Original Article

SENSITIVITY PATTERN OF METHICILLIN RESISTANT AND METHICILLIN SENSITIVE *STAPHYLOCOCCUS AUREUS* ISOLATES, AGAINST SEVERAL ANTIBIOTICS INCLUDING TIGECYCLINE IN IRAN: A HOSPITAL BASED STUDY

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ABSTRACT

Objectives: Methicillin resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen causing significant morbidity and mortality. The aim of this study was to evaluate in vitro activity of tigecycline and other new agents against MRSA, isolated from surgical wound and soft tissue infections in an Iranian 1000-bed tertiary hospital.

Methodology: In vitro activity of tigecycline and other antibiotics were tested against 102 strains of *Staphylococcus aureus* isolated from different patients hospitalized at Milad hospital from May 2008 to June 2008. All strains were identified according to routine bacteriological methods. Susceptibility testing was performed by disk diffusion methods as recommended by Clinical Laboratory Standards Institute (CLSI). Cefoxitin (30µg) disk used for detection of methicillin resistant strains of *S.aureus*.

Results: One hundred two strains of *S.aureus* were isolated from patients admitted to our hospital. The majority of patients was from surgical wards including open heart, orthopedic ward and had post operation wound infections. Of 102 strains 36(35.3%) isolates were MRSA. All isolates including MRSA strains were susceptible to tigecyline, linozolide and vancmycin. Of 102 isolated strains 96 (94.1%) were susceptible to teicoplanine and six stains (5.9%) were intermediate. Resistant rate to other antibiotics including clindamycinm erythromycin pencillin, co-trimoxazole, rifampicin and ciprofloxacin were 35.3%, 27.7%, 97.7%, 26.5%, 16.7%and 33.7% respectively.

Conclusion: All strains of *S.aureus*, isolated from wound and soft tissue in our hospital were susceptible to tigecyline, linozolide and vancomycin.

KEY WORDS: Methicillin Resistance, Staphylococcus aureus, Wound infection, Antimicrobial agents.

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INTRODUCTION

Staphylococcus aureus is a Gram-positive, non-motile catalase positive, coagulase positive, facultative anaerobe microorganism involved in causing a number of disease including boils pustules, impetigo osteomyelitis, mastitis, septicemia, wound infections, meningitis, food poisoning and toxic shock syndrom.[1,2] It

produces a variety of pyrogenic toxins and superantigens which contribute to their overall virulence. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a multidrug-resistant pathogen. Resistance to the macrolides, lincosamides, aminoglycosides, and all beta-lactam agents is also seen with MRSA.[3]

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a nosocomial pathogen that causes morbidity and mortality worldwide.[4,5] The options for the treatment of MRSA infections are limited to antibiotics such as vancomycin, tigecycline and lineozolid. Tigecycline, a member of the glycocycline class of antibiotics provides good activity against a broad range of gram-positive and gram-negative bacteria, with exception of *Pseudomonas aeruginosa*, *Proteus mirabilis* and indol positive *Proteus* spp.[3,5,6]

Tigecycline binds to the bacterial 30S ribosome, blocking the entry of transfer RNA. This ultimately prevents protein synthesis but halting the incorporation of amino acids into peptide chains and thus limits bacterial growth.[8] The aim of this study was to determine susceptibility of MRSA strains isolated from wound and soft tissue infection to commonly used and new antibiotics such as tigecycline in an Iranian 1000-bed tertiary hospital.

METHODOLOGY

Clinical isolates of *S.aureus* from wound and soft issue infections between May 2008 to June 2008 in Milad hospital of Tehran were studied .Milad hospital is 1000-bed non teaching tertiary hospital. In total 102 strains of *S.aureus* isolated from patients admitted to Milad hospital were studied. The majority of patients were hospitalized patients. Briefly, the samples were cultured aerobically in blood and MacConky agar. The plates were incubated overnight. All isolates were identified using gram stain, biochemical tests including ctalase, coagulase and DNase.[9] Susceptibility testing were performed by disk diffusion method as recommended by Clinical laboratory Standards Institute (CLSI). Antibiotics were used for susceptibility testing were, tigecycline (15µg), linezolid (30µg), teicoplanin (30µg), penicillin (10 units), oxacillin (1µg), cefoxitin (30µg), vancomycin(30µg), erythromycin(15 µg), clindamycin (2µg), ciprofloxacine, (5µg), Cotrimoxazole, (25µg) and rifampicin. (5µg). All antibiotics disks were provided from Mast diagnostic groups (UK) and tigecyclin provided from Oxoid Company.

Briefly after identification of *S.aureus* we made a suspension from overnight growth and adjusted turbidly by 0.5MacFarland standard. Sterile swabs were used to inoculate the test organism onto Mueller- Hinton agar. Sterile forceps were used to carefully distribute the antibiotic disks evenly on the inoculated plates. All plates incubated at 35°C for 18 hour. For detection of MRSA all plates were incubated at 35°C for 24 hours. In addition of disk diffusion methods, oxacillin screen agars were used for confirmation of MRSA. The inhibition zone diameter were measured in millimeters and interpreted as a susceptible (S) intermediate (I) or resistant (R) as recommended by CLSI.[10] *S.aureus* ATCC 25923, *E.coli* ATCC 25922 and *P aeruginosa* ATCC 27853 were used as a control strains for quality control of antibiotic disks in susceptibility testing .*S. aureus* ATCC 29213 was used as reference MRSA strain for quality control in MRSA detection process.

