

The Prevalence of Methicillin resistant *Staphylococcus aureus* among Surgical Patients at Kosti Teaching Hospital, Kosti – Sudan

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Abstract:

Background: *Methicillin Resistant Staphylococcus aureus* is a serious clinical and epidemiological problem with limited treatment options

Objectives: *The aim of this study was to determine the prevalence and antimicrobial susceptibilities of Methicillin Resistant Staphylococcus aureus nasal carriage and wound infections among patients at Kosti teaching hospital, Sudan.*

Methods: *The study was carried out from March 2013 to December 2014. It is a prospective descriptive cross sectional study. Two hundred and thirty two patients were enrolled in the study. A nasal swab and a wound swab were obtained for culture, from each participant. Staphylococcus aureus strains were isolated and identified by the conventional techniques. Methicillin Resistant Staphylococcus aureus was confirmed by cefoxitin disc diffusion test and Methicillin Resistant Staphylococcus aureus- latex agglutination test. Antimicrobial susceptibilities to 8 antibiotics were performed by CLSI disc diffusion technique.*

Results: *The prevalence of Methicillin Resistant Staphylococcus aureus in interior narses was 19/232 (8.2%), and it was 26/232 (11.2%) in wound infections, most isolates of MRSA 32/45(71.1%) were hospital acquired. There was a significant*

relationship between Methicillin Resistant Staphylococcus aureus wound infections and nasal carriage (P = .002). There was no effect of gender or age on distribution of Methicillin Resistant Staphylococcus aureus in interior narses or wound infections. Methicillin Resistant Staphylococcus aureus showed high susceptibility to vancomycin 94.3%, followed by clindamycin 88.6%. However, resistant to cotrimoxazole, erythromycin, cephalothin and amoxicillin-clavulanic acid was significantly associated with the Methicillin Resistant Staphylococcus aureus (P≤ 0.001 for each).

Conclusion: *There is a high prevelance of Methicillin Resistant Staphylococcus aureus nasal carriage and wound infections among patients at Kosti teaching hospital. Most of the isolates are susceptible to vancomycin.*

Key words: Prevalence methicillin resistant staphylococcus aureus among surgical patients.

INTRODUCTION:

Staphylococcus aureus (S.aureus) is one of the most common causes of nosocomial or community-acquired infections, leading to serious illnesses with high rates of morbidity and mortality. Since the occurrence of methicillin resistance *S. aureus* (MRSA) strains the bacteria has become more problematic⁽¹⁾ due to the fact that MRSA strains are resistant to all beta-lactam antibiotics⁽²⁾. Resistance to other antibiotics is also common which limit the treatment options for MRSA.

Azoz *etal* were studded the acute superficial abscesses at Kosti teaching hospital, and reported that *S.aureus* was isolated in 51(82.2%) of specimens. All isolates were 100% sensitive to ciprofloxacin, amikacin and gentamycin.⁽³⁾

Methicillin resistance occurs when the organism has a *mecA* gene producing an altered penicillin binding protein, designated PBP2a (also known as PBP2'). Detection of the *mecA* gene by molecular methods, or its product, penicillin binding

protein PBP2a by latex agglutination test, were considered the gold standards for MRSA confirmation⁽⁴⁾. Recent investigations suggested that disk diffusion using cefoxitin is superior to most previously recommended phenotypic methods^(5, 6)

MRSA nasal carriage, reported in 0.2% to 2.8% of the United States population, and has been recognized as a risk for MRSA skin and soft tissue infections SSTI⁽⁷⁾. There was an increase in the incidence of *S. aureus* with an increase in community MRSA SSTI.⁽⁸⁾ Previous studies have demonstrated that individuals with *S. aureus* bacteremia and or had surgical site infections 80% to 90% of the time of infection they carriage of the same isolate in their anterior nares^(9,10, 11). *S. aureus* was reported as the most common organism in SSTIs in Europe (71% of cases) with 22.5 % being MRSA, the highest rate of MRSA (48.4%) was in Belgium.⁽¹²⁾ Concerning Africa, several countries reported MRSA as a problem^(13,14,15) but there is still a lack of good epidemiologic data. In Dessie Referral Hospital in Ethiopia 12.7% of all healthcare-workers were identified as MRSA carriers. The rate of methicillin resistance among all *S. aureus* isolates was 44.1% (15/34). The highest rate of MRSA carriers (57.1%) were workers of surgical wards.⁽¹⁾

Vancomycin was considered as a drug of choice for treatment of MRSA. Several studies reported that *S. aureus* isolates including MRSA were sensitive to vancomycin and linezolid (100%)⁽¹⁶⁾. Vancomycin is considered inferior to β -lactams for the treatment of *S. aureus* bacteraemia and endocarditis⁽¹⁷⁾. However, MRSA that resistant to vancomycin were reported.

