ORIGINAL RESEARCH PAPER

Microbiology

DETECTION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS IN A TERITIARY CARE HOSPITAL

KEY WORDS: *Staphylococcus aureus,* MRSA, *Vancomycin*

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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. 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). 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. . MRSA isolates were also highly resistant to other antibiotics that were tested.

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 -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 -lactam antibiotics, with the exception of glycopeptide antibiotics In 1980s, becauseof 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. MRSA strains are difficult to eradicate as they are multidrug-resistant leaving glycopeptides as the drugs of choice[1] Resistance has been reported to these drugs also from various parts of the country[4,5] Currently, the treatment options for MRSA infections are limited to a very few and expensive drugs like teicoplanin, vancomycin, and linezolid.. The knowledge of prevalence of MRSA and their antimicrobial-susceptibility pattern is a must for appropriate treatment of these infections. The present study was conducted to know the prevalence of MRSA in our hospital, which is a tertiary referral 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 37^0 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, a lawn culture was made on the MH plates. The 6-8antibiotic discs per plate were placed and inoculated plates were incubated at 37 ^{0}C. 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 37^{0}C for 24hrs. Vancomycin resistance was screened using Vancomycin (30µg) disc (Himedia).

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,infection burns, osteomyelitis	88(73.3%)
2	Urine	Urinary tract infection	4(3.3%)
3	Sputum	Respiratory infections	3(2.5%)
4	Blood	Septicaemias	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%),

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	Gentamycin	19 (30.2%)
4	Imipenem	29 (100%)
5	Pencillin	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 -lactam antibiotics. In the present study isolation of MRSA is 52.5%, which correlates with the study of Benu Dhawan *et al.;* [6]. In the present study vancomycin susceptibility was tested by both disc diffusion method (Kirby- Baure) All strains showed sensitivity to vancomycin in disc diffusion method. 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.;* [6]. Jae-hoon *et al.;* Bhateja P *et al.;* [7]. Dhanalakshmi *et al*[8]. Sandra M. Tallent *et al.;* [9]. have also reported 100% sensitivity to vancomycin by disc diffusion. VRSA and VISA isolates have been reported by other researchers like Hare Krishna Tiwari *et al.;* [10]. G.A. Menezes *et al.;* [11]. Rajendra Goud *et al.;* [12]. Biswajit Saha *et al.;* [13]. who stated that it was mainly due to excessive use of antibiotics in intensive care units and in other health care sectors.

CONCLUSION

To conclude, glycopeptides seems to be the only antimicrobial agents that may be used as the drug of choice to treat MRSA infections. The high prevalence of MRSA and glycopeptide use, both thought to be risk factors for VRSA, make the widespread dissemination of these organisms an alarming and realistic possibility once it happens to emerge. So, glycopeptides must be kept reserved for life-threatening infections caused by MRSA. The most effective way to prevent MRSA infections is by doing continuous surveillance of antibiotic resistance profiles of local S. aureus isolates to formulate antibiotic policies and effective infection control practices.

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