Emergence of Vancomycin Resistant and Methcillin Resistant *Staphylococus aureus* in Patients with Different Clinical Manifestations in Khartoum State, Sudan

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Abstract: *Staphylococcus aureus* (*Staph. aureus*), a major cause of potentially life-threatening infections acquired in healthcare and community settings, has developed resistance to most classes of antimicrobial agents as determined by the dramatic increase The present study aimed to determine the prevalence of MRSA, and VRSA in patients with different clinical manifestations in Khartoum state. The study population (n, 426) were males and females with different age categories, suffering either from wound infections (105), ear infections (121), or UTI (101), in addition to nasal carriers of medical staff (100). Cultures, Gram staining and other biochemical tests were performed for conventional identification. Modified Kirbry-Bauer disk diffusion method was applied and DNA was extracted from MRSA and VRSA isolates and PCR was then performed for amplification of arc, mecA, VanA and VanB genes. The results confirmed the existence of *Staph. aureus* in 49/426 (11.5%) cases among which MRSA were isolated from 34/49 (69.4%) when modified Kirbry-Bauer disk diffusion method was applied. Ten out of these 34 MRSA were confirmed as VRSA by cultures on BHI agar containing 6µg/ml vancomycin according to NCCLS criteria. PCR revealed that out of the 34 MRSA isolates, 26 were mecA positive (76.5%) while 8 (23.5%) were arcC positive. No vanA or VanB genes were detected. Molecular method confirmed the results for MRSA through the presence of either arcC or mecA genes while it failed to approve the occurrence of VRSA since neither VanA or VanB genes were detected. Thus, VRSA may be attributed to other factors.
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1. Introduction

Over the last decade, methicillin resistant *Staph. aureus* (MRSA) strains have become endemic in hospitals worldwide. In addition, it is now incipient community pathogen in many geographical regions (Lowy, 1998). Glycopeptides such as vancomycin (come from *Streptomyces orintalis*) are frequently the antibiotics of choice for the treatment of infections caused by MRSA (Tiwari and Sen, 2006). Vancomycin continues to be used as a first-line antimicrobial agent for the treatment of infection with MRSA. Because alternative treatments are limited, development of resistance to vancomycin can make treatment of MRSA infections increasingly difficult (Tiwari and Sen, 2006).

Although vancomycin resistance was first reported for enterococci in 1988, the first clinical isolate of high-level vancomycin-resistant *Staph. aureus* (VRSA) was not isolated until June 2002 (Michigan, MIC = 1024 µg/ml) (CDC.2002 ; Weigel *et al.*,2003). This was closely followed by another VRSA isolated in Pennsylvania in September 2002 (MIC = 32 µg/ml) (CDC.2002). These isolates were associated with chronic skin ulcers, and vancomycin resistance was mediated by Tn1546-like elements, most likely acquired from co-infecting strains of vancomycin-resistant enterococci (VRE). Genetic exchange of antimicrobial resistance determinants among enterococci and Staphylococci is well documented (Francia, and Clewell, 2002; Nobel *et al.*, 1992). The resistance genes are typically found on conjugative plasmids or transposons. One requirement for conjugative transfer of mobile genetic elements are cell-to-cell contact between donor and recipient. To facilitate this contact, enterococci have highly-evolved conjugative systems that are responsible for the dissemination of antimicrobial resistance and virulence factors (Stewart and Costerton, 2001).

Methicillin resistant *Staph. aureus* has an additional genetic material known as the mecDNA, not found in methicillin sensitive strains, which encodes penicillin binding protein (PBP2) having a reduced affinity for beta lactam antibiotics. The mecA is found as a part of a mobile genetic element found in

all MRSA strains designated as SCCmec (Staphylococcal cassette chromosome mec). SCCs have been found to show great geographical variation which makes cassette chromosome typing essential for complete characterization of MRSA. (Jeshina and Surekha, 2009).

2. Materials and Methods

This study was approved by the National Ethics Committee, Ministry of Health, Sudan. Written consent was obtained from every patient before being enrolled in the study.

This study is a descriptive cross sectional laboratory based study It was conducted between April 2010 and May2011. In different hospitals in Khartoum state (Military Hospital, AL-Rebat University Hospital, Khartoum Teaching Hospital and Khartoum North Teaching Hospital.) Four hundred and twenty six (n= 426) different clinical samples were collected from eligible patients attending the above mentioned clinical centers during the period from April 2010 to May 2011.Clinical samples collected for this study included 101urine samples, 105 wound swabs, 121 ear swabs and 100 swabs from nasal discharges.