OBJECTIVES:

The aim of this study was to determine the prevalence and antimicrobial susceptibilities of Methicillin Resistant

Staphylococcus aureus nasal carriage and wound infections among patients at Kosti teaching hospital, Kosti- Sudan.

PATIENTS AND METHODS:

Patients:

This study carried out at Kosti teaching hospital, Kosti – White Nile State -Sudan from March 2013 to December 2014. It is a prospective cross sectional descriptive study. Samples were collected from 232 patients (a nasal swab and a wound swab from each) at Kosti teaching hospital surgical units and diabetic foot clinic in Abdalla Mohammed Salih Specialized Centre. Inclusion criteria were presence of abscesses or wound with pus or exudates as sign of infection. All swabs were transported in Amies transport media to microbiology laboratory for investigations.

Isolation and identification:

All swabs were inoculated on blood agar and MacConkey agar media and incubated aerobically for 24 h at 35°C. The colonies then identified by using Gram staining technique, and conventional tests including catalase, coagulase. DNase tests and mannitol fermentation test. MRSA were screened and identified by cefoxitin disc diffusion and detection of PBP2' (MRSA-latex agglutination test). *S.aureus* ATCC 25923 was used as control species

Cefoxitin disc diffusion test

Susceptibility of 155 *S. aureus* isolates were tested to cefoxitin (30µg) by the (CLSI) agar disc diffusion method using Mueller-Hinton agar. All plates were incubated at 35°C for 24 h before reading the results. Isolates were considered as MRSA when the inhibition zone diameter was ≤ 22 mm.

MRSA- latex agglutination test:

S. aureus isolates also were tested by MRSA-latex agglutination test (Oxoid PBP2'). The test was performed according to the manufacturer instructions. For each strain, a sterile 5µl loop used to remove sufficient growth of *S. aureus* colonies grown on Mueller-Hinton agar to fill the internal diameter of the loop suspended in four drops of extraction reagent 1 into a microcentrifuge tube. The suspension was boiled for 3 minutes then microcentrifuge tube was allowed to cool to room temperature and one drop of extraction reagent 2 added and mixed well. The mixture was centrifuged at 1500 × g for 5 minutes. A 50 µl of the supernatant was added to each of the test circle and the control circle on a disposable test card and mixed with one drop of the test latex (anti-PBP 2a monoclonal antibody sensitized latex) and one drop of the negative control latex, respectively. The contents on the card then mixed and rocked for 3 minutes. The presence of agglutination concerned positive.

Antimicrobial susceptibility testing:

Susceptibility of 70 *S. aureus* isolates (35 of Methicillin Sensitive *Staphylococcus aureus* (MSSA) and 35 of MRSA) were tested to erythromycin (15µg), Penicillin(10 IU), ampicillin (10µg), clindamycin (2µg), vancomycin (30µg), amoxicillin-clavulanic acid (30µg), cotrimoxazole (25 µg), and cephalothin (30 µg), by the (CLSI) agar disc diffusion method using Mueller-Hinton agar . Overnight *S. aureus* cultures were adjusted to turbidity of 0.5 McFarland standards. The bacterial suspensions were spread on Mueller-Hinton agar. All plates were incubated at 35°C for 24 hours before reading the results. According to zones of inhibition isolates reported as sensitive or resistant.

RESULTS:

In this study 232 patients were enrolled in the study from whom nasal and wound swabs were collected for culture. The patients' age ranged from 5 to 80 years with mean 41.96 years, 142 (61.2%) were males and 90(38.8%) were females. Patients were distributed in Kosti teaching hospital surgical units and diabetic foot clinic in Abdalla Mohammed Salih Specialized Centre as follows: Outpatient surgical clinic 57 patients, Female surgical ward 25, Male surgical ward 52 and diabetic foot clinic in Abdalla Mohammed Salih Specialized Centre 98 patients. Culture results revealed that 125/232 (53.9%) of the patients were either colonized or/and infected with *S.aureus* from whom 155 *S. aureus* isolates were isolated. MSSA accounted for 110/155 (71%) and MRSA for 45/155 (29%).The most isolates of MRSA 32/45(71.1%) were hospital acquired.

