*van*B Positive Vancomycin- Resistant *Staphylococcus aureus* among Clinical Isolates in Shendi City, Northern Sudan

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Abstract: *Staphylococcus aureus* is associated with different infections ranging from skin and soft tissue infections to endocarditis and fatal pneumonia. *S. aureus* is still the most common bacterial species isolated from inpatient specimens and the second most common from outpatient specimens. The aims of this project were out to estimate the prevalence of vancomycin resistant *Staphylococcus aureus* (VRSA) and also to determine which genes are responsible for VRSA phenomenon. A total of 123 methicillin resistant *S. aureus* (MRSA) were isolated from 200 clinical samples. The VRSA were tested using the Kirby-Bauer disc diffusion method. Out of the 123 isolates, 6.5% were VRSA. The resistivity of *S. aureus* to other antibiotics was also adopted by Kirby-Bauer disc diffusion method. All of the 8 VRSA isolates were found to be resistant to nitrofurantoin, penicillin, ampicillin, kanamycin, clindamycin, ofloxacin, ciprofloxacin, erythromycin and gentamicin. All VRSA isolates were confirmed to carry vanB gene. The study concluded that PCR assay was rapid and accurate technique for the identification of vanB gene of VRSA strains as compared to the conventional methods since the time was taken is less and can help efficiently in controlling and management of the emergence of multi drugs resistant pathogen such as *S. aureus*.
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1. Introduction

Staphylococcus aureus is one of the most common causes of nosocomial infections, especially pneumonia, surgical site infections and blood stream infections and continues to be a major cause of community-acquired infections. Methicillin-resistant *S. aureus* (MRSA) was first detected approximately 40 years ago and is still among the top three clinically important pathogens (Van Belkum *et al.,* 2001; Deresinski *et al.,* 2005). The emergence of high levels of penicillin resistance followed by the development and spread of strains resistant to the semisynthetic penicillins (methicillin, oxacillin, and nafcillin), macrolides, tetracycline, and aminoglycosides has made the therapy of staphylococcal disease a global challenge (Maranan *et al.*, 1997).

The glycopeptide vancomycin was considered to be the best alternative for the treatment of multi drug resistant MRSA (Wootton *et al.*, 2001). However, there are increasing numbers of reports indicating the emergence of vancomycin-resistant *S. aureus* (VRSA) strains exhibiting two different resistance mechanisms. Initially vancomycin-intermediate *S. aureus* (VISA) noted in Japan in 1996 and subsequently in United States in 1997, was believed to be due to the thickened cell wall (Cui *et al.,* 2006), where many vancomycin molecules were trapped within the cell wall. The trapped molecules clog the peptidoglycan meshwork and finally form a physical barrier towards further incoming vancomycin molecules (Cui *et al.,* 2006). Subsequent isolation of VISA and VRSA isolates from other countries including Brazil (Oliveira *et al.,* 2001) France (Poly MC *et al.,*1998), United Kingdom (Howe *et al.,* 1998), India (Tiwari *et al.*,2006; Assadullah *et al.,* 2003) and Belgium (Pierard *et al.,* 2004) has confirmed that the emergence of these strains is a global issue.

The aim of the present study was to identify the emergence of vancomycin resistant *S. aureus* (VRSA) isolates from patients attending different Hospitals in Shendi City, Sudan, and to also it aimed to determine which genes are responsible for VRSA phenomenon among enrolled subjects.

2. Materials And Methods

Clinical Isolates

A total of 123 Staphylococci isolates were collected from patients attending various hospitals and medical centers at Shendi City, Northern Sudan after obtaining their informed consent. Clinical samples which included wound swabs, urine, nasal secretions and ear swabs were collected from April 2013 to October 2014. Swabs samples were added in sterile tubes of Brain Heart Infusion Broth (HIMEDIA) while urine samples were inoculated on MacConkey's and Blood Agars and then all primary cultures were subcultured on Mannitol Salt Agar (ALPHA), and

identified primarily by routine laboratory procedures which included microscopic morphology and biochemical tests including β-hemolysis on blood agar, catalase 3%, oxidase, urease and DNase. Colonies grown were cultured into Nutrient Agar (ALPHA) and sensitivity to novobiocin disk for further testing according to the National Committee for Clinical Laboratory Standards (1990b).

Antibiogram

Susceptibility test was done for all the two hundred *S. aureus* isolates against the following antibiotics: oxacillin, penicillin, gentamicin, ampicillin, tetracycline, clindamycin, amoxicillin, linezolid, sulfamethoxazole- trimethoprim, imipenem and vancomycin (HiMedia) by Kurby-Bauer disk diffusion method according to the NCCLS guidelines (23, 24). Furthermore, all methicillin (oxacillin) resistant strains were identified and subjected to MICs against oxacillin which was determined by E-test (AB, Biomerieux, Marcy l'Etoile, France) that was performed according to the manufacturer's instructions.

