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RESEARCH ARTICLE

NASAL CARRIAGE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS AMONG MEDICAL STAFF AT OMDURMAN TEACHING HOSPITAL

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ABSTRACT

Background: methicillin-resistant *Staphylococcus aureus*(MRSA) is a strain of Staphylococcus aureus which is resistant to methicillin and other antibiotics. *Staphylococcus aureus* is an organism that colonizes mainly in the nose, skin folds, hairline, perineum and navel in human.

A patient becomes clinically infected if the organism invades the skin or deeper tissues and multiplies, other cases may remain subclinical. MRSA is prevalent in health care personal because their vulnerability to infection is higher than general population.

Methods: This was descriptive cross sectional study in which atotal of 200 nasal swabs were collected from medical staff, out of which 102 were from the nursing staff , 78were from clinical Staff and 20 from lab technician Sterile cotton swabs moistened with sterile saline were used for sample collection. Swabs were cultured on mannitol salt agar, incubated at 37^{O}Cfor 24 hrs. *Staphylococcus aureus* was identified by morphology, Gram stain, catalase test, coagulase test, and mannitol salt agar fermentation. Methicillin resistance was detected by Kirby Bauer disk diffusion method. All methicillin-resistant isolates were examined for the existence of the mecA gene by conventional PCR.

Results: Of the 200 samples screened 33 (16.5%) isolates of *Staphylococcus aureus* were identified, out of which 10 (30.3%) were methicillin resistant *Staphylococcus aureus*(MRSA) while 23 (69.7%) were methicillin sensitive *Staphylococcus aureus*(MSSA). The overall carriage rate of methicillin resistant *Staphylococcus aureus* in our study was the highest rate being seen among the nursing staff (51%) and clinical staff (39%) and laboratory technician (10%) were slightly less than nursing staff.

Conclusion: Our study revealed that nursing staff were the potential colonisers of methicillin resistant *Staphylococcus aureus* when compared to clinical staff.

In the present study about 5% of medical staff in Omdurman teaching hospital had methicillin resistant *Staphylococcus aureus*.

Keywords: Methicillin resistant *staphylococcus aureus*, Nasal carriage medical staff, Omdurman teaching hospital, PCR.

INTRODUCTION:

Staphylococcus aureus is an opportunistic pathogen often carried asymptomatically on the human body. Methicillin-resistant *S. aureus*(MRSA) includes those strains that have acquired a gene (MecA) giving them resistance to methicillin and essentially all other beta-lactam antibiotics[(1)]. The mecA gene is found on a large mobile genetic element called the staphylococcal chromosomal cassette mec (SCCmec). [(2)] At least 8 SCCmec types (SCCmec I through SCCmec VIII) have been identified, although some are more common than others. MRSA carrying SCCmec type I spread across the world in the 1960s, SCCmec II in the 1970s, SCCmec III in the 1980s, and SCCmec type IV in the 1990s. Different SCCmec types tend to occur in human hospital-associated and community-associated MRSA.[(3)]The principal mode of MRSA transmission within an institution is from patient to patient via the transiently colonised hands of hospital personnel who acquire the organism after direct patient contact or after handling the contaminated materials .[(4)]

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Following the introduction of penicillin in the 1940 s, strains of *S. aureus* unaffected by penicillin were reported in 1945.[5,6]Methicillin was introduced in 1959 to treat these infections, but in 1961, shortly after the introduction of methicillin, *S. aureus* isolates that had acquired resistance to methicillin (methicillin-resistant *S. aureus*, MRSA) were reported.[7]

MRSA was first reported as a nosocomial pathogen in human hospitals. Although these organisms cause the same types of infections as other *S. aureus*, hospital-associated strains have become resistant to most common antibiotics, and treatment can be challenging.[8] Since the 1990s, MRSA has also become a concern in people who have not been hospitalized or recently had invasive procedures, the strains that cause such infections are called community-acquired or community-associated MRSA.[9]

