

Original Research Article

Isolation, Identification and Antibiotic Susceptibility Profile of Some Bacteria Associated with Deterioration of Infected Wound

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Abstract: Wound infection is a significant health problem in many parts of the world especially, Africa. It complicates the recovery of the patient, increases trauma care, prolongs hospital stay and has economic consequences on the patient. This study was aimed to isolate, Identify and determine the antibiotic susceptibility profile of some bacteria associated with deterioration of infected wound in Kano State, Northern Nigeria. The study involved 170 patients attending Murtala Muhammad Specialist Hospital Kano for treatment of infected wound. Isolation and identification of the isolates was conducted using standard microbiological techniques. The bacteria isolates were subjected to antibiotic susceptibility testing using the agar disk diffusion method. The results showed that total of 6 different species were identified. *S. aureus* has the highest frequency with total of occurrence 90 (26.9%), followed by *Pseudomonas* with total frequency of 66 representing (20.3%), then *E. coli* with frequency of 54 (16.1%). *Klebsiella*, *S. epidermidis* and *Proteus* spp has percentage occurrence of 15.2%, 12.5% and 09.0% respectively. Generally, antibiotic resistivity of isolates to commonly used antibiotics was low; the results showed that 15 out of 355 isolates were multidrug resistant representing 4.5%. Ciprofloxacin, Augmentin and Gentamycin. It is concluded that several bacteria were responsible for wound infection.

Keywords: Antibiotics, Bacteria, Identification, Kano, Wound Infection.

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INTRODUCTION

The skin, being the outermost and first line of defense, is easily exposed to physical agents and different pathogens leading to various infections and wounds (Gantwerker and Hom, 2011). Wound, which is a breakage of the skin, results in the loss of continuity of epithelium with or without the loss of underlying connective tissue. Physical, chemical, thermal, microbial, and immunological factors may be responsible for causing wounds in human and animals (Raina *et al.*, 2008). Skin infections and topical wounds require special attention as they make human and animal prone to bacterial, fungal, and viral contaminations, thereby making them further susceptible to other types of secondary complications (Tiwari *et al.*, 2011). The most common pathogens isolated from wounds are *Streptococcus* spp., *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus* spp., *Klebsiella*, *Enterobacter*, *Enterococci*, *Bacteroides*, *Clostridium*,

Candida, *Peptostreptococcus*, *Fusobacterium*, and *Aeromonas* (Henry and John, 2001).

According to Sanjay *et al.*, (2010), Gram-positive cocci such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* spp. and Gram-negative bacilli such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus species* are the most common pathogenic bacteria isolated from wounds. These pathogens can seriously delay wound healing process by disrupting the normal clotting mechanisms and promoting disordered leukocyte function and poor quality granulation tissue formation, reduce tensile strength of connective tissue, and impair epithelization (Annan and Houghton, 2000).

The importance of wound infection in human and economic terms is enormous; it complicates illness, causes anxiety, increases patient discomfort and can lead to death (Falanga *et al.*, 1994) The delay in recovery and

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subsequent increased length of hospital stay have financial consequences on the patient (Plowman, 2000). The management of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and a greater incidence of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and several gram-negative rods (Dionigi *et al.*, 2001). Recognition of potential bacterial pathogens of wound is an essential guide to selecting suitable antimicrobial therapy that will aid in the prompt healing of wounds (O'Meara *et al.*, 2001). This study was therefore carried out to isolate, Identify and determine the antibiotic susceptibility profile of some bacteria associated with deterioration of infected wound in Kano State, Northern Nigeria.

Ethical Consideration

An approval for the study with reference number NHREC/17/03/2030 was obtained from Research and Ethic committee Kano State Ministry of Health through Health Service Management Board Kano in conjunction with the approval from Ethical Committee of Murtala Muhammad Specialist Hospital (MMSH).

