Incidence and Antibiotic Susceptibility Profile of *Staphylococcus aureus* Isolates from Wounds of Patients at Specialist Hospital, Sokoto, Nigeria

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ABSTRACT

Background: *Staphylococcus aureus* is an important human pathogen causing varieties of mild to life threatening community and hospital on-set infections. This study was carried out to determine the antibiotic susceptibility profile of *Staphylococcus aureus* isolated from wounds of patients at a tertiary healthcare facility in Sokoto, Nigeria.

***Methods*:** All wound swabs obtained from patients with wound infections during the study period were cultured on mannitol salt agar media. The isolates were identified using standard microbiological methods. Antibiotic susceptibility test was carried out on the identified isolates using the modified Kirby-Bauer disc diffusion method and methicillin resistant *Staphylococcus aureus* (MRSA) test was carried out using Oxacillin agar screen test as described by Clinical and Laboratory Standard Institute (CLSI, 2016).

***Results*:** A total of twenty (20) *Staphylococcus aureus* were isolated from thirty-eight (38) wound specimens investigated. Out of which, five (25.0%) were found to be MRSA. The isolates were resistant to most of the antibiotics tested and susceptible only to Gentamicin (85%), Norfloxacin (80%) and Amoxiclav (50%).

***Conclusion*:** The high incidence of *Staphylococcus aureus* isolates resistant to the commonly used antibiotics in the hospital calls for urgent need to put in place measures to curtail the spread of MRSA infections in the hospital.

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Introduction

Staphylococcus aureus are cluster-forming, facultative aerobic, Gram-positive cocci (1). It exhibits intrinsic ability to ferment carbohydrates with white to deep yellow pigmentation on solid culture media (2). Also, this bacterium ferments mannitol which turns Mannitol Salt Agar (MSA) yellow. Another key characteristic of this important human and animal pathogen is the production of deoxy-ribonuclease (DNase) and catalase enzymes along with production of coagulase protein (1, 2).

S. aureus is inherently resistant to many antibiotics. This is complicated by the acquisition of many resistance genes (1). Prior to the development and introduction of methicillin, *S. aureus* resisted the biocidal action of penicillins through the production of drug inactivating enzymes, the beta-lactamases. The introduction of methicillin as a penicillinase resistant β-lactam antibiotic was immediately followed by emergence of *S. aureus* strains resistant to this drug through the expression of an altered Penicillin Binding Protein (PBP) (3, 4). This alteration of PBPs is mediated by *mecA* and recently *mecC* genes, which are located on a large DNA fragment called Staphylococcal chromosomal cassette *mec* (SCCmec) (4, 5). This genetic cassette may harbour other antibiotic resistance genes thereby making this pathogen multiple antibiotic resistant (2).

Methicillin resistant *S. aureus* (MRSA) is one of the pathogens posing serious threat to the global health care of patients in both community and hospital settings (3, 6-8). MRSA causes infections such as skin and wound infections, pneumonia, blood stream infections (BSI), meningitis, toxic shock syndrome (TSS), etc. (6-9).

According to Clinical Laboratory Standard Institute (CLSI, 2016), any *S. aureus* with *mecA* mediated resistance to methicillin should be regarded as resistant to all other β-lactams, β-lactam/β-lactamase inhibitor combinations and cephamycin (5).

Studies in Nigeria have shown that *S. aureus* is the most frequently isolated microorganism from wounds and pus samples (10-12). While several studies have been conducted on the incidence and susceptibility profiles of *S. aureus* isolates in Nigeria, data is however scanty on it in Sokoto-Nigeria. The present study therefore aimed at providing baseline information on the occurrence and antibiotic resistance pattern of *S. aureus* isolates from wounds of patients attending tertiary healthcare centre in Sokoto, Nigeria.

Materials and Methods

Study Site

The study was carried out at Specialist Hospital Sokoto (SHS). The hospital is located within Sokoto metropolis. Specialist Hospital Sokoto is the apex State Government owned healthcare centre in the state offering specialist medical and surgical services to the residents of Sokoto metropolis and referral cases from primary and secondary healthcare centres within the state and adjoining states of Niger, Zamfara and Kebbi.

Study Design

The study was a prospective, cross-sectional observational study, conducted between 1st of June, 2016 and 30th of August, 2016.

Study Population

The study population consists of all patients with signs and symptoms of wound infections attending the hospital during the study period, and who consented willingly to participate in the study.

Sample Size and Sampling

A convenient sampling technique was used in the study and a total of thirty-eight (38) infected wounds of patients attending Specialist Hospital, Sokoto within the study period were investigated. Wounds swab samples were collected by the

attending Physicians or Nurses from the depth of the wounds of the hospitalized patients with wound infection by using the standard operational procedures. The collected samples were labelled and transported immediately to Microbiology Laboratory of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto for culturing and antimicrobial susceptibility testing.

