Annals of Microbiology and Infectious Diseases
Volume 1, Issue 1, PP 1-6

Characterization and Determination of Antibiotic Sensitivity Pattern of Bacteria from Infected Wound

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ABSTRACT

The study was aimed to characterize and determine the antibiotic sensitivity patterns of bacterial isolates from infected wound of patients attending Kura General Hospital from March–July 2017. A total of 32 male adult patients with various degree of infected wound were involved in the study. Clinical samples from the infected wound were collected using sterile swab and analyzed using standard microbiological techniques. Organisms were identified by their colonial morphology, Gram staining and biochemical characterization. Antibiotic susceptibility patterns of the bacterial isolates were determined using modified Kirby Bauer method. Out of 32 infected wound samples examined, 106 organisms were isolated. Staphylococcus aureus was the most frequent isolate (27%), followed by Klebsiella sp (24%), Pseudomonas aeruginosa (20%), Escherichia coli (15%), Proteus mirabilis (9) and Streptococcus (7%). On the sensitivity pattern of the isolates against the antibiotics used, some of the antibiotics are active against the isolates. P. aeruginosa is resistant to Norfloxacin, Amoxicillin, Rifampicin, Ampicillin and Chloramphenicol. Streptococcus also showed resistivity to Ciprofloxacin Norfloxacin and Gentamicin. Klebsiella is resistant to Amoxicillin, Rifampicin and Ampicillin. S. typhi is susceptible to the entire antibiotics but resistant to Rifampicin and Ampicillin. Statistical analysis of the results showed significant different on the susceptibility of the isolates to the antibiotics used at $p < 0.05$. There is the present of resistance to the commonly used antibiotics due to emergence of multi-drug resistant isolates. There is need for proper diagnosis to monitor the susceptibility pattern of wound isolates which will guide the use of antibiotics

Keywords: *Bacteria, infected wound, antibiotic, susceptibility pattern.*

INTRODUCTION

Wounds infection has been a recognized as the most critical problem especially in the presence of foreign materials that increases the risk of serious infection even with relatively small bacterial infection [1]. Wound infection is a major concern among healthcare practitioners, not only in terms of increased morbidity to the patient but also in view of its burden on financial resources and the increasing requirement for cost-effective management within the healthcare system. The control and management of infection is a complex and important aspect of wound care. Although, antibiotics have been of great value in treatment and in prophylaxis to prevent infections, the timing of administration, choice of antimicrobial agent, durations of administration have clearly defined the value of antibiotics in reducing wound infections [2]. The most common bacterial genera infecting wounds are *Enterococci, Escherichia, Pseudomonas, Klebsiella, Enterobacter, Proteus and Acinetobacter* [3]. Wound infection patients are subjected to several factors that may be associated with multidrug resistant microorganism carriage such as inappropriate antibiotic treatment, chronic course of the wound and frequent hospital admission [4].

A study on aerobic bacterial profile and antimicrobial susceptibility pattern of wound isolates in a South Indian tertiary care hospital revealed *Staphylococcus aureus* (24.29%) was the most common isolates, followed by *Pseudomonas aeruginosa* (21.49%), *Escherichia coli* (14.02%), *Klebsiella pneumoniae* (12.15%), *Streptococcus pyogenes* (11.23%), *Staphylococcus epidermidis* (9.35%) and *Proteus* species (7.47%) [5]. Another study on isolation of different types of bacteria from wound revealed also *Staphylococcus aureus* to be the predominant microorganism (40%) followed by *Klebsiella* species (33%), *Pseudomonas* species (18%), *Escherichia coli* (16%), and *Proteus* species (7%) [6]

The emergence of bacterial antimicrobial resistance has made the choice of empirical therapy more difficult and expensive [7]. Hence there is need for regular screening of organisms causing various infections and to characterize their antimicrobial susceptibility pattern to commonly used antibiotics at the hospital, regional, national and global levels to guide the clinicians to select a relevant antimicrobial for empirical treatment of infections. The objective of this study was to isolate and characterize various bacterial isolates from infected wounds, and determine their susceptibility to some antibiotics.

