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Original Article

Microbiology Section

Changing Trends in Resistance Pattern of Methicillin Resistant *Staphylococcus aureus*

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ABSTRACT

Background: Methicillin resistance in *Staphylococcus aureus* is associated with multidrug resistance, an aggressive course, increased mortality and morbidity in both community and health care facilities. Monitoring of newly emerging and prevalent Methicillin Resistant *Staphylococcus aureus* (MRSA) strains for their resistance patterns to conventional as well as novel drugs, are essential for infection control.

Aims: To study the changing trends in resistance patterns of MRSA at our hospital.

Settings and Design: This cross sectional study was carried out in a 750 bed tertiary care hospital in south India.

Material and Methods: One hundred and two clinical isolates of MRSA which were obtained in 2004-2011 were identified by using oxacillin, cefoxitin disc diffusion test and oxacillin screening agar test. Antibiotic susceptibility test was done for commonly used non beta lactam anti-Staphylococcal drugs, as well as for anti-MRSA drugs like vancomycin, linezolid, mupirocin and rifampicin. Minimum inhibitory concentration (MIC) of vancomycin was determined by using Vancomycin HiComb strip (Himedia, Mumbai, India).

Statistical Analysis which was done: Chi-square test and proportions were used to compare the two groups.

Results: MRSA isolates showed high resistance to co-trimoxazole (82.3%), ciprofloxacin (76.4%), gentamicin (64.7%) and tetracycline (49%) as compared to other drugs. High prevalence of ciprofloxacin resistance was detected, particularly among outpatients. Multi resistant MRSA with a ≥ 3 non-beta lactam agent resistance was 79%. All MRSA isolates were sensitive to vancomycin, linezolid, mupirocin and rifampicin. MRSA had displayed increase in resistance to most antibiotics except tetracycline in recent years.

Conclusions: Taking into consideration the prevalence of multidrug resistance in MRSA, resistance patterns should be evaluated periodically and antibiotic therapy should be guided by susceptibility testing.

Key words: Methicillin resistant *Staphylococcus aureus*, Vancomycin, Multidrug resistance

INTRODUCTION

Historically, *S. aureus* has been one of the most common pathogens which caused pyogenic local and systemic infections in both hospitals and community. By virtue of a battery of virulence factors, *S. aureus* has propensity to cause a wide spectrum of infections which involve several organ systems, some of which, especially meningitis, endocarditis and blood stream infections, are frequently fatal in nature [1]. Methicillin Resistant *S. aureus* (MRSA), are strains of *S. aureus* which express an altered penicillin binding protein (PBP2a), thus conferring resistance to beta lactam antibiotics. MRSA strains had caused several documented outbreaks of hospital cross infections throughout the world in 1970s and since then, they have drawn special attention in hospital acquired infections [2]. Severe and drug resistant infections which were predominantly restricted to hospitals are now becoming rampant in community, as novel MRSA strains, which have been described as community acquired MRSA (CA-MRSA) [3]. MRSA are notorious for their wide variations in antibiotic resistance patterns. They not only develop chromosomal resistance to penicillins and cephalosporins but also frequently show resistance to a wide range of antibiotics which are commonly used in hospitals [4]. Within a country, there may be local variations in predominant hospital and community strains of MRSA [5, 6]. The resistance pattern of CA-MRSA is essentially different from that of hospital acquired MRSA (HA-MRSA). Unlike CA-MRSA, hospitals strains display more drug resistance in an attempt to survive in hospital environment. The resistance patterns of prevalent MRSA strains in any setup are liable to continuous changes over a period of time, owing to changes in antibiotic prescription patterns, infection control measures and awareness among healthcare workers. As a result of increasing antibiotic pressure in hospitals, new strains with higher antibiotic resistance emerge and they replace the previous strains. While methicillin resistance in *S. aureus* is less in countries like Norway and Sweden (1%), Netherlands (2%) and Canada (5-10%), it is 25-50% in the United States, 54% in Portugal and 43%-58% in Italy [7]. High prevalence of MRSA is an emerging problem in India. Several authors have reported a substantial increase in MRSA prevalence in India. It has increased from 12% in 1992 to 40% in 2009 [8, 9]. Increasing resistance of MRSA in recent years has had a significant impact on several aspects of patient care and infection control. Antibiotic policies need to be updated regularly, along with comprehensive monitoring of antibiotic prescribing and antibiotic consumption in healthcare settings. These facts clearly highlight the need of a characterization of MRSA strains at a regular basis at all levels. Therefore, this study was done to determine changing patterns of MRSA infection in our hospital over past seven years, with a special focus on resistance pattern.

