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Research Paper

Methicillin Resistant Staphylococcus aureus (MRSA) – A Prospective Study in a Teaching Hospital of Semiurban Setup

Medical Science

KEYWORDS : Methicillin Resistant Staphylococcus aureus (MRSA), Antibiogram, Modified Kirby-Bauer, Multi drug resistant.

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ABSTRACT

Background: Staphylococcus aureus is a well known nosocomial pathogen and a superbug. In the present study,we determined the prevalence of from pus samples of various aetiology and their in vitro susceptibility pattern to various antimicrobial agents to record the current status of MRSA response to commonly used anti Staphylococcal antimicrobials in our area. Design and Duration: A two year cross sectional prospective study from February 2014 to January 2016. Setting: Gayatri Vidya Parishad Institute of Health Care and Medical Technology, which is a Teaching Hospital situated in a semiurban area of Madhurawada, Visakhapatnam, Andhra Pradesh, India. Patients: One thousand six hundred and eighty four pus samples obtained from in and out patients of various clinical departments were included. Methodology: All the pus samples were screened for Staphylococci for possible organism of Staphylococcus by Gram stain, cultured and identified Staphylococcus aureus. MRSA identification was done by using cefoxitin (30 µg) disc. The antibiogram was determined for all these MRSA by modified Kirby-Bauer disc diffusion technique. Results: The prevalence rate of MRSA in our study was 56.38%. Isolation rate of MRSA was more in males (55.66) than females(44.34) with a male female ratio of 1.4:1. The least resistant antimicrobials were vancomycin (0%), linezolid (02.36%) and teicoplanin (04.25%) where as the most resistant being penicillin (100%), cloxacillin (97.64 %,) and erythromycin (91.98%,). Majority of the isolates were multidrug resistant (46.22%).

INTRODUCTION

Staphylococcus aureus is a well known nosocomial pathogen and a superbug. Multi drug Resistant Organisms (MDR) have become a global problem and infections with these organisms make the patient management very difficult. The introduction of beta-lactamase resistant semi-synthetic penicillins in the early 1960's provided temporary relief which ended with the emergence of Methicillin (oxacillin) Resistant *Staphylococcus aureus* (MRSA), discovered shortly after methicillin became available for clinical use[1].

The prevalence of MRSA worldwide is alarmingly increasing. Available literature indicates that the prevalence can range from 3-66%,[2] and many such studies are reported from India[2, 3, 4, 5,6,7] as well as from abroad.[7,8,9,10,11] Many of these MRSA isolates are becoming multidrug resistant and superbugs and are susceptible only to glycopeptide antibiotics such as vancomycin.[12] The prolonged hospital stay, indiscriminate use of antibiotics are the possible predisposing factors of MRSA emergence.[4] Serious endemic and epidemic MRSA infections occur globally as infected and colonized patients in hospitals mediate the dissemination of these isolates and hospital staff assists further transmission.[13] Therefore, the knowledge on prevalence of MRSA and their current antibiogram became necessary in the selection of appropriate antimicrobial for empirical treatment of these infections. In present study, we determined the prevalence of from pus samples of various etiology and their *in vitro* susceptibility pattern to various antimicrobial agents to record the current status of MRSA response to commonly used anti *Staphylococcal* antibiotics in our area.

