Screening for Vancomycin Intermediate - Resistant *Staphylococcus aureus* among Clinical Isolates of MRSA

Dear Editor,

*Staphylococcus aureus* that are intermediate-resistant to vancomycin (VISA) with MIC 8 µg/mL were first reported in Japan in 1996 and continue to be reported from various parts of the world. Resistance is mainly due to overuse and improper use of vancomycin and due to cross-resistance from other glycopeptides like teicoplanin. As documented evidence of VISA in India are very few, we intended to find out the occurrence of VISA in clinical methicillin resistant *Staphylococcus aureus* (MRSA) isolates of our hospital.

A total of 224 MRSA isolates from wound swabs, frank pus, blood, urine, respiratory samples, fluids, intravascular devices, Foley’s catheters and other swabs received in microbiology Department of LTMG Hospital, Mumbai, were studied during a 12-month period for vancomycin susceptibility by disc diffusion method of Kirby-Bauer and MIC by broth dilution method. For MIC, 2-fold dilutions of vancomycin (Alkem Laboratories Ltd.) were prepared in supplemented Mueller Hinton Broth (SMHB), ranging from 2 µg/mL to 64 µg/mL. To each tube 1 mL of actively growing suspension of MRSA isolate (matched with 0.5 McFarland standard) and 1 mL of the antibiotic dilution were added and incubated at 37°C for 18-24 hours. *Staphylococcus aureus* NCTC 6571 was used as reference strain. MIC was calculated as the highest dilution of the drug inhibiting the growth and showing no turbidity. Growth in tubes ≥ 4 µg/mL was taken as VISA. Growth in tubes ≥ 32 µg/mL was taken as vancomycin resistant *Staphylococcus aureus* (VRSA).

Fifty MRSA isolates were also screened for hetero-VISA. To brain heart infusion agar (BHIA) incorporated with 4 µg/mL vancomycin, 10 µg/mL of overnight culture of test suspension was added after matching with 0.5 McFarland standard. Plate was incubated at 37°C for 48 hours. If cell growth was not apparent within 48 hours, it was VSSA. If countable number of colonies (1-30) are seen within 48 hours, it is possible hetero-VISA. Hetero-VISA status was confirmed if the strain produced subclone (s) with a vancomycin MIC of ≥ 4 µg/mL upon selection with vancomycin, with stability of the strain persisting beyond 9 days in a drug-free medium. If confluent growth of cells was seen within 24 hours, it was a potential VRSA.

Out of 224 isolates, 92.4% were from adults and 7.6% from children. 88.8% were from wards and 11.2% from intensive care units (ICUs). All the 224 isolates were sensitive to vancomycin (100%) by disc diffusion method. All the isolates showed no turbidity even in the tube containing 2 µg/mL of vancomycin by broth dilution method. Of the 50 isolates tested for hetero-VISA, 3 (6%) showed one colony each in BHIA incorporated with 4 µg/mL vancomycin. These three strains were further processed for detection of MIC by broth dilution method with concentrations of vancomycin being 2 µg/mL, 4 µg/mL and 8 µg/mL. None of the three showed any turbidity in the broth dilutions.

Appearance of VISA since 1996 became a grave concern to the clinicians. This not only revealed the therapeutic failure of vancomycin against MRSA infections, but also brought out the fact that there is no effective alternate therapy for the same.

Due to the difficulty in treating the patients with VISA, screening of all MRSA isolates for detection of VISA is really essential in all hospital setups. In the present study, vancomycin sensitivity was 100% by Kirby-Bauer method and MIC of vancomycin of all the 224 MRSA isolates were < 2 µg/mL. Thus none of our isolates were VISA. Assadullah et al from Srinagar has reported vancomycin MIC 8 µg/mL in 15% MRSA isolates and 16 µg/mL in 3.3% cases. In 1997, Japanese hospitals described heterogenous intermediate-resistance to vancomycin in clinical isolates of *Staphylococcus aureus* (Hetero-VISA) with an incidence of 9.3% in university hospitals and 1.3% in non-university hospitals.

Hetero-VISA is also detected in strains with MIC vancomycin even 1 µg/mL. Therefore we studied 50 MRSA isolates for detection of heterogenous resistance. Possible
hetero-VISA strains in this study was 6% (3/50). On repeating vancomycin MIC of these colonies, none of the three grew even in broth containing 2 µg/mL of vancomycin, therefore they were not confirmed hetero-VISA. Though no VISA was encountered in this study, reduction of overuse and misuse of antibiotics will decrease the risk of emergence of MRSA and also restriction of vancomycin use, to prevent spread of VISA in India. This study will help in providing guidance for choosing effective therapy against MRSA isolates. Antibiotics should be administered only on clinical suspicion of sepsis. Perioperative short-term antibiotic therapy can be given. Strict hand washing before and after handling each patient and restriction of movements of personnel within the ICUs and wards should be implemented. The high rise of morbidity and mortality and difficulty in treating these VISA cases, necessitates the screening for VISA and hetero-VISA strains in all hospitals.

References


*Corresponding author (email: dralkasonavane@yahoo.co.in)

Received: 24-02-05
Accepted: 08-11-06