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Original Article

In vitro vancomycin susceptibility amongst methicillin resistant *Staphylococcus aureus*

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ABSTRACT

Background: Vancomycin is drug of choice for treatment of Methicillin Resistant *Staphylococcus aureus* (MRSA) infections. *S. aureus* with reduced vancomycin susceptibility (SA-RVS) is on rise. Current guidelines of detection of SA-RVS are based on MIC (Minimum Inhibitory Concentration) by broth or agar dilution methods. Vancomycin MIC by E test (Epsilometer Test) is an alternative. A study was undertaken to know the prevalence of SA-RVS and compare vancomycin MIC by agar dilution and E test.

Methods: A prospective study was undertaken at tertiary care hospital; 232 clinical MRSA isolates were included. Vancomycin MIC was undertaken by agar dilution method and E test.

Results: All isolates were sensitive to Linezolid. Two MRSA isolates had vancomycin MIC ≥4 μg/ml; vancomycin MIC50 and MIC90 of MRSA isolates was 0.5 and 0.2 μg/ml respectively by agar dilution method. There was agreement over 93.5% isolates in vancomycin susceptibility by agar dilution and E test. E test had sensitivity and positive predictive value of 1.0 (CI – 0.34–1.0) and 0.5 (CI – 0.17–0.83) respectively compare to agar dilution method.

Conclusions: MRSA isolates continues to be susceptible to vancomycin and Linezolid. E test was found equally suitable in initial screening for vancomycin susceptibility. Due to geographic variation in prevalence, there is need of ongoing surveillance of SA-RVC.

Introduction

Methicillin Resistant *Staphylococcus aureus* (MRSA) is responsible for a sizable number of infections globally.[1] A multi centric study from India reported a MRSA prevalence of 41% in 2008–2009 from 17 participating tertiary care hospitals from different parts of India.[2] Vancomycin is treatment of choice for infections caused by MRSA. With increasing prevalence of MRSA infections, vancomycin use has increased many fold.[3] There was emergence of vancomycin resistance enterococci in 1980s; leading to fear of wide spread vancomycin resistance

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in *S. aureus*. However, the first report of reduced susceptibility to vancomycin in clinical isolates of *S. aureus* was reported in 1996.[4] Since then, there are many reports of reduced susceptibility to vancomycin from all over the world including India.[3,5–8] There is a necessity of surveillance for *S. aureus* with reduced vancomycin susceptibility (SA-RVS); *however*, there are roadblocks since most microbiology laboratories perform disc diffusion test for antibiotic susceptibility which is not reliable for vancomycin testing.[3] This is probably one of the reasons that many laboratories are not undertaking vancomycin susceptibility testing routinely. A survey by Centers for Diseases Control and Prevention (CDC) published in 2000, indicated that many laboratories participating in Emerging Infections Program were not using methods that can detect SA-RVS.[9] CLSI (Clinical and Laboratory Standards Institutes) and EUCAST (European Committee on Antimicrobial Susceptibility Testing) guidelines for diagnosis of VSSA (Vancomycin Susceptible *S. aureus*), VISA (Vancomycin Intermediate *S. aureus*) and VRSA (Vancomycin Resistant *S. aureus*) are based on MIC (minimum inhibitory concentration) by micro-dilution or agar dilution methods.[3,10,11] Susceptibility testing by micro-dilution or agar dilution methods is technically demanding. E test is also reported as one of the screening tests by CDC.[12] The study was undertaken to know the prevalence of SA-RVS amongst a MRSA isolates at tertiary care hospital and compare agar dilution method and E test in diagnosis of SA-RVS.

