Bangladesh Pharmaceutical Journal ***15****(2): 159-163, 2012*

Vancomycin Sensitivity of Clinical Isolates of *Staphylococcus aureus* from Patients in Dhaka City, Bangladesh

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA), resistant to all antibiotics including **v**ancomycin, 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 *Stap. aureus* in Dhaka. A total of 122 clinical isolates were collected from different hospitals, clinics and diagnostic centers of the city for about 12 months starting from August 2010 to July 2011.They were identified using standard bacteriological methods. Sensitivity to recommended antibiotics was determined by disc diffusion method. In the present study 74% of total isolates were found to be beta-lactamase producers by iodometric methods, whereas with Nitrocefin® sticks 80% of the isolates were found to be beta-lactamase producers**.** All the multiple drug resistant strains were beta-lactamase producers. Out of 122 isolates, although no strains were found vancomycin resistant, 93.44% were found intermediate and only 6.56% showed sensitivity. This study reveals the growing antimicrobial resistance in Bangladesh and refers not to use the antimicrobial drugs that show insufficient sensitivity against *Stap. aureus* to prevent resistance and associated treatment failure.

Key words: MRSA, vancomycin, resistance, *S. aureus*, intermediate

Introduction

Staphylococcus aureus (*S. aureus*) is a facultative anaerobic Gram-positive coccal bacterium. It is the most common species of staphylococci which can cause a range of illnesses from minor skin infections, such as pimples, impetigo, carbuncles, abscesses etc. to life-threatening diseases, such as pneumonia, meningitis, osteomyelitis, endocarditis, sepsis etc. Each year an estimated 500,000 patients in American hospitals contract staph infections (Bowersox, 1999). *S. aureus* causes a variety of suppurative (pus-forming) infections and toxinoses in humans. It is a major cause of hospital acquired (nosocomial) infection of surgical wounds and infections associated with indwelling medical devices. It also causes food poisoning by releasing enterotoxins into food. Methicillin-resistant *S. aureus* (MRSA) have been entrenched in hospital settings for several decades, MRSA strains have recently emerged outside the hospital becoming known as community associated- MRSA (CA-MRSA). Data from the Centers for Disease Control and Prevention (CDC) shows that more people now die of MRSA infection in US than of HIV/AIDS and tuberculosis combined (Boucher *et al.,* 2009). For clinicians, the spread of these methicillin-resistant strains has been critical as the therapeutic outcome of infections that result from MRSA is worse than those from methicillin-sensitive *S. aureus* (MSSA) (Cosgrove *et al.,* 2003).

Beginning with the use of the penicillin in the 1940's, drug resistance has developed in the staphylococci within a very short time after introduction of an antibiotic into clinical use (Todar, 2011). In the 1970s, MRSA resistant to orally administered antibiotics and sensitive only to vancomycin was reported (Boyce, 1998). Since the 1970s MRSA has become a nosocomial problem in hospitals for both children and adults (Karamat *et al.,* 1996). MRSA is the most frequently identified antimicrobial drug resistant pathogen (Raymond *et al.,* 2007). In the 1980s, due to the widespread occurrence of MRSA, empiric therapy for staphylococcal infections (particularly nosocomial sepsis) was changed to vancomycin in many health-care institutions (Tortora *et al.,* 2007). MRSA may be sensitive to some other antibiotics, such as clindamycin, macrolides, tetracyclines, trimethoprim-sulfamethoxazole