The result in study of nasal carriage of MRSA among the clinical staff and health care worker of ateaching hospital of Karnataka, India at 2012 is200 nasal swabs were collected of which 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 methicillin resistant *Staphylococcus aureus* and 16(11.4%) strains were methicillin sensitive *Staphylococcus aureus*(MSSA).[10] Community-associated MRSA first appeared in high-risk populations such as intravenous drug users, people in nursing homes, and people who were chronically ill, but they are now reported even in healthy children.[11]

There was study in sudan of healthcare workers including doctors ,nurses and medical technologists in Soba university hospital and from the adult community members in Khartoum state, sudan the results were of the 114 *S.aureus* isolated,20.2% represented MRSA with 32.7% among healthcare worker and 8.5% among community individuals. The occurrence of MRSA were significantly higher among healthcare worker than community individuals. [12]

MRSA isolates are genetically heterogeneous. Some strains, which are called epidemic strains, are more prevalent and tend to spread within or between hospitals and countries.[11]

METHOD AND MATERIALS:

Design:

The present study was descriptive cross sectional study in which consenting healthy medical staff in Omdurman teaching hospital during July to November 2014, 200 of medical staff was involved.

Subject selection:

Healthy Medical staff (nurses, physicians and lab technicians) in Omdurman teaching hospital in July to November, 2014, was included in this study. Non healthy medical staff was excluded from the study.

Approval was taken from faculty of Ethical Board; an informed or consent was obtained from each individual, every participant was informed about the research purpose before specimen collection the nasal samples and filling the questionnaire.

EXPERIMENTAL WORK:

Collection of specimens:

Nasal swabs were collected from the anterior naries by using a sterile cotton wood swab moistured with sterile normal saline; all the steps were conducted under aseptic conditions.

Culture of speciemen:

After collection, nasal swabs were streaked on Mannitol Salt Agar under aseptic condition and then incubated overnight at 37^{O}C for 24hrs.

Identefication of suspected colonies:

S. aureus was identified using standard methods based on colony morphology, gram stain, catalase test, mannitol fermentation and coagulase test. Methicillin resistance was tested using Kirby Bauer disk diffusion method. Inoculum turbidity from each isolate equivalent to that of 0.5 McFarland standard was prepared then cultured in Muller- Hinton Agar Disc of methicillin (Me) (5µg) was used, Inhibition zones were measured in mm after 24h of incubation at 37^{O}C and compared with chart and interpreted as sensitive ,resist or moderate.

Mec A gene detection:

DNA Extraction:

A chromosomal DNA was extracted from pure cultures of Methicillin resistant *Staphylococcus aureus* (MRSA) isolates by using physical method (Heating).

Briefly, 2 to 3 colonies of a pure culture of *S. aureus* are resuspended in 50 µL of distal water(D.W) in a 0.2 mL microfuge tube and vortexed to ensure a homogenous suspension. The suspension is then incubated at 100^{0}C for 10 minutes, quick chilled on liquid nitrogen and repeated three time prior to centrifugation at 13,000 rpm for 2 minutes. 2 µL of the supernatant was used as template in the PCR assay. PCR assay included 25 µl final reaction mixture which consisted 5 µl blue master mix (intron biotechnology, korea) which consisted (taq polymeras ,reaction buffer, and DNTPs and 2 µl of each 10 P mol forward and reverse primer spisefic for amplification mecA gene ,5 µl from DNA template and 13 µl from DW(H_2O) to complete the volume to 25 µl final reaction mixture .The reaction tube incubated in thermal cycler(techne, Japan) under condition denaturation 94C^{O}

for 10 min, followed by 10 cycles 94C^{O} for 45sec anneling 55 C^{O} for 45 sec and extension at 72 C^{O} for 75 sec and another 25 cycles 94C^{O}for 45 sec anneling 50C^{O} for 45 sec and extension at 72 C^{O} for 75 sec and final extention for 10 min. the amplicons were resolved and screened using 1.5% agarose gel electrophoresis method. All PCR reactions were performed with appropriate negative and positive controls which are size band 325 bp to avoid any false negative and positive results.

RESULTS:

A total of 200 nasal swabs were collected of which 51 %(n=102) were from the nursing staff, 39 %(n=78) were from the physician and 10%(n=20) from lab technician. Of 200 nasal swabs *S.aureus*16.5%was isolated; from 69.7% of them were methicillin resistant *staphylococcus aureus* (MRSA). The highest S.aureus nasal carriage was observed among nursing staff (63.6%) figure (1) and among 30-40age range figure (4).

