

# Investigation on Vancomycin Resistance (VRSA) among Methicillin Resistant *S. aureus* (MRSA) in Khartoum State, Sudan

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**Abstract Background:** *Staphylococcus aureus* is one of the most important and frequent cause of nosocomial infections worldwide. This study was carried out to investigate the prevalence of Vancomycin-Resistant *Staphylococcus aureus* and antibiotic sensitivity pattern in clinical isolates in Khartoum. **Methods:** One hundred and thirty three various samples were collected from some hospitals in Khartoum over a period of 5 months. The samples were cultured on bacteriological media for the isolation of *Staphylococcus aureus* using standard methods of isolation and identification of bacteria. The *Staphylococcus aureus* were tested for Methicillin susceptibility using 5 µg Oxacillin disc and Oxacillin E-test, with Resistance defined as an MIC of  $\geq 4\mu\text{g/ml}$ . **Results:** In this study all MRSA isolates displayed an Oxacillin MIC of  $\geq 256\mu\text{g/ml}$ . The MRSA strains were 41.0% while the Resistance to vancomycin was examined by vancomycin E-test, with resistance defined as an MIC of  $\geq 16\mu\text{g/ml}$ . In this study all VSSA isolates displayed vancomycin MIC of  $\leq 2\mu\text{g/ml}$  "except three intermediate resistant isolate MIC between 4-8 µg/ml". The percentage of the VISA strains was 12.0%. **Discussion:** The unprevalence of resistance to vancomycin may be related to the low usage of this antibiotic in this study area The majority MRSA isolates were multidrug-resistant (MDR) to different classes of antibiotics including; amoxicillin /clavulanic acid, ampicillin, tetracycline, erythromycin clindamycin and ciprofloxacin. Nitroforantoine exhibit no resistant pattern while 92% of MRSA isolates were susceptible to chloramphenicol. The unprevalence of resistance to Vancomycin may be related to the low usage of this antibiotic so Vancomycin might be used as a drug of choice for the treatment of MRSA infection.

**Keywords:** prevalence, MRSA, VRSA, Khartoum, Sudan

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## 1. Introduction

*Staphylococcus aureus* is one of the most important a frequent cause of nosocomial infections worldwide [1]. Emergence of Methicillin-resistant *S. aureus* strain (MRSA) in 1961 made staphylococcal infections as a major therapeutic challenge [2]. Methicillin Sensitive *S. aureus* (MSSA) and Methicillin Resistant *S. aureus* (MRSA). MRSA strains have been associated with a or hospital acquired infections world over and have also emerged as an important cause of community acquired infections [3]. MRSA infections are a problem across the whole health economy, and have been shown to be associated with a poorer outcome and higher mortality

than similar infections caused by Methicillin-sensitive strains of *S. aureus* (MSSA).

Many of these isolates are becoming multidrug resistant and are susceptible only to glycopeptides antibiotics such as Vancomycin [4] Vancomycin-resistant *Staphylococcus aureus* (VRSA) infections, which are always methicillin-resistant, are a rare but serious public health concern [5]. In 1988, vancomycin-resistant *enterococci* (VRE) were first reported. These organisms quickly became endemic in hospital intensive care units. In vitro conjugative transfer of the *vanA* gene from *enterococci* to *S. aureus* was demonstrated in 1992. However, it was not until 1996, when the first case of vancomycin intermediate *S. aureus* (VISA; MIC, 8–16 µg/mL) was detected, that decreased susceptibility to vancomycin became a clinical reality. None of the VISA strains identified contained the *vanA* gene or any of the other vancomycin-resistant genes found

in VRE. In vitro studies suggest that, with prolonged vancomycin exposure, VISA organisms produce a thickened cell wall matrix, limiting drug penetration [6,7]. Recently, however, due to the use of Vancomycin for treatment of hospital-based infections, there has been growing resistance to Vancomycin by species such as *Enterococcus* [8]. The emergence of Vancomycin resistance in *Enterococcus* gave rise to the possibility of horizontal transmission of resistance elements to *S. aureus*. In the laboratory, Vancomycin resistance has been transferred from *Enterococcus faecalis* to *S. aureus* [9]. The objective of our study was to determine the prevalence of Vancomycin resistance *Staphylococcus aureus* (VRSA) among Methicillin resistant *S. aureus* (MRSA) in Khartoum State.