MATERIALS AND METHODS

Ethical Approval

Ethical approval was obtained from Kano State Hospital Management Board based on the consent of Kura General Hospital ethical committee.

Study Area

Kura Local Government Area is geographically located in the southern part of Kano state along Zaria – Kano expresses with a distance of about 35 Kilometer from the State capital. It is located at Latitude 11^0 46'17" N and Longitude 8^0 25' 49" E. It covers an area of about 206 Km^2 of land and population of about 144,601 according to 2006 census [8]. Kura Local Government share common boundaries with Garun-mallam (West), Dawakin-kudu (East), Bunkure (South) and Madobi Local Government (North)[8]

Sample Collection

This study involved 32 male adult patients attending Kura General Hospital for treatment of infected wound between March–July 2017. The wound swab samples were obtained before cleaning of the wounds and were processed for isolation and identification of bacterial pathogens according to the standard microbiological techniques [9]. All the samples collected were immediately transferred under aseptic conditions to Microbiology Laboratory of Kano University of Science and Technology Wudil for isolation and identification.

Isolation and Identification of Isolates

The clinical wound swab samples were inoculated onto Nutrient agar (Life save Biotech, USA), Mannitol salt agar (Biomark, India) and Mac Conkey agar (Life save Biotech, USA) plates and incubated aerobically at 37^0 C for 24 hours. After incubation bacterial growth was observed for colony appearance and morphology. Each colony was re-inoculated into freshly prepared agar plates until a pure colony was obtained. For identification, each pure colony was Gram stained and subjected to further biochemical tests [9]. Results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute [10].

Gram Staining

A drop of normal saline was placed on a well labeled clean grease-free glass slide using a sterile inoculating loop; a colony of an overnight culture of the bacterial isolate was emulsified with the normal saline to make a thin smear. The smear was air dried and then heat fixed. The slide was flooded with crystal violet (primary stain) for 30 seconds after which the stain was rinsed from the slide with water. The smear was flooded with Lugol's iodine (mordant) to fix the primary stain. The iodine was rinsed with water after 60 seconds**.** The slide was then flooded with a decolorizer (acetone) and rinsed off almost immediately**.** The counter stain; safranin was added and left for 30 seconds before being rinsed off. The stained smear was air dried, and then observed under the microscope using X100 oil immersion objective lens of the microscope [11].

Biochemical Characterization

The isolates were also characterized by biochemical tests viz. IMViC reactions i.e. indole test, Methyl Red test, Vogues Proskauer test catalase test, oxidase test and Citrate utilization test, as well as Lactose and Mannitol fermentation reaction test by standard method given by Sherman [11] and Holt *et al*. [12].

Antibiotic Sensitivity Test

The bacteria isolates were subjected to antibiotic susceptibility testing using the agar diffusion method as described by Bauer *et al*. [13]. Mueller Hinton agar (MHA) plates were inoculated with overnight culture of each isolate by streak plating. The standard antibiotic sensitivity discs were then aseptically placed at equidistance on the plates and allowed to stand for 1 hour.

The plates were then incubated at 37°C for 24 hours. Sensitivity pattern of the isolates to Augmentin (30 µg/disc), Erythromycin (10 µg/disc), Streptomycin (30 µg/disc), Amoxicillin (30 µg/disc), Gentamicin (20 µg/disc), Oxacillin (10 µg/disc), Ofloxacin (30 µg/disc), Neomycin (20 µg/disc), Ciprofloxacin (10 µg/disc) and Septrin (30 µg/disc), produced by Abtek pharmaceutical limited, were determined. Isolates were divided into three groups based on the zone of inhibition produced by the antibiotic disc; susceptible, intermediately susceptible and resistant according to the Clinical and Laboratory Standards Institute (CLSI) guideline; Performance Standards for Antimicrobial Susceptibility Testing [10].