Study Sites

The study was conducted at Microbiology Department of Murtala Muhammad Specialist Hospital (MMSH). Kano State is located in the North-western Nigeria, it is coordinated at latitude 11° 30' N and longitude 8° 30' E (Wikipedia, 2021). It shares borders with Kaduna State to the South-West, Bauchi State to the South-East, Jigawa State to the East and Katsina State to the North. It has a total area of 20,131km² (7,777sqm) and estimated population of 13.4 million (NPC, 2014).

Sample Size

Sample size for the study was determined from a standard formula for the calculation of minimum sample size. Sample size was given by the formula; $N = (Z_{1-\alpha})^2 \times (p) \times (1-p)/d^2$.

Where N= minimum sample size, $Z_{1-\alpha}$ = value of standard normal deviate which at 95% confidence interval has found to be 1.96, p = the best estimate of prevalence obtained from literature review (12.3%) and d = difference between the true population rate and sample that can be tolerated, this is the absolute precision (in percentage) on either side of the population = 0.05.

Therefore, $N = (1.96)^2 \times (0.123) \times (1-0.123)/(0.05)^2 = 165.758$ as the minimum number of samples for the study. Therefore, a total of 165 with 3% (5) of this subject was added to the research for attrition, making a total of approximately 170 samples.

Samples Collection

The study involved 170 patients attending Murtala Muhammad Specialist Hospital Kano for treatment of infected wound. The infected wound swab samples was obtained before cleaning the wounds and

were processed for isolation and identification of bacterial pathogens according to the standard microbiological techniques (Cheesbrough, 2012). All the samples collected were immediately transferred under aseptic conditions to Microbiology Laboratory of School of Technology, Kano State Polytechnics for isolation and identification.

Isolation and Identification of the Isolates

The clinical infected wound swab samples were inoculated onto the surface of freshly prepared Nutrient agar (Life save Biotech, USA), Mannitol salt agar (Biomark, India) and Mac Conkey agar (Life save Biotech, USA) plates and incubate aerobically at 37°C for 24 hours. After incubation bacterial growth were 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 (Cheesbrough, 2012). Results was interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (2010).

Antibiotics Susceptibility Pattern

The bacteria isolates were subjected to antibiotic susceptibility testing using the agar disk diffusion method, as adopted by Ali *et al.*, (2024). Mueller-Hinton agar plates were inoculated with an overnight culture of each isolate by streak plating. The standard antibiotic sensitivity discs were aseptically placed at equidistance on the plates and allowed to stand for 1 hour. The plates were incubated at 37°C for 24 hour. Sensitivity pattern of the isolates to different antibiotics belonging to different classes. Isolates are to be divided into two groups based on the zone of inhibition produced by the antibiotic disc; susceptible and resistant according to the Clinical and Laboratory Standards Institute guideline; performance standards for antimicrobial susceptibility testing (CLSI, 2010).

RESULTS

Risk Factors Associated with Wound Infection

Table 1 presented the risk factors associated with wound infection among the study subjects. From the table, male has the highest frequency with total of 99 subjects representing 58.2% of the study subjects while female has total of 71 subjects accounted for 41.8%. The age group of most of the study subjects were between 16 to 30 years (41.1%), followed by 0 to 15 years (27.6%) while least was those aged above 61 years (8.2%). Subjects with open wound were the majority of the study respondents representing 57.6%, followed by those with burn wound 35 (20.6%) while those with post-operative wound have a total of 22 subjects representing 13%. In majority of the study subjects, road accident was the cause of wound with total of 47 representing 27.6%, followed by domestic accident with frequency of 38 accounted for 22.4%. The least category was those with

sporting activities as their cause of wound with 14 subjects representing 8.2%.