MATERIALS AND METHODS

It is a prospective cross sectional study for a period of two years from February 2014 to February 2016 at Gayatri Institute of Health Care and Medical Technology, which is a teaching hospital setup in a suburban area of Visakhapatnam,Andhra Pradesh. A total of 1684 pus samples were collected aseptically from various patients attending the outpatient and inpatient of various departments by 2 sterile swabs and processed. Gram's staining was done for all the samples for the likely organism by one swab and then the other pus swab was inoculated onto Nutrient agar, Blood agar, MacConkey agar & a selective media (Mannitol salt agar) obtained from Hi-Media Laboratories Pvt. Ltd, Mumbai and incubated at 37 ^{0}C for 24 hours aerobically. The suspected colonies of *Staphylococci* were taken and Gram's Staining was done again, all the Gram–positive cocci in clusters were further confirmed using a battery of standard biochemical reactions including the production of bound and free coagulase enzymes using slide and tube coagulase tests based on standard testing methods.[14] *Staphylococcus aureus* ATTC-25923 included as control. The recent literature showed that MRSA testing is more sensitive and specific by using cefoxitin (30 µg) disk rather than Oxacillin (30 µg) disc.[15,16] For further confirmation, all the isolates were subjected to disc diffusion test using a cefoxitin (30 µg) disc. A 0.5 McFarland standard suspension of the isolate was made and lawn culture was done on Muller-Hinton Agar plate. Plates were incubated at 35°C for 18 h and zone diameters were measured using a special scale obtained from Hi-Media Laboratories Pvt. Ltd, Mumbai. An inhibition zone diameter of ≤ 21 mm was reported as Methicillin resistant and ≥ 22 mm was considered as Methicillin sensitive[17] (Figure-1). MRSA ATCC **s**train No. 43300 was included as a control strain.

Further, the antibiotic susceptibility pattern of Methicillin Resistant *Staphylococcus aureus* strains was determined on the day of their isolation by Modified Kirby-Bauer disc diffusion method on Muller-Hinton agar using the criteria of standard zone sizes of inhibition to define sensitivity or resistance to different anti-

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microbials. The panel of antibiotics used were penicillin, cloxacillin, erythromycin, azithromycin, amikacin, gentamicin, ciprofloxacin, gatifloxacin, tetracycline, cephalexine, cefuroxime, cefoperazone, linezolid and vancomycin. *Staphylococcus aureus* ATCC 29213 was used as control strain for the standardization of antibiotic susceptibility.

RESULTS

In our present study, total of 1684 pus samples were analyzed, out of which 376 *Staphylococcus aureus* were isolated. Among these *Staphylococcus aureus,* 212 were Methicillin Resistant *Staphylococcus aureus* (MRSA). Hence, the prevalence rate of

MRSA in our study is **56.38%.** Isolation rate of MRSA was more in males (55.66) than in females(44.34) [Table-1] with a male female ratio of 1.4:1.

Antimicrobial susceptibility in MRSA: The drugs showing greater resistance to pencillin(100%), cloxacillin (97.64 %,), erythromycin (91.98%,), azithromycin

(71.70%,) ciprofloxacin (67.92 %), gatifloxacin (82.55 %), cephalexine (75.94 %), cefuroxime (71.70%)and cefoperazone (78.77 %,) [Table-2] where as gentamicin (G) (38.21%), amikacin (AK) (35.85%),tetracycline (TE) (22.17%), teicoplanin (04.25%),linezolid (LZ) (02.36%) showing lesser resistance and vancomycin (VA) was showing 100% sensitivity in our study (Table-2).

DISCUSSION:

Staphylococcus aureus is a major pathogen in hospital acquired infections. In recent years, there has been an alarming increase in *Staphylococcus aureus* strains showing resistance to methicillin , multi resistance and reduced susceptibility to vancomycin. The potential reservoirs of MRSA include infectious patients, hospital personnel and hospital environment. We isolated 212 MRSA among 376 *Staphylococcus aureus* strains from 1684 pus specimens obtained from various patients, specially from surgical units and septic ward and septic operation theatre. In our present study, prevalence of MRSA was 56.38%. The prevalence of MRSA varies between geographical regions and between study centres.

