Vancomycin sensitivity of Staphylococcus aureus isolates from hospital patients in Karachi, Pakistan

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Abstract: Methicillin-resistant Staphylococcus aureus (S. aureus) (MRSA), resistant to all antibiotics including Vancomycin, has been reported in Japan, USA, Canada and Brazil. Hence, the main objective of this study was to evaluate the possible presence of Vancomycin resistant or intermediate S. aureus in Karachi. A total of 850 clinical isolates were collected from two civil hospitals in the city between February 2006 and January 2007. They were identified using standard bacteriological methods. Sensitivity to recommended antibiotics was determined by disc diffusion, agar dilution, and E-test quantitative minimum inhibitory concentration (MIC). Susceptibility to natural or semi-natural products was determined by the agar dilution method. Out of 850 isolates, 250 were MRSA, of which 22% were resistant to 4 g/ml Vancomycin, 24% to 8 g/ml, 15.2% to 16 g/ml, 10% to 20 g/ml, and 13.2% to 30 g/ml; the remaining 15.6% were sensitive to all used concentrations. Although we did not detect any Vancomycin-resistant S. aureus (VRSA), we found that 13% of the strains were intermediates (VISA), i.e. resistant to 30 g/ml of Vancomycin. Because of the continuously increasing prevalence of VISA, it is imperative to minimize the use of Vancomycin. Indeed, the drug should only be prescribed for the treatment of documented, culture-proven infections with MRSA that are not susceptible to routine or alternative agents. This should help avoid the consequences of the development of Vancomycin resistant S. aureus (VRSA) in our environment.

Key Words: MRSA, VISA/VRSA, CMCSA, Vancomycin.

Introduction

In the 1970s, MRSA resistant to orally administered antibiotics and sensitive only to Vancomycin was reported [7]. Since the 1970s MRSA has become a nosocomial problem in hospitals for both children and adults [26]. MRSA may be sensitive to some other antibiotics, such as Clindamycin, Macrolides, Tetracyclines, Trimethoprim-Sulfamethoxazole and Quinolones, or it may be resistant to all antibiotics except Vancomycin [8]. Vancomycin remained the only predictable active antibiotic against all strains of S. aureus, and MRSA in particular [24]. Until recently no strains of S. aureus have failed to respond to Vancomycin, providing Vancomycin could reach the site of infection [9]. The recent discovery of VRSA has been to many microbiologists a nightmare scenario [10]. In May 1997, the Center for Disease Control & Prevention (CDC) confirmed that it has documented the first failure of Vancomycin in Japan [11,25]. In New York (1998) a man died of the same VRSA [12,18]. VRSA was first reported in Brazil in 1998 [13]. These strains are generally in the intermediate level of resistance to Vancomycin, with moderately raised minimal inhibitory concentrations (MICs), previously described as Vancomycin intermediate strains of S. aureus (VISA). However, they are frequently also resistant to the other glycopeptides used in clinical practice (teicoplanin) and are therefore more accurately described as GISA (glycopeptide intermediate S. aureus). They appear to have developed from strains of MRSA [10,15,19,20].

We are continuously isolating increasing numbers of MRSA, especially from hospitalized patients [16]. Fortunately, we have therapeutic options besides Vancomycin but it is almost inevitable that with time these strains will become resistant to all currently available agents. Hence, the main objective of this study was to monitor the current status of Vancomycin susceptibility for the possible presence of Vancomycin resistant or intermediate strains of S. aureus in Karachi, especially in hospitalized patients. These isolates were tested not only against recommended antibiotics, but also against different natural or semi–natural products such as caffeine, neem leaf extract (Azadirachta indica) and Amoxycassia (Amoxil + Cassia fistula). Caffeine has antioxidant and antibacterial properties, whereas neem is already in use in alternative medicine for chronic malarial fever, liver problems and skin ulcers [21].

Material and methods

A total of 850 clinical isolates of Staphylococcus aureus from different specimens including purulent drainage, wound swabs, urine, ear swabs and blood were selected for this study between February 2006 and January 2007. This selection was made on the basis of Spot tests: 1) morphological characteristics (Gram positive cocci arranged in bunches and scattered, non-capsulated, non-sporulated and a-flagellated), 2) biochemical characteristics (catalase positive, coagulase positive by the slide method, thermonuclease positive and beta-lactamase producer), and 3) colonial characteristics, i.e. round, off- white to golden yellow pigment, convex, entire margin on nutrient agar, mannitol fermenter, mostly beta hemolytic, DNase producers, pink to mauve colonies of coagulase positive S. aureus (CoPSA) and off-white to cream colonies of MRSA on CMCSA (CHROMagar Rambach, France).
To make bacterial suspensions, four to five colonies of pure growth from overnight cultures of target strains were transferred into a tube containing four to five ml of trypticase soy broth (Merck, Germany), and incubated at 37°C to match the turbidity with Mcfarland index of 0.5 (usually 2-6 hours). Lawns of each bacterial suspension were made on Mueller Hinton's agar (MHA) (Merck, Germany) using sterile cotton swabs. Antibiotic discs (Gentamicin, Methicillin, Erythromycin, Oxacillin, Ampiclox, Amikacin, and Tetracycline) (Oxoid) were positioned at appropriate distances on the bacterial lawns and incubated at 37°C for 24 hours. The growth inhibition zones were carefully measured with calipers and recorded according to the standard Kirby Bauer disc diffusion method and NCCLS guidelines (Staphylococcus spp. for which the MIC of Vancomycin is ≤4 µg/ml are considered susceptible). Isolates for which the MIC is 4-8 µg/ml are intermediate and those for which the MIC is ≥16 µg/ml are resistant [32]. The 250 isolated MRSA strains were checked for sensitivity to Vancomycin using E-test strips (AB Biodisk, USA). Bacterial lawns were made on MHA and then the E-test strips were positioned on the agar surface with sterile forceps, and incubated at 37°C for 24 hours. The MICs of Vancomycin from E-Test strips were noted according to the manufacturer’s guidelines (AB-biodisk, USA) and the NCCLS standards of 2003 [33].