Table 1: Risk Factors Associated with Wound Infection

Characteristics	Number (n)	Percentage (%)	P value
Gender			
Male	99	58.2	0.20740*
Female	71	41.8	
Total	170	100	
Age (Years)			
0 – 15	47	27.6	0.00001**
16 – 30	58	34.1	
31 – 45	23	13.6	
46 – 60	20	11.8	
61 – Above	14	08.2	
Total	170	100	
Total	170	100	
Types of wound			
Burn wound	35	20.6	0.00311**
Post-operative	22	13.0	
Open wound	98	57.6	
Bite wound	15	08.8	
Total	170	100	
Cause of wound			
Road accident	47	27.6	0.00038*
Diabetic foot	22	13.0	
Domestic accident	38	22.4	
Occupational trauma	14	8.2	
Fire accident	35	20.6	
Sporting activities	14	8.2	
Total	170	100	

Key: * Result is not significant at $p < 0.005$; **Result is significant at $p < 0.005$

Morphology and Biochemical Characteristics of Isolates

Table 2 represents the morphological and biochemical characteristics of the bacteria isolated from wound samples of the patients. Isolates were

characterized based on gram staining, biochemical test and lactose fermentation. A total of 6 different species were identified. They include *Staphylococcus aureus*, *E. coli*, *Klebsiella*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Proteus* spp.

Table 2: Biochemical Profile of Isolates from wound Samples of Patients

Code	GR	Shape	IND	MR	VP	CIT	CAT	COA	LF	Expected isolate
IS ₁	+	Cocci	+	+	-	-	+	+	+	<i>S. aureus</i>
IS ₂	-	Rod	-	+	-	-	+	-	-	<i>E. coli</i>
IS ₃	-	Rod	-	-	+	+	+	-	+	<i>Klebsiella</i> spp
IS ₄	+	Cocci	+	+	-	-	+	-	-	<i>S. epidermidis</i>
IS ₅	-	Rod					+	-		<i>Pseudomonas</i>
IS ₆	-	Rod	+	-	+	+	+	-	-	<i>Proteus</i> spp

Key: GR = Gram reaction, IND = Indole, MR = Methyl-red, VP = Voges Proskauer, CIT = Citrate utilization, CAT = Catalase, COA = Coagulase, LF = Lactose fermentation

Distribution of Bacteria Isolate

The distribution of the bacteria isolated from the wound samples of the study subjects is presented in Table 3. The result showed that *S. aureus* has the highest frequency with total of occurrence 90 (26.9%), followed

by *Pseudomonas* with total frequency of 66 representing (20.3%), then *E. coli* with frequency of 54 (16.1%). *Klebsiella*, *S. epidermidis* and *Proteus* spp has percentage occurrence of 15.2%, 12.5% and 09.0% respectively.

Table 3: Distribution of Bacteria species from Study Subjects

Bacterial species	No. of Species Isolated	Percentage (%)	P-value
<i>S. aureus</i>	90	26.9	0.00001*
<i>E. coli</i>	54	16.1	
<i>Klebsiella</i> spp	51	15.2	
<i>S. epidermidis</i>	42	12.5	
<i>Pseudomonas</i>	68	20.3	
<i>Proteus</i> spp	30	09.0	
Total	335	100	

Key: * = There is statistical significant difference in the number of bacteria isolates isolated in the study. Hence, the result is significant at $p < 0.05$.

Antibiotic Susceptibility of the Isolates

The number and percentage of resistant pattern of the isolates is presented in table 4. The results indicated majority of the isolates showed less resistant to the antibiotics used. *S. aureus* was more resistant to gentamicin (24%) and erythromycin (23.3%) but less resistant to ciprofloxacin (13.3%) and augmentin (16.6%). *E. coli* was highly resistant to streptomycin

(31.4%) while more sensitive to ciprofloxacin (14.8%). On the other hand, *Klebsiella* was more resistant to ofloxacin (31.5%) and erythromycin (29.6%) but less resistant to ciprofloxacin (18.5%). *Pseudomonas* was more resistant to streptomycin (25%) and septrin (25%) but less resistant to ciprofloxacin (17.6%) and augmentin (17.6%). *S. epidermidis* and *Proteus* showed less resistant to the antibiotics used.

