). Antibiotic treatment and wound care represent two critical factors for the management of the infection (Jeschke *et al.,* 2020). Inappropriate and continued use of systemic and topical antimicrobial agents has provided the selective pressure that has led to the emergence of antibiotic resistant strains (Gautam *et al.,* 2013). The majority of the isolates from infected wounds are known to be resistant to ampicillin and amoxicillin. Large numbers of *S. aureus* are methicillin-resistant *S. aureus* (MRSA) and most bacteria isolated are sensitive to quinolones, aminoglycosides and monobactam (Etok *et al.,* 2012).

The detection of the different microbial species colonizing a wound, as well as their susceptibility to the antimicrobials, can provide an indication for a more appropriate therapy to be administered to patients, significantly reducing the health care costs (Vaez and Beigi, 2013).
Therefore, the aim of this study was to isolate and identify bacteria associated with wound of patients attending Ahmadu Bello University Teaching Hospital, Shika, Zaria-Nigeria and determine their antibiotic susceptibility patterns.

MATERIALS AND METHODS

Study Area and Ethical Approval

This research work was carried out at Ahmadu Bello University Teaching Hospital, Shika Zaria-Nigeria. Ethical approval was given by the Management of Ahmadu Bello University Teaching Hospital (ABUTH), Shika, Zaria-Nigeria for collection of samples.

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Sample Size and Sample Collection
One hundred Samples were collected from consented wound patients of ABUTH. Sterile swab sticks were used to swab the wound surface, the swabs sticks were replaced into their jackets and transported in icepacks to the Department of Microbiology Laboratory, Ahmadu Bello University, Zaria, Nigeria, for Microbiological investigations.

Administration of Structured Questionnaire
Questionnaires were administered to the consented patients in order to determine the risk and demographic factors, and symptoms associated with the wound infection.

Isolation and Identification of Bacteria
Cultural tests, Microscopy/Gram staining and Biochemical tests were carried out to isolate and identified respectively the bacteria implicated in wound infections. After 24 h of incubation period at 37^{o}C the various inoculated media were observed. Colonies that were golden yellow on Mannitol Salt Agar (MSA) were suspected to be *Staphylococcus,* and thereafter subjected to Microscopic, Catalase, Coagulase and DNase tests for Confirmation. Bluish black colonies with green metallic sheen on Eosine Methylene Blue Agar (EMB) were suspected to be *E. coli.* Large pink mucoid colonies on MacConkey agar were suspected to be *Klebsiella*, while blue-green colonies on Centrimide were suspected to be *Pseudomonas* spp. The suspected Gram negative bacteria were subjected to Microscopic, and indole, methyl red, voges proskauer and citrate utilization tests for confirmation.

Antibiotic Susceptibility Test
Antibiotic susceptibility test was performed using the Kirby–Bauer disk diffusion method as described by (Akubuenyi *et al.,* 2011). The test organisms were standardized by picking three colonies of each of them and inoculated into 9 ml of normal saline water in a test tube, and their turbidity was compared with that of McFarland standard of 0.5 Barium sulfate solution. A sterile swab was dipped into the suspension of the standardized inoculum contained in normal saline, squeezed free from excess fluid against the side of tube and spread over the Mueller–Hinton agar plate. The plate was allowed to dry; the antibiotic discs were introduced into the medium using sterile forceps then incubated at 37^{o}C for 24h. Zones of inhibition were observed and measured in millimetre using a metre rule. The measured zones of inhibition were interpreted as Intermediate, Susceptible and Resistant using Clinical and Laboratory Standards Institute (CLSI) guidelines. The commercially prepared antibiotics used were chloramphenicol (30µg), Ampicillin (10µg), Ofloxacin (30µg), Trimethoprim/Sulfamethoxazole (25µg), Tetracycline (30µg) and Penicillin (10µg).

Statistical Analysis
Data were analyzed using the Statistical Software SPSS for windows version 20. *P value* < 0.05 was considered statistically significant and > 0.05 insignificant.

