RESEARCH ARTICLE

Characterization and Determination of Antibiotic Susceptibility Pattern of Bacteria Associated with Untreated Infected Wound

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ABSTRACT

Background: Infection due to the wound has been a major health concern worldwide. **Objectives:** The study was aimed to characterize and determine antibiotic susceptibility patterns of some bacteria associated with untreated wound infection among secondary school student at Kura Local Government, Kano State, Nigeria. **Methodology:** A total of 24 samples were collected from the subjects with untreated infected wound from July 2018 to December 2018. The wound swab samples were inoculated onto Nutrient agar, Blood agar, and MacConkey agar plates and incubated aerobically at 37°C for 24 h. Each colony was re-inoculated into freshly prepared agar plates until a pure colony was obtained. Isolates were identified using Gram staining and biochemical tests. Antibiotic susceptibility test was done using agar disk diffusion method. **Result:** The result showed that a total of 124 isolates were recovered belonging to seven different species, namely, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, coagulase negative *staphylococcus* species (CoNS), and *Streptococcus faecalis*. The most prevalent isolate was *S. aureus* with 24 isolates which accounted for 19.4% of the total isolates recovered. The antibiotics susceptibility pattern of the isolates showed that *S. aureus* is susceptible to the entire antibiotic used except amoxicillin. *Streptococcus* also showed resistivity to all the antibiotics except gentamicin. *E. coli* showed resistivity to augmentin and amoxicillin. *Klebsiella* is resistant amoxicillin, *P. mirabilis* showed resistivity to erythromycin and amoxicillin while CoNS was resistant to amoxicillin and ciprofloxacin. **Conclusion:** It is concluded that bacteria are one of the etiological agents of wound.

Key words: Antibiotics, bacteria, resistance, susceptibility pattern

INTRODUCTION

Wound can be resulted by a break in the structure of an organ or tissue by an external agent. Therefore, wound is a breach in the skin and the exposure of subcutaneous tissue following loss of skin integrity provides a moist, warm, and nutritive environment that is conducive to microbial colonization and proliferation.[1]

Wound provides a moist, warm, nutritive environment conducive to microbial colonization, proliferation, and infection.[2,3] Wound infection refers to the deposition and multiplication of bacteria in tissue with an associated host reaction. This may be characterized by the classic signs of redness, pain, swelling, and fever.[4] Wound infection is characterized by the presence of pus in lesions with pyrexia, pain, and induration. Infection occurs when virulence factors produced by the microorganisms overwhelms the host natural resistance.[1] Infection in wound delays healing and may cause wound breakdown, herniation of the wound and complete wound dehiscence.[5] Many different bacterial species live on human skin, in the nasopharynx, gastrointestinal tract, and other parts of the body with little potential for causing disease, because of the first line of defense within the body.[6,7] Despite this, any breach in the skin surface whether trauma, accident, surgical operation, or burn provides an open door

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for bacterial infections.[6] Bacterial wound infections are important because they can slow down the healing process, lead to wound breakdown, prolonged hospital stay, and increase in the cost of treatment.[8] Wounds are the third most frequent nosocomial infections.[9] In developing and resource-poor countries, traumatic and surgical site infections are reasons for high morbidity and mortality rates.[10]

The progression of a wound to an infected state is likely to involve a multitude of microbial or host factors including the type, site, and depth of wound, the extent of non-viable exogenous contamination, the general health and immune status of the host, the microbial load, and the combined virulence expressed by the types of microorganisms involved.[1] The common wound pathogens include bacteria, fungi, protozoa, and viruses.[11] Common bacterial pathogens associated with wound infection include *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Proteus* species, *Streptococcus* species, and *Enterococcus* species.[12-14] Although the majority of wounds are polymicrobial involving both aerobes and anaerobes, aerobic pathogens such as *S. aureus*, *P. aeruginosa*, and beta-hemolytic Streptococci have been most frequently reported as the cause of delay wound healing.[15,16]

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 bacteria, polymicrobial flora, and by fungi. The knowledge of the causative agents of wound infection has therefore proved to be helpful in the selection of empiric antimicrobial therapy and on infection control measures in health institutions. Therefore, the knowledge of the causative agents of wound infection has proved to be helpful in the selection of empirical therapy, on infection control measures in a health institution, and in formulating rationales of antibiotic policy.[16] It is therefore important to characterized and determine antimicrobial resistivity of bacteria pathogens from an infected wound. Thus, the aim of the research was to characterize and determine antibiotic susceptibility patterns of some bacteria associated with untreated wound infection among secondary school students at Kura Local Government, Kano State, Nigeria.

