

Vancomycin resistance among methicillin resistant *Staphylococcus aureus* isolates from intensive care units of tertiary care hospitals in Hyderabad

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Background & objectives: Multidrug resistant methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial and community acquired infections and is on the rise. The glycopeptide vancomycin has been proposed as the drug of choice for treating such infections. The present study aimed at identifying the vancomycin resistance both phenotypically and genotypically among the MRSA isolates from two tertiary care hospitals in Hyderabad, south India.

Methods: MRSA were isolated and identified from different clinical samples collected from ICUs of tertiary care hospitals in Hyderabad using conventional methods. Antibigram of the isolates and vancomycin MIC were determined following CLSI guidelines. *vanA* was amplified by PCR using standard primers.

Results: All vancomycin resistant *S. aureus* (VRSA) isolates were MRSA. The VRSA isolates were positive for *vanA* gene, except one which was negative. All VRSA had a vancomycin MIC in the range of 16-64 mg/l.

Interpretation & conclusions: The increase in vancomycin resistance among MRSA and excessive use of antimicrobial agents have worsened the sensitivity. Larger studies need to be done in various geographical regions of the country to better define the epidemiology, mechanism of vancomycin resistance in *S. aureus* and its clinical implications.

Key words MRSA - *Staphylococcus aureus* - *vanA* gene - vancomycin - VRSA

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^{1,2}. 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 challenge³.

The glycopeptide vancomycin was considered to be the best alternative for the treatment of multi drug resistant MRSA⁴. 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 wall⁵, 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 molecules⁶. The second, noted in United States in 2002⁷ among *S. aureus*, was identical to the mechanism seen in vancomycin-resistant *Enterococcus*⁸. Vancomycin-resistant *Enterococcus faecium* harbours the *vanA* operon, which contains five genes, *VanS*, -R, -H, -A and -X⁸. But Tiwari and Sen have reported a VRSA which is *van* gene-negative⁹. Subsequent isolation of VISA and VRSA isolates from other countries including Brazil¹⁰, France¹¹, United Kingdom¹², Germany¹³, India^{9,14} and Belgium¹⁵ 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 MRSA among *S. aureus* isolates from tertiary care hospitals in Hyderabad, south India, and to determine the sensitivity of these isolates to different antimicrobial agents. Further search is also conducted for the *vanA* gene in VRSA strains.

Material & Methods

Bacterial isolates: A total of 358 *S. aureus* isolates were obtained randomly from clinical samples (blood, urine and throat swabs, wound and ear swabs) of ICUs of Osmania hospital (OH) and Durgabhai Deshmukh Hospital and Research Center (DDHRC), Hyderabad, between March and October 2008. The study was done at department of microbiology, Gulberga University, Gulberga, Karnataka. *S. aureus* was identified by colony morphology, Gram stain, DNase, catalase and coagulase tests and fermentation of mannitol by conventional methods.

Antibiotic susceptibility testing: The antibiotic-resistance profile was determined by the disc agar diffusion (DAD) technique using different antimicrobial agents; penicillin G (10 U), oxacillin (1 µg), methicillin (30 µg), ampicillin (10 µg), ceftazidime (30 µg), gentamicin (10 µg), clarithromycin (15 µg), erythromycin (15 µg), clindamycin (2 µg), co-trimoxazole (25 µg), chloramphenicol (30 µg), rifampin (5 µg), tetracycline (30 µg) (Hi-media,

Mumbai) according to the guidelines recommended by Clinical and Laboratory Standards Institute (CLSI)¹⁶. Resistance to the aminoglycoside streptomycin was assessed similarly but the results were interpreted as per the guidelines of the manufacturer (Hi-Media). The standard *S. aureus* strains NCTC 12493 and ATCC 29213 were used as reference strains (department of microbiology, University of Gulberga) for MRSA and VSRA, respectively.

Determination of MIC: Minimal inhibitory concentration (MIC) of vancomycin was determined by agar dilution method using CLSI guidelines¹⁶. Briefly, gradient plates of Mueller-Hinton agar (Hi-media) were prepared with vancomycin (0.5-256 mg/l, Sigma-Aldrich). 0.5 McFarland equivalent inoculum prepared using 18-24 h old culture was spotted on to gradient plates. Plates were incubated overnight at 35°C for 24 h before assessing the visible growth.

PCR amplification for *van A*: The primer synthesis and the PCR amplification of the *vanA* were done at Bioserve Biotechnologies, Hyderabad, using Gene Amp (Applied Biosystems, USA) thermocycler. The primers and the PCR conditions were as described¹⁷. The primers for amplification of *vanA* were; Forward ATGAATAGAATAAAAGTTGC and Reverse TCACCCCTTTAACGCTAATA. The amplification conditions were initial denaturation at 98°C for 10 sec, annealing at 50°C for 1 min, polymerization at 72°C for 1 min; and final extension at 72°C for 5 min.

Results

Among the 358 clinical isolates of *S. aureus*, 285 (79.6%) were identified as methicillin resistant *S. aureus* (MRSA) by disc diffusion method.

The MIC for 335 of 358 isolates (93.57%) for vancomycin was ≤ 2 mg/l indicating that all were sensitive to vancomycin. Sixteen isolates showed an MIC range between 4-8 mg/l, indicating vancomycin intermediate resistance. For the remaining seven isolates, the MIC was in the range of 16-64 mg/l indicating that these seven isolates were vancomycin-resistant (VRSA). These were all MRSA and resistant to majority of the other antibiotics tested. All these seven isolates showed resistance to a minimum of six other antibiotics including vancomycin and methicillin (Table).