RESULTS
Out of 100 consented wound patients attending Ahmadu Bello University Teaching Hospital, 43 had bacterial infections, given rise to a total prevalence of 43% of wound infection (Figure 1).
Figure 2 Shows occurrence of bacteria in the infected wounds. *Staphylococcus aureus* was the highest encountered bacteria (58.1%), while *Klebsiella* spp was the least encountered (18.6%).
Table 1 shows prevalence of wound infection based on demographic factors. Out of 45 male patients, 17 (37.8%) had wound infection, while 26 (47.3%) female patients had the infection. Age group 21-40 years had the highest prevalence of wound infection (48.3%) and this was follow by age group > 40 years with rate of 44.4%. The least was observed among 10-20 years (33.3%)
Table 2 shows prevalence of wound infection based on symptom. The infection was found to be higher among individuals with pus (46.8%) compare to those without pus (39.1%). Patients experiencing pain had (58.8%) wound infection, while those that were not experiencing pain had (39.8%) rate.
Table.3 shows prevalence of wound infection based on risk factors. Patients with diabetic had infection rate of 63.6%, while those without had 40.4% infection rate. Smoker patients had 37.5% infection rate while non-smokers had 43.5% rate. Cancer and HIV patients were not encountered in this study.
Percentages of antibiotic susceptible, intermediate and resistant isolated bacteria are shown in table 4. Table 5 shows the number and percentages of the isolates resistance to classes of antibiotics. The isolates were 69.8%, 7% and 23.3% resistance to 1, 2 and 3 classes of antibiotics respectively. All the *Pseudomonas* isolates were 100% resistance to 3 of the antibiotic classes used. Figure 3 shows the percentages of multi-drug resistant isolates. All the isolated *Pseudomonas* species were multi-drug resistant, while 88% of the isolated *Staphylococcus aureus* were non multi-drug resistant.

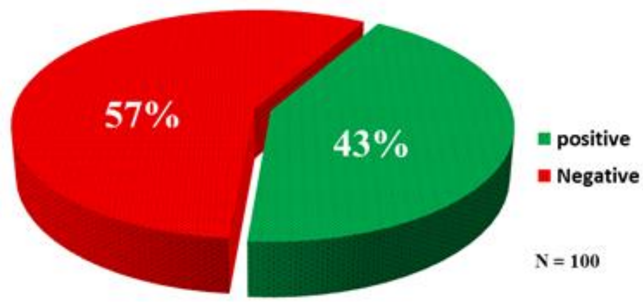

Figure 1: Prevalence of Bacterial Infections among Wound of Patients attending Ahmadu Bello University Teaching Hospital, Shika

Key: N=number of sample

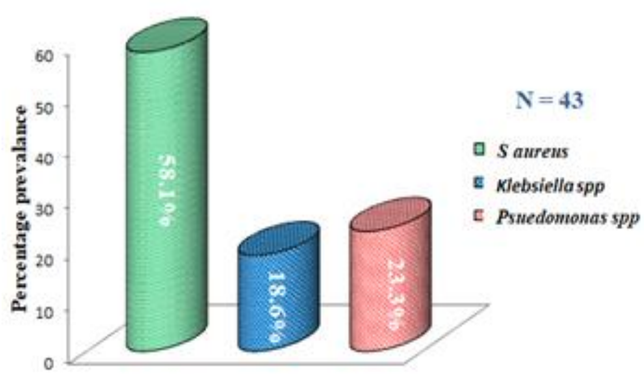

Figure 2: Occurrence of Bacteria in Wound Infections
Key: N = Total Number of Bacteria Isolated from Wound Infections

Table 1: Prevalence of Wound Infection Based on Demographic Factors

Variables	No. examined	No. positive (%)
Sex		
Male	45	17(37.8)
Female	55	26(47.3)
Age group (years)		
< 10	14	6(42.9)
10-20	21	7(33.3)
21-40	29	14(48.3)
> 40	36	16(44.4)

Table 2: Prevalence of Wound Infection Based on Symptoms

Symptom	No. examined	No. positive (%)	R^2 *value*	*P- value*
Pain			0.021	0.151
Yes	17	10(58.8)		
No	83	33(39.8)		
Fever			0.017	0.202
Yes	31	15(48.4)		
No	69	28(40.6)		
Pus			0.003	0.627
Yes	47	25(53.2)		
No	53	18(34.0)		

Table 3: Prevalence of Wound Infections Based on Risk Factors

Risk factors	No. examined	No. positive (%)	P- value
Diabetic			0.146
Yes	11	7(63.6)	
No	89	36(40.4)	
Hypertensive			0.087
Yes	16	10(62.5)	
No	84	33(39.3)	
Cancer			
Yes	0	0	
No	100	43(100)	
Smoking			0.746
Yes	8	3(37.5)	
No	92	40(43.5)	
HIV positive			
Yes	0	0	
No	100	43(100)	