Statistical Analysis

The data of average zone of inhibition produced by the isolates against the antibiotics used was analyzed using One-Way ANOVAs and the statistical program SPSS 21.0 (Statistical Package for the Social Sciences). The results were presented as the means ± standard deviation. Significance level for the differences was set at $p<0.05$.

Results

Biochemical Characterization of the Isolates

The biochemical characterization of the isolates from infected wound is presented in Table 1. The results showed that six different isolates were obtained namely; *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus sp, Klebsiella pneumoneae* and *Proteus mirabilis.*

Table1. *Biochemical Characterization of the recovered isolates*

Code	GS	IN	MR	VP	CI	CA	CO	OX	LF	MF	Isolates
IS_1	-	+	+	-	-	+	-	-	+	NA	*Escherichia coli*
IS_2	-	-	-	+	+	+	-	-	+	NA	*Klebsiella pneumoneae*
IS_3	-	-	+	-	+	+	-	-	-	NA	*Proteus mirabilis*
IS_4	+	NA	NA	NA	NA	+	+	-	NA	+	*Staphylococcus aureus*
IS_5	-	-	-	-	+	+	-	+	-	NA	*Pseudomonas aeruginosa*
IS_6	+	NA	NA	NA	NA	+	-	-	NA	-	*Streptococcus sp*

Key: *GS = Gram Staining, IN=Indole, MR=Methyl Red, VP=Vogues Proskauer, CI=Citrate, CA=Catalase, OX=Oxidase CO=Coagulase, LF=Lactose Fermentation, MF=Mannitol Fermentation. NA = Not applicable.*

Incidence of Isolates

The incidence of isolates recovered from 32 infected wound male patients is presented in table 2. Total of 106 isolates were recovered. The most common isolate was *Staphylococcus aureus* 28 (27%) followed by *Klebsiella pneumoneae* 25 (24%), *Pseudomonas aeruginosa* 21 (20%), *Escherichia coli* 16 (15%), *Proteus mirabilis* 9 (8%), and *Streptococcus sp* 7 (6%).

Table2. *Incidence and percentage of isolates recovered from infected wound*

Isolates	Number isolated	Percentage (%)
Escherichia coli	16	15
Klebsiella pneumoneae	25	24
Proteus mirabilis	9	8
Staphylococcus aureus	28	27
Pseudomonas aeruginosa	21	20
Streptococcus sp	7	6
Total	**106**	**100**

Antibiotic Sensitivity Test

The mean zone of inhibition of antibiotic sensitivity disc against the bacterial isolates is presented in table 3. Most of the antibiotics are active against the isolates. *S. aureus* is susceptible to the entire antibiotic used Streptomycin. *P. aeruginosa* is resistant to Norfloxacin, Amoxicillin, Rifampicin, Ampicillin and Chloramphenicol. *Streptococcus sp* also showed resistivity to Ciprofloxacin

Norfloxacin and Gentamicin. *Klebsiella* is resistant Amoxicillin, Rifampicin and Ampicillin. *Proteusmirabilis* is susceptible to the entire antibiotics but resistant to Rifampicin and Ampicillin.

Table3. *Mean zone of inhibition of antibiotics against bacterial isolates*

	Antibiotics (µg/disc)/ Average mean zone of inhibition (mm)									
Isolates	**CIP (10)**	**NOR (10)**	**GEN (10)**	**AMO (20)**	**STR (30)**	**RIF (20)**	**ERY (30)**	**AMP (20)**	**LEV (20)**	**CHL (20)**
Escherichia coli	21	22	19	10	17	10	21	10	20	19
Klebsiella	20	21	22	10	18	10	20	10	19	21
Proteus mirabilis	18	17	20	21	15	10	13	10	18	22
S. aureus	21	19	14	18	15	19	22	19	20	21
P. aeruginosa	20	10	21	10	20	10	15	10	11	10
Streptococcus sp	10	10	10	19	16	17	20	19	13	18

Key: *CIP = Ciprofloxacin, NOR = Norfloxacin, GEN = Gentamicin, AMO = Amoxil, STR = Streptomycin, RIF = Rifampicin, ERY = Erythromycin, AMP = Ampicillin, LEV = Levifloxacin, CHL = Chloramphenicol*

Susceptibility and Resistivity Status of the Isolates

The sensitivity pattern of the isolates against the antibiotics used is presented in Table 4.