Of the 102swabs from nursing staff 63.6 %(n=21) strains of *Staphylococcus aureus* were isolated. Out of which 24.2 %(n=8) strains were methicillin resistant *Staphylococcus aureus* and 39.4 %(n=13)strains were methicillin sensitive *Staphylococcus aureus*(MSSA). Of the 78 swabs from clinical staff 33.3 %(n=11) strains of *Staphylococcus aureus* were isolated. Out of which 3 %(n=1) were methicillin resistant *Staphylococcus aureus* and 30.3% (n=10) were methicillin sensitive *Staphylococcus aureus* (MSSA).Of the 20 swabs from lab technician 3 %(n=1) strains of *Staphylococcus aureus* were isolated and 3%(n=1)were methicillin resistant *Staphylococcus aureus*.

So of the 200 samples collected n=33(16.5%) strains of*Staphylococcus aureus* were isolated. Out of which 30.3%(n=10)were methicillin resistant *Staphylococcus aureus (*MRSA) and 69.7%(n=23) were methicillin sensitive *Staphylococcus aureus*(MSSA) Figure No(2)**.** , among whom 18.2% (n= 6) were males, 12.1 %(n=4) females. Figure (3)

Molecular detection of methicillin resistant strains for mecA gene showed all MRSA there possess the mentioned genes Figure (5).

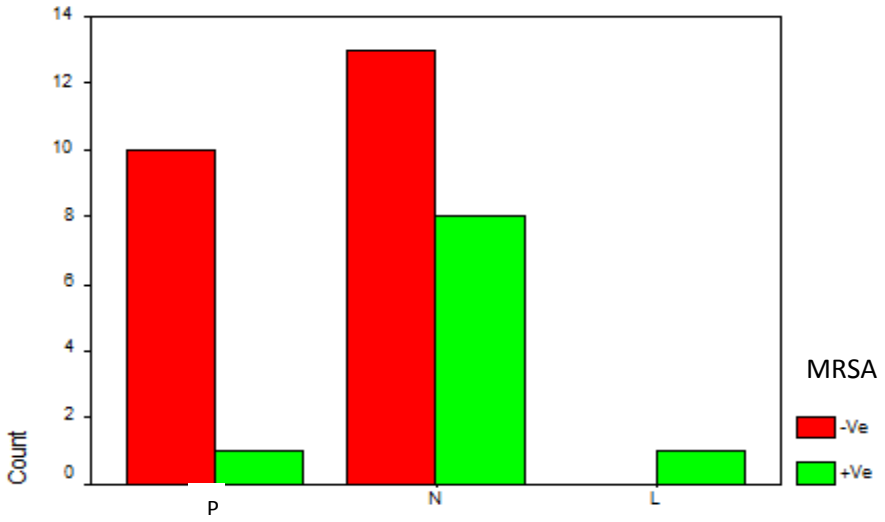

Figure 1: Frequency percentage of methicillin resistant *S.aureus* MRSA among study population according to their occupations