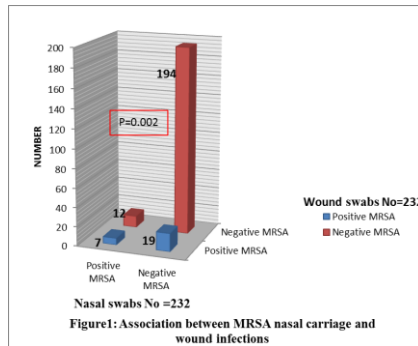
The prevalence of *S.aureus* nasal carriage was 84/232 (36.2%) and MRSA was 19/232 (8.2%). The highest high proportion of MRSA nasal carriage was noted in male surgical ward 7(13.5%), table 1 shows distribution of MSSA and MRSA nasal carriage and wound infections in surgical and diabetic foot clinic.

The prevalence of *S. aureus* in wound infection was 71/232 (30.6%) of which 26/232(11.2%) were MRSA. There was a significant relationship between MRSA wound infections and nasal carriage (Figure 1, P value =.002).

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Table 1: shows distribution of MSSA and MRSA nasal carriage and wound infections in surgical wards and units

Ward or unit	Total of tested patients	Nasal swabs No 232		Wound swabs No232	
		MSSA	MRSA	MSSA	MRSA
Outpatient surgical clinic	57	21/57 (36.8%)	4/57 (7.0%)	22/57 (38.6%)	7/57 (12.3%)
Female surgical ward	25	6/25 (24.0%)	2/25 (8.0%)	2/25 (8.0%)	3/25 (12.0%)
Male surgical ward	52	13/52 (25.0%)	7/52 (13.5%)	3/52 (5.8%)	6/52 (11.5%)
Diabetic foot clinic	98	25/98 (25.5%)	6/98 (6.1%)	18/98 (18.4%)	10/98 (10.2%)
Total	232	65/232 (28.0%)	19/232 (8.2%)	45/232 (19.4%)	26/232 (11.2%)



There was no effect of gender on distribution of MRSA in nasal carriage or wound infections as shown in table 2, where the P values were 0.481 and 0.296, respectively. No effect of age in distribution of MRSA (table 3). MRSA susceptibility to vancomycin was the highest one 94.3%, followed by clindamycin 88.6%, cotrimoxazole 40%, erythromycin 22.9 %, cephalothin 11.4 %, amoxicillin-clavulanic acid 2.9%, each of ampicillin and penicillin 0 % .Resistant to cotrimoxazole, erythromycin, cephalothin and amoxicillin-clavulanic acid was significantly associated with MRSA compared to MSSA ($P \leq 0.001$). Over All *S.aureus* isolates susceptibility to vancomycin was the highest one 97.1%, followed by clindamycin 92.9%, cotrimoxazole 61.4%,

cephalothin 54.3 %, erythromycin 52.9 %, amoxicillin-clavulanic acid 37.1%, each of ampicillin and penicillin 7.1%, (table 4).

Table 2: distribution of MRSA in nasal carriage and wound infections according to gender

Species	Nasal carriage		Wound infections	
	Females No 90	Males No 142	Females No 90	Males No 142
Non staph	58(64.4%)	90(63.4%)	59(65.6%)	102(71.8%)
MSSA	27(30%)	38(26.8%)	22(24.4%)	23(16.2%)
MRSA	5(5.6%)	14(9.8%)	9(10.%)	17(12%)
	P value = 0.481		P value = 0.296	

Table 3: distribution of MRSA in nasal carriage and wound infections according to age

Age groups (Total 232)	MRSA nasal carriage No 19	MRSA in Wounds No 26
≤ 40 years (No = 118)	8 (42.1%)	11(42.3%)
> 40 years (No =114)	11(57.9%)	15(57.7%)
	P value = 0.426	P value = 0.354

