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ANTIMICROBIAL SUSCEPTIBILITY OF METHICILLIN RESISTANCE *STAPHYLOCOCCUS AUREUS* TO VARIOUS ANTIBIOTICS WITH SPECIAL REFERENCE TO PRISTINAMYCIN

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Abstract

Methicillin-resistant Staphylococcus aureus (MRSA) is a major nosocomial pathogen worldwide. Vancomycin is the traditional drug of choice, but decreasing susceptibility to vancomycin and other glycopeptides has been reported since 1996. The present study was carried out to test the in vitro activity of other antimicrobial agents with special reference to pristinamycinagainst MRSA isolates recovered from hospitalized patients. We tested 100 MRSA isolates recovered from hospitalized patients. Staphylococcus aureus was identified by Gram's staining, catalase and coagulase test; followed by cefoxitin test for identifying MRSA. Susceptibility to other antibiotics was tested by the disk diffusion method. Antibiogram to MRSA isolates shows high resistance to Erythromycin (89%) and low resistance to Chloramphenicol (11%) and for the remaining antibiotics the resistance frequencies were: Pristinamycin (79%), Cotrimoxazole (67%) Gentamicin (57%), Tetracycline (34%), Clindamycin (31%) and Chloramphenicol (11%). The alarmingly increasing trend of MRSA infection calls for the implementation of an effective infections control policy which helps in counter acting the bioterror, if not completely eradicating it. Minimizing the antibiotic pressure is essential to control the emergence of resistance strains in the hospital and in the community.

INTRODUCTION

Staphylococcus aureus is facultative anaerobic Gram positive cocci. It is frequently found as part of the normal skin flora or skin and nasal passages. *S. aureus* can cause wide range of illnesses like skin infection, cellulitis, pneumonia, osteomyelitis, endocarditis etc. by producing extracellular virulence factors and cell wall associated proteins that are important for colonization.[1] The treatment of infections due to *Staphylococcus aureus* was revolutionized in 1940s by the introduction of the antibiotic penicillin. However most of the strains are now resistant to penicillin because *S. aureus* makes a substance called β lactamase that degrades penicillin and destroys its antibacterial activity.[2] In the early 1960s a new type of penicillin antibiotic called methicillin was developed. Methicillin was not degraded by β lactamase and hence used for treating *S. aureus* infections; but shortly MRSA strain emerged and its first major appearance was in USA in 1981 among intravenous drug users. MRSA is after referred in the media as a "superbug".[3] Resistance to methicillin is mediated via the mec operon, part of the staphylococcal cassette chromosomes MEC (SCC MEC). Resistance is conferred by *Mec A* gene which codes for an altered penicillin-binding protein (PBP2g or PBP2') that has lower affinity for binding beta lactams (penicillin's, cephalosporins and carbapenerns) and allows resistance to all β- lactams.[4]

The majority of MRSA infection can be classified as Community Acquired MRSA and Hospital Acquired MRSA. HA-MRSA infection occurs in healthcare setup such as hospitals, nursing homes. CA-MRSA occurs due to excessive use of antibiotics in healthy individuals in the community setting which leads to resistance among the microorganisms.[5]The present study includes detection of MRSA strain from various patient samples and to determine the Antibiogram pattern of MRSA isolates to various antibiotic disc with special reference to pristinamycin.

MATERIALS & METHODS

The study was undertaken at the Microbiology department in a tertiary care hospital from October 2011 to march 2012.

Various clinical specimens submitted for culture and sensitivity were processed and suspected staphylococci isolated from these clinical samples were included in the study and processed further. The samples include blood, urine, various body fluids, sputum and pus which were received at the microbiology department. The samples were processed by streaking onto a MacConkey agar medium and blood agar medium, and incubated at 35°C-37°C for 24 hrs.

Staphylococcus aureus was identified by Gram's staining, catalase and coagulase test; followed by cefoxitin test for identifying MRSA.[6]The MRSA strains were then subjected to antimicrobial susceptibility testing using various non-β-lactam antibiotics and also to pristinamycin. Antibiotics such as Chlormphenicol, Cotrimoxzole (Trinethoprim + sulphamethoxazole') Ciprofloxacin (quinolones), Gentamicin (aminoglycoside), Clindamycin (lincosamide), Erythromycin (macrolide), and tetracycline were tested along with the newer antimicrobials pristinomycin.

RESULTS AND DISCUSSION

Total numbers of methicillin resistant *Staphylococcus aureus*isolates were 100 and they were isolated from various samples. These included sample of pus (50%), catheter tips (14%), and blood (13%), body fluid (8%, sputum (8%) and urine (7%) Out of 100 samples, majority of the isolates of MRSA was from pus (50%) and lowest was from urine sample (7%) [Table-I]. Antibiogram to MRSA isolates shows high resistance to Erythromycin (89%) and low resistance to Chloramphenicol (11%) and for the remaining antibiotics the resistance frequencies were: Pristinamycin (79%), Cotrimoxazole (67%) Gentamicin (57%), Tetracycline (34%), Clindamycin (31%) and Chloramphenicol (11%) [TableII].