MATERIALS AND METHODS

Ethical approval

Ethical approval was obtained from the 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 a distance of about 35 km from the State capital. It is located at Latitude 11°46'17" N and Longitude 8°25' 49" E. It covers an area of about 206 km^2 of land and population of about 144,601 according to 2006 census.[17] Kura Local Government shares common boundaries with Garun Mallam to the West, Dawakin Kudu (East), Bunkure (South), and Madobi Local Government (North).[17]

Sample collection

The study population consisted of 24 secondary school students aged between 10 and 16 years with untreated wound infection. A total of 24 samples were collected from the subjects with untreated infected wounds among secondary school students within Kura Local Government Kano from July 2018 to December 2018. During collection, a sterile swab stick was used and the wound swab samples were obtained before cleaning the wounds.[18] Samples collected were transported to the Microbiology Laboratory of Bayero University Kano for bacteriological analysis.

Isolation and identification of isolates

The wound swab samples were inoculated onto Nutrient agar (Life save Biotech, USA), Blood agar (Biomark, India), and MacConkey agar (Life save Biotech, USA) plates and incubated aerobically at 37°C for 24 h. 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 (Indole, Methyl-red, Voges-Proskauer, Citrate utilization Catalase, Coagulase, and Oxidase) tests, nitrate reduction, sugar fermentation, and motility test.[19] Each plate was graded as positive or negative. Results were interpreted according to the guidelines of Bergey's Manual of Systemic determinative Bacteriology by Holt *et al*.[20] and Sherman.[21]

Antibiotic sensitivity test

The bacteria isolates were subjected to antibiotic susceptibility testing using the agar disk diffusion method, as described by Bauer *et al*.[22] Mueller-Hinton agar plates were inoculated with an 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 h. The plates were then incubated at 37°C for 24 h. Sensitivity pattern of the isolates to five different antibiotics belonging to different classes, namely, Erythromycin (10 µg/disc), Augmentin (Amoxicillin + clavulanic acid) (30 µg/disc), Amoxicillin (30 µg/disc), Gentamicin (20 µg/disc), and Ciprofloxacin (30 µg/disc) produced by Abtek Pharmaceutical Limited, was 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 guideline; performance standards for antimicrobial susceptibility testing.[23]

Statistical analysis

The data of the average zone of inhibition produced by the isolates against the antibiotics used were 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. The significance level for the differences was set at $P < 0.05$.

RESULTS

Cultural characteristics of the isolates

The cultural characteristics and Gram staining reaction of the isolates are presented in Table 1. The result revealed the presence of seven different isolates; four Gram-negative and three Gram-positive bacteria and their morphological characteristics in different types of media ranging from Nutrient agar, MacConkey, and Blood agar.

Biochemical characterization of the isolates

The biochemical characterization of the isolates from the infected wound is presented in Table 2. The isolates were tested for indole, methyl red, Voges-Proskauer, citrate utilization, catalase, oxidase, coagulase, lactose fermentation, and motility tests. The results showed that seven different isolates were obtained, namely, *E. coli*, *S. aureus*, *P. aeruginosa*, *Proteus mirabilis*, *K. pneumoniae*, coagulase-negative *Staphylococcus aureus* (CoNS), and *Streptococcus faecalis.*

Table 1: Cultural characteristics and Gram staining reaction of the isolates

Isolate code	Gram staining	Cultural characteristics
IS_1	Negative	White glistering and moist growth
IS_2	Positive	Produce opaque cream-yellow growth
IS_3	Negative	White growth turning media light green
IS_4	Negative	Produce smooth, shiny cream colony
IS_5	Negative	Produce shiny mucoid/viscous colony
IS_6	Positive	Golden yellow on nutrient agar plate
IS_7	Positive	Produce semi-transparent mucoid colony

Prevalence of bacteria isolates

The prevalence of bacteria isolated from 24 untreated infected wounds is presented in Table 3. The result showed that a total of 124 isolates were recovered belonging to seven different species. The most prevalent isolate was *S. aureus* with 24 isolates which accounted for 19.4% of the total isolates recovered. This is followed by *P. aeruginosa* 20 (16.1%), *K. pneumoniae* 19 (15.3%), *E. coli* 18 (14.8%), CoNS 17 (13.7%), and *P. mirabilis* 16 (12.9%) and the least prevalent isolate is *S. faecalis* 10 (8.1%).