Table 4: Antibiotic Susceptibility of the Isolates

Number (%) and resistant pattern of the Isolates						
Antibiotics (µg)	<i>S. aureus</i> (n = 90)	<i>E. coli</i> (n = 54)	<i>Klebsiella</i> (n = 51)	<i>S. epidermidis</i> (n = 42)	<i>Pseudomonas</i> (n = 68)	<i>Proteus</i> spp (n = 30)
AUG (30)	15 (16.6)	12 (22.2)	15 (27.7)	07 (16.6)	12 (17.6)	02 (6.6)
ERY (10)	21 (23.3)	15 (27.7)	16 (29.6)	10 (23.8)	14 (20.1)	03 (10)
STR (30)	19 (21.1)	17 (31.4)	15 (27.7)	12 (28.6)	17 (25.0)	05 (16.6)
AMO (30)	16 (17.7)	09 (16.6)	12 (22.2)	09 (21.4)	12 (17.6)	05 (16.6)
GEN (20)	22 (24.4)	12 (22.2)	16 (29.6)	12 (28.6)	14 (20.6)	04 (13.3)
OXA (10)	16 (17.7)	12 (22.2)	15 (27.7)	07 (16.6)	13 (19.1)	06 (20.0)
OFL (30)	17 (18.8)	13 (24.1)	17 (31.5)	06 (14.3)	13 (19.1)	05 (16.6)
NEO (20)	20 (22.2)	13 (24.1)	13 (24.1)	10 (23.8)	15 (22.1)	07 (23.3)
CIP (10)	12 (13.3)	08 (14.8)	10 (18.5)	05 (11.9)	12 (17.6)	04 (13.3)
SPT (30)	20 (22.2)	14 (25.9)	12 (22.2)	06 (14.3)	17 (25.0)	05 (16.6)

Key: AUG = Augmentin (30 µg/disc), ERY = Erythromycin (10 µg/disc), STR = Streptomycin (30 µg/disc), AMO = Amoxicillin (30µg/disc), GEN = Gentamicin (20 µg/disc), OXA = Oxacillin (10µg/disc), OFL = Ofloxacin (30 µg/disc), NEO = Neomycin (20µg/disc), CIP = Ciprofloxacin (10 µg/disc) and SPT = Septrin (30 µg/disc)

Distribution of Resistant Isolates

The distribution of resistant isolate is presented in Table 5. The results showed that 15 out of 335 isolates were resistant to 3 or more classes of antibiotics representing 4.5%. *S. aureus* has the highest number of

resistant isolate with total of 9 (2.7%), followed by *Pseudomonas* 3 (0.9%), *E.coli* has 2 resistant isolates with total of 2 representing 0.6% and *Klebsiella* spp has 1 resistant isolate (0.3%).

Table 5: Distribution of Resistant Isolates

Bacterial species	No. of species isolated	No. of resistant isolates	Prevalence (%)	P-value
<i>S. aureus</i>	90	9	2.7	0.00001*
<i>E. coli</i>	54	2	0.6	
<i>Klebsiella</i> spp	51	1	0.3	
<i>S. epidermidis</i>	42	0	0	
<i>Pseudomonas</i>	68	3	0.9	
<i>Proteus</i> spp	30	0	0	
Total	335	15	4.5	

Key: * = There is statistical significant difference in the number of bacteria isolates isolated in the study. Hence, the result is significant at $p < 0.05$.