## 2. Methods

This cross sectional study was conducted during the period of March to October 2015. Swabs of wound, skin (axilla, groin), sputum, ear, and throat, also urine, blood and body fluids and sputum specimens were collected. Patients in Intensive Care Unit (ICU) and wards, nasal swabs were collected from different Khartoum hospitals after obtaining consent. Patients were aged between days to 84 years from both sexes. Mannitol Salt Agar, Müeller Hinton agar, Nutrient agar, Nutrient broth and DNAase Agar media were prepared in laboratory depending on the manufacturers' instruction. *S. aureus* were identified by fermentation of mannitol, colony morphology, Gram stain, Catalase test and sub-cultured on nutrient agar for coagulase test and deoxyribonuclease (DNase) test by conventional methods. All MRSA strains were determined by disk diffusion method using and confirmed by MIC (bioMerieux) method using the Oxacillin E-test then by Vancomycin E-test for VRSA strains according to National Committee for Clinical Laboratory Standards [10]. The following antibiotic disks were tested: Oxacillin, (OX 5µg), Vancomycin (VA 30µg), Ampicillin/Cavulinic acid (AMC 10µg), Amoxicillin (AMX 10µg), Tetracyclin (TE 30µg), Erythromycin (E 15µg), Gentamycin (GEN

10µg), Ciprofloxacin (CIP 5µg), Clindamycin (CD 2µg), Chloramphenicol (C 30µg) Ceftriaxone (CTR 30µg), Trimethoprim-sulphanimide (COT 30µg, Nitrofurantoin (NIT 300µg) and Meropenem (MEM 10 µg). Potential anti-MRSA synergy was measured by fractional inhibitory concentration indices (FICI) This could be calculated as: The FIC of drug 'A', given by  $FICA = MICA$  combination / MICA alone while The FIC of drug 'B', given by  $FICB = MICB$  combination /MICB alone The FIC index of the combination in each tube is given by the sum of the FICs for each of the drugs present in the tube:  $FIC\ index = FICA + FICB$  [11].

### 2.1. Statistical Analysis

Statistical analyses were conducted using SPSS (version 20; SPSS Inc., Chicago, IL) software. Descriptive analyses of percentages of categorical variables were reported using chi square  $\chi^2$ . p value of < 0.05 denoted a statistically significant difference in all statistical comparisons.

### 2.2. Ethical Clearance

This study was approved by ethical clearance committee, Department of Microbiology- Tropical Medicine Research Institute., Khartoum, Sudan.

## 3. Results

Out of the 135 bacteria isolated from clinical specimens, 79(58.5%) were identified to be *Staphylococcus* species. Only 61 (97.2%) were Identified biochemically to be *S. aureus*, out of this 36(59%) Methicillin Sensitive *S. aureus* (MSSA) and 25(41%) Methicillin Resistant *S. aureus* (MRSA), Figure 1, Figure 3. MRSA were collected from patients in ICU units and wards from different Khartoum hospitals. Resistance to Methicillin was determined by two methods: Agar diffusion test to detect oxacillin ( $\leq 10$  mm) was considered resistant (3.9) and E-test for determination of Oxacillin Minimum Inhibitory Concentration (MIC).

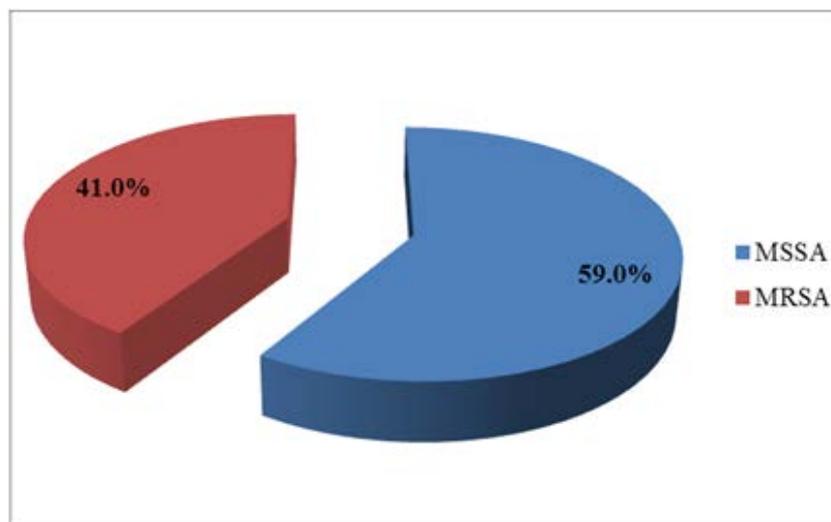


Figure 1. Distribution of *S. aureus* isolates (MRSA/MSSA)

The resistance defined as an MIC of  $\geq 4$  µg/ml (10). In this study all MRSA isolates displayed an Oxacillin MIC of