Antibiotic sensitivity test

The antibiotic sensitivity of the isolates expressed as a mean zone of inhibition is presented in Table 4 while the susceptibility percentage isolates against the antibiotics tested are presented in Table 5. The isolates were tested against erythromycin, augmentin, amoxicillin, gentamicin, and ciprofloxacin. The result showed that most of the antibiotics are active against the isolates. *S. aureus* is susceptible to the entire antibiotic used except amoxicillin. *Streptococcus* also showed resistivity to all the antibiotics except Gentamicin. *E. coli* showed resistivity to augmentin and amoxicillin. *Klebsiella* is resistant amoxicillin, *P. mirabilis* showed resistivity to erythromycin and amoxicillin while CoNS was resistant to amoxicillin and ciprofloxacin.

DISCUSSION

Infection due to the wound has been a major health concern worldwide due to its increased trauma to the patients and increasing the requirement for cost-effective management within the health-care system. The present study was aimed

Table 2: Biochemical characterization of the isolates

Code	IN	MR	VP	CI	CA	CO	OX	LF	MO	Isolates
IS_1	+	+	−	−	+	−	−	+	+	*E. coli*
IS_2	−	+	+	+	+	+	−	+	−	*S. aureus*
IS_3	−	−	−	+	+	−	+	−	+	*P. aeruginosa*
IS_4	−	+	−	+	+	−	−	−	+	*P. mirabilis*
IS_5	−	−	+	+	+	−	−	+	−	*K. pneumoniae*
IS_6	−	−	+	−	+	−	−	+	−	CoNS
IS_7	−	−	+	−	−	−	−	+	−	*S. faecalis*

IN: Indole, MR: Methyl red, VP: Vogus-Proskauer, CI: Citrate utilization, CA: Catalase, OX: Oxidase CO: Coagulase, LF: Lactose fermentation, MO: Motility test, CoNS: Coagulase-negative *Staphylococcus* species, *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*, *P. mirabilis*: *Proteus mirabilis*, *K. pneumonia*: *Klebsiella pneumonia*, *S. faecalis*: *Streptococcus faecalis*, *P. aeruginosa*: *Pseudomonas aeruginosa* to characterize and determine antibiotic susceptibility patterns of some bacteria associated with untreated wound infection among secondary school students at Kura Local Government, Kano State, Nigeria. The microbiological analysis of the wounds swab reveals that a total of 124 isolates were recovered belonging to seven different species. The most prevalent isolate was *S. aureus* with 24 isolates which accounted for 19.4% of the total isolates recovered. Several studies were conducted on a different part of the world.[24-27] The finding of this study was in conformity with that of Ali *et al*.[18] these findings also agree with a study done in Uganda that identified *S. aureus* as the most common causative agent of septic post-operative wounds.[28] 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 that *S. aureus* (63%) was the most frequently isolated pathogenic bacteria, followed by *E. coli* (12%), *Pseudomonas* species (9.5%), *Klebsiella* species (5%), *Proteus* species (3.5%), and coagulase-negative *Staphylococcus* species (3.5%).[29] The result of this study was in contrast with that of Motayo *et al*.[30] On wound isolates, the organism with the highest frequency of isolation was *P. aeruginosa* with 25.4%.

Most of the untreated wound infections in this study are polymicrobial in nature and in most cases, associated

Table 3: Prevalence of isolates recovered from the infected wound

Isolates	Frequency (n)	Prevalence (%)
E. coli	18	14.5
S. aureus	24	19.4
P. aeruginosa	20	16.1
P. mirabilis	16	12.9
K. pneumoniae	19	15.3
CoNS	17	13.7
S. faecalis	10	08.1
Total	124	100

CoNS: Coagulase-negative *Staphylococcus* species, *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*, *P. mirabilis*: *Proteus mirabilis*, *K. pneumonia*: *Klebsiella pneumonia*, *S. faecalis*: *Streptococcus faecalis*, *P. aeruginosa*: *Pseudomonas aeruginosa*

Table 4: Mean zone of inhibition of antibiotics against bacterial isolates

	Antibiotics/average zone of inhibition (mm)				
Isolates	ERY (10 µg/d)	AUG (30 µg/d)	AMO (30 µg/d)	GEN (30 µg/d)	CIP (30 µg/disc)
E. coli	22±1.5[b]	16±1.1[b]	13±1.0[a]	18±1.2[a]	21±1.5[b]
S. aureus	21±0.7[b]	23±1.2[c]	15±0[a]	24±1.1[b]	23±1.2[b]
P. aeruginosa	18±1.6[a]	20±1.7[b]	14±0.7[a]	20±1.9[b]	17±1.3[a]
P. mirabilis	16±1.3[a]	19±0.9[b]	14±1.4[a]	23±1.4[b]	23±0.5[c]
K. pneumoniae	23±2.1[b]	17±1.6[b]	15±1.8[a]	18±0.8[a]	21±0.8[b]
CoNS	19±0.7[a]	18±1.8[b]	16±1.2[b]	23±0.9[b]	16±1.2[a]
S. faecalis	18±1.8[a]	10±1.2[a]	19±1.1[b]	22±1.0[b]	24±1.4[c]

