Journal of Pharmaceutical and Scientific Innovation
www.jpsionline.com
Short Communication

A PROSPECTIVE STUDY OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN A TEACHING HOSPITAL OF RURAL SETUP

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Received on: 15/03/12 Revised on: 20/04/12 Accepted on: 25/04/12

ABSTRACT
Methicillin Resistant *Staphylococcus aureus* (MRSA) is an important nosocomial pathogen. We report the prevalence and antimicrobial susceptibility pattern of MRSA in a Teaching Hospital of Rajivgandhi Institute of Medical Sciences (RIMS), Srikakulam, Andhra Pradesh, India. A total of 4840 clinical specimens were collected from different patients and subjected to MRSA screening using conventional microbiological methods. Subsequently the antibiotic sensitivity test was performed for the confirmed MRSA isolates. Out of 280 strains of *Staphylococcus aureus* isolated from clinical samples, 146(52%) were found to be Methicillin Resistant. Predominant MRSA isolates were from Pus (64.38%) followed by sputum/Throat swabs (24.66%). All (100%) clinical MRSA strains were resistant to penicillin, least resistance to lenezolid (4.11%) and all strains of MRSA were sensitive to Vancomycin (100%). Multidrug resistance was observed among 76% of clinical MRSA isolates with 36.% isolates were resistant to 8 drugs. The determination of prevalence and antimicrobial sensitivity pattern of MRSA will help the treating clinicians for empirical treatment in places where antimicrobial sensitivity testing facilities were not available or even available, till the receipt of the report.
Keywords: Methicillin Resistant *Staphylococcus aureus* (MRSA), Methicillin Sensitive *Staphylococcus aureus* (MSSA), Antimicrobial Sensitivity, Multidrug Resistance, Vancomycin

INTRODUCTION

Staphylococcus aureus has been reported as a major cause of community and hospital acquired infections.[1] Methicillin resistant *Staphylococcus aureus* (MRSA) strains were initially described in 1961 and emerged in the last decade as one of the most important nosocomial pathogens which was reported just one year after the launch of methicillin.[2] Many of these MRSA isolates are becoming multidrug resistant and are susceptible only to glycopeptide antibiotics such as vancomycin.[3] The prolonged hospital stay, indiscriminate, irregular use of antibiotics, lack of awareness, receipt of antibiotics before coming to the hospital etc. 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.[5] Therefore, the knowledge of prevalence of MRSA and their current antimicrobial profile become necessary in the selection of appropriate empirical treatment of these infections as antimicrobial testing laboratories are uncommon in many parts of this area. We determined the prevalence of MRSA from different clinical samples 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 and around Srikakulam, Andhra Pradesh.

MATERIALS AND METHODS

A total of 4840 clinical specimens such as Pus, Sputum/Throat Swabs, Urine, Blood, Ear Swabs, Vaginal swab and Pleural Fluid were collected for *Staphylococcus aureus* screening. The clinical samples (4840) were obtained from various patients attending various departments of Rajivgandhi Institute of Medical Sciences (RIMS) Government General Hospital, Srikakulam, Andhra Pradesh, India from March., 2010 to February, 2012. All the samples were aseptically handled and processed. The morphotypes were done for all the samples based on the Gram staining method to determine the likely organism present. Subsequently, the clinical specimens were inoculated on to blood agar plates (aerobic with 5% CO_2), MacConkey agar for further subculturing and incubated at 37ºC for 24 hours. The colonies of 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 test based on standard methods.[6] *Staphylococcus aureus* ATTC-25923 of known coagulase production was included as control strain. All the confirmed *Staphylococcus aureus* strains were subsequently tested for methicillin resistance based on recommendations of Clinical Laboratory Standard Institute[7] (CLSI), formerly National Committee for Clinical Laboratory Standards (NCCLS) using oxacillin discs (1µg) obtained from Hi-Media Laboratories Pvt. Ltd, Mumbai. The isolates were considered Methicillin (Oxacillin) resistant if the zone of inhibition was 13 mm or less. Further, the antibiotic susceptibility pattern of methicillin resistant *Staphylococcus aureus* strains was determined on the day of their isolation by disc diffusion method on Muller Hinton agar using the criteria of standard zone sizes of inhibition to define sensitivity or resistance to different antimicrobials[7] Penicillin(P), Erythromycin(E), Gentamicin(G), Ciprofloxacin(CF), Azithromycin(AT), Piperacillin + Tazo(PT), Tetracycline(T), Tetracycline(T), Gatifloxacin(GF), Cefoperazone(CS), Cephalexine(CP), Cefuroxime(CU), Cloxacillin(CX), Linezolid(LZ) and Vancomycin(VA). *Staphylococcus aureus* ATCC 29213 was used as control strain for the standardization of antibiotic susceptibility testing.

