

Staphylococcus aureus from the microbial load of open wounds from patients attending aminu kano teaching hospital, Kano, Nigeria

Abstract

Background: *Staphylococcus aureus* is the causative agent of a wide variety of infections in humans including diseases of the skin and soft tissues. It causes disease like skin pustules, impetigo and also form part of microbial community in wounds which can also lead to serious infections such as bacteremia.

Objective: The objective of the study was to determine the relative proportion of *S. aureus* among the microbial community of open wound.

Methods: A total of 264 open wound samples were used for the study. The organisms isolated were characterized and identified phenotypically using conventional biochemical tests and the tested for antibiotic susceptibility testing using disc diffusion technique.

Results: Of the total wound samples, 245(92.8%) were cultured positive, of which 107(40.53%) were Gram positive cocci and 138(52.26%) were Gram negative bacilli. Of the 107 Gram positive cocci isolated, 69(64.49%) were *S. aureus*, 14(13.08%) were other *Staphylococcus* species, while 24(22.43%) were *Streptococcus* species. Out of the 69 (28.16%) *S. aureus* isolates, 21 (30.43%) were found to be positive for the production of beta lactamase enzyme. Age group 0-10years had the highest percentage of the *S. aureus* isolates with 37.68% while the least was 71-80 with 4.35 % respectively. Antibiotic susceptibility test of the sixty nine (69) *S. aureus* isolates revealed ciprofloxacin and erythromycin to be more effective with sensitivity rates of 75.56% and 75.76% respectively. Cotrimoxazole and amoxycyclav sharply resisted with 68.42% and 64.71% as their resistant rates respectively.

Conclusion: The study aid in isolating the *Staphylococcus aureus* isolates from open wound with its proper identification using standard biochemical tests. Erythromycin and ciprofloxacin are the most effective antibiotics against *Staphylococcus aureus* isolates from open wounds in Kano.

Keywords: *staphylococcus aureus*, open wound, microbial load, antibiogram

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Introduction

Staphylococci are part of human flora found in the axillae, the inguinal, perineal areas, other parts of the skin, and the anterior nares.¹ Persistent carriage is more common in children than in adults. Nasal carriers may be divided into persistent carriers with high risk of infection and intermittent or non carriers with low risk of infection.² It was reported that, among healthy adults, carrier rates of 11-32% were detected in the general population, and a prevalence of 25% was detected in hospital personnel.³ Wertheim et al.,⁴ stated that between 25% and 35% of healthy human carry *S. aureus* on the skin or mucous membranes. It is also one of the most common pathogens that causes skin infections and when left untreated, disorders associated with this organism may progress into a wider range of conditions like tissue infections, pneumonia, wound, joint, and/or bone infections.⁵⁻⁷ Most staphylococcal infections can be easily treated with antibiotics; however, in recent years *Staphylococcus* found its way to resist the commonly used and effective antibiotics; like macrolides, lincosamides, tetracycline, gentamicin, and beta-lactams, particularly methicillin.⁵⁻⁹ *Staphylococcus aureus* is a versatile pathogen capable of growth and infection under diverse conditions. It has an extremely elastic genome capable of high variability and is known to acquire

genes from closely related species. It causes a wide spectrum of afflictions.¹⁰ It is the leading cause of skin and soft tissue infections such as folliculitis and furunculosis, and it is the pathogen most often implicated in wound infections resulting from surgery or trauma. Thus, *S. aureus* is of major significance in both community-onset and healthcare-associated infections.¹¹

Attachment and colonization are the first step for *S. aureus* pathogenesis. Biofilm formation allows the bacteria to resist higher concentrations of antimicrobial agents, environmental conditions and the host immune responses.¹² The part and essentialness of microorganisms in wound mending has been a topic for a long time, a few specialists consider the microbial thickness to be basic in anticipating wound recuperating and disease while others consider the sorts of microorganisms to be of more prominent hugeness. Albeit wound contaminations are brought on by microorganisms, broad debate still exists with respect to the system by which they cause disease. The greater part of open injury colonization is polymicrobial.¹³⁻¹⁵