Blood (13%) isolates showed more resistance to ciprofloxacin (12%) and Erythromycin (12%) and low resistance to chloramphenicol. Urine samples (7%) showed more resistance to Erythromycin (12%) and ciprofloxacin (12%) and low resistance to chloramphenicol. Pus isolates (50%) showed more resistance to Erythromycin and low resistance to chloramphenicol.Catheter tips isolates

(14%) showed more resistance to Erythromycin and low resistance to chloramphenicol.Body fluids isolates (8%) showed more resistance to Erythromycin and low resistance to chloramphenicol.

DISCUSSION

The staphylococcal isolates of the present study included all the multi resistant MRSA infections. The susceptibility pattern was determined by Kirby- Bauer disc diffusion on 100 MRSA isolates from different samples received at microbiology department and showed 79% isolates resistant to pristinamycin indicating the high frequency resistance to pristinamycin among MRSA. The first staphylococcal isolate resistant to the pristinamycin was reported in France in 1975.[7] Staphylococcal resistance always pertains to dalfopristin but not necessary to quinupristin. Quinupristin resistance to staphylococci is mediated by methylation of 23r RNA inactivation of drug. Resistance to dalfopristin is mediated by plasmid genes like *vat, vat B, vat C, vat D* and *sat A*. Two cases were reported in India from Allahabad with pristinamycin resistance of neonatal septicemia, caused by MRSA which showed primary *in vitro*pristinamycin resistance.[8]The minimum inhibitory concentrations for pristinamycin in these 2 cases were 30µg and 25µg respectively. It is interesting to find pristinamycin resistant *Staphylococcus aureus* in our country where pristinamycin is not available for patient management. So it may be postulated that resistance against pristinamycin is plasmid mediated. Various genes have been identified which are responsible for causing streptogramin resistance like erm, VgbA, vgbB and msr genes.[9]

Emergence of *Staphylococcus aureus*resistant to pristinamycin has been observed in Tunisia and characterization of the mechanism of resistance to macrolides and streptogramins were recorded from the department of dermatology, Tunisia University hospital from skin samples after oral use of pristinamycin between 2004 and 2007.[10] Resistance was observed due to carriage of vga and vat genes and this is due to selective pressure of pristinamycin. Among the remaining antibiotics which were included in our study chloramphenicol (11%) showed lowest rate of resistance to the MRSA isolates, since chloramphenicol is cheap and easy to manufacture it is frequently used in the third world. However

due to safety concerns it is no longer a first line agent for infection.

This study has highlighted the very low efficiency of pristinamycin against MRSA isolates at a tertiary care center. As this antibiotic is not available in India and hence seldom used, the high frequency of resistance is a matter of concern. There is no pressure for selective emergence of resistance to the antibiotic. There may be horizontal transfer of genes coding for streptogramin resistance from other Gram positive and Gram negative bacteria in our setting. As and when the antibiotic comes in commercial use in India proper testing and surveillance is necessary to prevent treatment failure if this agent is to be used as an empiric therapy.

CONCLUSION

Antibiotics have traditionally been known as miracle drugs, but there is growing evidence that they are becoming ever worked miracles. Although the development of antibiotic resistance may be inevitable, the rate at which it develops may be reduced by the rational use of antibiotics.

The observation of the present study and various other studies, infer that staphylococcal infections are common among hospitalized patients and continue to be a growing thorn for the medical personnel. Despite the recent reports that Gram negative bacteria have overtaken staphylococci as the leading cause of nosocomial infections, MRSA continues to be the main threat in the health care setting. Extensive surgical procedures indiscriminate use of antibiotics, lack of barrier nursing practices and prolonged hospitalization paves way for the staphylococci to cause infections and to develop drug resistance.

The alarmingly increasing trend of MRSA infection calls for the implementation of an effective infections control policy which help in counter acting the bioterror , if not completely eradicating it. Antimicrobial resistance to penicillin, methicillin or pristinamycin is an unavoidable consequence of the selective pressure of antibiotic exposure. Minimizing the antibiotic pressure is essential to control the emergence of resistance strains in the hospital and in the community.

TABLE 1

RESISTANT PATTERN OF MRSA TO VARIOUS ANTIBIOTICS AND SAMPLE DISTRIBUTION

Name of the antibiotic disc	*Pristina-mycin (15µg)*	*Chloroam-phenicol (30µg)*	*Cotrimoxa-zole (23.75µg)*	*Cipro-floxacine (5µg)*	*Gentamicin (10µg)*	*Clindamycin (2µg)*	*Erythromycin (15µg)*	*Tetracycline (30µg)*	*Linezolid*	*Vancomycin*
Types of Specimen received, N=100										
Blood (N=13)	11	3	8	12	5	6	12	4	0	0
Urine (N=7)	5	0	4	7	1	1	7	2	0	0
Sputum (N=8)	5	0	5	6	6	4	7	2	0	0
Pus (N=50)	39	4	34	41	29	11	42	16	0	0
Catheter tip (N=14)	13	2	12	13	12	7	13	9	0	0
Body Fluid(N=8)	6	2	4	7	4	2	8	1	0	0
Total Resistance (%)	79	11	67	86	57	31	89	34	0	0

TABLE 2

ANTIBIOGRAM OF MRSA ISOLATES FROM VARIOUS SAMPLES (N=100)

Antibiotic	Resistance %
Erythromycin	89%
Ciprofloxacin	86%
Pristinamycin	79%
Co-trimoxazole	67%
Gentamicin	57%
Tetracyclin	34%
Clindamycin	31%
Chloramphenicol	11%
Linezolid	0%
Vancomycin	0%

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