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The Prevalence of Methicillin Resistance *Staphylococcus aureus* Nasal Carriage among Medical Interns in the Academic Charity Teaching Hospital, Khartoum 2016

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Abstract:

Background: *Nasal carriage of Staphylococcus aureus constitutes an increasing problem among healthcare workers, medical interns and the population at large in most countries in the world.*

Objective: *This study aimed to determine the prevalence of methicillin-resistant staphylococcus aureus (MRSA) among medical students in the Academic Charity Teaching Hospital – Khartoum, Sudan (ACTH).*

Material and Methods: *In this cross-sectional study, samples of 100 nasal swabs collected randomly from medical students studying at ACTH, were screened for MRSA. Samples were cultured and the isolated S. aureus strains were tested for their susceptibility for*

methicillin using disc diffusion method. The data was analyzed using SPSS version 21.

Results: *Out of 100 screened intern in ACTH 38 were S.aureus nasal carrier, the prevalence of methicillin susceptible S. aureus (MSSA) was 78.6% whereas 6 (21.4%) were methicillin resistant S. aureus (MRSA) carriers, specifically in MLS and dentistry colleges.*

Conclusion: *The present study demonstrates that interns can carry certain MRSA and contribute to the spread of MRSA between the community and hospital. Medical students must receive sufficient knowledge regarding control measures to avoid spread of this infection in hospitals.*

Key words: MSSA, *S. aureus,* MRSA, Students.

INTRODUCTION

Staphylococcus is a genus of facultative anaerobic, gram +ve bacteria with spherical shape that are clustered in grapelike arrangements, it's the most common cause of folliculitis and associated infections of the skin[1,7].

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of *Staphylococcus aureus* that is resistant to methicillin and other related antibiotics of the penicillin class, MRSA strains can be treated with glycopeptide antibiotics, such as vancomycin and teicoplanin in serious systemic infections, such as pneumonia, bacteremia, and endocarditis, Methicillin resistant *S.aureus* (MRSA) has become more common in hospitals, so it's imperative that health care workers take precautions against introducing such bacteria into patients [2].

There are both the hospital acquired MRSA and the community acquired MRSA strains and infections[4]. Asymptomatically colonized patients and health care workers (HCW) are the major sources of MRSA in the hospital environment, with the latter being more commonly identified as the link in the transmission of MRSA between the patients[4].

Global studies have documented *S.aureas* nasal colonization. In Saudi Arabia, the nasal carriage rates of *S. aureus* were 25.3% among medical students[7]. In Nigeria, the reported rate is 35.6% among healthcare workers. In India, 33(23.6%) were *S.aureus* nasal carriers out of which 17 (12.2%) were MRSA[11].

This study aimed to determine the prevalence of methicillin resistant *staphylococcus aureus* MRSA among medical students in ACTH and to assess the susceptibility of *S. aureus* isolates to methicillin using disk diffusion method.

JUSTIFICATION

Major concerns of substantial infection from patients with MRSA are confronting health care workers and medical students (internship), the source of many of these infections is thought to be the patient's endogenous flora [11].

Research is needed to fuel the necessary knowledge to all HCW and internship students, since the strain is emerging as a main concern in wound infections and systemic manifestation such as pneumonia, etc.

Medical students is a key target group to introduce awareness of hospital acquired infections[7]. Therefore, prevalence studies need to be carried out to screen this group to assess their carriage status during their clinical rotations. Medical students must receive sufficient knowledge regarding control measures to avoid spread of this infection in hospitals, through hygienic procedures such as hand washing, the regular use of antiseptics[7], etc.

Measures to screen and control the emergence and spread of MRSA are justified because there are fewer options available for the treatment of MRSA infections and because these strains spread amongst vulnerable at risk patients. Patients with MRSA bloodstream infection are twice as likely to

die from their infection, compared to patients with bloodstream infection caused by methicillin-sensitive *S. aureus* [12].