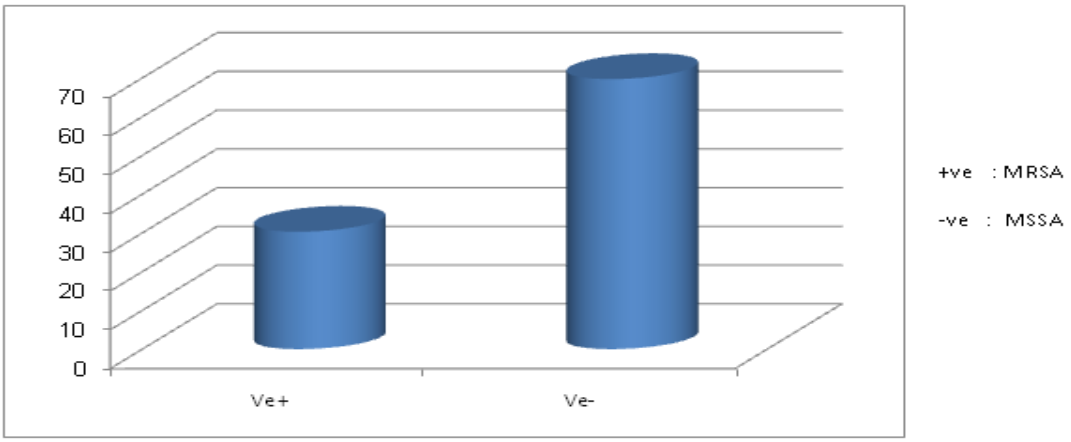

Figure 2: MRSA (+Ve) and MSSA (-Ve) amonge isolated *S.aureus* in the study group

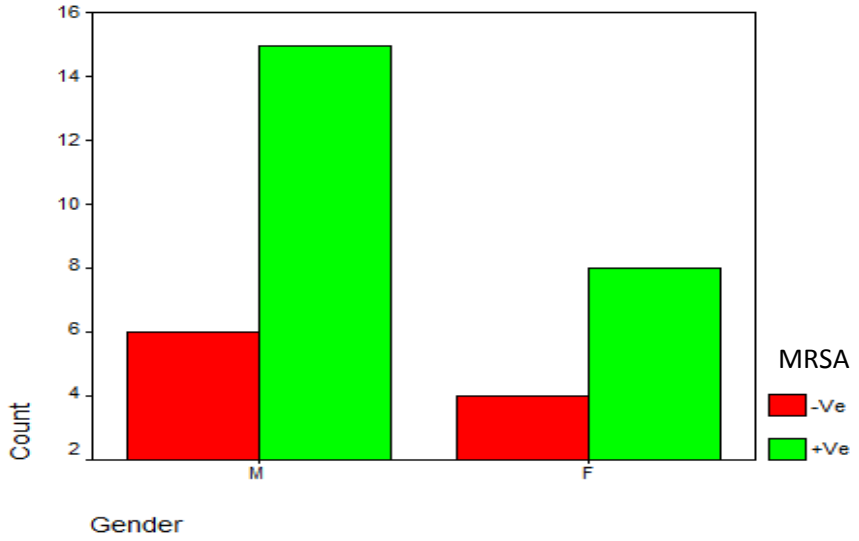

Figure 3*:* **isolated *S.aureus* among study group according to their gender**

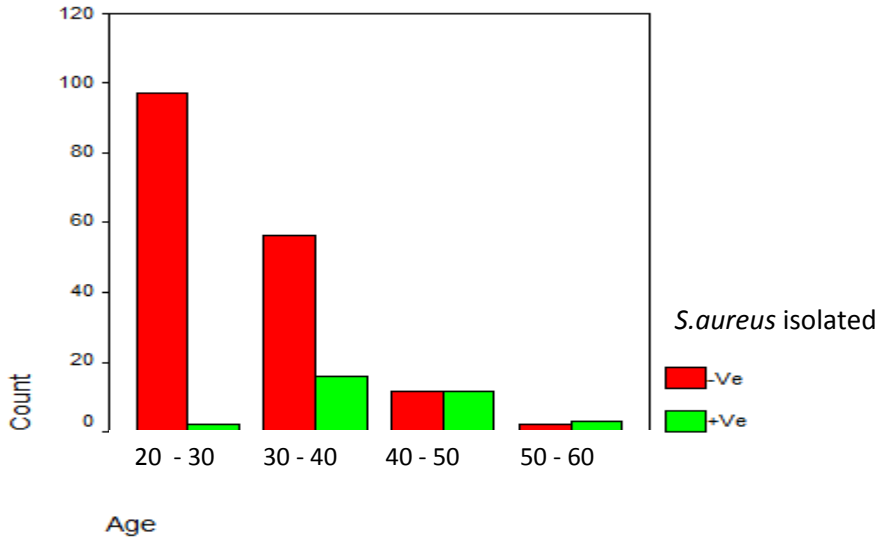

Figure 4: *S. aureus* among study group according to their age group.