Cultures

The samples were inoculated onto blood agar (Hi-Media, India) and MacConkey agar (Hi-Media, India). Cultures were incubated over night aerobically at 37º C. CLED agar (Hi-Media, India) was also used for urine samples. Gram staining and other biochemical tests were also performed according to (Mackie and McCartney *et al*., 2002) for conventional identification.

All isolates were identified as *Staph. aureus* based on morphology, positive catalase, positive coagulase and fermentation mannitol.

Antibiotic Susceptibility Test

Antibiotic susceptibility test was done for all isolates of *Staphyloccus aureus* using Kirbry-Bauer disk diffusion method. Discs of Vancomycin (VA)30 µg, from (Himedia, India and Abteck, UK.) and mecthicillin (ME) 5 µg from (Himedia, India) were used.

DNA Extraction

Phenol choloroform method was used for DNA Extraction as described by (Aziz et al., 2004)

Primers

The primers used in the study (Table1) were purchased from Metabion International AG-Germany.

Table 1. Sequences of Primers used in the PCR

Primer specifity	primers	Primer pair Sequence (5' ---3')	Product size (bp)	Ann. temp	Reference
Carbamate kinase (arcC)	arcC-Up	5'TTGATTCACCAGCGCGTATTGTC3'	456	62 C^0	(Enright *et al*., 2000)
	arcC-Dn	5' AGGTATCTGCTTCAATCAGCG3'			
mecA	mecA F	5'GTAGAAATGACTGAACGTCCGATGA3'	310	55 C^0	(Hare and Malay, 2006)
	mecA R	5'CCAATTCCACATTGTTTCGGTCTAA3'			
Van A	vanA F	5'CATGAATAGAATAAAAGTTGCAATA3'	1030	64 C^0	(Hare and Malay, 2006).
	vanA R	5'CCCCTTTAACGCTAATACGACGATCAA 3			
Van B	van B F	5'GTGACAAACCGGAGGCGAGGA 3'	433	59 C^0	(Hare and Malay, 2006)
	vanB R	5'CCGCCATCCTCCTGCAAAAAA 3'			

PCR

A 25µl reaction was prepared according to Eisenach et al, (1990) for each of the genes (arcC, mecA and vanA,vanB Genes) which contained 2.5µl 10x PCR buffer (PROMEGA) 2.5 µl MgCl2(25mM)(promega), 1µl from dNTP mix (10mM) (VIVANTIS-Malisya),0.2 µl of each primer (Metabion-Germany),0.5µl Taq polymerase (5U/µl) (PROMEGA) and 17.1 µl distilled water. 1µl template DNA was added separately to each reaction tube with a final volume of 25µl/ Reaction.

PCR product was visualized on 2% agarose gel and band size as compared to DNA marker (100 bp). A positive results of *Staphylococcus aureus* will

produce a band of 456 bp for arcC, 310 bp for mecA, 1030 for Van A and 433 for Van B genes.

3. Results

A total of four hundred and twenty six patients (n = 426) with symptoms of wound infection, urinary tract infection and ear infection as well as nasal smears from medical staff (nurses and lab technicians), were included in this study, the frequency of males were 211(49.5%) while 215(50.5%) were females, All candidates were classified into three age groups; age group one less than 10 years old with moderate frequency of 93(21.8%), age group two (11 – 49 years old) with the highest frequency of 273(64.1%) and age group three (more than 50 years old) with the lowest frequency of 60 (14.1%), this study confirmed clearly the existent of *Staph. aureus* in 49/426 (11.5%) cases. The Kirby-Bauuer disk diffusion method indicated that MRSA were isolated from 34/49 (69.4%), while VRSA were also detected among enrolled subjects when referring to NCCL and CDC criteria with a significant frequency 10/49 (20.4%).

PCR Results

arcC gene was detected in 8/34 (24%) strains as shown in figure 1. Regarding mecA gene, 26/34 (76%) of isolates revealed a band of 310 bp similar to the positive required size (Fig. 2). However, both VanA and VanB genes were negative in PCR.

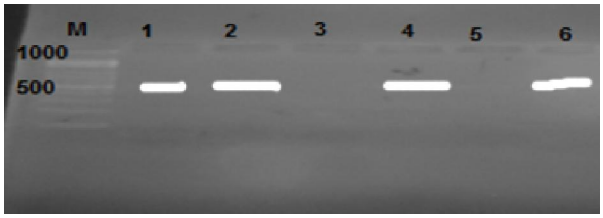

Figure 1. PCR result of arcCgene on 2% agarose gel: lane M= 100 bp DNA marker; 1= positive control ; 2, 4, 6 =patients sample showing positive arcCgene (456 bp); 3,5=negative control.