Contributing to human health and well-being is the goal of this research and to increase the awareness among HCW and medical interns.

METHODS

This cross-sectional study was conducted between December 2015 and April 2016, at the Academic Charity Teaching Hospital – Khartoum, Sudan (Latitude: 15. 552353, Longitude: 32. 542445°). The number of students participating in the study was selected by simple random sampling technique and the sample size was 100, out of which 21 were collected from dentistry internships, 10 from anesthesia internships, 11 from medicine internships and 58 from medical laboratory internships, as shown in table (1-1).

Collection and processing of specimens:

A nasal swabs was collected by cotton swab, and transported to the lab using Amies transport media. Swab was cultured in blood agar followed by mannitol salt agar, *S.aureus* (mannitol fermenting) was isolated and fully identified, then their susceptibility to methicillin was determined by disk diffusion method using (Oxicillin and methicillin).A total 100 samples were collected by means of sterile cotton swabs from the deep nostrils by rotating the swab 360 degree in each of the nostrils from medical students.

Isolation methods:

- **Inoculation:**

Under aseptic condition near Bunsen burner the samples were inoculated primarily in mannitol salt agar.

- **Incubation**:

The inoculated plates were transferred to incubator and incubated for 18-24 hours in 35°c.

- **Isolation**:

Only the growth that showed characteristic colonial morphology 1-2 mm and mannitol fermentation were selected. The resultant isolates were stored in crayotubes in refrigerator at 4°c.

Identification:

Primary identification:

- **Colonial morphology:**

Yellow colonies were studied. Size, shape & color were evaluated i.e. moderate smooth yellow colonies.

Secondary identification:

- **Biochemical tests:**

Catalase test: With an applicator wooden stick a portion from the center of a zig-zag transferred to a 3% H_2O_2 glass tube. The formation of bubbles (release of oxygen) indicates a positive test, compared with positive control[5].

Coagulase test: A coagulase slide test was run with a negative control to confirm the absence of auto agglutination. Double drops of normal saline were added onto the glass slide labeled with a sample number, [T] test and [C] control. The 2 saline drops were emulsified with the test organism using a loop. A plasma drop (plasma of rabbit anticoagulated with EDTA solution is recommended) is put on the inoculated normal saline drop corresponding to test, and mixed well, then the glass slide is shacked gently for about 10-15 seconds.

- If 'positive', macroscopic clumping would be observed in the plasma within 10 seconds, with no clumping in the saline drop.
- If 'negative', no clumping will be observed.

Control:

- Positive *Staphylococcus aureus* strain and coagulase negative *S.epidermidis* served as control organisms. Each reconstituted vial of plasma was tested within the control organisms.
- Control organism: *S.aureus* ATCC 29213

Antibiotic susceptibility tests:

Antibiotic susceptibility test was done for all isolates of *S.aureus*, Using Kirby-Bauer (Disc diffusion) method. Antibiotics tested were Oxacillin (1.0µg) and Methicillin (5 µg).

McFarland standard:

- The 0.5 Mcfarland solution is prepared as follow:

1. Adding 1 ml of concentrated H_2SO4 to 99 ml of distilled water in a conical flask or beaker and mix well. In this way, a 1 % v/v solution of H_2SO4 is prepared.
2. Dissolve 0.5 g of dehydrated barium Chloride salt (BaCl2.2H2O) in 50 ml of distilled water. In this way a 1 % w/v of BaCl2 is prepared.
3. Adding 0.6 ml of $BaCl_2$ solution to 99.4 ml of H_2SO4 solution to make up to 100 ml.
4. Mixing the solution well. This is the stock solution of the 0.5 McFarland turbidity standards.
5. Transfer about 2-3 ml of the solution into capped tubes and store at room temperature until use.

❖ This standard solution has the turbidity of the suspension of approximately $1.5*10^8$CFU/ml[1].

