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Original Research Article

Detection of Methicillin Resistance and Vancomycin Resistance among Clinical Isolates of *Staphylococcus aureus* in a Tertiary Care Hospital at Tirupati

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Abstract: *Staphylococcus aureus* is one of the important causes of community acquired and hospital acquired infections worldwide. Methicillin resistant *Staphylococcus aureus* is one of the major antibiotic resistant organisms. The present study was undertaken to determine the antibiotic sensitivity of *Staphylococcus aureus* isolates from various clinical samples and to evaluate the possible presence of MRSA, VISA (vancomycin intermediate *Staphylococcus aureus*) and VRSA (vancomycin resistant *Staphylococcus aureus*). A total of 120 isolates of *S. aureus* which were isolated from various clinical samples were tested for methicillin resistance using the oxacillin disc diffusion test (1µg) and cefoxitin disc diffusion test (30µg). All isolates were subjected to minimum inhibitory concentration (MIC) testing with agar dilution method according to the CLSI (Clinical Laboratory Standards Institutes) guidelines. Disc diffusion method was also used to determine the susceptibility of strains to common antibiotics. 64(53.3%) isolates were found to be MRSA by the cefoxitin disc diffusion method and 63(52.5%) isolates were found to be MRSA by oxacillin disc diffusion method. No VISA&VRSA isolates were detected by using the MIC agar dilution method. MRSA isolates were also highly resistant to other antibiotics that were tested.
Keywords: *Staphylococcus aureus*, MRSA, Vancomycin Resistance

INTRODUCTION

Staphylococcus aureus is implicated as one of the important causes of community acquired and hospital acquired infections worldwide. *Staphylococcus aureus* causes a variety of infections ranging from relatively benign skin infections like folliculitis, furuncles, impetigo, abscesses and carbuncles to life threatening systemic illnesses like toxic shock syndrome, bronchopneumonia, septicemia, endocarditis, and meningitis etc. *Staphylococcus aureus* has long been recognized as a major pathogen of hospital acquired infections. Methicillin resistant *Staphylococcus aureus* is one of the major antibiotic resistant organisms. Treatment of infection caused by *S. aureus* has become more problematic since the occurrence of methicillin resistance, as MRSA strains are resistant to all β-lactam antibiotics thereby significantly limiting the treatment options [1-3].

Over the last decade, methicillin resistant *Staphylococcus aureus* (MRSA) strains have become endemic in hospitals worldwide. In addition, it is now incipient community pathogen in many geographical regions .The relentless spread of antibiotic resistance among strains of *Staphylococcus aureus* is one of the greatest challenges faced by clinicians today. In addition to being methicillin resistant, most strains are also resistant to other β-lactam antibiotics, with the exception of glycopeptide antibiotics. In 1980s, because of widespread occurrence of MRSA, empiric therapy for staphylococcal infections (particularly nosocomial sepsis) was changed to vancomycin in many health care institutions. Vancomycin use in developed countries is also increased during this period because of the growing numbers of infections with Clostridium difficile and Coagulase negative Staphylococci (CoNS) in healthcare institutions. Thus, the early 1990s have shown a discernible increase in vancomycin use. As a consequence, selective pressure was established that eventually lead to the emergence of strains of *S. aureus* and other species of staphylococci with decreased susceptibility to vancomycin and other glycopeptides. In 1996, the first clinical isolate of *S. aureus* with reduced susceptibility to vancomycin was reported from

Japan [4]. The vancomycin minimum inhibitory concentration (MIC) result reported for this isolate was in the intermediate range (vancomycin MIC=8 µg/mL) using interpretive criteria defined by the National Committee for Clinical Laboratory Standards (4). As of June 2002, eight patients with clinical infections caused by vancomycin-intermediate *S. aureus* (VISA) have been confirmed in the United States [5, 6]. This report describes the first documented case of infection caused by vancomycin-resistant *S. aureus* (VRSA) (vancomycin MIC >32 µg/mL) in a patient in the United States [5, 6, 7].

The present study was undertaken to determine the antibiotic sensitivity of *Staphylococcus aureus* isolates from various clinical samples and to evaluate the possible presence of MRSA, VISA (vancomycin intermediate *Staphylococcus aureus*) and VRSA (vancomycin resistant *Staphylococcus aureus*), in a tertiary care hospital.

MATERIALS AND METHODS:

A total of 120 consecutive isolates of *Staphylococcus aureus*, were included in this study which were isolated from clinical samples of patients who were admitted in a tertiary care hospital (SVRR Govt Gen Hospital, Tirupati). The samples were inoculated on Nutrient agar, Blood agar and Mac Conkey agar. The inoculated plates were incubated at 370 C for overnight. If any growth was seen on the plates, it was processed according to the standard bacteriological techniques. The colonial appearance and morphological characters of the isolated bacteria was noted. On nutrient agar colonies were large, convex, smooth, shiny, and opaque and most of the strains produce yellow pigment. On blood agar colonies were smooth, low convex, glistening, opaque and sometimes surrounded by a narrow zone of beta haemolysis. On Mac Conkey agar small, pink colonies were observed. The isolated colonies were subjected to preliminary tests like Grams staining, Catalase test and Oxidase test. These preliminary tests were followed by coagulase test and biochemical reactions for the identification of *Staphylococcus aureus*.

The antibiotic susceptibility pattern of isolated *Staphylococcus aureus* was done by Kirby Bauer disc diffusion method on Mueller-Hinton (MH) agar plates using commercially available antibiotic discs (Hi Media). The *S. aureus* suspension was made by inoculating 4-5 isolated identical colonies in peptone water. After 2 hours of incubation, the turbidity was standardized by using0.5 McFarland standards. By using sterile swab, alawn culture was made on the MH plates. The 6-8antibiotic discs per plate were placed and inoculated plates were incubated at 37^0C. The results were read after overnight incubation and compared with the standard chart. The Cefoxitin disc is used for detection of methicillin resistance. The Oxacillin disc was also used in parallel on a separate MH plate and was incubated at 35^0C for 24hrs.