Isolates were divided into three groups based on the zone of inhibition produced by the antibiotic disc; Susceptible S (above 18mm), intermediately susceptible I (11-17mm) and Resistant R (below 11mm).

Table4. *Susceptibility and resistivity status of the isolates*

	Antibiotics (µg/disc)/ Average mean zone of inhibition (mm)									
Isolates	**CIP (10)**	**NOR (10)**	**GEN (10)**	**AMO (20)**	**STR (30)**	**RIF (20)**	**ERY (30)**	**AMP (20)**	**LEV (20)**	**CHL (20)**
Escherichia coli	S	S	S	R	I	R	S	R	S	S
Klebsiella	S	S	S	R	S	R	S	R	S	S
Proteus mirabilis	S	I	S	S	I	R	I	R	S	S
S. aureus	S	S	I	S	I	S	S	S	S	S
P. aeruginosa	S	R	S	R	S	R	I	R	I	R
Streptococcus sp	R	R	R	S	I	I	S	S	I	S

Key: *CIP = Ciprofloxacin, NOR = Norfloxacin, GEN = Gentamicin, AMO = Amoxil, STR = Streptomycin, RIF = Rifampicin, ERY = Erythromycin, AMP = Ampicillin, LEV = Levifloxacin, CHL = Chloramphenicol, S = Susceptible, I = intermediately susceptible, R = Resistance*

Discussion

Antimicrobial resistance has increased drastically in recent years in both developed and developing countries and it has rapidly become a leading public health concern [14]. In the presence study, a total of 106 isolates were recovered. The most common isolate was *Staphylococcus aureus* 28 (27%) followed by *Klebsiella pneumoneae* 25 (24%), *Pseudomonas aeruginosa* 21 (20%), *Escherichia coli* 16 (15%), *Proteus mirabilis* 9 (8%), and *Streptococcus sp* 7 (6%). This result was inconformity with several studies conducted on bacteria isolates responsible for wound infection. A study done in a University teaching hospital in Nigeria, revealed *Staphylococcus aureus* (42.3%), *Pseudomonas aeruginosa* (32.9%), *Escherichia coli* (12.8%) and *Proteus mirabilis* (12.8%) are associated with surgical wound infections [15]. Another study on isolation of different types of bacteria from pus revealed also *Staphylococcus aureus* to be the predominant microorganism (40%) followed by *Klebsiella* species (33%), *Pseudomonas* species (18%), *Escherichia coli* (16%), and *Proteus* species (7%) [6]. These findings agree with those reported in Kenya on surgical site infections, that *Staphylococcus aureus* was the most prevalent bacterial isolate [16]. These findings also agree with a study done in Uganda that identified *Staphylococcus aureus* as the commonest causative agent of septic post-operative wounds [17]. A cross-sectional study designed to determine the distribution of the bacterial pathogens and their antimicrobial susceptibility from suspected cases of post-operative wound infections, also revealed *Staphylococcus aureus* (63%) was the most frequently isolated pathogenic bacteria, followed by *Escherichia coli* (12%), *Pseudomonas* species (9.5%), *Klebsiella* species (5%), *Proteus* species (3.5%) and coagulase negative *Staphylococcus* species (3.5%) [18].This is inline with the presence study. The result of this study was in contrast with that of Motoya *et al*. [19] on wound isolates, the organism with the highest frequency of isolation was *Pseudomonas aureginosa* with 25.4%. This was followed by *Escherichia coli* with 23.8% and *Klebseilla sp* with 20.3%. *Staphylococcus aureus* was the only gram positive organism isolated with a frequency of 14.7%,