Testing for the Vancomycin

The antibiotic-resistance profile was determined by the disc agar diffusion (DAD) technique use vancomycin with 30 µg (in Mueller-Hinton agar (Hi-media) according to the guidelines recommended by Clinical and Laboratory Standards Institute (CLSI) (Wayne., 2007).

DNA Extraction

DNA was extracted from pure *S. aureus* culture using the standard method of phenol chloroform according to (Jain *et al*., 2002).

Detection of arcC Gene

All *S. aureus* isolates were subjected to PCR to detect *arcC* as described by Al-Abbas 2012.

Detection of vanA and vanB Genes

All *S. aureus* isolates were subjected to PCR searching for the presence of *van*A and *van*B gene using two sets of primers for each gene according to Elimam *et al.,* 2014, as shown in table 1.

Table 1. Sequences of vanA and vanB used in the detection of VRSA isolates

Primer	Primer sequencing	Ampilicon size (bp)
*van*A	Forward:5'CATGAATAGAATAAAAGTTGCAATA 3' Reverse:5'CCCCTTTAACGCTAATACGACGATCAA 3'	1030
van B	Forward: 5' GTGACAAACCGGAGGCGAGGA 3' Reverse: 5'CCGCCATCCTCCTGCAAAAAA 3'	433

3. Results

Identification of the Isolates

Identification scheme confirmed that all subjected samples (n=123) were belonging to the species *S. aureus* as illustrated in Figure 1.

Detection of *arcC* Gene

All 123 methicillin-resistant *S. aureus* (MRSA) strains were tested positive for *arcC* genes as illustrated in Figure 2.

Antibiotics Susceptibility Testing

Among the 123 MRSA isolates, 8 (4%) were identified as vancomycin resistant *S. aureus* (VRSA). Five out of the eight VRSA isolates were wound while the remaining three isolates were urine samples (Fig. 3 and Table 2).

Table 2. Distribution of drug resistance, the presence of VRSA among 123 MRSA isolates from Shandi State, North Sudan.

Serial no	Type of specimen	Hospital	Sex	Age/ (years)	MIC for VRSA
1	Urine	Elmak Nemir	Female	62	16µg/ml
2	Urine	Markaz 15	Male	72	32 µg/ml
3	Wound	Elmak Nemir	Female	70	32µg/ml
4	Wound	Eltalymey Hospital	Female	50	32µg/ml
5	Wound	Elmak Nemir	Female	74	32µg/ml
6	Wound	Eltalymey Hospital	Male	38	32µg/ml
7	Wound	Eltalymey Hospital	Female	49	16µg/ml
8	Urine	Out patient	Female	39	32µg/ml