Table 4:Antibiotics Susceptibility Pattern of MSSA and MRSA

Antibiotic	MSSA		MRSA		Total susceptible		P values
	No	%	No	%	No	%	
Clindamycin	34	97.1	31	88.6	65	92.9	0.356
Erythromycin	29	82.9	8	22.9	37	52.9	P ≤ 0.001
Cotrimoxazole	29	82.9	14	40.0	43	61.4	P ≤ 0.001
Ampicillin	5	14.3	0	0	5	7.1	0.054
Penicillin	5	14.3	0	0	5	7.1	0.054
Amoxicillin-clavulanic acid	25	71.4	1	2.9	26	37.1	P ≤ 0.001
Vancomycin	35	100	33	94.3	68	97.1	0.493
Cephalothin	34	97.1	4	11.4	38	54.3	P ≤ 0.001
Total tested <i>S. aureus</i>	35	100	35	100	70	100	

DISCUSSION

In this study The prevalence of MRSA nasal carriage was 19/232 (8.2%),and the rate of MRSA among *S. aureus* nasal carriage was 19/84 (22.6%).The research in MRSA nasal carriage in Sudan is so limited that prevalence rates were not well known, it was reported in 1998 at Soba University Hospital (Khartoum, Sudan) that nasal carriage of *S. aureus* at time of admission of 414 patients was 98(24%) and among the

staff personnel screened nasal carriage rate was 26.8% , but MRSA was not reported. ⁽¹⁸⁾This may be because that MRSA was not emerged or was not common bacterium in Khartoum at that time. However, many studies from other countries showed high prevalence of MRSA nasal carriage. MRSA nasal carriage was reported in 0.2% to 2.8% of the United States population ⁽⁷⁾. In Dessie Referral Hospital in Ethiopia 12.7% of all healthcare-workers were identified as MRSA carriers. The rate of methicillin resistance among all *S. aureus* isolates was 44.1% (15/34) ⁽¹⁾. In Iran one study reported that on hospitalized patients nasal carriage of *S. aureus* were 38/100 (38%) and MRSA were 20/38 (52.6%). ⁽¹⁹⁾ Other study found among hospital personnel 64/300 (21.33%) were nasal carriers for *S. aureus*; 16/64 (25%) of the carriers were MRSA ⁽²⁰⁾.

In this study the prevalence of MRSA in wound infections was 26/232(11.2%) and the rate of MRSA was 26/71(36.6%) of isolated *S.aureus*. MRSA susceptibility to vancomycin was 94.3%, the result is comparable with that reported in three hospitals Khartoum Teaching Hospital, Khartoum North Teaching Hospital and Omdurman Teaching Hospital(Sudan), where MRSA were 15 / 163 (9.2%), and showed susceptibility to vancomycin ⁽²¹⁾. However, very high prevalence of MRSA in skin infections 78 / 223 (35.3%) also reported in Khartoum hospital (Sudan), with 9% resistant to vancomycin ⁽²²⁾. A very high percentage was reported in gastrointestinal tract (GIT) surgical ward in Ibn Sina hospital, Khartoum, Sudan 25/35 (71.4%) of isolated *S. aureus* from surgical samples were MRSA and resistance to vancomycin was 4% ⁽²³⁾

In this study there was a significant relationship between MRSA wound infections and nasal carriage (P value =.002), Croft group ⁽²⁴⁾ demonstrated that MRSA nasal colonization was a predictor of subsequent MRSA infection among trauma patients. Stevens and colleagues ⁽²⁵⁾ reported

that skin infections were more likely to develop in MRSA carriers. In this study the most isolates of MRSA 32/45(71.1%) were hospital acquired, this result is in agreement with that published by Lucet *etal.* ⁽²⁶⁾, and Cavalcanti, *etal.*⁽²⁷⁾ who observed a significant association between MRSA and hospitalized patients . However the result disagrees with those reported by Korn and his colleagues ⁽²⁸⁾ who observed no significant association between MRSA and hospitalization. It is possible that the patients had been hospitalized for an insufficient period of time for them to become contaminated with *S. aureus* or MRSA.

CONCLUSION:

There is a high prevalence of Methicillin Resistant *Staphylococcus aureus* nasal carriage and wound infections among patients at Kosti hospital. Most of the isolates were susceptible to vancomycin. However they were highly resistant to cotrimoxazole, erythromycin, cephalothin amoxicillin-clavulanic acid, ampicillin and penicillin.

RECOMMENDATIONS:

- The antibiotic susceptibility test tables have to be updated from time to time and from place to place to cope with microorganisms changing antibiotic resistant.
- Measures to improve hygiene like hands washing can possibly reduce the incidence of infection by MRSA among people
- Additional research and studies are needed to provide more information about MRSA infections and prevalence.

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