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Nasal and Hand Carriage of *Staphylococcus Aureus* Amongst Some Healthy Medical Laboratory Science Students of The University of Jos Nigeria.

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Abstract

Methicillin resistant Staphylococcus aureus (MRSA), have been reported to contribute to longer hospital stay of patients due to their resistance to antibiotics to which they were previously sensitive to. Knowledge of carriage of this bacterium would help in reducing the burden of hospital acquired infection. The objective of this study was to isolate S. aureus from the nose and hands of Medical Laboratory Science students of the University of Jos, Nigeria and to determine whether the strains were MRSA using Cefoxitin antibiotic. Nasal and hand swabs were collected with the aid of Sterilin commercial swab sticks. Samples were inoculated onto Mannitol salt agar plates aseptically and incubated at 35°C for 18-28 hours. Culture plates were then examined for characteristic colonial appearance. Suspected S. aureus colonies were subjected to Gram stain reaction, coagulase and catalase tests. Furthermore, confirmed colonies were subjected to antibiotic sensitivity test with Cefoxitin antibiotic discs(30µg) to determine MRSA as well as multidrug antibiotics. Out of the 100 students sampled, the age range 25-29 years were most frequently sampled. Only 12 samples (12%) were culture positive with confirmed S. aureus. There was significant difference in the carriage of S. aureus in the age ranges between male and female gender (<0.05). Antibiogram test with Cefoxitin showed that these colonies were all Cefoxitin resistant and thus MRSA. In addition, multi-drug antibiogram showed that the MRSA strains were also sensitive to Tarivid, Refloxacin, Ciprofloxacin, Augmentin, Gentamicin, Streptomycin, Ceporex, Nalidixic acid, Septrin

and Ampicillin with a frequency of 47. There was carriage of MRSA by some Medical Laboratory Science students. This carriage rate was low, suggesting that students could have been observing hand washing guidelines. The few cases recorded was a sign of a possibility of students infecting patients or fellow students.

Keywords: Staphylococcus aureus, MRSA, Cefoxitin, Carriage, Infection, Hospital

Introduction

Staphylococcus aureus (S. aureus) is a Grampositive bacterium reported to be responsible for many contagious diseases such as boils, septicaemia, kidney disease to mention a few (Schaumburg and Becker, 2014). They can be found on the hands, skin and nose of healthy individuals where they exist as commensals until the right conditions are provided which makes them pathogenic (Kluytmans et al., 1997). In addition, they have been isolated from door handles, floor, blankets and towels (Udobi et al.,2013). Over the years, strains of S. aureus were reported to be resistant to penicillin and later methicillin (MRSA) through the mediation of the mecA gene (Charles et al., 2015). This became a serious epidemiological problem considering risk factors such as HIV, poor sanitation, poverty, inadequate health care facilities, leading to prolonged hospital admission for patients. In addition, it could result in increased cost of treatment and or mortality (Obianuju et al., 2015). It was reported that hospital acquired S. aureus infection were multidrug –resistant, while community acquired strains were not (Clarence et al., 2005). Unfortunately, epidemiological data in Nigeria is

very scanty. Kesah et al. (2002), reported highest rates of 21 – 30 % for Nigeria, Kenya and Cameroon but the incidence has been reported previously by Obianuju et al. (2015) to be on the increase. Consequently, it was necessary to isolate S. aureus from the hands and nose of healthy individuals in order to ascertain the MRSA strains which are potentials for flare up of infections, especially as Medical Laboratory Science students are exposed to infected surfaces and bacteria in the course of their practical posting to the medical laboratories and the hospital in general. Thus, this asymptomatic carriage in these healthy students could be harboured for a long time. This cross-sectional study used Cefoxitin as substitute antibiotic to determine methicillin resistance (Clarence et al., 2005).

Materials and Methods. Study design and setting.

This study was conducted amongst students in the Department of Medical Laboratory Science, University of Jos Nigeria.

Collection and Treatment of Samples.

