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Antimicrobial Susceptibility Pattern of Methicillin Resistant *Staphylococcus aureus* (MRSA) in and around Trivandrum, India

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A B S T R A C T

Staphylococcus aureus continues to be a dangerous pathogen for both community-acquired as well as hospital-associated infections. Strains of *S. aureus* resistant to methicillin were reported soon after its introduction in October 1960.The antimicrobial chemotherapy for this species has always been empirical, because of its resistance to many therapeutic agents. This study was carried out in and around Trivandrum, Kerala to isolate MRSA from a total of 3934 clinical samples comprising of urine, pus, throat swab/sputum, nasal swab and blood. The percentage of MRSA in this study was 35.41, which is considered to be very high compared to the prevalence of MRSA in most of other published studies. In this study all the strains of MRSA were susceptible to linezolid and vancomycin and resistant to all other antibiotics trimethoprim, gentamycin, amikacin, ciprofloxacin, erythromycin and clindamycin. An antibiotic policy and the monitoring of susceptibility patterns of MRSA may also help in decreasing the prevalence of MRSA and antibiotic resistance.

Introduction

Staphylococcus aureus continues to be a dangerous pathogen for both community-acquired as well as hospital-associated infections. The antimicrobial chemotherapy for this species has always been empirical, because of its resistance to many therapeutic agents (Jun *et al.*, 2004). The emergence of methicillin resistant *Staphylococcus aureus* (MRSA), was reported just one year after the launch of methicillin (Qureshi *et al.*, 2004). Many of these MRSA isolates are becoming multidrug resistant and are susceptible only to glycopeptide antibiotics such as vancomycin (Mehta *et al.*, 1998) Low level resistance even to vancomycin has been reported (Assadullah *et al.*, 2003). The prolonged hospital stay, indiscriminate use of antibiotics, lack of awareness, receipt of antibiotics before coming to the hospital etc. are some of the possible reasons for the emergence of MRSA (Anupurba *et al.*, 2003). Serious endemic and epidemic MRSA infections occur globally as infected and colonized patients in hospitals mediate the dissemination of these isolates and

the hospital staff assists further transmission (McDonald, 1997). The development of resistance to multiple antibiotics and control of disease transmission by MRSA isolates in hospitals/communities have been recognized as the major challenges as the bacterial population that expresses the resistance phenotype varies according to the environmental conditions (Qureshi *et al.,* 2004). Hence the present study was carried out to determine the prevalence of MRSA isolated from different clinical samples and to record the current status of MRSA response to commonly used anti *Staphylococcus* antibiotics in and around Trivandrum, since this city is surrounded by eleven villages.

Materials and Methods

A total of 3934 clinical specimens such as urine, pus, sputum/throat swab, nasal swabs and blood were collected in sterile containers for the isolation and identification of *Staphylococcus aureus.* The clinical samples were obtained from various private hospitals and private pathological laboratories situated in and around Trivandrum from July 2014 to June 2015. All the samples were aseptically handled and were examined individually for the presence of *S. aureus* by plating them on Mannitol salt agar (HiMedia) and incubated at 37 °C for about 24 hr. The characteristic colonies were aseptically isolated and the bacterial strains were sub cultured on nutrient agar slants and stored at 4°C for further use.

The isolated strains were identified up to their species level by Gram staining and standard biochemical tests such as catalase, urease, oxidase, citrate utilization, indole, methyl red and Voges Proskauer test. Identification of *S.aureus* isolates was confirmed by direct-tube coagulase test with plasma. The haemolytic activity of the S. aureus isolates were determined by blood agar plate assay (Breneder and Janda, 1987). All strains were further tested for the production of free coagulase enzyme using tube coagulase test based on standard methods. [7] *Staphylococcus aureus* ATTC-25923 of known coagulase production was included as control strain. A total of 384 isolates were found to be the strains of *S. aureus*, out of which 136 isolates were classified as MRSA and the remaining 248 isolates were MSSA.

The antibiotic susceptibility testing was performed at different study sites by the Kirby Bauer‘s’ disc diffusion technique and minimum inhibitory concentration (MIC) testing, using Clinical and Laboratory Standards Institute (CLSI) recommendations (CLSI document M100-S18, 2008). All the confirmed *S. aureus* strains were subsequently tested for methicillin resistance based on Kirby-Bauer disk diffusion method using Cefoxitin (30 µg) discs obtained from Hi-Media Laboratories Pvt. Ltd. The isolates were considered methicillin resistant if the zone of inhibition was 10 mm or less. The other antibiotics tested included penicillin (10 units), gentamicin (10 µg), co-trimoxazole (1.25/23.75 µg), ciprofloxacin (5 µg), erythromycin (15 µg), clindamycin (2 µg), vancomycin (30 µg) and linezolid (30 µg). Discs from Hi-media (Mumbai) were used in this study. Inoculum was prepared by making a direct saline suspension of isolated colonies selected from an 18- to 24-h blood agar plate. Turbidity of the suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard and five discs were applied on a 100mm Mueller Hinton agar plate as per CLSI guidelines. *S. aureus* ATCC 25923 was used as the quality control strain for disc diffusion.

Results and Discussion

Out of 3934 clinical samples comprising of urine, pus, throat swab/sputum, nasal swab and blood, 384 isolates were found to be *S.*

aureus (9.76%). The maximum number of strains of *S. aureus* was isolated from pus (31.03%). The percentage of isolation of *S. aureus* form urine, throat swab/sputum, nasal swab and blood was found to be 1.57, 6.46, 3.16 and 9.64 respectively. Out of 384 strains of *S. aureus*, the maximum number of MRSA isolates was confirmed from throat swab/sputum (42.30%). The percentage of MRSA was found to be 16.66, 36.6, 28.57 and 28.4 from clinical samples of urine, pus, nasal swab and blood respectively (Table 1).

