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RESEARCH ARTICLE

VANCOMYCIN RESISTANCE AMONG METHICILLIN RESISTANT *Staphylococcus aureus* ISOLATES IN KHARTOUM-SUDAN

***Mohammed Nafi and Waleed Eldaif**

Department of Microbiology, AL-Neelain University, Faculty of Medical Laboratory Sciences, P.O. Box: 12702, Cod 11121, Khartoum – Sudan

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ABSTRACT

Background: Glycopeptides such as vancomycin are frequently the antibiotics of choice for the treatment of infections caused by methicillin resistant *Staphylococcus aureus*. For the last 10 years incidence of vancomycin intermediate *S. aureus* and vancomycin resistant *S. aureus* has been increasing in various parts of the world
Objective: The aim of this study was to determine the prevalence of vancomycin intermediate *S. aureus* and vancomycin resistant *S. aureus* and their antimicrobial susceptibility pattern among hospital and community acquired Methicillin Resistant *Staphylococcus aureus* isolates.
Methods: This is a cross sectional study. *Staphylococcus aureus* strains were isolated and identified from patients suffering from skin and wound infections using conventional microbiology techniques. Methicillin resistant strains were investigated by detection of *mec*A gene using PCR. Strains were also tested for antimicrobial resistance using disc diffusion technique and vancomycin resistance using E test.
Results: Out of 223 *S. aureus* strains 35.3% were found to be methicillin resistant. 37.2% out of 78 MRSA strains were community acquired; while 62.8% out of 78 MRSA strains were hospital acquired. Out of 78MRSA strains 9% were found to be vancomycin resistant and 28.2% of strains of MRSA have shown to be vancomycin intermediate strains.

INTRODUCTION

Staphylococcus aureus has long been recognized as a major pathogen of hospital acquired infections. Over the last decade, methicillin resistant *S. aureus* (MRSA) strains have become endemic in hospitals worldwide. In addition, it is now incipient community pathogen in many geographical regions [(1)]. MRSA is important because, in addition to being methicillin resistant, most strains are also resistant to other β-lactam antibiotic, with the exception of glycopeptide antibiotics such as vancomycin [(2)]. Vancomycin has served as the cornerstone of therapy for MRSA infections for 50 years [(3)]. The growing prevalence of MRSA has increased the use of the glycopeptide antibiotic vancomycin over the past 3 decades [(4)]. As a consequence, selective pressure was established that eventually lead to the emergence of strains of *S. aureus* with decreased susceptibility to vancomycin and other glycopeptides [(5)]. In 1997, the first strain of vancomycin-intermediate *S. aureus* (VISA) was reported from Japan [(6)]. Shortly after, two additional cases were reported from the United States. However, first clinical isolate of VRSA was reported from United States in 2002 [(7)]. Subsequent isolation of VISA and VRSA isolates from United States and other countries including Brazil [(8)], France [(9)], United Kingdom [(10)], Germany [(11)], India [(12;13)] and Belgium [(14)] has confirmed that emergence of these strains is a global issue. The aim of current study was to determine the prevalence of vancomycin intermediate *S. aureus* and vancomycin resistant *S. aureus* and their antimicrobial susceptibility pattern among hospital and community acquired Methicillin Resistant *Staphylococcus aureus* isolates.

***Corresponding author:** Mohammed Nafi*
Department of Microbiology, AL-Neelain University, Faculty of Medical Laboratory Sciences, P.O. Box: 12702, Cod 11121, Khartoum – Sudan

METHODS

Study population

Between September 2010 and September 2011, 400 patients suffering from various skin and wound infections, 150 (37.5%) with no history of hospitalization or any contact with a hospital during the past twelve months and 250 (62.5%) hospitalized patients, were enrolled in this study.

Collection of bacterial isolates

A total of 223 strains of *S. aureus* were isolated by inoculation of pus materials on Mannitol slat agar after aerobic overnight incubation at 37°C. The isolates were identified based on colony morphology, Gram's stain, catalase, coagulase, and DNase according to NCCLS [(15)]. *S. aureus* strain ATCC 25923 was included as control strain. The isolates were sub-cultured onto nutrient agar and incubated at 37°C for approximately 18 hours prior to testing.

Extraction of bacterial DNA

DNA extraction was done using bacteria DNA preparation kit *Jena Bioscience, Germany*. The extracted DNA was stored at -20°C.