One isolate of *S. aureus* had vancomycin MIC of 4 mg/l and considered VISA according to recent CLSI breakpoints for vancomycin. However, this isolate

Table. Description of VRSA (n=7) including antibiotic susceptibility profile as determined by disc diffusion method

Isolate no.	Specimen	Ward	vanA	Resistance to	Susceptibility to
VRSA1	Wound swab	Trauma ICU	+	S, G, Ca, R, Co, E	C, Cd, Cw, T
VRSA2	Wound swab	Trauma ICU	+	Ca, R, E, Co	S, G, T, C, Cw, Cd.
VRSA3	Urine	Renal ICU	+	S, T, C, Ca, R, Co	G, E, Cd, Cw
VRSA4	Ear swab	Medical ICU	+	Ca, R, S, G, E, Cw	T, C, Cd, Co
VRSA5	Blood	Medical ICU	-	G, Ca, R, E, Co, Cw	S, T, C, Cd
VRSA6	Throat swab	Respiratory	+	S, C, Ca, R, Co	G, T, E, Cd, CW
VRSA7	Urine	Renal ICU	+	G, Ca, R, E, Co	S, T, C, Cd, Cw

Ca, ceftazidime; G, gentamicin; Cw, clarithromycin; E, erythromycin; Cd, clindamycin; Co, co-cotrimoxazole; R, rifampin; C, chloramphenicol; T, tetracycline; S, streptomycin; ICU, intensive care unit

was not distinguished from vancomycin susceptible *S. aureus* in the disc diffusion test because it has shown >20 mm zone of inhibition. All isolated VRSA were resistant to ceftazidime, rifampicin, and were inducible for clindamycin resistance. About 86 per cent of the isolates were susceptible to tetracycline and 71.4 per cent each were susceptible to chloramphenicol and clarithromycin.

PCR amplification for *vanA* among the seven VRSA showed that six contained *vanA*, whereas all the isolates expressed *mecA* (unpublished data). One VRSA isolate was negative for *vanA* by PCR (Fig.).

Discussion

Infections caused by methicillin-resistant *S. aureus* have been associated with high morbidity and mortality rates. In Indian hospitals, MRSA is one of

the common causes of hospital-acquired infections and 30 to 80 per cent methicillin resistance in *S. aureus* based on antibiotic sensitivity tests has been reported from different hospitals¹⁸. The present study showed 79.6 per cent of MRSA. 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 countries¹⁹. Many reports from north India also recorded the emergence of low level and intermediate vancomycin resistance^{9,14,20-23}.

The MIC values indicated that only 1.9 per cent isolates were resistant to vancomycin. One isolate with intermediate resistance as per the MIC was recorded as sensitive in the disk diffusion test. There has been increasing evidence that isolates with a vancomycin MIC of 4 mg/l behave similarly in the clinical settings to VRSA and clinical failure generally results if treatment with vancomycin is continued²⁴. Thus, the previous breakpoints for vancomycin [<4 mg/l (S); 8-16 mg/l (I); >32 mg/l (R)] have recently been revised [<2 mg/l (S); 4-8 mg/l (I); >16 mg/l (R)]¹⁶.

Vancomycin-resistant *S. aureus* 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 mortality²⁴. In the present study, VRSA showed resistance to a wide range of antimicrobial agents. Though all the isolates were phenotypically sensitive to clindamycin, these showed inducible clindamycin resistance. This may be due to the increased use of antibiotics in the intensive care units. Linezolid and quinupristin/dalfopristin were recently approved by the

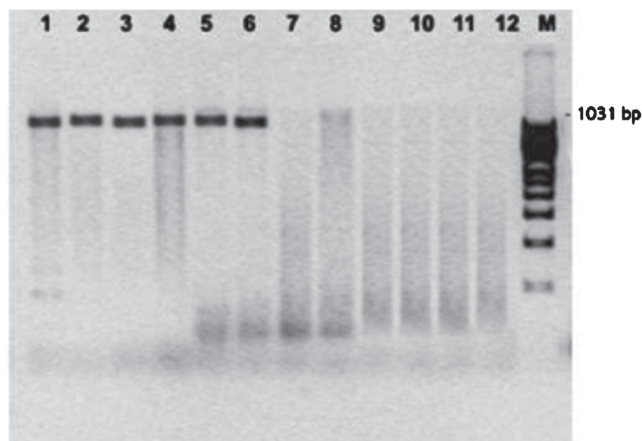


Fig. PCR amplification of the *vanA* gene for vancomycin resistance in methicillin resistant *S. aureus*. Lanes 1-6 *vanA* positive VRSA; Lane 7: *vanA* negative VRSA and Lanes 8-12: *vanA* negative VSSA, M-100 bp ladder.

Food and Drug Administration and are antimicrobials with activity against glycopeptides-resistant Gram positive microorganisms such as VRSA²⁵.

The genetic mechanism of vancomycin resistance in VRSA is not well understood. Several genes have been proposed as being involved in certain clinical VRSA strains²⁶⁻²⁸. The experimental transfer of the *vanA* gene cluster from *E. faecalis* to *S. aureus*²⁹ has raised fears about the occurrence of such genetic transfer in clinical isolates of methicillin resistant *S. aureus*. In this study, all the VRSA isolates contained *mecA*, but only six contained *vanA*. Tiwari & Sen⁸ have also reported a *van* gene-negative VRSA.

In conclusion, the results of this study showed the occurrence of *vanA* gene-negative VRSA in south India. The increase of vancomycin resistance among MRSA and excessive use of antimicrobial agents have worsened the sensitivity, which call for further epidemiological studies.

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