Inoculums preparations and application:

- To obtain a reproducible result, a standard number of colonies were selected and taken using sterile loop and transferred into a sterile normal saline and suspended, the inoculum turbidity was then adjusted by using Mcfarland standard tube turbidity.
- Within 15 minutes of preparing inoculums, a sterile swabs was dipped into adjusted suspension, excess

solution were removed by pressing and rotating the swab firmly against the side of the tube above the level of liquid.

- The swab was streaked over the surface of the Muller Hinton agar plate three times, the plate was rotated through an angle of 60 degree each time to ensure the distribution of the inoculum in all surfaces and the edge of the plate by passing the swab round it[1].

Disc application:

- The antimicrobial discs are placed on the inoculated plates using a sterile forceps. Make sure the disc is pressed gently down to insure even contact with the medium; the plates were then placed in an incubator at exactly 35^{o}c for 18-24 hours[17].

Reading and interpretation:

- After overnight incubation the diameter of each zone was measured and record in mm, using the rule on the under-surface of the plates. Zones of inhibition were uniformly circular, the diameter of the zones were recorded to the nearest millimeter. The end point of inoculation was generally judged by naked eye at the edge where growth starts. The zone margin should be taken as the area showing no obvious growth that was detected unaided eye[17].
- The zone of inhibition around the oxacillin disk was examined for light growth using transmitted light (plate held up to light); any discernable growth within the zone of inhibition of oxacillin is indicative of oxacillin resistance as described in the CLSI document[6].

Statistical Analysis:

The data was coded and processed using the Statistical Package for Social Sciences (SPSS) software. Descriptive analysis, using

standard statistical methods was performed. Graphics and frequency tables were used to present the findings.

Ethical Considerations:

Ethical approval was obtained from the UMST Ethics Committee to undertake the study (a copy is attached in the Appendix). I also obtained the approval of the manager of ACTH. A verbal consent to the study was obtained from the medical students (interns) before recruiting them in the study.

RESULTS:

Out of the 100 sample collected, 21 were collected from dentistry internships, 10 from anesthesia internships, 11 from medicine internships and 58 from medical laboratory internships, as shown in table (1-1).

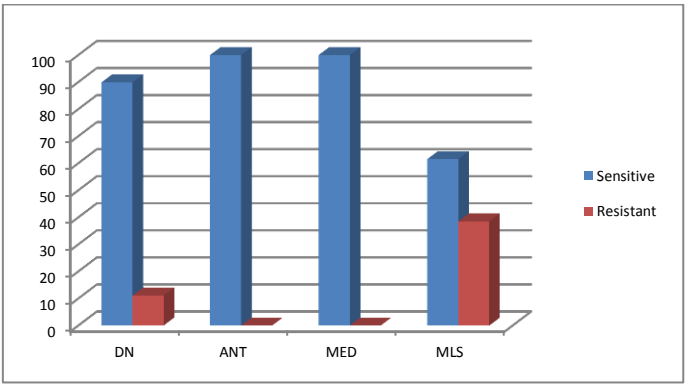
S. aureus were detected in 28 (28%) of the total samples. The 28 *S. aureus* isolates screened for the presence of MRSA strains, 6 (21.4%) were found to be MRSA, 22(78.6%) were found to be MSSA, table (1-1).

Out of the 21 samples collected from dentistry faculty, 9(42.9%) were *S. aureus* carriers, of the 10 swabs collected from anesthesia, 3(30%) were found to be *S. aureus* carriers, 3 (27%) *S. aureus* isolates were found among 11 medicine students and 13 (22%) *S. aureus* were isolated among 58 medical technologists. The carriage differed significantly in the 4 different colleges.The MRSA carriers were distributed in two faculties; Dentistry and Medical Laboratory Sciences, figure (1-1).