RESULTS

In our present study, we isolated 146 MRSA strains from 280 isolates of *Staphylococcus aureus* from 4840 clinical specimens obtained from various patients. The prevalence of MRSA was 52%. The prevalence of MRSA was different among various clinical specimens and was found that 64.38%

of these isolates were from Pus followed by Sputum/Throat Swabs(24.66%), Urine(4.11%), Blood(2.74%), Ear Swabs(1.24%) and Vaginal swab(1.37%). (Table-**1**). The antimicrobial sensitivity pattern was shown in Table-**2**. All (100%) clinical MRSA strains were resistant to penicillin, least resistance to lenezolid (4.11%) and all strains of MRSA were sensitive to Vancomycin (100%).

DISCUSSION

Staphylococcus aureus is a major human pathogen that is very common and highly virulent. Increased antimicrobial resistance for such an organism is, therefore a cause of concern. As new antistaphylococcal agents have become available, there has been a subsequent increase in *Staphylococcus aureus* resistance to them. In recent years there has been an alarming increase in the *Staphylococcus aureus* strains showing resistance to methicillin and reduced susceptibility to Vancomycin. The potential reservoirs of MRSA include infectious patients, hospital personnel and hospital environment. This has driven the search for even more drugs and for ways to control the spread of the organisms.[8]

In our present study, we isolated 146 MRSA and 134 Methicillin Sensitive *Staphylococcus aureus* (MSSA) among 280 *Staphylococcus aureus* strains from 4840 clinical specimens obtained from various patients. The prevalence of MRSA was 52% . Similar prevalence rate of MRSA was obtained from other workers in India and abroad include – Mujumder D et al[22] (52.9); Anupurba S., et al[23] (54.8); Dal Ja et al[24] (54.85) where as a lesser prevalence rate was recorded by Mehta AA et al[9] (42.5,47.1): Hanumantappa et al[10] (43): Gourni M et al[11] (41.45): Chakravarthy et al[12] (6.9): Pal N et al[13] (22.8): Pulimood et al[14] (2.4): Mehta AA et al[9] (26.6) Nishi V et al[15] (35); Rajaduraipandi K et al[16] (31.1): Orrett FA et al[17] (9.8): Merlino et al[18] (34): Pantazatou et al[19] (33.3); Naimi TS et al[20] (12) and Adebayo O shittu et al[21] (26.9) and Bandaru Narasinga Rao et al[25]

A higher prevalence rate was obtained by some other workers in their study – Borg M et al[26] (65). The details of these findings were shown in Tables **3** and **4**.

Our predominant MRSA isolates were from Pus (63%) followed by sputum/Throat swabs(21.44%). Similar findings were observed by other workers in their study – Anupurbha et al[23] 52.5%, Tahnkiwal SS et al[27] 26.92% , Quereshi from Pakisthan[28] 83% where as a lower incidence was reported by Rajaduraipandi et al[16] 33.6%. But Rajaduraipandi et al[16] reported a higher percentage from sputum/Throat swabs(35.7%).

The drug resistance patterns of MRSA isolated from clinical specimens were found to be highly variable. All the 146 MRSA strains (100%) were resistant to penicillin, 94.52% to Erythromycin, 92.47% to Azithromycin, Gentamicin and Cephalexine 84.25% each, Cloxacillin 81.50%, Piperacillin + Tazobactum 78.08% ,Cefoperazone 76.71%, Gatifloxacin 66.44%, Cefuroxime 64.38%, Tetracycline 60.96% and least resistant to Linezolid(4.11%). However, all (100%) MRSA strains were sensitive to vancomycin, which was followed by 95.99% to linezolid.

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[2] found resistance to gentamicin, tobramycin, netilmicin and amikacin to be more than 90% , Pulimood T B et al[17] reported 85.5% resistance to gentamicin. Majumder D et al[9] reported 94.1% resistance to gentamicin and 20.5% by Rajaduraipandi K et al.[19]

MRSA strains are 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[2] in 1989 recorded 90% resistance to erythromycin.

Maple et al[2] reported 17% resistance to ciprofloxacin , Pulimood T B et al[14] reported 90% resistance and Majumder D et al[9] reported 22.8% resistance and 12.8% by Rajaduraipandi K et al.[19]

All the above studies show that the MRSA isolates are 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 linezolid and Vancomycin were found to be useful drugs in treating MRSA infections and similar findings were observed by Rajaduraipandi K et al[19] with 100% sensitivity for both the drugs.

