Characterization of a Vancomycin Intermediate-Resistant Staphylococcus aureus Isolated from a Hospital
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ABSTRACT
A Vancomycin intermediate resistant strain of Staphylococcus aureus (S. aureus) labeled as CP2 (MIC 16 µg/ml) was isolated from an in-patient of local Cardiac Hospital of Karachi. CP2 showed typical characters of Vancomycin intermediate resistant S. aureus (VISA) i.e. high level of oxacillin resistance, thickened cell wall with rough surface and reduced autolytic activities associated with murein hydrolase (MH) enzyme. This strain may have acquired vancomycin resistance due to long term exposure to antibiotic during the treatment of the patient. Therefore, it implicates the importance of monitoring the usage and also to control of the abuse of antibiotics for prevention of any further proliferation of this type of notorious bugs.

Key words: Vancomycin. Staphylococcus aureus. Resistance.

VISA strain of S. aureus was isolated from blood sample of a postoperative cardiac patient hospitalized in a public hospital of Karachi. The subject patient acquired post operative methicillin resistant S. aureus (MRSA) infection, and was prescribed with vancomycin 500 mg/day. According to the case history, after one month of vancomycin treatment, a strain of VISA was recovered from the blood of the patient by culturing on blood agar. The β-hemolytic colonies were identified as S. aureus by gram staining, biochemical analyses and successive sub-culturing on selective and differential media i.e. Baird Parker agar with egg yolk telurite (Merck), Manitol Salt agar (Merck), Staphylococcus aureus chromo agar (Oxoid) and DNase agar (Merck). Vancomycin and oxacillin sensitivity profiles were determined by E-test (AB-Biodisk) broth and agar dilution methods according to Clinical and Laboratory Standards Institute (CLSI) guidelines.1 Briefly, colonies were taken from overnight blood agar plates, and inoculated to sterile Brain Heart Infusion (BHI) broth (Merck) to make a 0.5 McFarland equivalent suspension and 0.1 ml inoculum of this mixture was inoculated to Brain Heart Infusion (BHI) broth supplemented with (2 fold) dilutions of vancomycin and incubated at 35°C. The growth was monitored after 24 and 48 hours of incubation. The MH (autolytic) activities was analyzed through Triton X-100-induced autolysis assay in glycine buffer as described by Sieradzki and Tomasz.4

The CP2 showed typical characteristics of VISA strains reported earlier from different parts of the world i.e. high level of oxacillin resistance [Minimum Inhibitory Concentration (MIC) > 500 µg/ml], slow growth rate and heterogeneous type of vancomycin resistance (MIC) 16 µg/ml. In addition, CP2 also produced extracellular matrix material (Figure 2b) and exhibited reduced MH activities. It was also observed that CP2 produced a mixture of colonies i.e. small and large (Figure 1) on vancomycin containing BHI agar. Fred et al.2 also described that VISA isolates generate diversified colonies on vancomycin containing agar media.

Scanning electron microscopy has shown some significant changes in morphology of CP2 i.e. the production of amorphous extracellular matrix material (Figure 2b) and un-detached cells even after complete cross wall (septa) formation (Figure 2a).

These characters in CP2 might have been appeared due to decreased activity of murein MH or autolysins that play physiologic roles in cell separation, penicillin-induced lysis, and ongoing peptidoglycan remodeling.3

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Figure 1: Colonial characters of CP2 in the presence of 14 µg/ml Vancomycin on Brain-Heart Infusion agar plate.

The resistance to Triton X-100-induced autolysis (MH activity) was observed in CP2 after exposure to 8 µg/ml vancomycin. This is in agreement with Sieradzki and Tomaz.4

Vancomycin and oxacillin interfere with the cell wall synthesis in gram positive bacteria.5,6 It is hypothesized that, in case of VISA, cell multiplication is inhibited by vancomycin, but the synthesis of cell wall monomers continues, which are not used in the proper architecture of cell wall. Consequently, the newly synthesized monomers are excreted out and deposited on the surface, where they form a thick layer of matrix material. These extracellular molecules may create hindrance in vancomycin influx by reducing the cell wall porosity in vancomycin resistant S. aureus. Similarly, inhibition of MH in CP2 might have been the result of increased cell wall alterations rather than the decreased production of the enzyme.

REFERENCES


Figure 2a: Undetached cells of CP2 after complete cross wall/septum formation.

Figure 2b: Production of extra-cellular matrix material by CP2 in presence of vancomycin.