Isolation and Identification of Bacteria

All the samples collected were cultured on mannitol salt agar and the plates were incubated aerobically at 37 °C for 24 hours. Subsequently, the morphological characteristics of the emergent colonies were observed. The isolates that grew on mannitol salt agar with the fermentation of mannitol and acid production to give yellowish colonies were further characterized using Gram staining reaction, Catalase and Coagulase tests as described by (13). This was then sub-cultured on Nutrient agar slant and preserved at 4 °C.

Identification of Methicillin Resistant isolates

All bacterial isolates which were identified as *S. aureus* were screened for susceptibility to methicillin using Oxacillin in agar screen test (5). Isolates from a solution adjusted to 0.5 McFarland standards were spot inoculated onto Mueller Hinton agar supplemented with 6µg/mL Oxacillin (Sigma-Aldrich, USA) and 4% NaCl. The plates were incubated at 35 °C for 24 hours and then observed for growth. Plates that showed more than one colony were considered methicillin resistant (5).

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed on the isolates using the modified Kirby-Bauer disc diffusion method (5). A sterile swab stick was used to inoculate the standard inoculum (1.5×10^8 CFU/ml) of the test organisms evenly on the surface of Mueller Hinton agar and was allowed to stand for three minutes to dry. Commercially prepared discs of norfloxacin (10 µg), erythromycin (15 µg), ciprofloxacin (5 µg) and amoxiclav (10/20µg), from Oxoid Limited were placed on the inoculated Mueller Hinton agar 25mm away from each other. The plates were then incubated at 35 °C for 18–24hrs after which the zones of inhibition for each of the antibiotic were observed and recorded. The results were interpreted using the interpretative chart provided by Clinical Laboratory Standard Institute (CLSI, 2016) (5).

Data Processing and Analysis

Data was analyzed using IBM SPSS statistical software, version 24. Proportions for categorical variables were expressed in percentages and compared using chi-square test of independence. In all cases, p-values less than 0.05 were considered statistically significant.

Ethical considerations

Ethical approval for the study was obtained from the Research and Ethics Committee of the Specialist Hospital, Sokoto. Written informed consent was obtained from each of the participants after adequately informing them about the background and the objective of the study. The confidentiality, privacy and autonomy of the patients were preserved throughout the study period.

Results

The study participants consist of 38 patients comprising 12 (35.6%) females and 26 (68.4%) males. The rate of recovery of *S. aureus* among the various age groups and gender of patients enrolled for the study is presented in the Table 1. The rate of isolation of *S. aureus* was higher among the female patients (58.3%) than among the male patients (50%). Among the various age groups of patients observed, paediatric patients (0-17 years)

had the highest isolation rate followed by patients aged 37 years and above. Analysis of data on the rate of isolation of *S. aureus* among the gender and age categories using chi-square test of independence showed that there was no significant statistical association between the genders, age categories and the rate of isolation of *S. aureus.*

A total of 20 (52.6%) *S. aureus* were isolated from wounds of patients attending the Specialist Hospital Sokoto. The majority of the isolates used in this study were obtained from post-operative wound and burn wound (Table 1) with no significant statistical difference between the rate of isolation of *S. aureus* and the sources of specimens.

As shown in the Figure 1, the result of antibiotics susceptibility test revealed that the isolates were highly resistant to erythromycin (100%) and ciprofloxacin (70%), moderately susceptible to amoxiclav (50%) and highly susceptible to gentamicin (85%) and norfloxacin (80%). Of the twenty (20) *S. aureus* isolates, 25% were Methicillin resistant (Figure 2).

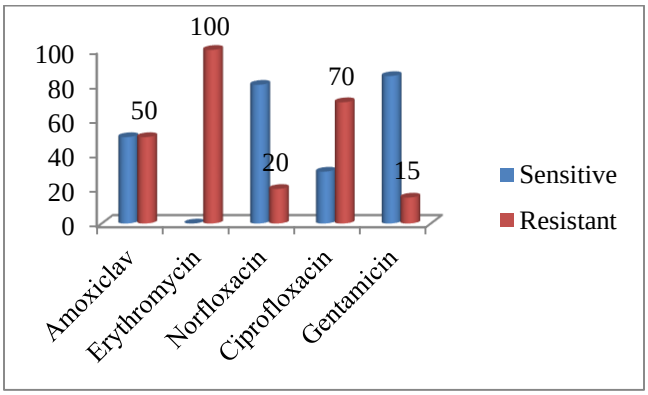

Figure 1. Antibiotics susceptibility profile of *S. aureus* isolated from wounds of patients attending Specialist Hospital, Sokoto, Nigeria .