In the present study of the 146 MRSA strains isolated, 32.09% were resistant to 8 antimicrobials,. Hence these MRSA are multi drug resistant *Staphylococcus aureus.* The present study coincides with Majumder et al[9], Anupurba et al[10] and Rajaduraipandi K et al.[19] 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 antibiotic sensitivity pattern of MRSA and formulation of definite antibiotic policy may be helpful for reducing the incidence of MRSA infections. the degree of resistance or sensitivity of MRSA towards commonly used antibiotics is recognized to be diverse from region to region and Vancomycin seems to be the only antimicrobial agent which showed 100% sensitivity and may be used as the drug of choice for treating multidrug resistant MRSA infections. One has to keep in mind that the emergence of Vancomycin Intermediate Resistant *Staphylococcus aureus* (VISA) and Vancomycin Resistant *Staphylococcus aureus* VRSA stains were reported which can not be identified by conventional antimicrobial sensitivity testing methods and such strains need special testing methods for their identification. However, regular monitoring of vancomycin sensitivity and routine testing of other newer glycopeptides like teicoplanin should be carried out.

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Table 1: Sex wise Distribution among MRSA* Isolates (n=146)

Sex	Total	MRSA Isolates	
		No.	Percentage
Male	156	89	60.96
Female	124	57	39.04
TOTAL	**280**	**146**	**100.00**

***** MRSA : Methicillin Resistant *Staphylococcus aureus*

Table 2: Specimen wise distribution of MRSA* (n=146)

Clinical Specimens	MRSA	
	No.	Percentage
Pus	94	64.38
Sputum/Throat Swabs	36	24.66
Urine	6	4.11
Blood	4	2.74
Ear Swabs	4	2.74
Vaginal Swab	2	1.37
Pleural Fluids	Nil	Nil
Total	**146**	**100**

*** MRSA :** Methicillin Resistant *Staphylococcus aureus*

Table 3: Antibiotic susceptibility pattern of MRSA* (n=146)

Antibiotic	Disc content	Resistance (%)	Sensitivity (%)
Penicillin(P)	10 U	146(100.00)	0(0.00)
Erythromycin(E)	15 mcg	138(94.52)	8(5.48)
Azithromycin(AT)	15 mcg	135(92.47)	11(7.53)
Ciprofloxacin(CF)	10 mcg	133(91.09)	13(8.91)
Cephalexine(CP)	30 mcg	123(84.25)	23(15.75)
Gentamicin(G)	50 mcg	123(84.25)	23(15.75)
Cloxacillin(CX)	5 mcg	119(81.50)	27(18.50)
Piperacillin + Tazo(PT)	100/10 mcg	114(78.08)	32(21.92)
Cefoperazone(CS)	75 mcg	112(76.71)	34(23.29)
Gatifloxacin(GF)	5 mcg	97(66.44)	49(33.56)
Cefuroxime(CU)	30 mcg	94(64.38)	52(35.62)
Tetracycline(T)	30 mcg	89(60.96)	57(39.04)
Linezolid(LZ)	30 mcg	6(4.11)	140(95.99)
Vancomycin(VA)	30 mcg	0(0.00)	146(100.00)

*** MRSA:** Methicillin Resistant *Staphylococcus aureus*

Table 4: MRSA* isolated from clinical samples: Indian studies

1988	Chakravarthy et al[12]	Delhi	6.9
1990	Pal N et al[13]	Chandigarh	22.8
1993	Pulimood et al[14]	Vellore	2.4
1996	MRSA surveillance study group by Mehta AA et al[9]	Mumbai, N. Delhi, Bangalore	26.6, 42.5, 47.1
2001	Majumder D et al[163]	Assam	52.9
2002	Hanumathappa et al[10]	Davangere	43
2003	Anupurba S., et al[43]	Varanasi	54.8
2004	Nishi V et al[15]	Mangalore	35
2006	Rajaduraipandi K et al[16]	Tamilnadu	31.1
2006	Dar JA et al[24]	Uttar Pradesh	54.85
2009	Bandaru Narasinga Rao et al[25]	Visakhapatnam	45
2012	Present Study	Srikakulam	52

*** MRSA :** Methicillin Resistant *Staphylococcus aureus*

Table 5: MRSA* isolated from clinical samples: International studies

Year	Author	Place	MRSA%
1999	Orrett FA et al[17]	Trinidad	9.8
2001	Gourni M et al[11]	Cyprus	41.45
2002	Merlino et al[18]	Sydney	34
2003	Pantazatou et al[19]	Greece	33.3
2004	NaimiTS et al[20]	U.S.A.	12
2006	Adebayo O shittu et al[21]	South Africa	26.9
2006	Borg M et al[25]	Jordan	65

*** MRSA :** Methicillin Resistant *Staphylococcus aureus*

Source of support: Nil, Conflict of interest: None Declared