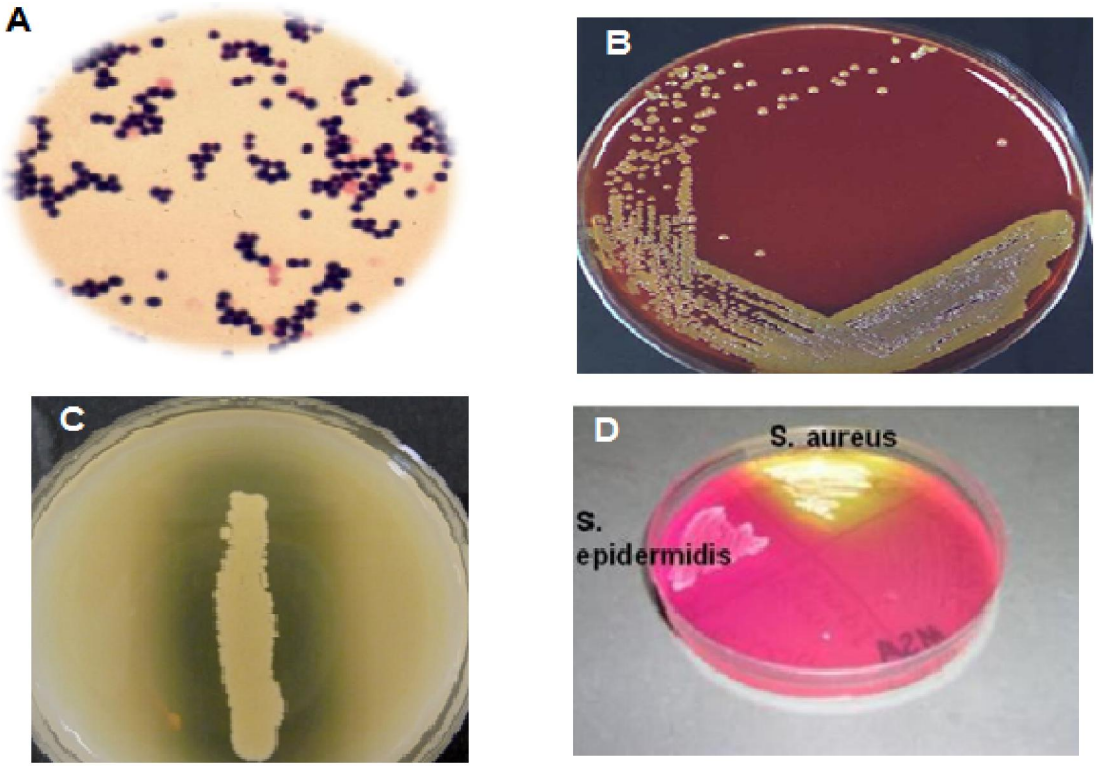

Figure 1. Identification of the isolates (A); *Staphylococcus aureus* under microscope with X100 objectives**, (B);** Overnight growth of *Staph. aureus* on blood agar medium which produces yellow color, (**C**)**;** Growth of *Staph. aureus* on DNase medium showing positive result with clear zone area around the colonies**, (D);** Fermentation reaction of *Staph. aureus* on MSA medium.

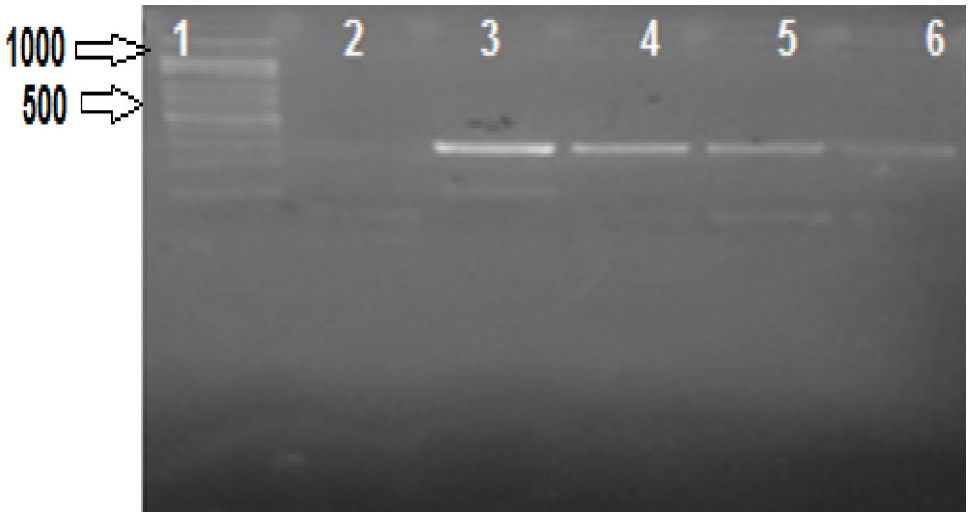

Figure 2. 2% agarose gel electrophoresis of PCR products. Lane 1: 100 bp molecular weight marker, Lanes 2: negative for *arc*C gene, Lanes: 3,4,5,6 are specimens under test showing positive results for *arc*C as indicated by 456 bp PCR ampilicon**.**