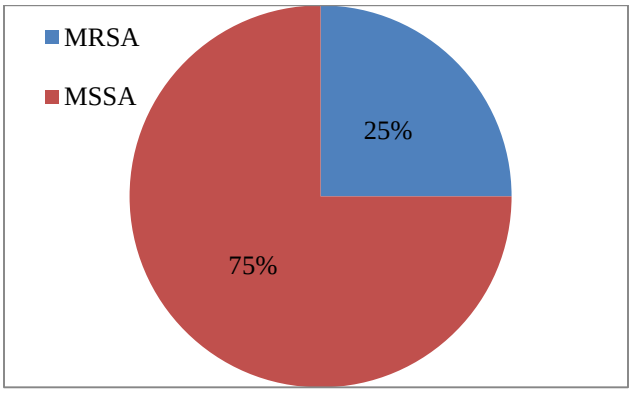

Figure 2. Incidence of methicillin resistant *S. aureus* (MRSA) and methicillin susceptible *S. aureus* (MSSA).

Discussion

The wound infection is a significant clinical challenge in hospitals particularly in developing countries where proper healthcare delivery is hindered by limited resources (14). In this study, the 52.6% recovery rate of *S. aureus* is consistent with several other reports where similarly *S. aureus* was found to be the most frequently isolated bacteria from wounds of patients (10, 15-17).

The results of antibiotic susceptibility test showed that gentamicin has the highest sensitivity profile followed by norfloxacin and Amoxiclav. This is in line with the results reported at tertiary healthcare facility in Kano and Port Harcourt, northwest and south-south Nigeria respectively (15,18). The 100% resistance of the *S. aureus* isolates to erythromycin concurs with the findings from another study (17).

This study describes for the first time, to the best of our knowledge, the occurrence of MRSA isolates among the bacteria detected in wounds of patients attending the Specialist Hospital, Sokoto-Nigeria. The high prevalence of MRSA (25%) recorded is comparable to similar reports from other centres across the country (19-21). Similar to the finding of this study, high rate of MRSA has also been reported in other countries (17, 22).

Table 1. The distribution and the rate of isolation of *S. aureus*.

Variables		No of *S. aureus* isolates	Rate of recovery (%)	χ^2- value	p-value
Gender	Male *(n=26)*	13	50	0.229	0.632
	Female *(n=12)*	7	58.3	-	-
Age Group	0-17 (n=10)	7	70	2.931	0.231
	18-36 (n=14)	5	35.7	-	-
	≥37 *(n=14)*	8	57.1	-	-
Sources of specimen	Post-operative wounds *(n=23)*	12	52.2	0.583	0.747
	Burn wound *(n=8)*	5	62.5	-	-
	Others *(n=7)*	3	42.9	-	-

The high rate of isolation of antibiotic resistant *S. aureus* including the MRSA from the infected wounds of patients in the hospital may delay wound healing and increase financial burden on the patient and the healthcare centre. Compared to non-MRSA infections, infections caused by MRSA are associated with a higher rate of morbidity and mortality (23).

The high incidence of MRSA wound infections in the hospital may be due to overcrowding of the healthcare facility, unhindered access of patients' relatives to the patients and low healthcare personnel-patient ratio, which can all facilitate nosocomial transmission of the infection in the hospital (24).

The findings of this study underscore the need to strengthen infection control measures and improve surveillance of antibiotic resistant bacteria particularly *S. aureus* in the hospital as MRSA has been implicated in both nosocomial and community-onset infections which has shown increasing endemic and epidemic spread in the last four decades (23,25). Further exacerbating the problem is the increasing multidrug resistant phenotype of many isolates of *S. aureus*, thereby limiting therapeutic options available for the management of its infections (23, 25).

Since patient-to-patient transmission in healthcare settings, usually via hands of healthcare personnel has been demonstrated to be responsible for increase in incidence and prevalence of MRSA infections and those due to other multidrug resistant organisms, the first and the most effective means of curbing further transmission in healthcare setting is breaking the chain of transmission through proper hand hygiene, use of personal protective equipment (PPE), such as disposable gloves, isolation gown, face masks, goggles, face shields, etc (25). This will help to protect mucous membranes, airways, skin, and clothing from contact with infectious agents thereby preventing dissemination of resistant organisms from personnel to patients.

Another factor identified for increase prevalence of MRSA and other resistant bacteria infections is the inappropriate use of broad-spectrum antibiotics (9). Therefore, chemotherapy should be guided by sensitivity of the probable causative organism.

Lastly, alongside the education and training of medical and other healthcare personnel,

enlightenment should be created about the risk factors and routes of transmission of infection in the community and the healthcare settings (25).

In order to have a better understanding of the incidence and susceptibility of *S. aureus* in the hospital, an expanded study covering all clinical specimens in all service units in the hospital over an extended period of time should be conducted. Also, effort should be made in future studies to investigate the molecular basis underlying resistance of *S. aureus* in the hospital as data is currently unavailable on this in the hospital.

Conclusion

In the light of the findings of this study, it can be concluded that *S. aureus* is a major agent of wound infection at the Specialist Hospital Sokoto, Nigeria. The *S. aureus* isolates exhibited resistance to most of the antibiotics with the exception of gentamicin, norfloxacin and amoxiclav. A high proportion of the *S. aureus* isolates were MRSA.

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Conflict of interest

None declared.

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