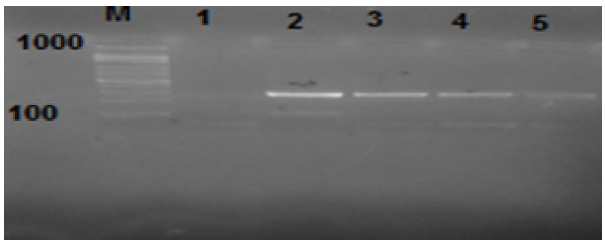

Figure2. PCR result of mecA gene on 2% agarose gel: Lane: M,=100-bp DNA ladder; lane1= negative control lanes 2,3,4 = Patients sample showing positive mecAgene (310 bp).Lanes 5= positive control.

4. Discussion

Staphylococcus aureus is an important pathogen in human infections and is implicated in a wide variety of infections, from mild skin infections to more serious and invasive infections (Tenover *et al*., 2000; Holmes *et al*., 2005).The percentage of *Staph. aureus* observed in this study (11.5%) is lower than that obtained by Misk Elyamen *et al*., 2014 who proved the presence of *Staph. aureus* were 15% among Sudanese patients. Similarly high percentage observed in Uganda where *Staph. aureus* was isolated from 28.7% of target population. However, in the same study, MRSA was isolated from 31.5% which is much lower than what was observed in this study (69.4%) as well as one of our previous findings where we reported 78% (Misk Elyamen *et al*., 2014). In Europe, MRSA prevalence ranges from over 50% in Portugal and Italy to below 2% in Switzerland and the Netherlands, where infection control measures have been shown to work (Verhoef *et al.*, 1999). In Asia, the prevalence lies around 50%, with extremely high rates in Hong Kong (75%) and Japan (72%) (Diekema *et al.*, 2000). In other studies comprising of many African Hospitals the prevalence of MRSA was found 15 % with Kenya and Nigeria having the highest prevalence of 21-30 % (Gorwitz *et al.*, 2006). In Uganda, about 10% of the surgical procedures become septic which account for an increasing morbidity and mortality with the commonest organism isolated being *Staph. aureus,* though no MRSA had been isolated by 1999.(Ojikan,1978 ; Mugisa,1988 ; Olaro, 1999). The 11.5 % *Staphylococcus aureus* observed in this study is similar to 8.8% prevalence in deep-seated recurrent genital ulcers in Nigeria (Agwu *et al.,* 2007) and 10.7% prevalence in wound infections in Iran (Rastegar *et al*., 2005). The 11.5% *Staphylococcus aureus* reported in the present study may: depict the level of *Staphylococcus aureus* carriage in this locality; be attributed to the level of contamination arising from the habit of some of the volunteers to treat their wound aseptically before seeking appropriate medical attention and may also be due to low personal hygiene and poor health education. In this study and out of 34 isolates positive for MRSA, 20 (58.8%) male patients were infected with MRSA more than 14 (41.2%) females.It appears there is a decline in the overall ability of different healthcare settings to stop or reduce the spread of MRSA. In developing countries, it has always been contended that the inappropriate use of antibiotics for community infections may increase the prevalence of resistant bacteria infection (Agwu *et al.,* 2005) including MRSA. It has been reported that 60% of the *Staph. aureus* appeared not to have originated from nasal carriage of *S. aureus* by the patient, suggesting an

exogenous source (Farr and Jarvis, 2002; Perl et al, 2002).

In conclusion, *Staph. aureus* is highly prevalent in Khartoum hospitals, Sudan. MRSA is highly (77.6%) prevalent among populations of *Staph. aureus* isolated from different clinical specimens in different hospitals in Khartoum State, Sudan, with most of the MRSA isolates being from wound infections.. This study has opened a broad research horizon that will enable future researchers to investigate: the source of MRSA; the link between MRSA acquisition and various factors like age, sex, occupation, ethnicity, geographical location, hospitalization, antibiotic usage, surgery and distinction between community-acquired MRSA and hospital acquired MRSA. Moreover, the use of NCCL and CDC criteria highlighted a considerable presence of VRSA in Sudan, which were primarily confirmed as MRSA. All these findings ensure that the use of antibiotics needs to be tightly controlled by health authorities since the presence of VRSA is a real indicator of antibiotic abuse.

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