Generation and Phenotypic Characterization of Vancomycin Resistant *Staphylococcus aureus*

ABSTRACT

Staphylococcus aureus appears as Gram positive cocci in clusters. *S. aureus* has long been recognized as an important human pathogen. *S. aureus* causes infections in all age groups and has been recognized as a major causative agent in surgical wound infections and epidemic skin diseases. The proportion of Methicillin resistant *S. aureus* (MRSA) has risen worldwide during the last two decades. Vancomycin has been a drug of choice in clinics for most MRSA infection over the last 30 years. In 1996, the first vancomycin intermediate *S. aureus* (hVISA) MU 3 followed by MU 50 (VISA) was isolated. Since then VISA isolates have been reported from USA, Europe and Far East. Three reports of vancomycin resistant *S. aureus* (VRSA) isolates with MIC of > 32 μg/ml in 2002 and 2004 from USA have added more serious concern.

Vancomycin resistance in *S. aureus* is difficult to define. At Present, MIC determination by broth or agar dilution or by E test is the gold standard for determining vancomycin susceptibilities, but these methods are not suitable for routine use in diagnostic laboratories. Several methods have been proposed including the simplified population analysis (PS), modified vancomycin agar screen, CDC method, population analysis profile (PAP) for detection of VISA or VRSA. Recently, PAP-AUC ratio criteria for determination of vancomycin resistance in *S. aureus* are used.

Combination therapy is the major theme for VRSA and VISA treatment. The combination is shown to have synergistic or additive effects against VISA and hVISA strains. Several studies suggested that β-lactam agents and vancomycin work synergistically against VISA.

In addition to the antibiotic combination therapy, still there is a need to explore new agents which might be active against VISA strains. One of the options is to explore natural products.

Cyanobacteria form an ancient and diverse group of Gram negative phototropic prokaryotic microorganism. Cyanobacteria have been identified as one of the most important groups of organism from which biochemically active secondary metabolites have been isolated. Secondary metabolites from cyanobacteria are associated with

toxic, hormonal, antiviral, antibacterial, antifungal, immunosuppressive and antineoplastic effects.

Thus realizing the importance of emergence of vancomycin resistant *S. aureus* strains in clinics, the present study was undertaken with the following objectives.

- To generate VRSA strains in the laboratory.
- To study the change in the phenotypic characters with increase in the vancomycin resistance
- To investigate the susceptibility of *S. aureus* and VRSA strains to other antibiotics and Cyanobacterial extracts.

Three methods were used to screen 160 *S. aureus* clinical isolates along with ATCC quality control strains, subsequently MIC of all these strains were determined by NCCLS methodology. PS method depicted false positives. 23 strains growing on 4 μg/ml of vancomycin proved to be susceptible according to the NCCLS MIC giving a specificity of 85% and positive predictability of 8%. Modified agar method showed positive predictability of 50% while the CDC method showed 100%, proving it to be the most reliable method for detection of VRSA or VISA strains

In the present study, *in vitro* generation of *S. aureus* with reduced susceptibility to vancomycin was performed. Spiral plating method shifted the MIC of the strains from 0.25 to 4 μg/ml after 20 passages. In the present study, 8 VISA strains were generated after serial passaging on vancomycin. NCCLS MIC of all the strains ranged from 8-16 μg/ml.

The present study with the 18 parent and passaged strains depicted that PS method showed the least specificity and positive predictability of 46% and 34%, respectively. Population analysis profile and PAP-AUC ratio were 100% sensitive in detection of VISA strains. PAP-AUC ratios of all parent and the passaged strains were calculated. It was observed that PAP-AUC ratios of all the 8 *in vitro* generated strains was found to be >1.3. However, it was seen that there was no correlation between MIC and PAP-AUC ratio for rest of the 18 strains used in the study.

The phenotypic and biochemical characterization of all the parent and passaged cultures were performed. It was observed that the colony size of all the VISA strains was smaller than the parent strains. All the strains were catalase and coagulase positive, VISA RB 5, 33 and B 1773 lost their methicillin resistance as they were

found to be oxacillin sensitive with increasing resistance to vancomycin. MICs of vancomycin and teicoplamin were affected by inoculum and temperature against the VISA strains. The doubling time shifted from 18.8-25.86, 20-25.75, 15-16.6, 15-26.0, 22.8-34.8, 13.8-26.76, 24.7-29.7 and 18.1-29.2 min for VISA 238 K, RB5, 1026/99, 29213, 33, B 1773, 5064 and 5063, respectively.

Few VISA strains lost their β-lactams and fluoroquinolone resistance. The loss of the fluoroquinolone resistance was probably because of the effect on the *Nor A* efflux pumps. Acqusition of vancomycin resistance in one of the MRSA strain triggered the *Mec A* gene deletion which led to the sensitivity of VISA strains to β-lactams and cephalosporins.

There have been few reports that β-lactams and vancomycin work synergistically against VISA strains. The present study was designed to investigate the synergy between vancomycin and β-lactams against three *in vitro* generated VISA strains by two methods. The result showed that vancomycin given in combination with β-lactams and cephalosporins demonstrated synergistic activity against the VISA strains FIC indices of <0.5 for vancomycin combination with both cephalexin and amoxicillin was seen against all the 3 VISA strains and MU 50.

Cyanobacteria have been identified as one of the source for new drugs. A study was performed to check the activity of aqueous and organic extracts of 9 cyanobacterial strains against the *in vitro* generated VISA strains. It was observed that aqueous extracts of all the cyanobacterial strains showed no activity against VISA strains. Methanol extract of all *Anabaena* cultures and *Aulosira fertillissima* showed MIC of 32- 64 and 64-128 μg/ml, respectively. There was a spark of activity seen against all the VISA strains and further work on fractionation of the cyanobacterial crude extracts is required. This will shed some light on the identification of new active molecular agents against the VISA strains.

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