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Original Article

Heterogeneous vancomycin-intermediate among methicillin resistant *Staphylococcus aureus*

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ABSTRACT

Background: Hetero-resistance vancomycin intermediate Staphylococcus aureus (hVISA) is phenotype, which on in-vitro susceptibility test is vancomycin susceptible (VSSA) but has a minority population of vancomycin intermediate (VISA). hVISA is responsible for vancomycin treatment failure. Population Analysis Profile- Area under Curve (PAP-AUC) is a test for detection of hVISA; however, this test is unsuitable for clinical microbiology laboratory. Tests, such as Brain Heart Infusion Agar with 6 μg/ml vancomycin (BHIA6V), E test and Macromethod E Test (MET) are available; however reported to have variable results.

Methods: 58 clinical isolates of Methicillin resistant S aureus (MRSA) having MIC of vancomycin more than 1 μg/ml by E test and agar dilution were analyzed by PAP-AUC, BHIA6V and MET.

Result: The prevalence of hVISA was 6.9%. hVISA isolates were having vancomycin E test MIC >2 μg/ml. Sensitivity of BHIA6V, MET and E test with MIC >2 μg/ml were 0.75, 0.67 and 1.0 respectively; however, positive predictive values (PPV) were 0.43, 0.4 and 0.27 respectively with PAP-AUC. PAP-AUC ratio correlated with MIC by E test and MET.

Conclusions: There is need for screening MRSA isolates showing in-vitro vancomycin susceptibility ≤2 μg/ml by agar dilution method for detection of hVISA. PAP-AUC test is unsuitable for routine laboratory testing. BHIA6V, MET and E test can be used for screening, however have low PPV.

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Introduction

Vancomycin is treatment of choice for infection caused by Methicillin Resistant Staphylococcus aureus (MRSA).[1] In screening vancomycin susceptibility, the Clinical and Laboratory Standard Institute (CLSI) in 2006 has redefined *S. aureus* strains as vancomycin-susceptible (VSSA), vancomycin-intermediate (VISA), vancomycin-resistant (VRSA) having vancomycin MIC (Minimum Inhibitory Concentration) as ≤2, 4–8 and ≥16 μg/ml respectively by micro-dilution method.[2] There has been interest in use of in-vitro vancomycin MIC results to predict the outcome in patients with serious *S. aureus* infections being treated with vancomycin.[3,4] These studies demonstrated that infection with VSSA isolates can have vancomycin treatment failure. Later, this phenomenon was demonstrated due to hetero-resistant vancomycin intermediate *S. aureus* (hVISA).[1] hVISA is defined as *S. aureus* isolates having in-vitro susceptibility test results within vancomycin susceptible range but having proportion of population in the vancomycin-intermediate range. The resistant population is present at frequency of $\leq 10^5$–10^6. As CLSI methods use inoculum of 5×10^4, they are not suitable to detect hVISA.[1] Population Analysis Profile- Area under Curve (PAP-AUC) as described by Wootton, et al[5] is a reference method of detection of hVISA. However, this method is technically demanding and not suitable for use in clinical microbiology laboratory settings. Various methods, such as Brain Heart Infusion Agar with 6 μg/ml vancomycin (BHIA6V) screening, vancomycin E test, and Macromethod E Test (MET) have been recommended; however, there is no unanimity over the vancomycin testing strategies for hVISA[1,6]. hVISA may be a precursor of VISA and clinically associated with treatment failure. Various studies have reported hVISA prevalence of 0–50% from clinical isolates.[1,7,8] Infections due to hVISA are associated with longer duration of bacteraemia, higher bacterial load, longer hospital stay and treatment failure.[1,7,8] We undertook a study to know the prevalence and compare various tests in in-vitro diagnosis of hVISA.

Materials and methods

Isolates: Non-repeat clinical MRSA isolates from patients of tertiary care hospital during the period from Sep 2010 to Mar 2013 were subjected for vancomycin MIC by agar dilution method and/or E test.[9] A total of 58 such isolates having MIC more than 1 μg/ml by any of the methods were collected and preserved at −80 °C. These isolates were revived. For each isolate various tests were carried out on same day. Isolates having borderline or indeterminate results were re-tested.