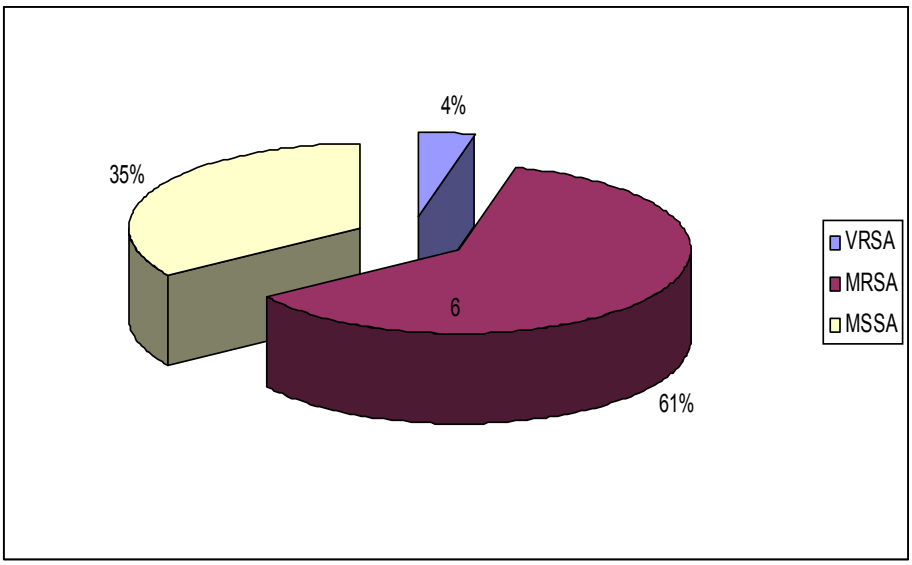

Figure 3. Percentage of VRSA versus MRSA among Study Subjects

Detection of *vans* Genes

Van A gene was not detected in any of the tested strains while vanB was detected only in 3/8 (38%) of the isolates, as indicated by a band of 433 bp. (Figure 4).

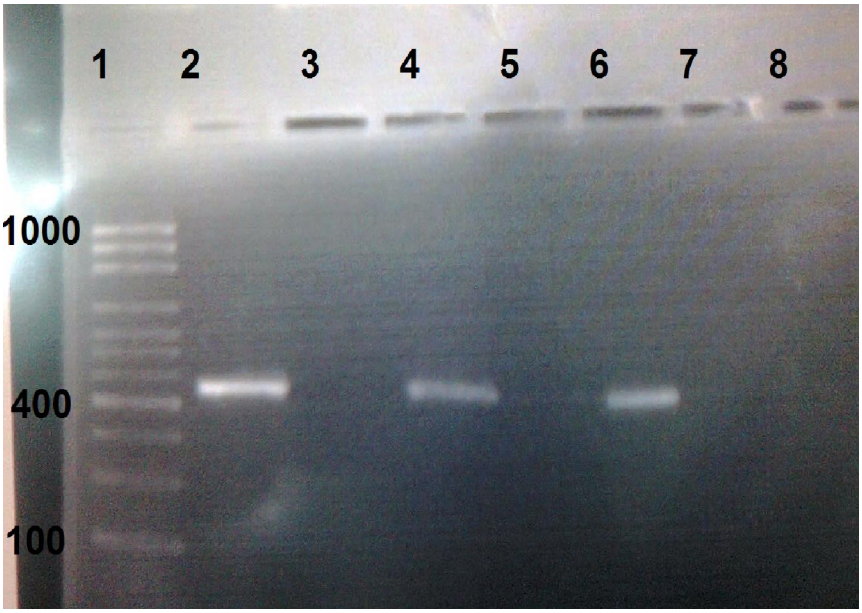

Figure 4. PCR amplification of the *van*B gene for vancomycin resistance S. *aureus*. Lanes2- 4-6 *van*B positive VRSA; Lane 3-5-7 *van*B negative VSSA and Lanes 1 M-100 bp ladder.

4. Discussion

Infections caused by vancomycin-resistant *S. aureus* have been associated with high morbidity and mortality rates. VRSA is one of the common causes of hospital-acquired infections (Anupurba *et al.*, 2003). Vancomycin is the main antimicrobial agent available to treat serious infections with MRSA but unfortunately, decrease in vancomycin susceptibility

of *S. aureus* and isolation of vancomycin-intermediate and resistant *S. aureus* have recently been reported from many countries (Benjamin *et al.,* 2010).

The present study showed clearly the existent of VRSA 6.5% among the enrolled subjects, these finding was suggested previously in Sudan by El imam *et al.,* 2014 and Ahmed *et al.,* 2014, in USA by Rohan *et al*., 2010 and in India by Bhateja *et al*., 2005 and Hare and Malay, 2006.

While it is so difficult to detect vancomycin resistance in clinical microbiology laboratory, it recommended to follow the CDC policy which adopted three criteria to identify VISA strains: Broth microdilution vancomycin MIC of 8-16µg/ mL, E test vancomycin MIC of >6µg/mL and growth on BHI agar containing 6µg/mL vancomycin within 24 hours (Bhateja et al., 2005).

The genetic mechanism of vancomycin resistance in VRSA is not well understood. Several genes have been proposed as being involved in certain clinical VRSA strains (Jansen *et al*., 2007; Maki *et al*., 2004).

In this study, all the VRSA isolates carry *mec*A, but only three contained *van*B. This may open the door to the researchers in this field to seek for other factors which may be responsible for VRSA phenomenon rather than vans genes.

Conclusion

This study demonstrates that only *van*B can be use as diagnostic tool for VRSA strains. This finding has important implications for the management and controlling outbreak and emerges of VRSA in Shendi community. On the basis of this finding, attention should also be given when using conventional disk diffusion method when evaluating resistant *S. aureus* isolates.

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