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and quinolones, or it may be resistant to all antibiotics except vancomycin (Brumfitt and Hamitton, 1989). Vancomycin remained the only predictable active antibiotic against all strains of *S. aureus*, and MRSA in particular (Rubeena *et al.,* 2001). Until now no strains of *S. aureus* have failed to respond to vancomycin, provided vancomycin could reach the site of infection (Maple *et al.,* 1989). The recent discovery of vancomycin-resistant *S. aureus* (VRSA) has been reported to many microbiologists a nightmare scenario (Moreno *et al.,* 1995). In May 1997, the first clinical isolate of *S. aureus* with reduced susceptibility to vancomycin was reported from Japan (Tortora *et al.,* 2007; Shahla *et al.,* 2000; Smith *et al.,* 1999). In New York (1998) a man died of the same VRSA (Hiramatsu *et al.,* 1997). VRSA was first reported in Brazil in 1998 (Sieradzki *et al.,* 1999). The first clinical infection with VRSA (minimal inhibitory concentrations, MIC ≥ 32 μg/mL) was reported in July 2002 (Heriech *et al.,* 2005) from Michigan with a second case in Pennsylvania reported shortly thereafter (Tortora *et al.,* 2007). These strains are generally in the intermediate level of resistance to vancomycin, with moderately raised MIC, previously described as vancomycin intermediate strains of *S. aureus* (VISA) (Heriech *et al.,* 2005). However, they are also frequently resistant to the other glycopeptides used in clinical practice (teicoplanin) and are therefore more accurately described as glycopeptide intermediate *S. aureus* (GISA). They appear to have developed from strains of MRSA (Moreno *et al.,* 1995; Waldvogel, 1999; Brunet *et al.,* 1990). We are continuously isolating increasing numbers of MRSA, especially from hospitalized patients. Fortunately, we have therapeutic options besides vancomycin but it is almost inevitable that these strains will become resistant to all currently available agents with time. Hence, the main objective of this study was to monitor the current status of vancomycin susceptibility for the possible presence of VRSA or VISA in Dhaka, especially in hospitalized patients. These isolates were tested not only against recommended antibiotics, but also against different natural or semi–synthetic products such as amoxicillin, cephalexin, cloxacillin, imipenem, and penicillin. Though all the strains were resistant against penicillin, it was used as a marker.

Material and Methods

A total of 122 clinical isolates of *S. aureus* from different specimens including purulent drainage, wound swabs, urine, ear swabs and blood were selected for this study between August 2010 and July 2011. Morphological characteristics, biochemical characteristics and colonial characteristics were done for the proper identifications of the isolates. 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 (Himedia, India), and incubated at 37°C to match the turbidity with Mcfarland index of 0.5. Lawns of each bacterial suspension were made on Mueller Hinton's Agar (MHA) (Himedia, India) using sterile cotton swabs. Antibiotic discs (amoxicillin, cephalexin, cloxacillin, imipenem, penicillin and vancomycin) (Oxoid) were positioned at appropriate distances on the bacterial lawns and incubated at 37^{o}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 as 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 (NCCLS, 2006).

Results and Discussion

This study was conducted on 122 clinical isolates of *S. aureus* obtained from different hospitals, clinics and diagnostic centers in the city. All of them were identified as MRSA, as determined by susceptibility to methicillin discs and growth on CMCSA agar. Beta-lactamase production among the tested strains was checked by iodometric methods and Nitrocefin® sticks (Oxoid). By iodometric methods 74% of total isolates were found to be beta-lactamase producers, whereas with Nitrocefin® sticks 80% of isolates were beta-lactamase producers. All the multiple drug resistant strains were beta-lactamase producers (Table 1).

Antimicrobial susceptibility of *S. aureus* by Kirby & Bauer's disk diffusion method showed that out of 122 isolates, low sensitivity was found to penicillin (0%), followed by amoxicillin (21.31%), cephalexin (21.31%), cloxacillin (70.49%). Finally 6.56% of the isolates showed

sensitivity to vancomycin. Highest sensitivity was shown by imipenem 95.90% (Figure 1). But 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). Figure 2 depicts the percentage of resistant, intermediate and sensitive *S. aureus* to vancomycin clearly.