PAP-AUC method: PAP-AUC method as described by Wootton, et al[5] was undertaken. Isolates were cultured on Trypticase soya broth for 24 h and were log diluted 10^{-3} to 10^{-8} by saline; 100 μl of each of suspensions were lawn cultured onto Brain Heart Infusion agar (BHI) containing 0.25, 0.5, 1, 2, 4, 6, 8 μg/ml of vancomycin and plain BHI Agar plates. Vancomycin analytical powder was commercially purchased (Hi-Media). The plates were incubated at 35 °C for 48 h. The colony counts (log10numbers of CFU/ml) were counted and were plotted against the vancomycin concentration on a graph paper. AUC was calculated for each isolate. A ratio was then calculated by dividing the AUC of the study isolate by the AUC of Mu3 strain (*S. aureus* ATCC 700698). Study isolates having ratio of >0.90–<1.3 were diagnosed as hVISA and those with ratio of ≥1.3 as VISA.

BHIA6V screening: In house BHI Agar plates with 6 mg/ml of vancomycin were prepared. 10 μl of 0.5 McFarland suspensions of each of the isolate was inoculated as spot of 15 mm in diameter. These plates were incubated at 35 °C for 24 and 48 h and were observed carefully in transmitted light for growth. Isolates were considered VISA/VRSA; hVISA or VSSA, if there was confluent growth, countable growth or no growth respectively after 48 h of incubation.

MET: Commercially available vancomycin E test strips (AB Biodisk) were used. A 2.0 McFarland standard suspension of MRSA isolates was prepared and 200 μl of suspension lawn cultured on the BHI agar. 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 and reading was undertaken after 24 and 48 h of incubations. A tear drop zone of inhibition was observed. The zone edge intersecting the graded strip at the minimum concentration of the antibiotic is interpreted as the MIC. Those isolates with MIC >8 mg/ml were considered as hVISA. Comparison of various tests were undertaken for parameters as sensitivity, specificity, positive & negative predictive value (PPV & NPV), positive & negative likelihood ratios (LR+ & LR−) with confidence Interval at 95% against PAP-AUC as a gold standard.

Results

A total 58 MRSA isolates having vancomycin MIC more than 1 μg/ml by either agar dilution method or E test were subjected for PAP-AUC, BHIA6V Screen and Macro E Test for vancomycin. PAP-AUC ratio of study isolates ranged from 0.5 to 1.2; with a mean of 0.70 (±0.17). A total of 54 (93.1%) isolates had ratio less than 0.9 and were diagnosed as VSSA; while 4 (6.9%) isolates had ratio ≥0.9 to 1.2 and were diagnosed as hVISA.

On BHIA6V screening, 51 (87.9%) isolates were identified as VSSA; while, 5 (8.6%) and 2 (3.5%) isolates were hVISA and VISA respectively. A comparative result of PAP-AUC and BHIA6V is presented in Table 1; isolates identified as VISA and hVISA by BHIA6V were clubbed together for statistical analysis. There was disagreement on results of 5 (8.6%) isolates; amongst them, 4 were identified as *S. aureus* with reduced vancomycin susceptibility (i.e. VISA or hVISA) and one as VSSA by BHIA6V were actually VSSA and hVISA respectively by PAP–AUC. On MET, a total of 53 (91.4%) and 5 (8.6%) isolates had vancomycin MIC <8 and ≥8 ug/ml respectively. The comparison of MET and PAP-AUC is given in Table 2. There was agreement on results of 53 (91.4%) isolates in correctly identifying as hVISA or VISA by MET against PAP-AUC.

We analyzed vancomycin MIC by E test of the study isolates[9] with PAP-AUC; the comparison is given in Table 3. There were 15 isolates having MIC more than 2 μg/ml; amongst them, 4 (26.7%) and 11 (73.3%) isolates were diagnosed as hVISA and VSSA respectively by PAP-AUC test. We used two