Burn wounds are a suitable site for multiplication of bacteria including *Staphylococcus aureus* and are more persistently richer

sources of infection than surgical wounds, mainly because of the larger area involved and longer duration of patient stay in the hospital.¹⁶ Microorganisms are still transmitted to the burn wound surfaces of recently admitted patients by the hands of personnel, by fomites, and to some extent by hydrotherapy. The common pathogens isolated from burn wounds are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and various *coliform bacilli*.¹⁷

Staphylococcus aureus is one of the frequently isolated pathogen in both community and hospital practices. The pattern of antimicrobial susceptibility of *S. aureus* and other organisms is a worldwide change, especially in developing countries making antimicrobial agents increasingly less effective.¹⁸

Methodology

Study area

The study was carried out in Aminu Kano Teaching Hospital (AKTH), Kano which is situated within Kano metropolis. The hospital was established in 1988, it serves a large number of patients from within and outside Kano State. Kano State is located in the North-western Nigeria. It lies between latitude 11°30'N and longitude 8°30'E.

Study population

The population involved in the study was patients with open wounds attending Aminu Kano Teaching Hospital. Ethical approval for this study was obtained from the ethical committee of Aminu Kano Teaching Hospital, in addition, individual patients consent was obtained and the purpose of study was made known to them in both English and local dialects prior to sample collection.

Sample collection and processing

All the wounds were judged as possibly infected by the presence of purulent material. Samples were collected before wound cleansing and dressing conducted, the exudates from each wound site were carefully taken using sterile swab stick and inoculated onto blood agar and incubated according to.¹⁹

Microbiological analysis

Various microbiology techniques were carried out to phenotypically characterize and identify the *staphylococcus aureus*, these included: Catalase test; Coagulase test (Slide method for bound coagulase; Tube method for free coagulase), Sugar Fermentation Tests (Mannitol salt agar (MSA) and MacConkey agar) for mannitol and lactose fermentation respectively, beta-lactamase production using cefoxitin disc (30mg) and Haemolysin Activity Test on blood agar.^{19,20}

Antimicrobial susceptibility

All isolates were tested for susceptibility to a panel of antimicrobials using Kirby Bauer agar disc diffusion method. The result of the susceptibility pattern of the antibiotics tested were read using the guideline of the National Clinical Laboratory Standard (NCLS, 2011).

Statistical analysis

Data derived was analyzed using Statistical package for social science (SPSS) version 20.0 in which frequency descriptive analysis was performed. Level of significance was set at 95% confidence limit (p-value<0.05).

Results

Of the two hundred and sixty four 264 wound samples collected, 245(92.80%) were positive for culture and sixty nine 69 (28.16%) isolates were found to be *Staphylococcus aureus*. Out of the 69 (28.16%) *S. aureus* positive samples, 39 (56.52%) were from male patients and 30 (43.48%) from female patients (Table 1).

Table 1 Distribution of *Staphylococcus aureus* isolates according to gender

Gender	Frequency	Percentage
Male	39	56.52
Female	30	43.48
Total	69	100

The result also showed that the age group 0-10years had the highest percentage of the isolates with 26(37.68%). followed by 21-30, 51-60, 61-70, 31-40, 11-20, 41-50 and 71-80years with 10(14.50%), 7(10.14%), 7(10.14%), 6(8.70%), 6(8.70%), 4(5.80%) and 3(4.35%) respectively (Table 2).