DISCUSSION

In this study, finding showed that *S. aureus* has the highest frequency with total of occurrence 90 (26.9%), followed by *Pseudomonas* with total frequency of 66 representing (20.3%), then *E. coli* with frequency of 54 (16.1%). *Klebsiella*, *S. epidermidis* and *Proteus* spp has percentage occurrence of 15.2%, 12.5% and 09.0% respectively. These results were in agreement with results of Ali *et al.*, (2024); Oladeinde *et al.*, (2013) and Imarenezor (2017) who reported that *S. aureus* was the most prevalent pathogen with the frequency of occurrence, followed by *P. aeruginosa*. On the other hand, this result contradicts that of (Isibor *et al.*, 2008) who reported *Pseudomonas aeruginosa* as the dominant isolate in wound infection. The high prevalence of *S. aureus* and *P. aeruginosa* among subjects may be attributed to contamination of wounds with soils, medical devices or the existence of locality variability. *P. aeruginosa* is an opportunistic human pathogen. It is "opportunistic" because it seldom infects healthy individuals. Furthermore, any *P. aeruginosa* already present on a burn victim's skin before the injury can transform from an innocuous organism on the surface of the skin to a source of infection in the bloodstream and body tissues of the same individual (Lyczak *et al.*, 2000). High rate of occupational, nutritional, unhealthy life styles related diseases, diabetics, poor hygiene and accessibility to health care facility may be responsible for the observed trend.

Other isolated in this study includes pathogens such as *E. coli* known to cause food poisoning, diarrhea, abdominal pain, fever, nausea, vomiting and about 75% to 95% of urinary tract infections. *E. coli* infection is contagious and can be spread from person to person by faecal contamination. *K. pneumonia* that causes health care-associated infections in form of pneumonia, sepsis, wound and urinary tract infections (Long *et al.*, 2017). It has caused a global explosion of drug-resistant and are increasingly difficult to treat because they are resistant to many of the available antibiotics (Long *et al.*, 2017).

Generally, antibiotic resistivity of isolates to commonly used antibiotics was low; the results showed that 15 out of 355 isolates were multidrug resistant representing 4.5%. Ciprofloxacin, Augmentin and Gentamycin were the most potent antimicrobial agents observed in our study. Meanwhile, a high level of resistance of isolates tested with Neomycin and Septrin was also observed. This may be attributed to the fact that Neomycin and Septrin derivatives have been widely abused and frequently implicated in self-medication in Nigeria. The multiple resistance of isolates, especially *S. pneumonia*, *K. pneumonia* and *P. aeruginosa* to commonly used antibiotics in the locality of the study calls for an immediate action on the controlled use of antimicrobials in the hospitals and the need to monitor resistance. Good antimicrobial use is necessary for effective wound management. The susceptibility rate of bacterial isolates observed in this study, agrees with the

reports of Ogba *et al.*, (2014), Sule *et al.*, (2002), Oladeinde *et al.*, (2013) and Pondei *et al.*, (2013) in Nigeria and Anguzu and Olila, (2007) in Uganda. Pondei *et al.*, (2013) also reported that high level of antibiotic abuse in Nigeria arise from self-medication which is associated with inadequate dosage and failure to comply with treatment regimen. These antibiotics are being sold over the counter with or without prescription (Ogba *et al.*, 2014).

CONCLUSION

In the present study, more males were infected with wound infection than female counterparts and the frequency of wound infection was more among subjects aged between 16 - 30 years. Findings of the study showed that six bacterial species were identified. These include *S. aureus*, *P. aeruginosa*, *E. coli* spp, *Klebsiella*, *S. epidermidis* and *Proteus* spp. *S. aureus* was found as the predominant isolate followed by *P. aeruginosa*. The antibiotic resistivity of isolates to commonly used antibiotics was low; the results showed that 15 out of 355 isolates were multidrug resistant representing 4.5%. Ciprofloxacin, Augmentin and Gentamycin were the most potent antimicrobial while, a high level of resistance of isolates tested with Neomycin and Septrin was also observed.

It is recommended that, wound specimens should be diagnosed for culture and susceptibility testing to help the clinicians before appropriate antibiotic selection and chemotherapeutic management.

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