A total of 100 students (of different age groups and gender) who consented to take part in this cross-sectional study after signing the written informed consent form were recruited as subjects. Ethical clearance was obtained from the Ethical Committee of the University of Jos Teaching Hospital Jos. Nasal and hand samples were collected with the aid of sterile cotton wool swab sticks (Sterilin, U.K) according to the method previously described by Solayide *et al.* (2016). Properly labelled sterile cotton wool swabs were then placed on ice packs and transported to the laboratory where they were processed within 6 hours.

Inoculation and Identification of Culture.

Specimens were inoculated on to mannitol salt agar (Oxoid, UK) plates aseptically and incubated in an incubator (Memmert, UK) at 37°C for 18 – 24 hours according to methods previously described by Cheesbrough (2010) after which colonies were observed for characteristic growth. In addition, specimens were smeared on to clean grease-free glass slides (75 x 25 mm), dried, fixed briefly over Bunsen flame and stained with Gram stain according to

methods previously described by Baker and Breach (1980). Locally confirmed *S. aureus* strains were used as control. Culture plates were examined for characteristic morphological appearance. Further Gram stain reaction was carried out as well as coagulase, catalase and DNase tests according to methods previously described by Baker and Breach (1980). Colonies that showed characteristic golden-white appearance, coagulase, catalase and DNase positive were confirmed as *Staphylococcus aureus* (Taiwo *et al.*, 2004).

Antibiotic Sensitivity Testing.

Antibiotic sensitivity testing was carried out on Muella-Hinton sensitivity agar (Oxoid, UK) by the disc diffusion method (NCCLS,2004; Baker and Breach 1980). Methicillin sensitivity using 30µg Cefoxitin disc (Oxoid, UK) was carried out according to the method previously described by Baker and Breach (1980). Plates were incubated for 18 – 24 hours at 35°C. Zone inhibition diameter was determined with the aid of a calliper and reported in millimetre (mm). Zone diameter ≤ 19mm was reported as resistant, while zone diameter ≥ 20mm was susceptible (NCCLS, 2004). Multi-drug sensitivity test using other antibiotics: Tarivid (10µg), Reflacine (10μg), Ciprofloxacin (5μg), Augmentin (30μg), Gentamycin (10µg), Streptomycin (30µg), Ceporex(10µg), Nalidixic acid (30µg), Septrin (25μg) and Ampicillin (10μg) was carried out aseptically by modified Kirby- Bauer disc diffusion method previously described by Cheesbrough (2010).

Statistical Analysis

Data were presented in Tables and significance determined using Chi-squared test as well as percentages. The level of significance was set at $p \le 0.05$.

Results

Students in the age range 25-29 years, representing 39% were most frequently sampled followed by the age range 20-24 years (35%). Though equal number of male and female were sampled, the female gender had higher frequency than the male gender in the age range 20-24 years (42%) and 25-29 years (42%) respectively (Table 1).



Table 1. Frequency and Percent of Nasal and Hand Swab Sampled according to Age and Gender.

Age-Range (Years)	Frequency	Gender		
		Male	Female	
15-19	06 (6) *	03	03	
20-24	35 (35)	14	21	
25-29	39 (39)	15	24	
30-34	14 (14)	13	01	
35-39	04 (4)	03	01	
≥ 40	02(2)	02	**	
Total	100	50	50	

Key: *Percent; **Not sampled.

Out of 100 hand swabbed, only 2(2%) were culture positive for *S. aureus* while 10(10%) of the 100 nasal swabs were culture positive for *S. aureus*. They were all confirmed pathogenic *S. aureus*. There was carriage of pathogenic *S. aureus* in both nasal (4) and hand (2) swabs respectively while there were 6 in the age range 30-34 years (Table 2).

Table 2. Nasal and Hand Swabs Culture Positive for Staphylococcus aureus in the Age-Range

Age-range (Years)	Culture	
	Nasal	Hand
15-19	*	
20-24	04 (33.3) **	02 (16.67)
25-29		
30-34	06 (50)	
35-39		
≥ 40		
Total	10	02

Key: *Culture negative; **Percent (p-value=0.0217)

Furthermore, there was a significant difference in the carriage of *S. aureus* in the age-ranges between the female and male gender at p < 0.05 (Table 3).

Table 3. Frequency of Nasal and Hand swabs culture positive for Staphylococcus aureus in Relation to Gender.