The susceptibility pattern of MRSA and MSSA against different antibiotics is tabulated in Table 2. The percentage of antibiotic susceptibility pattern of MRSA isolates was found to be variable.

All the isolates of MRSA were totally susceptible to linezolid and vancomycin and resistant to co-trimoxazole (90.45%), gentamycin (91.18%), amikacin (79.42%), ciprofloxacin (90.45%), erythromycin (88.98%), clindamycin (64.71%). All the MSSA isolates were found to be susceptible to most of the antibiotics such as cefoxitin (100%), co-trimoxazole (34.67%) amikacin (63.70%), erythromycin (64.43%), clindamycin (89.91%), linezolid (100%), and vancomycin (100%).

Staphylococcus aureus continues to be a dangerous pathogen for both community-acquired as well as hospital-associated infections. Strains of *S. aureus* resistant to methicillin were reported soon after its introduction in October 1960 (Jevons MP, 1961). Methicillin resistant *S. aureus* (MRSA) is now endemic in India. The percentage of MRSA in this study was 35.41, which is considered to be very high compared to the prevalence of MRSA in most of other studies. The incidence of MRSA varies from 25 per cent in western part of India (Patel *et al.*, 2010) to 50 per cent in South India (Gopalakrishnan and Sureshkumar, 2010). Community acquired MRSA (CA-MRSA) has been increasingly reported from India (D'Souza *et al.*, 2010). In this study the maximum number of MRSA was isolated from throat swab/sputum (42.30%) where as in case of pus it was 36.6 % only. The percentage of MRSA in case of nasal swab and blood was found to be 28.57 and 28.4 respectively. A high prevalence of MRSA (35% in ward and 43% in ICU) was observed from blood culture specimens in a study in Delhi (Wattal *et al.*, 2010) In a study conducted by Rajaduraipandi *et al.*, (2006), the prevalence of MRSA was significantly different among various clinical specimens and was found to be 35.7% isolated from throat swabs, followed by pus (33.6%).

Table.1 Isolation of *S.aureus* and MRSA from clinical specimens

Clinical Samples	Total samples (n=3934)	S. aureus (n=384=9.76%)	%	MRSA (n=136=35.41%)	%
Urine	1145	18	1.57	3	16.66
Pus	986	306	31.03	112	36.6
Throat swab/sputum	402	26	6.46	11	42.30
Nasal swab	221	7	3.16	2	28.57
Blood	280	27	9.64	8	28.4
Total	**3934**	**384**		**136**	

Table.2 Antibiotic susceptibility pattern of 136 strains of MRSA and MSSA

Antibiotics	MRSA n=136 (35.41%)	% of susceptibility	% of resistance	MSSA n=248 (64.58%)	% of susceptibility	% of resistance
Cefoxitin	0	0	100	248	100	0
Penicillin	0	0	100	22	8.87	91.13
Co-trimoxazole	13	9.55	90.45	86	34.67	65.33
Gentamycin	12	8.82	91.18	119	47.98	52.12
Amikacin	28	20.58	79.42	158	63.70	36.30
Ciprofloxacin	13	9.55	90.45	115	46.37	53.63
Erythromycin	15	11.02	88.98	183	64.43	35.57
Clindamycin	48	35.29	64.71	223	89.91	10.09
Linezolid	136	100	0	248	100	0
Vancomycin	136	100	0	248	100	0

The overall MRSA prevalence in the study conducted by INSAR was 42 per cent in 2008 and 40 per cent in 2009. The prevalence of MRSA in a study from Chennai (Gopalakrishnan and Sureshkumar, 2010) was reported as 40-50 per cent. *S. aureus* constituted 17 per cent of catheter related blood stream infections (CRBSIs) in that centre. A high prevalence of MRSA (35% in ward and 43% in ICU) was observed from blood culture specimens in a study in Delhi (Wattal *et al.,* 2010). Chatterjee *et al.,* (2009*)* found the overall prevalence of *S. aureus* nasal colonization was 52.3 per cent and that of MRSA was 3.89 per cent in the community. In a study from North India (Arora *et al.,* 2010), the prevalence of MRSA was 46 per cent and MRSA isolates were found to be more resistant to other antibiotics than MSSA.

In this study all the strains of MRSA were susceptible to linezolid and vancomycin and resistant to all other antibiotics such as trimethoprim, gentamycin, amikacin, ciprofloxacillin, erythromycin and clindamycin. Significant difference was observed in case of erythromycin, ciprofloxacin, gentamicin and amikacin. Vancomycin is considered inferior to β-lactams for the treatment of MSSA bacteremia and endocarditis (Liu *et al.,* 2011). Therefore, the first-generation cephalosporins are the drugs of choice for the treatment of MSSA infections in patients who are unable to tolerate antistaphylococcal penicillins. De-escalation of vancomycin to β-lactams should be encouraged in all cases of MSSA. With MRSA isolates being widespread, it is imperative that treating physicians de-escalate to β-lactams once the culture sensitivity results reveal a MSSA isolate. Preservation of glycopeptides and linezolid for use only in MRSA cases should be encouraged.

In conclusion, the study has shown that the prevalence of MRSA infections is high in comparison to studies done earlier. An antibiotic policy and the monitoring of susceptibility patterns of MRSA may also help in decreasing the prevalence of MRSA and antibiotic resistance.

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