Materials and methods

A prospective study was carried out at a tertiary care hospital of a teaching hospital from 01 Sep 2010 to 31 Mar 2013. Non-repeat clinical isolates of MRSA from various clinical specimens were included in the study. Isolates were identified as *S. aureus* based on morphology, colony characteristics and biochemical reactions as per the standard protocol.[13] All isolates were tested for their susceptibility to various antibiotics as Primary, Primary selective, Supplementary and Urine by Kirby Bauer method using CLSI 2009 guidelines.[10] Isolates of *S. aureus* were identified as MRSA by disc diffusion based on *mec*A mediated oxacillin resistance using cefoxitin disk as surrogate marker.[10] *S. aureus* ATCC 25923 and ATCC 43300 strains were used as negative and positive controls respectively for standardization of procedure and quality control.

MIC of vancomycin by agar dilution method: All MRSA strains were also tested for MIC of vancomycin by agar dilution method. Muller Hinton Agar (MHA) plates containing vancomycin concentrations of 0.25, 0.5, 1, 2, 4, 8, 16 and 32 μg/ml were prepared in house. MRSA isolates were inoculated in nutrient broth and incubated at 37 °C for 4 h. Adjusted 0.5 McFarland bacterial suspensions were inoculated onto these plates with the help of multipoint inoculators with 25 points. *S. aureus* ATCC 25923 and ATCC 700698 were included in all the test plates as control organisms. Plates were incubated at 35 °C for 24 h. Each spot was noted for the presence of growth or no growth. The least concentration of antibiotic that was able to inhibit visible growth of the organism was taken as MIC of the organism.

Table 1 – Specimen wise distribution of samples.

Nature of specimen	Number	Percentage (%)
Pus	178	76.7
Blood	9	3.9
Urine	12	5.2
Central line tip	9	3.9
Tracheal aspirate	7	3.0
Sputum	6	2.6
Joint aspirate	4	1.7
Other	7	3.0
TOTAL	232	100

Vancomycin MIC by E-test (Epsilometer Test): Commercially available vancomycin E-test strips (AB BIODISK) were used. A 0.5 McFarland standard suspension of MRSA isolates were prepared as described above. Suspensions of isolates were lawn cultured on the MHA plates. Vancomycin E- test strip was applied over the plate with the help of applicator within 5 min of lawn culture. Plates were incubated at 37 °C for 24 h. A tear drop zone of inhibition was observed. The zone edge intersecting the graded strip at the minimum concentration of the antibiotic was interpreted as the MIC.

Definitions: VSSA, VISA, VRSA were defined as isolates having vancomycin MIC by agar dilution method as ≤2, 4–8 μg/ml and ≥16 μg/ml respectively.[10] SA-RVS's were VISA or VRSA isolates. The MIC_{50} and MIC_{90} were defined as the vancomycin concentrations that inhibited growth of 50 and 90% of the isolates respectively.

Statistical analysis: Comparison of MIC of E test against agar dilution was undertaken for parameters as sensitivity, specificity, positive & negative predictive value (PPV & NPV), positive & negative likelihood ratios with confidence interval at 95%. Pearson product moment correlation coefficient was calculated to see the correlation between MIC's.

Results

A total of 232 non-repeat clinical isolates of MRSA were included in the study. Specimen wise distribution of these isolate is given in Table 1. It is apparent that the maximum MRSA isolates were from pus specimen. The location wise distribution of patients from whom MRSA were obtained is given in Table 2. A total of 26 (11.2%) and 48 (20.8%) of study subjects had more than 48 h stay in ICU or wards respectively indicative of hospital acquired infections. Antimicrobial susceptibility pattern of these isolates is presented in Table 3 and Fig. 1. Resistance to most of the commonly used antibiotics ranged from 60% to 90%. However, all isolates were sensitive to Linezolid.

Vancomycin MIC by agar dilution method: A total of 230 (99.1%) isolates were having vancomycin MIC ≤2 μg/ml i.e. VSSA; amongst them 80.9% (186/230) strains were having vancomycin MIC ≤1 μg/ml. Only 2 (0.9%) isolates were having MIC ≥2 μg/ml i.e. VISA. MIC_{50} and MIC_{90} of the study isolates were 0.5 and 2 μg/ml respectively.

MIC by E test: All isolates were also subjected to E test. A total of, 215 (92.7%) isolates were having MIC ≤2 μg/ml. 13