Table (1-1): Distribution of *S.aureus* carriage and sensitivity among participant Students according to College (n=100)

	Frequency	*S.aureus*	Sensitive	Resistant
Dentistry	21	9 (42.9%)	8(88.9%)	1(11.1%)
Anesthesia	10	3 (30%)	3(100%)	0(0%)
Medicine	11	3 (27%)	3(100%)	0(0%)
Medical lab sciences	58	13 (22%)	8(61.5%)	5(38.5)
Total	100	28	22	6

Figure (1-1): Results of the susceptibility to methicillin among students according to their College (n=28)



DN= Dentistry; ANT= Anesthesia; MED= Medical College; MLS= Medical Lab Sciences

DISCUSSION

The presence of *S. aureus* nasal colonization among healthcare personals and healthy community members known to be as a major risk factor for the development of both community-acquired and hospital-acquired nosocomial infections including MRSA[22]. Determination of colonization prevalence provides a useful estimate of the potential for development of *S. aureus* infections [9,16].

This study attempted to assess the *S. aureus* nasal carriage rates among medical students in a teaching hospital at Khartoum State, Sudan. The study revealed that the prevalence of S. aureus nasal colonization among medical

students at Academic Charity Teaching Hospital was 28%. These findings are almost similar to that previously reported in the Soba University Hospital during the period from March 2009 to April 2010 by Alaa *et al*. while the prevalence of MRSA was 22.8%.

In this study, our data showed that MRSA carriage rates were high among medical students. This finding is in agreement with other studies, which documented that the MRSA nasal carriage was higher among medical personnel than non-medical personnel [6,16].

MRSA carriage in these medical students may accelerate its spread in the community and among patients at hospitals. This study provides precious data in Khartoum on the prevalence of *S. aureus* nasal colonization in a healthy student population, the rate of MRSA nasal colonization carriage varied across different medical faculties.

Our finding indicated highest prevalence of *S. aureus* (n=13, 22%) in the medical lab students out of which 5 were MRSA reservoir, whom are more vulnerable due to their contact to lab equipment and settings, Weinstein *et al*. identified MRSA as a potential nosocomial infection to lab personnel[14].

Wagenvoort *et al*. reported 2 cases which involved two different main pandemic MRSA outbreak clones (multilocus sequence types 5 and 8) among medical technicians and thus such events could go unnoticed or not appreciated in laboratories elsewhere[13].

The other positive isolate was found in dentistry students in which (n=9, 42.9%) *S.aureus* were isolated and a single MRSA strain was found among them. A study by H. Kurita *et al.* in 2006, assessed the possibility of methicillin-resistant *Staphylococcus aureus* (MRSA) transmission via the surfaces of the dental operatory. MRSA was observed on the surfaces of dental operatory including the air-water syringe and

reclining chair, nosocomial infection or colonization of MRSA occurred in eight out of 140 consecutive patients who had no evidence of MRSA at admission, antibiograms of 30 antibiotics revealed that the isolates from the eight patients were of the same strain as those from the surface of dental operatory, suggesting that surface contamination of the dental operatory may be one of the causes of nosocomial infection with MRSA and the possibility of cross contamination through the dental operatory[15].

Numerous studies have been carried out to estimate the prevalence and risk factors of S. aureus among medical students and healthcare workers in many countries.

In China, 2010, Xiao Xue Maa[8], *et al*. screened the nasal carriage of methicillin-resistant *Staphylococcus aureus* among preclinical medical students. Out of 2103 medical students at the China Medical University, *Staphylococci* were found in 523(24%) specimens. Among them, 413(79%) were identified as coagulase-negative *staphylococci* (CNS) and 110(21%) were identified as *S. aureus*. In total, the carriage of MRSA among S. aureus was 9.4%[8].