PCR procedure for *mecA*-gene

Detection of staphylococcal *mec*A gene was performed by PCR, as described by Oliveira and de Lencastre [(16)]. The primer sequences were as follows: forward primer TCCAGATTACAACTTCACCAGG; reverse primer CCACTTCATATCTTGTAACG. The reaction was performed in a 50 µL volume using *Jena Bioscience, Germany* master

mix of thermostable DNA polymerase for PCR. Thermo-cycling conditions in a Techne thermocycler *(Bibby Scientific Limited, Beacon Road, Stone, Staffordshire ST15 0SA, UK)* were as follows: 94°C for 2 min, followed by 35 cycles of 94°C for 30 sec, 68°C for 30 sec and 72°C for 30 sec, with a final extension at 72°C for 5 min. The amplified products (5 µl) were separated by electrophoresis on 1.5% agarose gel and visualized by staining with ethidium bromide using UV gel documentation system. A 162-bp PCR product was amplified with the above *mecA*-gene specific primers.

Determination of Vancomycin Minimum Inhibitory Concentration (MIC) using E test

Using a sterile wire loop, 3 - 5 well isolated colonies of similar appearance to the test organism were emulsified. In a good light the turbidity of the suspension was matched with the turbidity of the standard of barium chloride. A plate of Mueller Hinton agar containing 2% NaCl was inoculated with the suspension using a sterile swab. The swab was evenly streaked over the surface of the medium in three directions, and the plate was rotated approximately 60° to ensure even distribution. The surface of the agar was allowed 5 minutes to dry. The e test vancomycin strip was placed using sterile forceps on the inoculated plate. The plate was incubated aerobically at 37°C for overnight. Vancomycin sensitive defined as MIC ≤ 4 µg/mL, vancomycin intermediate is defined as MIC 6 - 12 µg/mL, while vancomycin resistance is defined as MIC >16 µg/mL [17;18].

Antibiotic susceptibility testing of VISA and VRSA

The antibiotic susceptibility pattern of VISA and VRSA strains was determined by the modified Kirby Bauer disc diffusion method on Muller Hinton agar using the criteria of standard zone sizes of inhibition to define sensitivity or resistance to different antimicrobials according to NCCLS [15]. *S. aureus* ATCC 29213 was used as reference strain for the standardization of antibiotic susceptibility testing. The antibiotics used were Amoxyclav (30 mcg), Cefepime (30 mcg), Ciprofloxacin (5 mcg), Gentamicin (10 mcg), Tetracycline (30 mcg), Rifampicin (5 mcg), Vancomycin (30 mcg), Erythromycin (15 mcg), Clindamycin (2 mcg) and Imipenem (10 mcg).

Ethical Clearance

Approval was taken by the ethical review board of Faculty of Medical Laboratory Sciences Al-Neelain University. Verbal consent was taken from each study unit.

RESULTS

S. aureus strains were isolated from 55.8% (223/400) patients. 80% (120/250) of strains were community acquired *S. aureus*, while 41.2% (103/150) of strains were hospital acquired *S. aureus*. 35% (78/223) of *S. aureus* strains were methicillin resistant due to presence of *mecA*-gene. 29 (37.2%) out of 78 MRSA strains were community acquired; while 49 (62.8%) out of 78 MRSA strains were hospital acquired. VISA represented 7.7% and 20.5% among CA-MRSA and HA-MRSA respectively; while VRSA represented 2.6%, and 6.4% among CA-MRSA and HA-MRSA respectively.

Distribution of VSSA, VISA and VRSA among 78 isolates of HA-MRSA and CA-MRSA

	CA-MRSA No. (%)	HA-MRSA No. (%)	Total No. (%)
VSSA	21 (26.9)	28 (35.9)	49 (62.8)
VISA	06 (07.7)	16 (20.5)	22 (28.2)
VRSA	02 (02.6)	05 (06.4)	07 (09.0)
Total	29 (37.2)	49 (62.8)	78 (100.0)

MIC for all CA-MRSA and HA-MRSA isolates was above 1.5µg/ml, while MIC for only one HA-MRSA strain was 32µg/ml.