Values having the different script along each column are considered statistically different at $P<0.05$. ERY: Erythromycin, AUG: Augmentin, AMO: Amoxicillin, GEN: Gentamicin, CIP: Ciprofloxacin, CoNS: Coagulase-negative *Staphylococcus* species, *E. coli*: *Escherichia coli, S. aureus*: *Staphylococcus aureus, P. mirabilis*: *Proteus mirabilis, K. pneumonia*: *Klebsiella pneumonia, S. faecalis*: *Streptococcus faecalis, P. aeruginosa*: *Pseudomonas aeruginosa*

Table 5: Susceptibility percentage isolates against the antibiotics tested

	Antibiotics/susceptibility rate (%)				
Isolates	ERY (10 µg/d)	AUG (30 µg/d)	AMO (30 µg/d)	GEN (30 µg/d)	CIP (30 µg/disc)
E. coli	65	20	10	38	62
S. aureus	60	75	25	85	75
P. aeruginosa	35	60	15	56	40
P. mirabilis	30	55	15	75	80
K. pneumoniae	65	33	20	45	65
CoNS	55	40	25	70	32
S. faecalis	50	00	50	65	80

ERY: Erythromycin, AUG: Augmentin, AMO: Amoxicillin, GEN: Gentamicin, CIP: Ciprofloxacin. CoNS: Coagulase-negative *Staphylococcus* species, *E. coli: Escherichia coli, S. aureus*: *Staphylococcus aureus, P. mirabilis*: *Proteus mirabilis, K. pneumonia*: *Klebsiella pneumonia, S. faecalis*: *Streptococcus faecalis, P. aeruginosa*: *Pseudomonas aeruginosa* with *S. aureus* and other microorganisms such as *E. coli*, *P. aeruginosa*, *P. mirabilis*, *K. pneumoniae*, and CoNS and *S. faecalis*. The variety of organisms observed in this study support the need to obtain culture specimens from infected wounds for microbiological evaluation and antibiotic susceptibility determination so that adapted chemotherapy can be prescribed. The prevalence of a high rate of wound infection as well as the polymicrobial infection had also been reported by Shittu *et al*.[16] These isolates contribute to the pathology of the wound infection, for example, Streptococcal and Staphylococcal invasion of wound delays healing as well as results in deterioration of wounds.[31] *Pseudomonas* and *Proteus* species are responsible for extensive tissue destruction with poor blood circulation to the affected site especially diabetic foot ulcers.[32]

The antibiotic sensitivity pattern of the isolates revealed that *S. aureus* is susceptible to the entire antibiotic used except amoxicillin. *Streptococcus* also showed resistivity to all the antibiotics except Gentamicin. *E. coli* showed resistivity to augmentin and amoxicillin. *Klebsiella* is resistant amoxicillin, *P. mirabilis* showed resistivity to erythromycin and amoxicillin while CoNS was resistant to amoxicillin and ciprofloxacin. This implies that most of the isolates showed resistivity to augmentin (amoxicillin + clavulanic acid) and amoxicillin, and it can be due to the production of enzyme beta-lactamase which inactivates the beta-lactams antibiotics. This sharp increase in resistance patterns to beta-lactams antibiotics may be attributed to the widely abused and frequently implicated in self-medication in Nigeria. It has also reported that a high level of antibiotic abuse in Nigeria arises from self-medication which is associated with inadequate dosage and failure to comply with the treatment regimen. These antibiotics are being sold over the counter with or without prescription.[33] The fluoroquinolones (ciprofloxacin) and aminoglycoside (gentamicin) were more effective antibiotics tested against the isolates in this study. This agrees with the report of Mordi and Momoh.[34] These antibacterials should be used in the management of untreated wound infection.

CONCLUSION

Based on the findings of this study, the isolation rate of bacterial pathogens was high. The predominant isolates were *S. aureus*, *P. aeruginosa*, *Klebsiella* species, *E. coli*, CoNS, *P. Mirabilis,* and *S. faecalis*. *S. aureus* was the most prevalent isolate. Most of the isolates were sensitive to gentamicin, ciprofloxacin, and erythromycin but resistant to augmentin and amoxicillin**.** A good policy is recommended that will regulate the prescription of antibiotics.

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