$\geq 256$ µg/ml. Resistance to vancomycin was determined by two methods: Agar diffusion test to detect vancomycin

resistance ( $\leq 9$  mm) was considered resistant (12) and E-test for determination of vancomycin MIC. The resistance defined as an MIC  $\geq 16$   $\mu\text{g/ml}$  (5,10). In this study all VSSA isolates displayed vancomycin MIC of  $< 2\mu\text{g/ml}$  "except three intermediate resistant isolates MIC between 4-8  $\mu\text{g/ml}$ " The percentage of the VISA was 12.0%, Table 1, Figure 4. We found a VISA strain in a patient who had underlying conditions including immunosuppressive therapy, long-time hospitalization, and serious disease. The majority of the MRSA isolates were resistant to multiple antimicrobial agents, in this study out of 25 MRSA strains

isolated, exhibited high resistance to different classes of antibiotics including; Amoxicillin/Clavulanic acid and Ampicillin (100%), Ceftriaxone and Erythromycin (80%), Meropenem (60%), Ciprofloxacin, Clindamycin and Trimetoprim-Sulphanomide (56%), and Tetracycline (44%). Nitroforantoie exhibit no resistant pattern (100%) while 92% of MRSA isolates were susceptible to Chloramphenicol Table 2. No synergistic effects were detected from the above mentioned combined antibiotics according to FICI.

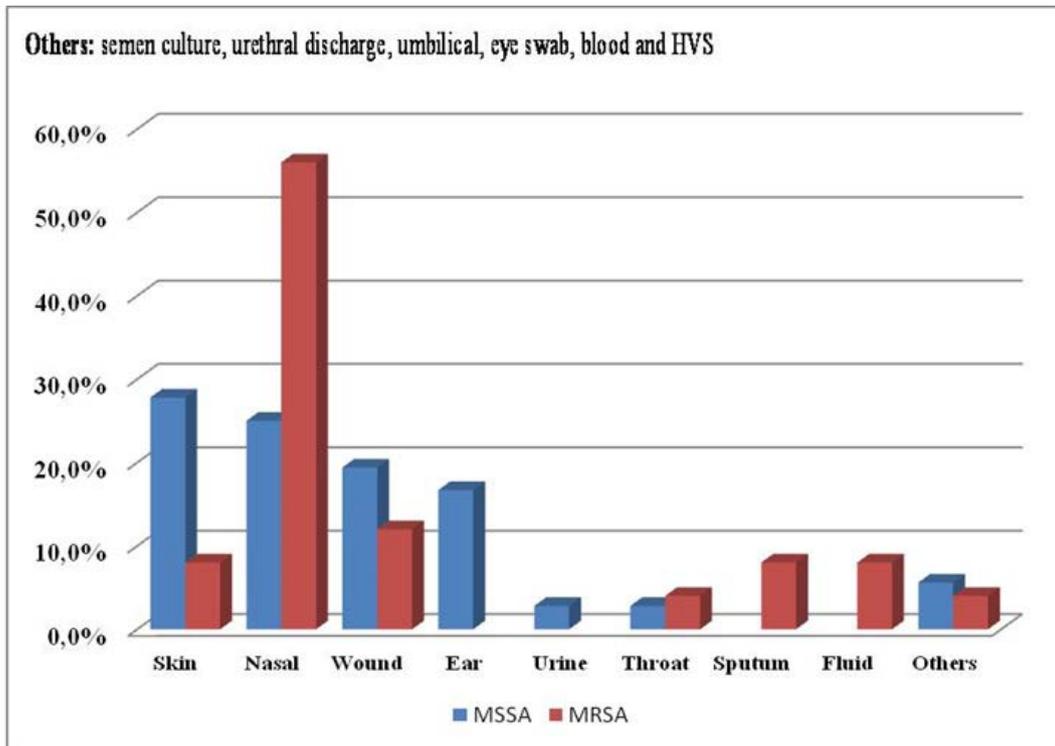


Figure 2. prevalence of MRSA/MSSA strains obtained from different clinical specimens in Khartoum state