Table 2 Distribution of *Staphylococcus aureus* Isolates according to Age groups

Age group	Frequency	Percentage
0-10	25	36.23
20-Nov	6	8.7
21-30	10	14.5
31-40	6	8.7
41-50	4	5.8
51-60	7	10.14
61-70	7	10.14
71-80	3	4.35

Gram positive cocci in clusters obtained in the wound isolates were 83 (33.88%) from the total 245 wound isolates, Gram positive cocci in chain had 24 (9.80%) while Gram negative bacilli (GNB) has the highest isolates with 138(56.33%) (Table 3).

Table 3 Distribution of the culture positive isolates and their gram staining reaction

Organism	Frequency	Percentage (%)
Gram Positive Cocci	83	33.88
In clusters	24	9.8
In Chains	138	56.33
Gram Negative Bacilli	245	100

The results of sugar fermentation and haemolysis revealed 60 (87.34%) positive for mannitol fermentation and 9(12.66%) did not fermented mannitol. For lactose, 64(92.75%) were lactose fermenters while 5(7.25%) were non lactose fermenters while for the haemolysis on blood agar 36(52.17%) showed beta hemolysis while 33(47.83%) showed gamma haemolysis (Table 4).

Of the total 107 Gram positive cocci isolated, 69(64.49%) were *S. aureus* while 14(13.08%) were found to be other *Staphylococcus* species and 24(22.43%) were *Streptococcus* species (Table 5). On the

bases of catalase and coagulase enzymes production, all the 69 (100%) *S. aureus* isolates were catalase positive. Sixty three 63(91.3%) were slide coagulase positive and 6 (8.7%) were negative. All the isolates 69(100%) were found to be positive for tube coagulase enzymes production; the remaining 14 were all catalase positive but coagulase negative (Table 6).

Table 4 Biotyping characteristics pattern of *Staphylococcus aureus*

Test	Frequency	% Positive	Frequency	% Negative
Mannitol	60	87.34	9	12.66
Lactose	64	94.73	5	5.27
Hemolysis	36	52.17(beta)	33	47.83(gamma)

Table 5 Distribution of gram positive cocci isolates

Isolates	Frequency	Percentage
<i>S. aureus</i>	69	64.49
<i>Streptococci Spp</i>	24	22.43
Other Staph Spp	14	13.08
Total	107	100

Table 6 Phenotypic characteristic patterns of *Staphylococcus aureus* and Coagulase negative staphylococcal isolates

Staphylococci	<i>S. aureus</i> isolates		Coagulase negative	
	% Positive	% Negative	% Positive	% Negative
Catalase	100	0	100	0
Coagulase	91.32	8.7	0	100
Coagulase	100	0	0	100

The result of betalactamase production test of the *Staphylococcus aureus* isolates showed that 21(30.43%) of the isolates were betalactamase producers while 48(69.57%) did not produced the betalactamase enzyme (Table 7). Ciprofloxacin and erythromycin were the most effective against the *S. aureus* isolates with 75.56% and 75.76% respectively while the less effective agent was cotrimoxazole 68.42% and amoxiclav 64.76% (Table 8).

Table 7 Distribution of Betalactamase Production among *Staphylococcus aureus* Isolates

Betalactamase production using cefoxitin disc (30µg)				
Isolates	Frequency	%Positive	Frequency	%Negative
69	21	30.43	48	69.57

Table 8 Antibiotic Susceptibility Pattern of *Staphylococcus aureus* Isolates

Antibiotics	% Susceptible	% Resistant
Tetracycline	62.86	37.14
Ciprofloxacin	75.56	24.22
Amoxyclav Clindamycin	35.29	64.71
Erythromycin	61.1	38.89
Cephalexin	75.76	24.24
Cotrimoxazole	41.18	58.82
Cloxacillin	31.58	68.42
	42.85	57.15

Discussion

The study revealed that male subjects had higher isolation rate of *staphylococcus aureus* from the wounds 39(56.52%) compared to the female subjects 30(43.48%). However, a study in Pakistan, conducted by Yasmeen et al.,²¹ reported 19(38%) and 31(62%) as males and females percentage frequencies respectively.

