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 37ºC 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 $\leq$ 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 $\geq$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).

Table 1: Strains of *Staphylococcus aureus* producing beta-lactamase

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.

References

1- Waldvogel FA. Staphylococcus aureus (including toxic shock syndrome) in Mandell GL, Bannett JE, Dolin R. Principles & Practice of Infectious Diseases. 1999 4th Ed; 2: 1745-1777.

2- Collignon, P et al. Antibiotic resistance: Is it leading to re-emergence of many infections from the past. Recent advances in Microbiology. Edited by Val Asche. Pp 203, 256.

3- Berber M, Rozwadowska-Dowzenko M. Infection by Penicillin–Resistant Staphylococci. Lancet.1948; 2:641-644.

4- Rolinson GN, Stevens S, Batchelor FR, Wood JC, Chain EB. Bacteriological studies on a new penicillin: BRL 1241. Lancet. 1960; 2:564-567.

5- Jevons MP. Celbenin-resistant Staphylococci. BMJ. 1961; 1:124-125.

6- Gutmann MM. Methicillin-resistant Staphylococcus aureus and Vancomycin-resistant Enterococci: Therapeutic realities and possibilities. Lancet. 1997; 349:1901-1906.

7- Boyee JM. Are the epidemiology and microbiology of methicillin-resistant Staphylococcus aureus changing? JAMA. 1998; 279:623-624.

8- Brumfitt W, Hamitton MJ. Methicillin resistant Staphylococcus aureus. N Eng J Med. 1989; 320:1180-1196.

9- Maple PAC, et al. Worldwide antibiotic resistance in methicillin-resistant Staphylococcus. Lancet. 1989; 1:537-540.

10- Moreno F, Crisp C, Jorgensen JH, Patterson JE. Methicillin-resistant Staphylococcus aureus as a community organism. Clin Inf Dis. 1995; 21:1308-1312.

11- Smith TL, Pearson ML, Wilcox KR, et al. Emergence of Vancomycin-resistant Staphylococcus aureus. N Eng J Med. 1999; 340:537-540.

12- Hiramatsu K, Aritaka N, Hanaki H, Kawasaki S, Hosoda Y, Hori S, Fukuchi Y, Kobayashi I. Dissemination in Japanese hospitals of strains of Staphylococcus aureus heterogeneously resistant to Vancomycin. Lancet. 1997; 350:1670-1673.

13- Sieradzki K, Roberts RB, Haber SW, Tomasz A.. The development of Vancomycin-resistance in patients with methicillin-resistant Staphylococcus aureus infection. N Eng J Med. 1999; 340:517-523.

14- Smith TL, Pearson ML, Wilcox KR, Cruz C, Lancaster MV, Robinson-Dunn B, Tenover FC, Zervos MJ, Band JD, White E, Jarvis WR. Emergence of Vancomycin-resistance in staphylococcus aureus. N Eng J Med. 1999; 340:493-501

15- Waldvogel FA. New resistance in Staphylococcus aureus. N Eng J Med. 1999; 340:556.

16- Lambert PH., Grady WF. 1992. Antibiotics & Chemotherapy. 6th Ed.

17- Collignon P, et al. Community acquired methicillin-resistant Staphylococcus aureus in Australia. Lancet. 1998; 352:145.

18- Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant Staphylococcus aureus clinical strain with reduced Vancomycin succeptibility. J Antimicrob Chemotherap. 1997; 40:135-136.

19- Brunet F, Vedel G, Dreyfus F, Vaxelaire JF, Giraud T, Schremmer B, Monsallier JF. Failure of teicoplanin therapy in two neutropenic patients with staphylococcal septicemia who recovered after administration of Vancomycin. Eur J Clin Microb Infec Dis. 1990; 9:145-147.

20- Tenover F, et al. Characterization of Staphylococci with reduced susceptibilities to Vancomycin and other glycopeptides. J Clin Microbiol. 1998; 34(4):1020-1024.

21- The Encyclopedia of plants. Andrew Chevalier, DK Publishing, London. 1996.

22- Lisa Waltz ND. The herbal encyclopedia: A practical guide to the many uses of herbs. (unpublished in traditional form, available as an electronic book (e-book) from earthnow. org. 1999-2000

23- Hafiz S, Hafiz AN, et al. Methicillin resistant Staphylococcus aureus: A multicentre study. J Pak Med Assoc. 2002; 52 (7): 312-314.

24- Rubeena H, Maleeha A, Shahbaz A. Incidence of Methicillin resistant Staphylococcus aureus in blood culture isolates a-retrospective study. Ann King Edward Med Coll. 2001; 7(4): 264-266.

25- Shahla L, Anwer MS, Chaudhry NA. Susceptibility pattern of Nosocomial methicillin resistant Staphylococcus aureus (MRSA) isolates to Vancomycin and other anti-staphylococcal antibiotics. Biomedica. 2000; 16: 32-35.

26- Karamat AK, Nadeem RA, Abbasi SA, Butt T, Usman J, Hussain AB. An outbreak of methicillin-resistant Staphylococcus aureus. Pakistan J Pathol. 1996; 7(1): 24-28.

27- Lisa M, Lysenko C, Marcinkowski Z, Gwiezdzinski Z. Performance of the chromogenic CHROMagar Staph aureus and the Staphychrom coagulase test in the detection and identification of Staphylococcus aureus in clinical specimens. J Clin Microbiol. 2001; 39(7): 2581-2583.

28- Merlino J, Leroi M, Bradbury R, Veal D, Harbour C. New Chromogenic identification and detection of Staphylococcus aureus and methicillin resistant S. aureus. J Clin Microbiol. 2000. 38(6): 2378-2380.

29- Denis O, Nonhoff C, Byl B, Knoop C, Bobin-Dubreux S, Struelens MJ. Emergence of Vancomycin-intermediate Staphylococcus aureus in a Belgian hospital: microbiological and clinical features. J Antimicrob Chemother. 2002; 50: 383 – 391.

30- No authors listed. Vancomycin-resistant Staphylococcus aureus. Pennsylvania, 2002. MMWR Morb Mortal Wkly Rep. 2002; 51: 902.

31- Noble WC, Virani Z, Cree RG. Cotransfer of Vancomycin and other resistant genes from Enterococcus faecalis NCTC 12201 to Staphylococcus aureus. FEMS Microbiol Lett. 1992; 72: 195 – 198.

32- Clinical and Laboratory Standards Institute/ NCCLS. Performance standards for Antimicrobial Susceptibility Testing. Sixteenth informational supplement. M100-S16. Wayne, PA: CLSI, 2006.

33- National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 6th ed. Approved standard, M7-A6. Wayne, Pennsylvania; 2003.