RESULTS

One hundred two strains of *S.aureus* were isolated from specimens including soft tissues, post operative and diabetic foot infections. Of 102 isolates 20 strains isolated from sternum infections flowing heart surgery. A few of patients had diabetic foot infections. Of 102 patients, 60(58/8%) were male and 42 (41.2%) were female. The age of patients ranged between one and 85 years old (mean 43.71, SD ± 20.04). Of 102 patients 84 (82.4%) were hospitalized and 18(17.6%) were outpatients. Twenty (19.6%) of patient were from heart ward, 68 (66. .7%) from other wards such as orthopedic, surgery and the remaining 14(15%) were outpatients.

The results of this study indicated that 36 (35.3%) of all isolates of *S.aueus* stains were

resistant to oxacillin and cefoxitin therefore these strain were methicillin resistant *S.aureus* (MRSA). All isolated strains of *S.aureus* including methicillin resistant (MRSA) and methiillin susceptible *S.aureus* (MSSA) were susceptible to tigecyclin.. Other two antibiotics including linozolid and vancomycins had the high efficacy against both MRSA and MSSA. There were no resistant strains against theses antibiotics. Of 102 isolated strains 96 (94.1%) were susceptible to teicoplanine and six stains (5.9%) were intermediate. There was no resistant strains to this antibiotic. Resistant rate to other antibiotics including clindamycinm, erythromycin pencillin, co-trimoxazole, rifampicin and ciprofloxacin were 35.3.%, 27.7%, 97.7%, 26.5%, 16.7% and 33.7% respectively Table-I.

DISCUSSION

MRSA is a major nosocomial pathogen causing significant morbidity and mortality. The important reservoirs of MRSA in hospitals/institutions are infected or colonized patients and transient hand carriage on the hands of health care workers.[4,5,10] In Islamic Republic of Iran, the significance of MRSA had been recognized relatively late and it emerged as a problem in the1980s and in the 1990s. Epidemic strains of these MRSA are usually also resistant to several other antibiotics. During the past 15 years, the appearance and world-wide spread of many such clones have caused major therapeutic problems in many hospitals, as well as diversion of considerable resources to attempts at controlling their spread.[11]

In our study, the prevalence of MRSA isolated from different wound infections was 35.3%. This is not in line with our previous reports.[12] Similar observation was made by Mehta, in India who in his study on control of MRSA in a tertiary care center, had reported an isolation rate of 33% from pus and wound swabs.[13] However, Qureshi from Pakistan reported a high isolation rate of up to 83% MRSA from pus. The result of this study revealed that all isolates of both MRSA and MSSA were susceptible to vancomycin, tigecycline and linozolid.[14] There is some reports regarding reduced susceptibility or resistance of *S.aureus* to vancomycin. The first reported isolation of vancomycine intermediate *S aureus* (VISA) occurred in Japan in 1997 and more than 100 VISA isolates have since been reported.[15] In 2002, three vancomycin-resistant *S. aureus* (VRSA) from clinical specimens of American patients were found to have high-level resistance to vancomycin [minimal inhibitory concentration (MIC) >32 µg/ml] although a few more cases of VRSA have since been described.[16] Fortunately, these isolates have not yet become widespread.[17] Linezolid is an oxazolidinone antibiotic with activity against gram-positive pathogens including vancomycin resistant (VRE), MRSA, and vancomycin intermediate (VISA). The unique mechanism of action involves inhibition of bacterial protein synthesis through binding to the domain V regions of the 23S rRNA gene.[18] Resistance to linezolid requires mutations of multiple gene copies. Linezolid is currently approved for skin

Table-I: Results of Susceptibility testing of *S.aureus*

Antibiotic	*Susceptible %*	*Intermediate %*	*Resistan %*
Tigecycline	102 (100)	0.0	0.0
Linozolide	102 (100)	0.0	0.0
Vancomycin	102 (100)	0.0	0.0
Teicoplanine	96 (94.1)	6 (5.9)	0 (0.0)
Oxacillin	66 (64.7)	0.0	36 (35.3)
Clindamycin	66 (34.7)	4 (3.9)	32 (31.4)
Erythromycin	37 (36.3)	37 (36.3)	28 (27.5)
Penicillin	2 (2.0)	0.0	100 (98)
Co-trimoxazole	75 (73.5)	0.0	27 (26.5)
Ciprofloxacin	56 (65)	1 (1.7)	29 (33.7)
Rifampin	83 (81.4)	2 (2)	17 (16.7)

and soft tissue infections and pneumonia due to susceptible pathogens.[9] Based on in vitro susceptibility data, tigecycline has a broad spectrum of activity against both gram-positive cocci including MRSA. Clinical trials have been conducted in patients with complicated skin and soft tissue infections and intra abdominal infections for which the drug has been approved by FDA.[20,21] In our county there is no published documents for treatment of soft tissue infections caused by MRSA. However these antibiotics can be used for treatment of infections caused by multidrug –resistant organisms such as MRSA.

CONCLUSION

Our study showed that nearly 36% isolates of *S.aureua* were methicillin resistant .Vancomycin, tigecycline and linozolid were effective against both methicillin resistant and susceptible strains of *S.aureus*.

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