**Results**

This study was conducted on 850 clinical isolates of *S. aureus* obtained from hospitalized patients in Karachi between February 2006 and January 2007. Of these, 250 isolates were identified as Methicillin resistant *S. aureus* (MRSA), as determined by susceptibility to Methicillin discs and growth on CMCSA agar.

Beta-lactamase production among tested strains was checked by iodometric methods and Nitrocefin® sticks. By iodometric methods 44% of total isolates were found to be beta-lactamase producers, whereas with nitrocefin sticks (Oxoid) 50% of isolates were found to be beta-lactamase producers. All the multiple drug resistant strains were beta-lactamase producers (Table 1).

| Test method   | β-lactamase positive | β-lactamase negative |
|---------------|----------------------|----------------------|
| Iodometric method | 44 %                | 56 %                |
| Nitrocefin method | 50 %                | 50 %                |

Antimicrobial susceptibility of *S. aureus* by Kirby & Bauer’s disk diffusion method showed that out of 850 isolates, 603 (70.9%) were resistant to Oxacillin, 416 (48.9%) resistant to Gentamicin, 250 (29.4%) resistant to Methicillin, 255 (30 %) resistant to Tetracycline, 518 (60.9%) resistant to Erythromycin, 255 (30 %) resistant to Amikacin and 425 (50 %) resistant to Ampiclox. No isolates were found to be resistant to Vancomycin as measured by CLSI or NCCLS standard disk diffusion interpretive criteria for Vancomycin (organisms for which the Vancomycin zone diameters are ≥ 15 mm are considered susceptible) (Table2).

Later, isolated Methicillin resistant strains of *S. aureus* (n=250) were tested specifically against different concentrations of Vancomycin by the agar dilution method and by E-test strips (Table 3).

**Discussion**

As strains of *S. aureus* with reduced susceptibility continue to emerge and evolve, perhaps to full resistance, there is a clinical need to fully characterize them and conduct well-designed research and epidemiological studies. Concern over development of VRSA emanates from the newly widespread occurrence of Vancomycin resistant strains of enterococci (VRE).

VRE is more likely to occur in long-term hospital patients, who are also frequently colonized with MRSA. The presence of van A genes in VRSA suggests that the resistance determinant is acquired from a Vancomycin-resistant *Enterococcus* [29]. In fact, experimental transfer of the van A genes from enterococci to *S. aureus* has been shown previously. Because Vancomycin remains one of the few (and in some cases the only) antibiotic to treat MRSA, there is concern that the DNA sequences encoding Vancomycin resistance in enterococci could be transferred to clinical isolates of MRSA.

A study by Denis, et al, showed that the proportion of hetero-VISA strains was 0.1% of *S. aureus* and 0.4% of MRSA strains, whereas the proportion of VISA strains was 0.1% of *S. aureus* and 0.3% of MRSA strains [29].

On the other hand, studies on Japanese and American isolates of VRSA showed that the mechanism of resistance in that case appears to be different from Vancomycin resistance in VRE. In some *S. aureus* strains resistance involves a markedly altered and thicker cell wall as well as changes in Penicillin binding proteins (PBPs) [30,31].
The present study did not detect any VRSA, but it is alarming that 38.4% of S. aureus isolates were resistant to >16 µg/ml of Vancomycin because of the risk of transmission of these organisms between patients.

We are frequently unable to accurately predict what will happen in the future. Microbes have always been able to come up with unexpected and novel mechanisms of resistance. The emergence of VRSA emphasizes the importance of appropriate Vancomycin dosing to ensure complete eradication of the bacteria.

We also should not neglect the use of combination therapy against MRSA. Besides, the effect of different natural or semi natural products combined with antibiotics can be tested against MRSA and VISA or VRSA.

Conclusion
What can we do about this? Unfortunately, once these strains have developed, the situation becomes more drastic and more difficult to deal with. We have to continuously monitor the resistance pattern of multi-drug resistant S. aureus.

We need to slow down the spread and amplification of these strains (VISA/VRSA) as much as possible through good infection control, conservative measures, prudent use of antibiotics, and good hygiene.

We should not use antibiotics unless needed, and when used, it is imperative that we employ an agent with the narrowest possible spectrum. This means in particular avoiding the use of agents such as Vancomycin, unless it is essential.

This situation translates into a need for new antibiotics and novel therapeutic approaches, but it also stresses the need for understanding the mechanisms of reduced susceptibility and ultimate resistance.

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