Age related distribution of the pathogens among the enrolled patients inclined more to the age group of 0-10 having 37(39.68%) followed by 21-30 age bracket 10 (15.87%) while the least frequency was recorded between the ages of 71 and 80 3(4.76%). In a study conducted in the same place (AKTH), similar pattern of isolation frequencies were observed in age limit of less than 11 (<11years), Nwankwo & Nasiru²² which indirectly means age group of 0-10years as used in this study, with frequency of 71(47.3%), also, as documented in this study, 21-30 was next in the isolation rate 24.(16.0%). They also had the least number of isolation in the age range of 51- 60 with 4(2.6%) which was just an isolate more than the 3 we had in the present study.

Erythromycin and ciprofloxacin were found the most effective in vitro against the isolated *Staphylococcus aureus*, with percentage efficacy of 75.76% and 75.56% respectively. While cotrimoxazole was found to have the lowest efficacy rate 31.58%, this, by implication means the most resisted antimicrobial agent in the study. In southern Jamaica Brown et al.,²³ in a study titled antimicrobial resistance in clinical isolates of *Staphylococcus aureus* from hospital and community sources in southern Jamaica, reported erythromycin to be effective rather than resisted against wound swab isolates of *Staphylococcus aureus*, it was also found to hinder growth of same species from abdominal aspirate in the same study. Incidentally, these isolates from these two sources resisted ciprofloxacin which was found one of the most effective in this study. This could be that they encounter hospital based strains pre-exposed to the drug, and/or due to locality differences.

Up to 69(64.49%) isolates of *Staphylococcus aureus* were recovered from the wound samples of the enrolled patients in the study while other *staphylococcus* species had 14(13.08%). Other group of pathogens encountered were *streptococcus* species 24(22.43%), which makeup the remaining component of the Gram positive cocci recovered in the study. Zayed et al.,²⁴ also reported, in burn and wound patients of some Egyptian Hospitals that pathogens from wounds were mostly *Staphylococcus aureus* with 35 isolates while other *Staphylococcus* species isolated were 7, all of which were *Staphylococcus epidermidis*. They also found few isolates (4) as *Streptococcus* species (*Streptococcus pyogenes*) among the wound pathogens during their study.

Lactose and mannitol fermentation as well as haemolysis on blood agar were characters exhibited by the *Staphylococcus aureus* isolated in the study. Among the 69isolates, 64 and 60 were lactose and mannitol fermenters while 36 produced haemolysis on blood agar. Favorable findings with regards to these sugars fermentation and haemolysis was also obtained in 2011, according to Adejuwon et al.,²⁵ in a study titled isolation of *Staphylococcus aureus* in septic caesarean wound, in Ile Ife, Nigeria, in which *Staphylococcus aureus* isolates were categorized in to three, based on their different colonial morphology on mannitol salt agar and coded as OD and K. All the three different appearance coded as OD and K were found lactose and mannitol fermenters with acid production (A) as a proof of that they were also found positive for haemolysis on blood agar.^{26,27}

Conclusion

The positive cultures found in this study showed 245(92.80%) which is highly enormous and signified high percentage of people with infected wounds, hence this study aid in isolating the *Staphylococcus aureus* isolates from open wound with its proper identification using standard biochemical tests. The result of antibiotic susceptibility testing showed that; erythromycin and ciprofloxacin were found to be the most active against *Staphylococcus aureus* isolates from an open wound samples. Thus it is believed that these antibiotics should be used in the treatment of *Staphylococcus aureus* in wound infections including Beta-lactamase producing *Staphylococcus aureus*.

Recommendation

Health workers should provide an awareness forum to enlighten peoples in maintaining proper hygiene. Proper antimicrobial susceptibilities should be done before therapy to prevent wrong use of drugs. Surveillance should be put in place to keep tract of drug resistant pathogens especially MRSA which develops resistance easily.

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Conflict of interest

The author declares no conflict of interest.

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