Distribution of vancomycin MICs for 78 isolates of HA-MRSA and CA-MRSA as determined by the E test:

MIC (µg/ml)	CA-MRSA No. (%)	HA-MRSA No. (%)	Total No. (%)
1	00 (00.0)	00 (00.0)	00 (00.0)
1.5	00 (00.0)	00 (00.0)	00 (00.0)
2	04 (05.1)	06 (07.7)	10 (12.8)
3	07 (09.0)	08 (10.2)	15 (19.2)
4	10 (12.8)	14 (18.0)	24 (30.8)
6	04 (05.1)	07 (09.0)	11 (14.1)
8	02 (02.6)	05 (06.4)	07 (09.0)
12	00 (00.0)	04 (05.1)	04 (05.1)
16	02 (02.6)	02 (02.5)	04 (05.1)
24	00 (00.0)	02 (02.6)	02 (02.6)
32	00 (00.0)	01 (01.3)	01 (01.3)
Total	29 (37.2)	49 (62.8)	78 (100)

VRSA strains showed 100% resistant to amoxyclav, cefepime, gentamicin and clindamycin; While multi-drugs resistant strains (MDRS) (i.e. strains resistant to 4 or more antibiotics) were represented 51%, 81.8% and 100% among VSSA, VISA and VRSA respectively.

Antimicrobial resistance among VSSA, VISA, and VRSA:

	VSSA No. (%)	VISA No. (%)	VRSA No. (%)	Total No. (%)
Amoxyclav	25 (51.0)	13 (59.1)	07 (100)	45 (57.7)
Cefepime	49 (100)	22 (100)	07 (100)	78 (100)
Ciprofloxacin	18 (36.7)	15 (68.2)	04 (57.1)	37 (47.4)
Gentamicin	04 (08.2)	12 (54.6)	07 (100)	23 (29.5)
Tetracycline	17 (34.6)	17 (77.3)	05 (71.4)	39 (50.0)
Rifampin	03 (06.1)	05 (22.7)	03 (42.9)	11 (14.1)
Erythromycin	24 (49.0)	13 (59.1)	06 (85.7)	43 (55.1)
Clindamycin	35 (71.4)	19 (86.4)	07 (100)	61 (78.2)
Meropenem	00 (0.0)	00 (00.0)	03 (42.9)	03 (03.8)
MDRS	25 (51.0)	18 (81.8)	07 (100)	50 (64.1)
Total	49 (62.8)	22 (28.2)	07 (09.0)	78 (100)

DISCUSSION

Infections caused by MRSA have been associated with high morbidity and mortality rates [19]. Vancomycin has been the most reliable and available therapeutic agent against serious infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) but unfortunately, decreases in vancomycin susceptibility of *S. aureus* and isolation of VISA and VRSA were recently reported from many countries [20-22;23]. Until now, no reports from Sudan were published about VISA and VRSA. In this study 49/78 (62.8%) of MRSA isolates with vancomycin MICs ≤ 4 (vancomycin sensitive), 22/78 (28.2%) of MRSA isolates with vancomycin MICs 6 - 12 (vancomycin intermediate); while 7/78 (9%) of MRSA isolates with vancomycin MICs ≥ 16 (vancomycin resistant). CA-MRSA isolates were more sensitive to vancomycin than HA-MRSA isolates. In MICs ≤ 4: 72.4% CA-MRSA isolates versus 57.1% HA-MRSA strains; In MICs 4 - 6: 20.6% CA-MRSA isolates versus 32.7% HA-MRSA strains; In MICs ≥ 16: 7% CA-MRSA isolates versus 10.2% HA-MRSA isolates. These results agree with Tenover *et, al.,* (2001) [24] reported that there is emergence of strains of *S. aureus* with decreased susceptibility to vancomycin and other glycopeptides. These results also agree with Howden *et al.,* (2004) [25], Moise-Broder *et, al.,* (2004) [26] and Sakoulas *et, al.,* (2004) [27] reported a high rate of treatment failure with vancomycin in patients with MRSA infections. On the other hand, VISA and VRSA tend to be multidrug resistant against a large number of currently available antimicrobial agents, compromising treatment options and increasing the likelihood of inadequate antimicrobial therapy and increase in morbidity and mortality [19]. In the present study, isolated VRSA and VISA showed resistance to a wide range of different antimicrobial agents. So newer therapeutic modalities are urgently needed. Isolation of VRSA and VISA in Sudan calls for the

implementation of a wide surveillance system to monitor presence of these strains in other regions in Sudan.

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