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

Tests	β - Lactamase positive	β- Lactamase negative
Iodometric	74%	26%
Nitrocefin	80%	20%

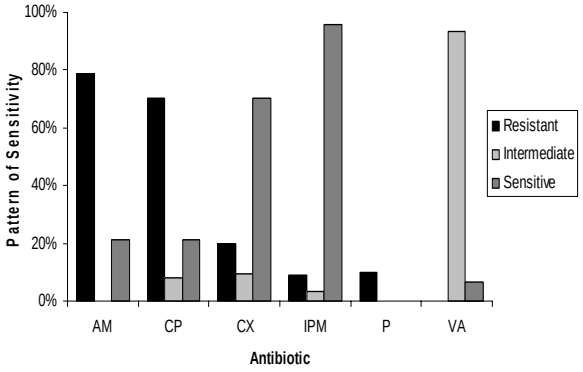

Figure 1. Percentage of resistance pattern of *Staphylococcus aureus* to different antibiotics including vancomycin

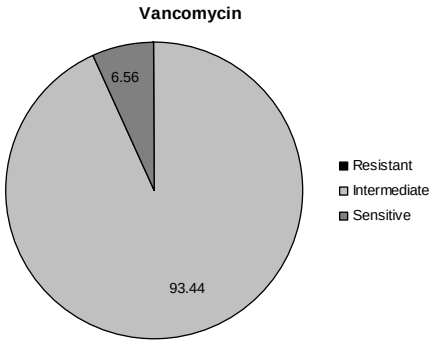

Figure 2. Percentage of *Staphylococcus aureus* resistant, intermediate and sensitive to vancomycin

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) **(**Hakim *et al.,* 2006**)**. 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 (Denis *et al.,* 2002). 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.

Literature revealed 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 (Denis *et al.,* 2002). In another study 13 clinical isolates of enterococci (13.4%) showed multi-resistance patterns (Maschieto *et al.,* 2004). Studies on Japanese and American isolates of VRSA showed that the mechanism of resistance 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 (PBP) (Noble *et al.,* 1992). In another study, out of 850 isolates, 250 were MRSA, of which 22% were resistant to 4 µg/ml vancomycin, 24% to 8 µg/ml, 15.2% to 16 µg/ml, 10% to 20 µg/ml, and 13.2% to 30 µg/mL; the remaining 15.6% were sensitive to all used concentrations. Although the study did not detect any VRSA, but found that 13% of the strains were VISA, i.e. resistant to 30 µg/mL of vancomycin (Hakim *et al.,* 2006).

Although the present study did not detect any VRSA, it is alarming that only 6.56% *S. aureus* showed sensitivity to vancomycin and 93.44% were intermediate. At this situation it is difficult to predict about future occurrence regarding this. 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 bacteria. We also should not ignore the use of combination therapy against MRSA. Besides, the effect of different natural or semi-synthetic products combined with antibiotics can be tested against MRSA and VISA or

VRSA. Rational drug policy should be in use before potent antibiotics are introduced to the country (Aseffa and Yohhannes, 1996). Antibiotic administration should follow certain minimal requirements (Wellington and Van elsas, 1992). To restore efficacy, to earlier antibiotics and to maintain the success of new antibiotics that are introduced, it is necessary to use antibiotics in a way, which assures an ecological balance that favors the predominance of susceptible bacterial flora (Bower, 1999). In Bangladesh, empirical therapy is the rule rather than the exception (Bennish, 1987) and in this context of changing the dynamics of resistance to antibiotics, it is imperative for optimal patient care that constant evaluation of antibiotic sensitivity pattern of pathogens for commonly used antimicrobial agents in a particular environment is carried out.

Conclusion

The variation found in the sensitivity pattern of commonly used drugs in present study could be attributed to the prevailing usage and abuse of the drugs in the area under study. 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. This further suggests the relation between antibiotic usage and the level of drug resistance encountered. The judicious use of antibiotic by the health professionals, efforts to control procurement and use of antibiotics officially in the locality will probably help to limit the increasing rate of drug resistance in the pathogens.

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