In 2012, a study was conducted by Lakshmi S. and Peerapur, on the prevalence of nasal carriage of MRSA among clinical staff and health care workers of a teaching hospital of Karnataka, India. A total of 200 nasal swabs were collected, 140 were from the nursing staff and 60 were from the clinical staff. Of the 140 swabs (nursing staff), 33 (23.6%) strains of *Staphylococcus aureus* were isolated, out of which 17(12.2%) strains were MRSA and 16 (11.4%) strains were MSSA, Of the 60 swabs from the clinical staff, 12 (20%) strains of *Staphylococcus aureus* were isolated, out of which 7 (11.7%) were MRSA and 5 (8.3%) were MSSA[11].

Shadi A. Zakai, studied the prevalence of methicillin-resistant *Staphylococcus aureus* nasal colonization among medical students at King Abdul-Aziz University (KAU) Jeddah,

Saudi Arabia. Using molecular approaches (PCR) to identify *Staphylococcus aureus* nuc gene, and an additional PCR was performed on S. aureus positive samples to detect the presence of mecA gene. Out of 150 students screened (150 internship and sixth-year medical students), 38 were nasal carriers of *S. aureus*. The prevalence of methicillin sensitive *S. aureus* (MSSA) carriers was 18.7%, whereas 10 students (6.7%) were mecA-positive, representing MRSA carriers. It was concluded that interns carry MRSA more than 6th year students and students who were not exposed to clinical work ($p<0.05$), while MSSA is found more in students who were not exposed to clinical work ($p<0.01$)[7].

MSSA colonization appears to be influenced more readily than MRSA colonization by many health and environmental factors, unlike the colonization by MRSA which is more dependent on the carriage state [10]. People with staphylococcal lesions should refrain from working with food, patients with open wounds, immunocompromised patients, and women in labor, and they should not work in nurseries or operating rooms. The most important measures for protecting against nosocomial infection are proper aseptic techniques and particularly correct hand antisepsis [3].

CONCLUSION:

MRSA has caused many hospital epidemics especially amongst patients in the ICU and has been responsible for many deaths. Moreover, our numbers indicate that minority of medical interns who are undertaking their clinical training at ACT hospital carry MRSA strains in their nasal cavities. Such findings indicate that those students might be a source for spreading MRSA in the hospital.

Recommendation:

- Since MRSA infections could arise in the hospital units through hospital personals carriers, good hand hygiene practice of hospital staff is a primary important factor to avoid dissemination of multi-drug resistant organisms in the hospital unit[16].
- In addition, the implementation of infection control measures in our hospitals is an important barrier to reduce transmission of MRSA carriage and is necessary to reduce the risk of subsequent infection[16].
- Also regular screen for MRSA among those students and affording treatment for the carrier should be a routinely practices.
- We suggested that more sessions of nosocomial prevention and hand hygiene may be essential for medical interns prior to starting hospital training, beside educational sessions on patient's safety, to increase awareness of hospital-acquired MRSA and other infections.