Table 4: Percentages of Antibiotic Susceptible, Intermediate and Resistant Bacteria

Antibiotics(µg)	S n (%)	I n (%)	R n (%)
	Staphylococcus aureus		
OFX(5)	25(100)	0(0)	0(0)
SXT(25)	23(92)	2(8)	0(0)
AMP(10)	14(56)	4(16)	7(28)
CH(30)	23(92)	2(8)	0(0)
P(10 µg)	0(0)	0(0)	25(100)
TET(30 µg)	25(100)	0(0)	0(0)
	Pseudomonas Species		
OFX(5)	10(100)	0(0)	0(0)
SXT(25)	0(0)	0(0)	10(100)
AMP(10)	0(0)	0(0)	10(100)
CH(30)	10(100)	0(0)	0(0)
P(10 µg)	0(0)	0(0)	10(100)
TET(30 µg)	0(0)	1(10)	9(90)
	Klebsiella Species		
OFX(5)	8(100)	0(0)	0(0)
SXT(25)	7(85.7)	0(0)	1(12.5)
AMP(10)	0(0)	0(0)	8(100)
CH(30)	8(100)	0(0)	0(0)
P(10 µg)	0(0)	0(0)	8(100)
TET(30)	8(100)	0(0)	0(0)

Key: R= Resistant; I=Intermediate; S=Susceptible; CH=Chloramphenicol; AMP=Ampicillin; OFX=Ofloxacin; SXT=Trimethoprim/Sulfamethozole; TET=Tetracycline, P= Penicillin, n= Number of Isolates

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Table 5: Number and Percentage of Isolates Resistance to Classes of Antibiotics

Isolates	N	RC1	RC2	RC3	RC4	RC5
Staphylococcus aureus	25	23(92.0)	2(8.0)	0(0.0)	0(0.0)	0(0.0)
Pseudomonas Species	10	0(0.0)	0(0.0)	10(100)	0(0.0)	0(0.0)
Klebsiella Species	8	7(87.5)	3(7.0)	0(0.0)	0(0.0)	0(0.0)
Total	**43**	**30(69.8)**	**3(7.0)**	**10(23.3)**	**0(0.0)**	**0(0.0)**

Key: N = Number of Isolates; R = Number of resistant isolate; C1-C4 = number of antibiotic classes in which isolates were resistance to; Values in parenthesis = Percentages

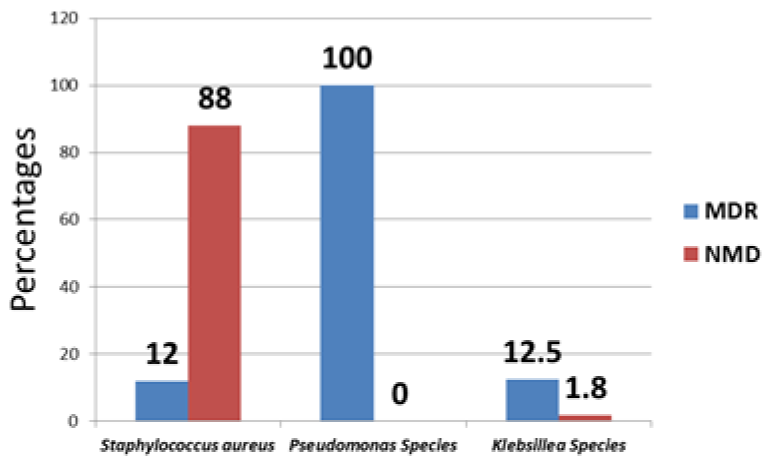

Figure 3: Occurrence of Multi-Drug Resistant Isolates
Key: MDR = Multi-drug Resistant; NMD = Non Multi-drug Resistant