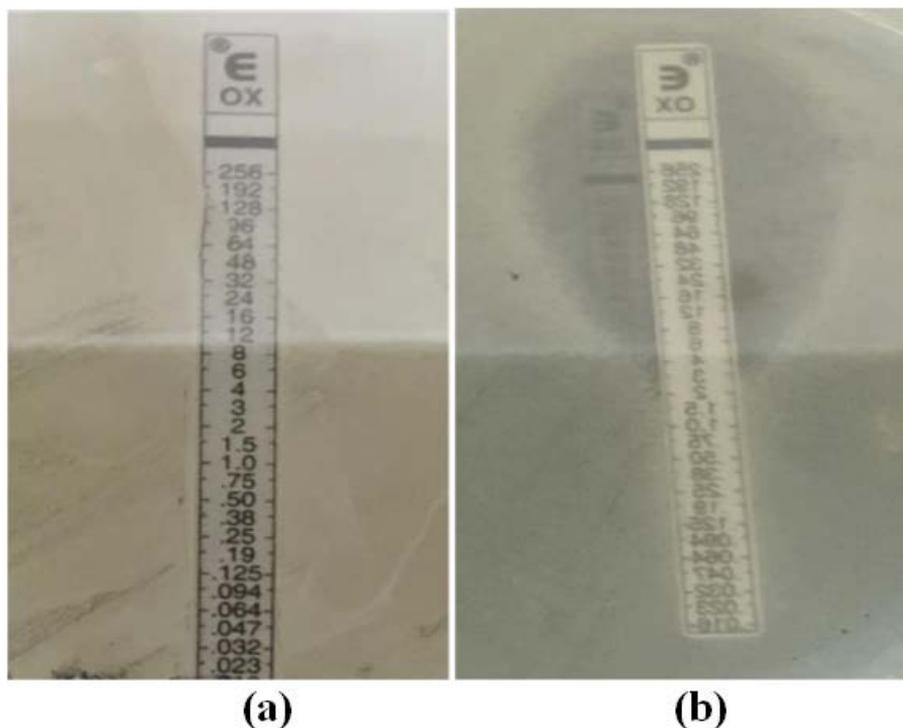


Figure 3. E-test technique for MRSA/MSSA strains (a) MRSA strain with MIC  $> 256\mu\text{g/ml}$  and (b) MSSA strain with MIC =  $2\mu\text{g/ml}$

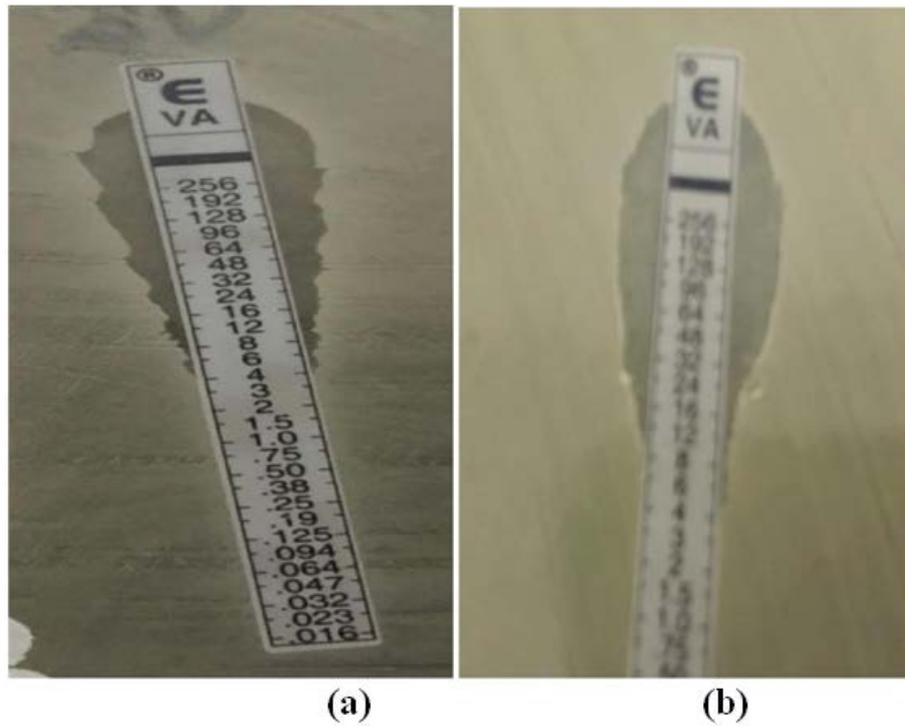


Figure 4. VISA strains by E-test method: (a) MIC =4 µg/ml, (b) MIC= 8 µg/ml

Table 1. Prevalence of VISA/VSSA obtained from clinical specimens according to E-test method (BiomMérieux) of vancomycin

Bacterial isolates	Resistant pattern to vancomycin	
	No.	%
VISA	03	4.9
VRSA	00	00
VSSA	22	88.0
Total	25	100

VRSA: ≥ 16 µg/ml

VISA: 4-8 µg/ml

VSSA: ≤4 µg/ml

Table 2. Antimicrobial resistance pattern of MRSA obtained from clinical specimens