Vancomycin resistance was screened using Vancomycin (30µg) disc (Himedia). Vancomycin sensitivity of S .aureus was further tested by using standard Minimum Inhibitory Concentration by agar dilution method. This was done by using MH agar and vancomycin powder with potency of 950mcg/1mg. Vancomycin was obtained from Himedia laboratories Ltd, Mumbai. Stock solution was prepared by dissolving 256mg of vancomycin powder in 100ml of sterile distil water. Vancomycin solutions were prepared in different dilutions so that their final concentrations in media were 0.25µg, 0.5µg, 1µg, 2µg, 4µg, 8µg, 16µg, 32µg, 64µg and 128µg. The sterile Mueller-Hinton agar plates were labeled according to the final concentration of the drug after pouring. After drying of the plates, 1µl of spot inoculation of the S .aureus was done on the plate. The S .aureus suspension was made by inoculating 4-5 isolated identical colonies on blood agar plate after 18-24 hrs incubation and the inoculum was standardized with 0.5 McFarland standards. The inoculated plates were incubated at 35^0c for 24hours and the results were read after 24hours and compared with CLSI standards [8].

RESULTS AND DISCUSSION:

A total of 120 consecutive isolates of *Staphylococcus aureus*, were included in this study which were isolated from clinical samples. Most of the isolates were from the pus samples (n=88, 73.3%), followed by other body fluids (n=18, 15%), blood (n=7, 5.8%), urine (n=4, 3.3%) and sputum (n=3, 2.5%) (Table 1).

Table 1: Distribution of the isolates of *Staphylococcus aureus* in various clinical samples

Sl No	Specimen	Specimen source	No of isolates (%)
1	Pus	wounds, postoperative wound infections, burns, osteomyelitis	88 (73.3%)
2	Urine	urinary tract infections	4 (3.3%)
3	Sputum	respiratory infections	3 (2.5%)
4	Blood	septicemias	7 (5.8%)
5	Other body fluids	Pleural fluid, cerebro spinal fluid etc	18 (15%)
		TOTAL =	**120**

Out of these isolates, 63 (52.5%) of the strains were resistant to Methicillin (Cefoxitin/Oxacillin). All MRSA strains were resistant to penicillin and cefoxitin. All MRSA strains were sensitive to vancomycin (Table 2). These MRSA isolates were resistant to several other antibiotics, including Cefoxitin (100%), Erythromycin (77.8%), Clindamycin (41.3%), Gentamycin (30.2%), Amoxyclav (70%), and Imipenem (31.7%). All 120 isolates of *Staphylococcus aureus* were tested for vancomycin sensitivity by both disc diffusion method and MIC. All strains were found to be vancomycin sensitive by both methods. None of them were resistant to vancomycin. No VISA strains were isolated.

Table 2: Antibiotic Resistance Pattern among the MRSA isolates (n=63)

Sl No	Antibiotic	Resistant isolates (%)
1	Erythromycin	49 (77.8%)
2	Clindamycin	26 (41.3%)
3	Gentamicin	19 (30.2%)
4	Imipenem	29 (46%)
5	Penicillin	63 (100%)
6	Oxacillin	63 (100%)
7	Cefoxitin	63 (100%)
8	Amoxyclav	44 (69.8%)
9	Vancomycin	0 (NIL)

Over the last decade, Methicillin resistant *Staphylococcus aureus* (MRSA) strains have become endemic in hospitals worldwide. In addition to being methicillin resistant, most strains are also resistant to other β-lactam antibiotics. In the present study isolation of MRSA is 52.5%, which correlates with the study of Benu Dhawan *et al.;* [9].

In the present study vancomycin susceptibility was tested by both disc diffusion method (Kirby- Baure), and MIC by Agar Dilution Method. All strains showed sensitivity in disc diffusion method and all were sensitive (<4µg/ml) by MIC- agar dilution method. Wide spread use of vancomycin among MRSA has been reported to result in reduced susceptibility to vancomycin. Vancomycin was not used clinically in treating infections in our hospital during the study period. This could be the possible reason for not detecting vancomycin resistance among *S. aureus* isolates inthe present study. Benu Dhavan *et al.;* [9], Jae-hoon *et al.;* [10], Bhateja P *et al.;* [11], Dhanalakshmi *et al.;* [12], Sandra M. Tallent *et al.;* [13], have also reported 100% sensitivity to vancomycin by both disc diffusion and by MIC. VRSA and VISA isolates have been reported by other researchers like Hare Krishna Tiwari *et al.;* [14], G.A. Menezes *et al.;* [15], Rajendra Goud *et al.;* [16], Biswajit Saha *et al.;* [17], who stated that it was mainly due to excessive use of antibiotics in intensive care units and in other health care sectors.

CONCLUSIONS:

Vancomycin resistance can be difficult to detect in clinical microbiology laboratory. Disk diffusion sensitivity testing by standard 30µg vancomycin frequently misclassifies intermediately susceptible isolates as fully susceptible. Presently MIC determinations by broth or agar dilution or by E test are the gold standard for determining vancomycin susceptibility but these methods are not suitable for routine use in the diagnostic laboratories. It is recommended that diagnostic laboratories screen their *S. aureus* isolates by the CDC method and submit strains to reference laboratories for confirmation of vancomycin resistance by determining the MIC. Clinicians should continue to exercise caution in their use of vancomycin in order to preserve this useful antibiotic and prolong its therapeutic usefulness.

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