DISCUSSION

Detection of bacterial infections is important to promote wound healing and curb chronic non-healing wounds. Our study demonstrated a high prevalence 43% of bacterial infections in wounds of the subject population. This finding was lower to the 81.1%, 60.20% and 92% reported by Kemebradikumo *et al.* (2013), Kc *et al.* (2014) and Khalim *et al.* (2022) respectively. The difference could be attributed the source of wound infections from which samples were obtained. Another reason that may explain this variation is the adopted protocol of infection control and antibiotic prophylaxis that may play a crucial role in bacterial growth. *Staphylococcus aureus* was the most frequently encountered isolates in the wound infections with occurrence of 58.1%, followed by *Pseudomonas* spp. (23.3%) while the least encountered was *Klebsiella* spp. (18.6%). *Staphylococcus aureus* is particularly high because the skin is a natural habitat for *staphylococcus species* hence a higher risk of wound been infected with it (Grice and Segre, 2011). Moreover, *S. aureus* are known to have a vast number of virulence factors that increase their ability to cause infections when compared to other bacteria (Manyahi, 2012). Similar to the pattern of bacteria occurrence in this study was that of Ogba *et al.* (2014) who reported that *Staphylococcus aureus* was the most prevalent pathogen 91 (30.8%) followed by *Pseudomonas aeruginosa* 51 (17.3%). In like manner, different studies confirmed the predominance of *S. aureus* isolated from wound infections (Mama *et al.,* 2014; Puca *et al.*, 2021). However, in the findings of Pandukur *et al.* (2020), *Pseudomonas aeruginosa* was the commonest isolates, accounting for 8 (26.7%), followed by *Staphylococcus aureus* and *Escherichia coli* with 7 (23.3%) and 6 (20.0%) respectively. It is well documented that bacteria such as *S. aureus* and *P. aeruginosa* produce very destructive virulence factors, responsible for maintaining infection and delay healing in chronic wounds. *S. aureus* causes clinically relevant infections mostly because of its virulence factors such as coagulase, catalase, clumping-factor A and leucocidines (Dissemond, 2009). Similarly, the production of an elastase by *P. aeruginosa* has been associated to its pathogenicity in the wound environment (Schmidtchen *et al.,* 2003). Thus, our results confirm the usual most prevalent microorganisms found in infected wounds.

Female patients had the highest prevalence of wound infections which accounted for 47.3% while that of their male counterpart was 37.8%. This finding contradicted that of Kc *et al.* (2014) and Chizurum *et al.* (2022) who reported (63.16% and 56.76%) and (80% and 20%) in male and female patients respectively. Age group 21-40 years had the highest prevalence (48.3%) of the infections, followed by >40 years (44.4%), <10 years (42.9%) while 10-20 years had the least rate of 33.3%. Ideally, the age of a patient seems likely to have a bearing on wound infection and healing, people at the extremes of life being more prone to wound infections, but in this study, bacteria were isolated more from age group 21-40 year. However, statistical analysis revealed by Khalim *et al.* (2022) showed that the prevalence of wound infection was not affected by gender and age. Similar studies in Niger delta University Teaching Hospital, a rural tertiary Hospital in Nigeria and health facilities in Cameroon also reported that gender and age of patients had no influence on the prevalence of wound isolates (Oladeinde *et al.,* 2013; Pondei *et al.,* 2013; Kihla *et al.,* 2014). What is generally agreed upon is that age of a patient may affect the ability of immune response to infection with the young and elderly patients being more prone to infections which may affect bacterial isolation rate in these age groups (Fuentes *et al.,* 2017). These findings showed no association between the prevalence of bacterial infections with the symptoms experienced by the patients ($p>0.05$). However, there was higher prevalence of bacterial infections among wound patients with pain (58.8%), fever (48.4%) and pus (53.2%) compared to those without these symptoms. The rate of bacterial infections among wounds with pus in our study was lower to the 59.55% reported by Kc *et al.* (2014). In our study, no significant association was found between the risk factors and wounds with positive culture ($p>0.05$). Out of the 11 diabetic patients encountered, 7(63.6%) had their culture positive for bacterial growth while 40.4% out of the 89 culture of non-diabetics had bacterial growth. The wound infections rate among diabetic patients in this study was lower compared the rate (100%, 6/6) reported by Akoachere *et al.* (2014). The higher rate of wound infections among diabetic patients in this study was not surprising because diabetes lower immune response to bacterial infections (Chalya *et al.,* 2011). Also, hypertensive patients had higher prevalence of wound infections (62.5%) than non-hypertensive (39.3%). Hypertension may lower immune system and hence aid bacterial infections. Cancer and HIV positive patients usually have their immune system compromised. However, these categories of patients were not encountered in this study.