Antibiotics	Antibiotics Resistance pattern					
	No.			%		
	S	I	R	S	I	R
ampicillin (10 µg)	--	--	25	--	--	100
amoxicillin clavulanic acid (10 µg)	--	--	25	--	--	100
ceftriaxone (30 µg)	4	1	20	16.0	4.0	80.0
clindamycin (2 µg)	11	--	14	44.0	--	56.0
chloramphenicol (30 µg)	23	--	2	92.0	--	8.00
ciprofloxacin (5 µg)	8	3	14	32.0	12.0	56.0
erythromycin (15 µg)	3	2	20	12.0	8.00	80.0
gentamycin (10 µg)	10	7	8	40.0	28.0	32.0
meropenem (10 µg)	4	6	15	16.0	24.0	60.0
nitroforantoie (300 µg)	25	--	--	100	--	--
tetracycline (30 µg)	9	5	11	36.0	20.0	44.0
trimetoprim-sulphanomide (30 µg)	11	--	14	44.0	--	56.0
vancomycin (30 µg)	22	3	--	88.0	--	12

S: susceptible

I: intermediate

R: resistant

### 4. Discussion

In our study Oxacillin disk could not detect all MRSA isolates but Oxacillin MIC test had determined these MRSA isolates. Our result is similar to a study conducted in Iran [13]. It showed that the sensitivity of Cefoxitin

disk and Oxacillin MIC test equal to PCR. Disc diffusion method of Vancomycin did not differentiate between VISA and VSSA strains. So the result is not considered unless the MIC of Vancomycin is used according to NCCLS. Besides those MRSA isolates, Coagulase Negative Staphylococcus (CoNS) was also isolated from clinical specimens in two patients. One of them was found to be methicillin and vancomycin resistant and the other

was methicillin and vancomycin susceptible. Methicillin resistant coagulase negative isolate may be a result of the horizontal transfer of *mecA* gene between MRSA and coagulase negative staphylococcal species. In our study, all isolates 'except three intermediate resistant isolates in which MIC values between 4-8 µg/ml' were susceptible to vancomycin. They displayed MICs of < 2 µg/ml). The unprevalence of resistance to vancomycin may be related to the low usage of this antibiotic in the study area. Also our result substantiated the result of the study carried out in Khartoum [14]. Antibiotics resistance has become a serious public health concern with economic and social implications throughout the world, being community acquired infections like Streptococcal infections, pneumonia, typhoid fever or hospital acquired infections due to MRSA, VRE, VISA or extended spectrum β -lactamase (ESBL) enzyme producing Gram -ve bacteria.

These infections lead to higher rates of hospitalization, longer hospital stay, and increased cost of treatment and thus increased economic burden on the community [15]. MRSA strains were sensitive to Nitroforantoie, Chloramphenicol (100%), (92%) respectively while they were highly resistant to antibiotics including; amoxicillin /clavulanic acid and ampicillin (100%), ceftriaxone and erythromycin (80%), meropenem (60%), ciprofloxacin, clindamycin and trimetoprim- sulphonomide (56%), and tetracycline (44%). Our results go with the results of a study conducted in Sudan [4] that revealed a cross-resistance of MRSA strains. The rapid proliferation of antibiotic-resistant pathogens has spurred the use of drug combinations to maintain clinical efficacy and combat the evolution of resistance. Drug pairs can interact synergistically or antagonistically, yielding inhibitory effects larger or smaller than expected from the drugs' individual potencies. The inhibitory effect of two drugs in combination can be larger or smaller than expected from their individual effects, corresponding to synergistic or antagonistic interactions between the drugs respectively. Synergistic interactions are usually thought of as advantageous since, for a given amount of drug. They inhibit the growth of drug-sensitive pathogens more effectively. However, in vitro studies have suggested that, for the same level of inhibition, more synergistic drug pairs may foster antibiotic resistance. Antagonistic drug combinations, on the other hand, are less effective at inhibiting drug-sensitive pathogens, but can reduce and even invert the selective advantage of single-drug resistant mutants, causing selection against resistance [16]. While this is so, we recommend more combinations of different antibiotics to solve this resistant problem.

## 5. Conclusion

In conclusion, *S. aureus* is highly prevalent in Khartoum hospitals, Sudan. MRSA is highly prevalent among populations of *S. aureus* isolated from different clinical specimens in different hospitals in Khartoum State, Sudan, with most of the MRSA isolates being from ICU patients.

The unprevalence of resistance to vancomycin may be related to the low usage of this antibiotic in the study area where it is used as a drug of choice for the treatment of MRSA infection.

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## Competing Interests

The authors declare that they have no competing interests.

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