Age-range (Years)	Gender		
	Male	Female	
15-19	*		
20-24	01	03	
25-29	04	04	
30-34			
35-39			
≥ 40			

Key: *Culture negative (p-value= 0.0743)

Antibiotic sensitivity test showed that all the confirmed pathogenic *S. aureus* strains (12) were resistant to *Cefoxitin*. However, there were strains that were sensitive (47) to other multiple antibiotics – Tarivid, refloxacin, ciprofloxacin, augmentin, gentamicin, streptomycin, ceporex, nalidixic acid, septrin, and ampicillin while 83 were resistant. In addition, there was a significant difference in the antibiotic sensitivity pattern at p = 0.05 (Table 4).

Table 4. Frequency of Cefoxitin and other Multi-drug susceptibility of confirmed *Staphylococcus* aureus.

Antibiotic	Number Sensitive	Number Resistant
Cefoxitin	*	12
Tarivid	09	03
Reflacine	08	04
Ciprofox	08	04
Augmentin	05	07
Gentamycin	04	08
Streptomycin	02	10
Ceporex	02	10
Nalidixic acid	03	09
Septrin	04	08
Ampicillin	02	10

Key: *Resistant (p-value = 0.0520)

All the 12 confirmed pathogenic *S. aureus* (Table 5) fulfilled the NCCLS (2004) guidelines and were Cefoxitin resistant and thus classified as methicillin resistant Staphylococcus aureus (MRSA).

Table 5. Frequency of Methicillin Resistant *Staphylococcus aureus* carriage from Nasal and Hand Swabs.

Swab	S. aureus (n=12)	MRSA (n=12)	MSSA
Nose (n=100)	10	10	*
Hand (n=100)	02	02	
Total	12	12	

Key: *none

Discussion.

The nasal and hand carriage of *S. aureus* amongst asymptomatic carriers was investigated. We found that some students harboured pathogenic MRSA *S. aureus* in their nose and hands. These students could have been exposed to poor living conditions in some of their hostels, as well as in the hospitals where they go for their clinical laboratory postings (Okamo *et al.*,2016; Radhakrishna *et al.*,2016). Although the agerange most infected were 25-29 years and 20-24 years respectively. Our finding is consistent with a previous study among young and healthy

students (Adebola *et al.*, 2013). Our finding is however contrary to the colonisation/infection pattern for MRSA.

In relation to gender, female students were most frequently sampled and thus most frequently colonised by MRSA than their male counterpart. Similar findings were reported by Gebreyesus *et al.* (2013). The confirmed isolates of *S. aureus* were all coagulase positive. This showed that these isolates were pathogenic (Charles *et al.*,2015). In addition, all the isolates were all resistant to cefoxitin and thus methicillin

resistant (Clarence et al., 2005). Since these Medical Laboratory Science students were asymptomatic carriers, the potential of infecting patients, colleagues and their families are high. In addition, when conditions are fulfilled, they could be sick. Out of the 12 confirmed MRSA, 10 were from nasal swabs confirming similar findings by Gebreyesus et al., (2013) and Okamo et al., (2016) and revealing that the nose is the niche for MRSA. The few strains of MRSA isolated from the hands could be due to poor hand washing by the students before leaving the hospital facility. However, some strains of MRSA were sensitive (47) to multiple antibiotics with Tarivid, Reflacine and Ciprofox having high frequency respectively while 83 were resistant to multiple antibiotics with Streptomycin, Ceporex and Ampicillin having highest frequency (Gebrevesus et al., 2013). This could limit the choice of antibiotics for treatment of MRSA.

Conclusion/Recommendations

MRSA potential of causing nosocomial infection is very high. Medical Laboratory Science Students are at danger of carrying this pathogen in their nose and hands from where they could infect patients leading to longer hospital stay. Students should be adequately educated on their potential role in infection prevention measures through observance of proper hygiene and frequent check-ups where nasal and hand swabs are taken to ascertain that they do not harbour MRSA. Where they do, prompt antibiotic treatment should be administered. In addition, Cefoxitin can be safely used as substitute for methicillin to determine methicillin-resistant S. aureus where this is not readily available (NCCLS, 2004).

Conflict of interest declaration

We do not have any conflict of interest.

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