Out of the 8 patients who were found to be smoking in this study, 3(37.5%) had their wound swab culture positive for bacterial infections while 40(43.5%) of the 92 non-smoking patients had wound infections. These findings contradicted that of Kc *et al.* (2014), who reported higher wound infections among smoker patients (65.52%) compared to non-smokers (55.47%). These contradictions could be due to differences in the sample sizes of the two studies.

Ofloxacin was the drug of choice in this study, all the isolates were 100% susceptible it. Kc *et al.* (2014) and Akoachere *et al.* (2014) reported that 100% of their isolated bacteria from wound were susceptible to ofloxacin which was in line with our findings. The isolates were also highly susceptible to chloramphenicol; 92% of the isolated *Staphylococcus aureus*, and 100% of the *Pseudomonas* species and *Klebsiella* species were susceptible to it respectively. These findings were however, in contradictions with that of Pandukur *et al.,* (2020) who reported high level of resistance of bacterial isolated from wounds to chloramphenicol. Also, Akoachere *et al.* (2014) recorded low susceptibility (6.5%) of bacteria isolated from wound to chloramphenicol. The outcome of susceptibility of our isolates to chloramphenicol could be as a result of the fact that it is hardly got abused or prescribed in our study area. All the *Staphylococcus aureus* and *Klebsiella* species were 100% susceptible to tetracycline in this study were. These findings contradicted the 51% and 57% resistance of *Staphylococcus aureus* and *Klebsiella* species respectively recorded in South-West Ethiopia by Mama *et al.* (2014) and with the ones done elsewhere (Taiwo *et al.,* 2002; Mulu *et al.,* 2012). However, 90% of our isolated *Pseudomonas* species were resistance to tetracycline. This was consistent with the study done in Ethiopia (Mama *et al.*, 2014). *Staphylococcus aureus* was 92% susceptible trimethoprim-sulfamethozol. This corresponded to the 94% reported by Mama *et al.* (2014). In the findings of Worku *et al*. (2023), *Staphylococcus aureus* were 75.8% susceptible trimethoprim-sulfamethozol, which also corresponded to the high susceptibility of the isolates to the antibiotic in our findings. Also in this study, *Klebsiella* species isolates were highly susceptible to trimethoprim-sulfamethozol with susceptibility rate of 85.7%. However, this contradicted the 85.7% resistance reported by Mama *et al.* (2014). *Pseudomonas* species was 100% resistance to trimethoprim-sulfamethozol in this study, which is similar to the 73% resistance reported by Mama *et al.* (2014). *Pseudomonas* and *Klebsiella* species isolates were 100% resistance to ampicillin. These findings were in agreement with the findings of Mama *et al.* (2014). Also, Bessa *et al*. (2015) and Worku *et al*. (2023) respectively reported 100% resistance of *Pseudomonas* and *Klebsiella* isolates to ampicillin. All the isolates from this study were 100% resistance to penicillin. The 100% resistance of our isolated *Staphylococcu*s *aureus* to penicillin is similar to the 90.2% reported by Worku *et al*. (2023). The increased bacterial resistance is probably due to irrational and inappropriate use of antimicrobial agents, disregard to hospital infection control policies and showing negligible regard to culture susceptibility pattern while administering antimicrobial agents (Verma *et al.* 2012).

Isolates that were resistance to two or more classes of antibiotics were considered multi-drug resistant (MDR). In our findings, 12% and 12.5% of the isolated *Staphylococcu*s *aureus* and *Klebsiella* isolates respectively were non-multidrug resistant. This contradicted the findings of Worku *et al*. (2023), where 100% of *Staphylococcu*s *aureus* were MDR. However, *Pseudomonas* isolates we encountered in this study were 100% multi-drug resistant. Multi-drug resistant bacteria in wound infections are great challenge in the effective management of the infections. There is an urgent monitoring of antimicrobial use by clinicians, microbiologists, and health and government agencies. Equipping Microbiology Laboratories will aid effective surveillance of antimicrobial resistance. We also advocate proper isolation and identification of bacteria implicated in wound infections, and testing for antimicrobial susceptibility before drug use.

Conclusion

The most frequently encountered bacteria in this study was *S. aureus* (58.1%) followed by *Pseudomonas* species (23.3%) and least was *Klebsiella* species (18.6%). These isolates showed high level of susceptibility to ofloxacin (100%). However, they were 100% resistance penicillin. All the isolated *Pseudomonas* species were 100% MDR while least MDR (12%) was observed in *S. aureus*.

Conflict of Interest

The authors declare no conflict of interest in this study.

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