Studies obtained from other workers in and abroad showed that there is a rise of

prevalence of MRSA in the studies of Bandaru N Rao et al[4] (52%), Chaudary A et al[18] (52.8%), Mujumder D et al[19] (52.9%), Anupurba S et al[7] (54.8%), Dar JA et al[20] (54.85%), Davoodi NR et al[21](56%), Singh S et al[22] (57.14%), Srinivas B et al[23] (58.81%), LeBlanc DM et al[24] (61%), Yilmaz S et al[25] (61.1%), Borg M et al[9] (65%), Mistry RD et al[26] (66%), Ho PL et al[27] (77%) and Alsayed S et al[28] (86.4%). where as a lesser prevalence rate of MRSA was recorded by Pulimood et al[3] (2.4%),

Chakravarthy et al[29] (6.9%), Ghebremedhin B et al[30] (7.3%), Orrett FA et al[31] (9.8%), Naimi TS et al[32]12%) Adam HJ et al[33] (19%), Shanmuga Vadivoo N et al[34] (19%), Pal N et al[35](22.8%), Ouko TT et al[36] (26.3%), Adebayo O shitter et al[37] (26.9%) Ho PL et al[27] (27.6%),Bhat G K et al[38](28%), Oommen S K et al[39] (28.7%), Rajaduraipandi K et al[40](31.1%), Stenehjem E et al[41] (31.8%), Pantazatou et al[11] (33.3%), Merlino et al[10] (34%), Khan MF et al[42] (34.8%) Nishi V et al[43] (35%), Gourni M et al[8] (41.45%), Hanumantappa et al[44] (43%), and Bandaru Narasinga Rao et al[45] (45%) and Mehta AA et al[12] (26.6%, 42.5% & 47.1%) in their studies.

In our present study, male predominance was observed in MRSA isolates with Male, Female ratio was 1.4:1(218:158).Similar findings were observed by Srinivas et al[23] and Khan MF et al.[42]

The drug resistance patterns of MRSA from clinical specimens were found to be highly variable in our study as observed between geographical regions and between study centers by other workers. In the present study, all the 212 MRSA strains were resistant to pencillin(100%), cloxacillin (97.64 %,), erythromycin (91.98%,), gatifloxacin (82.55 %), cefoperazone (78.77 %), cephalexine (75.94 %), azithromycin (71.70%), cefuroxime (71.70%), ciprofloxacin (67.92 %), and where as gentamicin (G) (38.21%), amikacin (AK) (35.85%),tetracycline (TE) (22.17%), teicoplanin (04.25%),linezolid (LZ) (02.36%) showing lesser resistance and vancomycin (VA) was showing 100% sensitivity.

Among aminoglycosides,gentamicin resistance in MRSA is world wide. Mechanism of resistance is drug inactivation by cellular transferase enzyme. Even when the organisms are sensitive either alone or with beta-lactams has proved to be less satisfactory for treatment of Staphylococcal infections. Maple et al[46] found resistance to gentamicin, tobramycin, netilmicin and amikacin to be more than 90% , Pulimood T B et al[13] reported 85.5% resistance to gentamicin. Majumder D et al[19] reported 94.1% resistance to gentamicin,Khan MF et al[42] 67.7%, Srinivas et al[46] 55.4%. Rajaduraipandi K et al[40] reported 20.5%, whose results were coinciding with our present findings (38.21%) for gentamicin and (35.85%) for amikacin. MRSA strains were also resistant to macrolides. Strains resistant to erythromycin are generally cross resistant to clarithromycin and azithromycin. Mechanism of resistance is target site alteration.Maple et al [46] in 1989 recorded 90% resistance to erythromycin and 17% to ciprofloxacin , Pulimood T B et al[13] reported 90% resistance whose results were in accordance to our findings (91.98%)and Majumder D et al[19] reported 22.8% resistance and 12.8% by Rajaduraipandi K et al[40]. All the above studies showed that the MRSA isolates were often resistant to multiple antibiotics. Therefore, treatment of infections due to this organism and its eradication is difficult and also use of beta lactam antibiotics in MRSA infections will increase antibiotic selection pressure. In the present study, the resistance pattern of linezolid(02.36%), vancomycin (00.00%) were found to be useful drugs in treating MRSA infections and similar findings were observed by Rajaduraipandi K et al[40] with 100% sensitivity for both the drugs where as Khan MF et al[42] (0.6%, 0.9%), Shanmuga Vadivoo N et al[34] (0.00%, 4%) showed similar results, but Srinivas B et al[23] (8.9%, 22.%) found more resistance for these two drugs. Teicoplanin showed 4.25% resistance where as Kaleem F et al[47] observed 6% in their study. In the present study of the 212 MRSA strains isolated, 46.22% were resistant to 8 antimicrobials. Hence, these MRSA were multi drug resistant Staphylococcus aureus. The present study is in concurrence with Majumder et al[19], Anupurba et al and Rajaduraipandi K et al[40], Khan MF et al[42], Shanmuga Vadivoo N et al [34] and Srinivas B et al[23].