Acknowledgement

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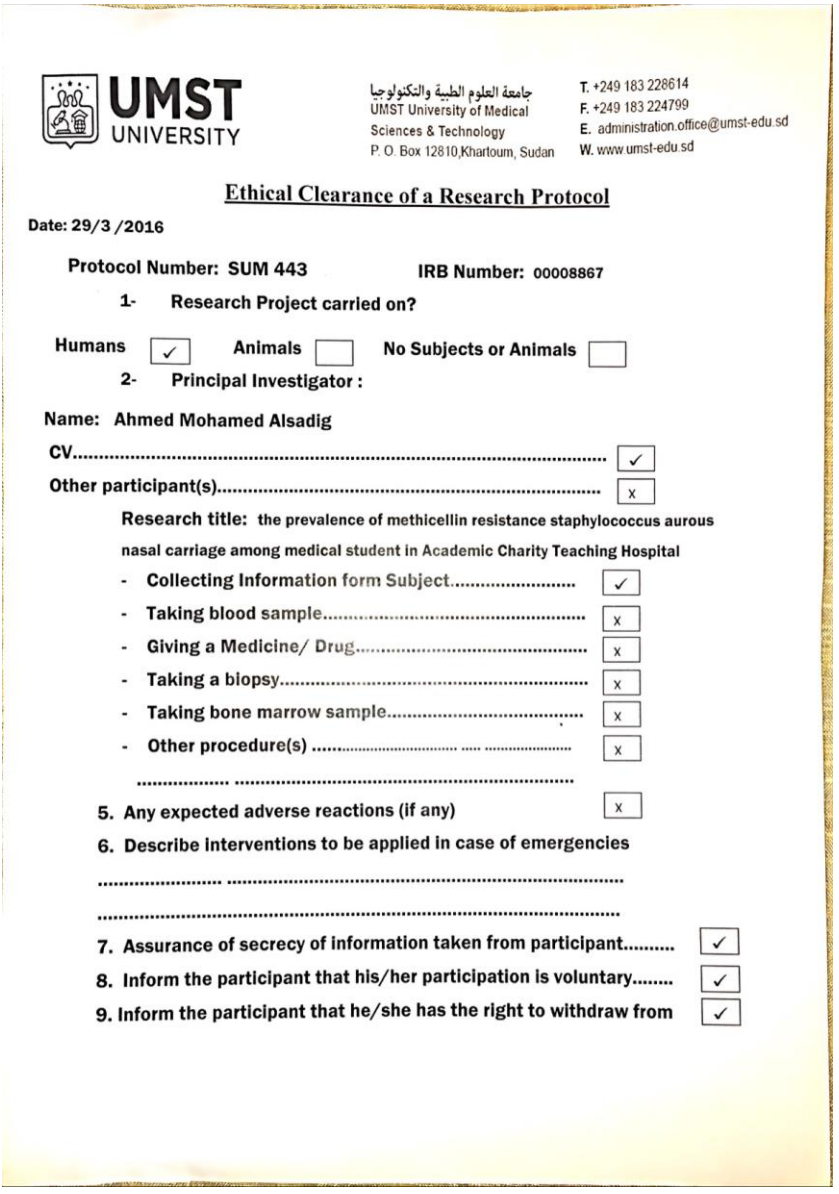
APPENDIX:

Ethical clearance:



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Ethical Clearance of a Research Protocol

Date: 29/3 /2016

Protocol Number: SUM 443 **IRB Number: 00008867**

1- Research Project carried on?

Humans ✓ **Animals** ☐ **No Subjects or Animals** ☐

2- Principal Investigator :

Name: Ahmed Mohamed Alsadig

CV..... ✓

Other participant(s)..... x

Research title: the prevalence of methicellin resistance staphylococcus aurous nasal carriage among medical student in Academic Charity Teaching Hospital

- **Collecting Information form Subject**..... ✓
- **Taking blood sample**..... x
- **Giving a Medicine/ Drug**..... x
- **Taking a biopsy**..... x
- **Taking bone marrow sample**..... x
- **Other procedure(s)** x

....

5. Any expected adverse reactions (if any) x

6. Describe interventions to be applied in case of emergencies

....

.....

7. Assurance of secrecy of information taken from participant..... ✓

8. Inform the participant that his/her participation is voluntary..... ✓

9. Inform the participant that he/she has the right to withdraw from ✓

The study .....

10. Participant consent form..... ✓

11. Proposal Details

Character	Place of Research	Duration of Research	Introduction	Objectives General	Objectives Specific
Present	✓	✓	✓	✓	✓
Absent					

Character	Type of Study	Variables	Data Collection Technique	Sample Procedure	***
Present	✓	✓	✓	✓	
Absent					

Ethical Committee decision:

Passed ☑ **Not passed** ☐

Date Approved: 29/3/2016

Expiry Date: 29/9/2016

Prof. Abdalla .O. Elkhawed
Chairman
Ethical Committee
UMST
Signature Abdalla awad
Date 29.3.2016

Dr. Hanan Tahir
Convener
Ethical Committee
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Signature H. Tahir
Date 29.3.2016