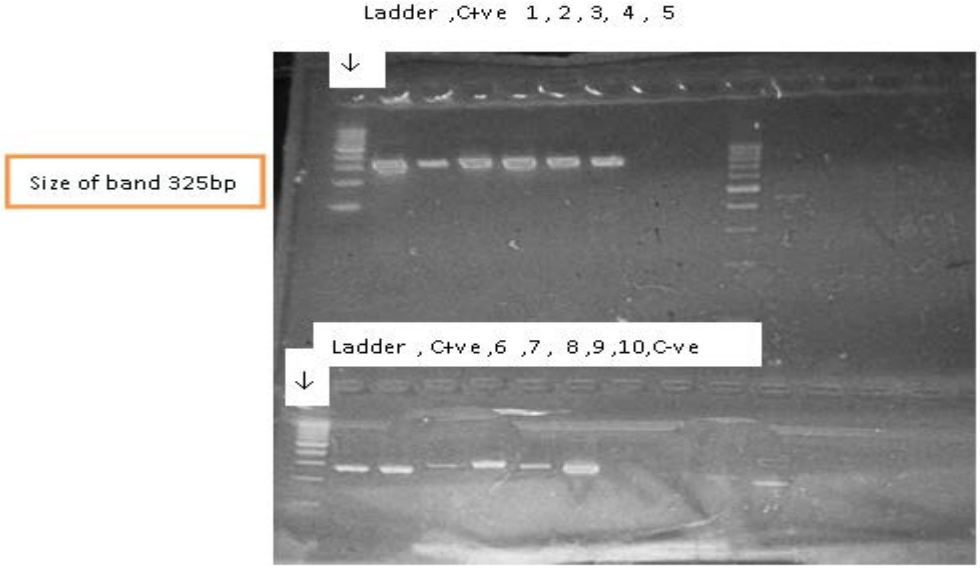

Figure 5: Gel electrophoresis detection of MecA gene by PCR

DISCUSSION:

This study revealed that 33(16.5%) of medical staff were carriers of *S. aureus* strains. Of these, 10(30.3%) were MRSA and 23(69.7%) were MSSA .The overall MRSA carriage rate in our study was 30.3% with the highest rate being seen among the nursing staff (39.4%). Carriage rate among the clinical staff was slightly less (30.3%), indicating that the nursing staff were the potential colonisers and disseminators of MRSA in the hospital settings.

MRSA is important nosocomial pathogen worldwide because of the increased rate of multidrug resistant

Strains among the hospital acquired MRSA. [(13)] Serious infections due to methicillin resistant *S. aureus* such as bacteraemia, osteomyelitis, and sepsis are more prevalent in the hospital settings ,but more importantly many cases of MRSA infections can be seen among previously healthy individuals with no exposure to health care setting, hence, these communities associated MRSA have become more important in daily practice.[(14)] MRSA are those strains of *S. aureus* that express *mec*A or another mechanism of methicillin resistance, such as changes in affinity of penicillin binding proteins. So all the strains of *S. aureus* that are highly resistant to methicillin produce an additional low affinity penicillin binding protein (PBP2a) encoded by the mecA gene.[(15)]

In the present study 200 participant were enrolled, the result showed 33(16.5%) *S.aureus* were isolated, out of them 10(30.3%)methicillin resistant *Staphylococcus aureus*(MRSA) and (100%)posses mecA gene .These result slightly similar to which done in india by .Lakshmi S. Kakhandki (2012)studied of nasal carriage of MRSA among the clinical staff and health care workers of a teaching hospital of Karnataka, of the 200 samples collected, 45(43.6%) strains of *Staphylococcus aureus* were isolated. Out of which 24(12%) were methicillin resistant *Staphylococcus aureus*(MRSA) .However it slightly differ from study done in india by Bidyashrestha ,Bharat Mani Pokhrel , Tribhuban M Mohapatra (2009) who found of 129 of health care worker 27.13% (n=35) were identified as nasal carriers of *S. aureus* out of them 2.32% (n=3) %)methicillin resistant *Staphylococcus aureus*(MRSA) .

The presence of MRSA may cause problems in hospital infection control program. Further studies are needed to evaluate whether enhanced environmental disinfection strategies can reduce transmission of nosocomial pathogens.

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