On the sensitivity pattern of the isolates against the antibiotics used, most of the antibiotics are active against the isolates. *S. aureus* is susceptible to the entire antibiotic used. *P. aeruginosa* is resistant to Norfloxacin, Amoxicillin, Rifampicin, Ampicillin and Chloramphenicol. *Streptococcus* also showed resistivity to Ciprofloxacin Norfloxacin and Gentamicin. *Klebsiella* is resistant Amoxicillin, Rifampicin and Ampicillin. *S. typhi* is susceptible to the entire antibiotics but resistant to Rifampicin and Ampicillin. This result support the study conducted by Tigist *et al*. [20] who found Sensitivity of *S. aureus* isolates from burn wound infections at a hospital in Ethiopia were 93.9% vancomycin, 90.9% clindamycin, 86.4% kanamycin and 86.4% erythromycin. Rao *et al*. [5], reported that out of 144 aerobic isolates from pus samples in post-operative wound infections 94.4% were sensitive to imipenem, 75.5% to amikacin, 27% to ciprofloxacin, 22.2% to gentamicin, 21.5% to cotrimoxazole, 12.5% to cefotaxime, 9.7% to ceftazidime and 6.25% to amoxicillin/clavulanic acid. All isolates were resistant to ampicillin. All gram positive cocci isolated were sensitive to vancomycin and all gram negative isolates were sensitive to imipenem. The result of this study supported the result of a study conducted by Dessalegn *et al*. [21]. They found that *E. coli* were resistant to ampicillin (100%). *Proteus* was resistant to ampicillin (100%) and *Pseudomonas* isolates were susceptible to ciprofloxacin.

Statistical analysis of the result on susceptibility of the organisms against the antibiotics used, showed that the isolates demonstrated an average zone of inhibition of 16.40±0.57mm with *S. aureus* and *Klebsiella* been the most susceptible organisms with average zone of inhibition of 18.80 and 17.10 mm respectively. However, on the other hand, least zones of inhibition were recorded in *P. aeruginosa* and *Streptococcus* with average zones of inhibition of 13.70 and 15.20 mm respectively. On analysis of variance of the result, the F- value was found to be 20.605 and significant probability of 0.00. This probability value is less than the critical probability value ($p = 0.05$) which implies that the species differed in their susceptibility to the antibiotics used and hence null hypothesis is rejected.

Conclusion

In conclusion, wound infections were found prevalent among patients attending Kura General Hospital and the most common isolate was *Staphylococcus aureus* 28 (27%) followed by *Klebsiella pneumoneae* 25 (24%), *Pseudomonas aeruginosa* 21 (20%), *Escherichia coli* 16 (15%), *Proteus mirabilis* 9 (8%), and *Streptococcus sp* 7 (6%). Bacterial isolates exhibited high to moderate levels of susceptibility against different classes of antibiotics used. *S. aureus* is susceptible to the entire antibiotic used. *P. aeruginosa* is resistant to Norfloxacin, Amoxicillin, Rifampicin, Ampicillin and Chloramphenicol. *Streptococcus* also showed resistivity to Ciprofloxacin Norfloxacin and Gentamicin. *Klebsiella* is resistant to Amoxicillin, Rifampicin and Ampicillin. *S. typhi* is susceptible to the entire antibiotics but resistant to Rifampicin and Ampicillin. A good policy is recommended that will regulate the prescription and purchase of drugs.

Acknowledgement

The authors wish to acknowledge the staff of Kura General Hospital Kano for their co-operation and sample provision. We also like to acknowledge Technical staffs of Microbiology department Kano University of Science and Technology Wudil for their support and use of the department's laboratory facilities. Sincere thanks to Kano State Government through Ministry of health for granting us with ethical clearance for the conduct of the research.

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