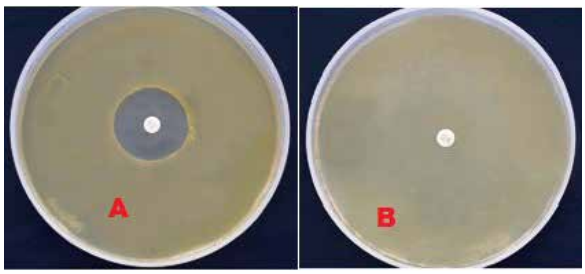
CONCLUSIONS

For life threatening Staphylococcal infections and specially for MRSA strains, vancomycin is the drug of choice. The resistance of MRSA to a wide range of antibacterials is well documented. This makes the empirical use of antibacterials effective against MRSA imperative. The regular surveillance of hospital associated infections including monitoring antimicrobial sensitivity pattern of MRSA and formulation of definite antimicrobial policy may be helpful for reducing the incidence of MRSA infections. The degree of resistance of MRSA towards commonly used antibiotics was recognized to be diverse from region to region, centre to centre and vancomycin was the only antibiotic found to give uniform sensitivity. When antimicrobials including vancomycin are considered for treatment, choice inevitably requires the need for *in vitro* susceptibility testing of every isolate of MRSA in the clinical laboratories as now a days vancomycin was also showing reduced susceptibility for these MRSA and the same testing by disc diffusion method may give false results because of the disc concentration being used in the procedure.[48]

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Figure – 1: MRSA Testing using cefoxitin (30 µg) disc.



A: Methicillin Sensitive, B: Methicillin Resistant

Table-1: Age-wise distribution of MRSA

SEX	TOTAL	MRSA* ISOLATES (n=212)	
		Number	Percent
Male	218	118	55.66
Female	158	94	44.34
Total	376	212	100.00

MRSA* - Methicillin Resistant *Staphylococcus aureus*

Table -2: Antibiogram of MRSA showing resistance to various antimicrobials

S.No.	Name of the Antimicrobial	Disc Concentration	MRSA	
			Number	Resistance%
	Amikacin (AK)	30 µg	76	35.85
	Azithromycin(AZM)	15 µg	152	71.70
	Cefoperazone(CPZ)	75 µg	167	78.77
	Cefuroxime (CXM)	30 µg	152	71.70
	Cephalexin (CN)	30 µg	161	75.94
	Ciprofloxacin (CIP)	5 µg	144	67.92
	Cloxacillin (COX)	5 µg	207	97.64
	Erythromycin (E)	15 µg	195	91.98
	Gatifloxacin (GAT)	5 µg	175	82.55
	Gentamicin (G)	10 µg	81	38.21
	Linezolid (LZ)	30 µg	5	02.36
	Teicoplanin (TEI)	30 µg	9	04.25
	Penicillin (P)	10U	212	100.00
	Tetracycline (TE)	30 µg	47	22.17
	Vancomycin (VA)	30 µg	0	00.00

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