MATERIAL AND METHODS

A cross sectional study was carried out, after obtaining institutional ethical committee clearance, in a 750 bed tertiary care hospital in south India, which caters to patients from Pondicherry and neighboring districts of Tamil Nadu. Informed consents were obtained from patients with MRSA infection, after explaining to them, all aspects of MRSA infection and their participation in a clinical study. A proforma was prepared for recording patient related information. Forty MRSA isolates from clinical materials which were preserved from March, 2004–December, 2009 and 62 new isolates which were recovered during January, 2010 to June, 2011, from

various clinical samples such as pus swabs and aspirates, blood, urine, sputum and endotracheal tube aspirates, were included in this study. Consecutive isolates of MRSA from same patients were excluded.

All clinical samples were processed in the laboratory as per standard guidelines. *S. aureus* isolates were identified by standard laboratory procedures. Disc diffusion test which used a 30µg cefoxitin disc and a 1µg oxacillin disc and oxacillin screen agar test (which contained 6 µg /ml of oxacillin and 4% NaCl) were performed as per Clinical and Laboratory Standards Institute (CLSI) guidelines, to detect MRSA stains [10]. A panel of commonly used anti-Staphylococcal antibiotics (Himedia, Mumbai, India) which comprised of ciprofloxacin (5µg), tetracycline (30µg), gentamicin (10µg), amikacin (30µg), netilmicin (30µg), co-trimoxazole (1.25+23.75 µg), chloramphenicol (30µg), erythromycin (15µg), vancomycin (30µg), linezolid (30µg), clindamycin (5µg), mupirocin (5µg) and rifampicin (5µg) were tested by Kirby Bauer disc diffusion method for susceptibility patterns. All isolates were subjected to MIC testing of vancomycin, which was determined by using HiComb Vancomycin strips (Himedia, Mumbai, India). *S. aureus* ATCC 25923 and ATCC 43300 were used as controls for antibiotic susceptibility test. The viability of test isolates was maintained by doing periodic subcultures in semisolid nutrient agar.

All data were entered in a Microsoft Excel 2007 spreadsheet and statistical analysis was done by using GraphPad In Stat, version 3.00 (San Diego, CA, USA). Chi-square test was used to compare the two groups. All p values which were < 0.05 were considered to be statistically significant.

RESULTS

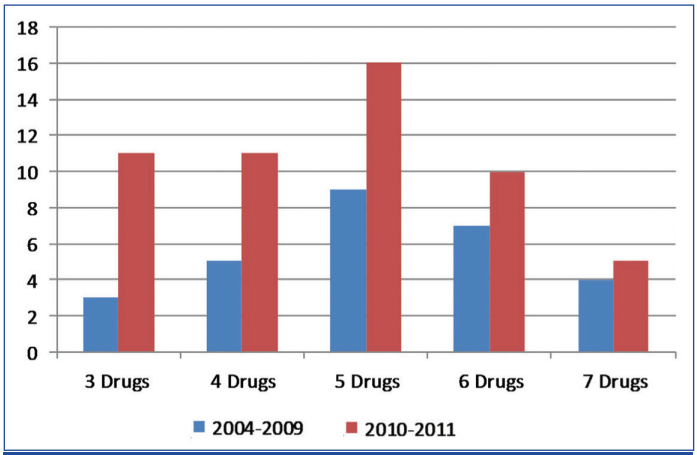
Amongst 102 cases, 39 were outpatients and remaining 63 were inpatients and they were predominately males (67.6%). A total of 75 patients (73.52%) were in 16 to 60 years age group. The most common samples were pus swabs from skin and soft tissue lesions and aspirates from superficial and deep abscesses, which constituted 58.8% and 22.5% samples respectively, followed by endotracheal aspirates (3.9%), biopsies (3.9%), blood (2.94%), urine (1.9%) and sputum (1.9%). Eighty one out of 102 patients had pyogenic lesions like superficial and deep abscesses, skin ulcers, postoperative infections, wound infections and other surgical or orthopaedic conditions like cellulitis, fistulas and bone and joint infections. MRSA infections were mainly associated with abscesses (17.64%), especially breast and thigh abscesses, which accounted for 2.9% of total cases each. Among ulcerative lesions, foot ulcers (13.72%) were predominant. In contrast to pyogenic lesions, 12 medical and 9 ICU patients had medical conditions like urinary tract infections, chronic obstructive pulmonary disease, pneumonia, organophosphate poisoning, puerperal sepsis, and endocarditis.

The resistance patterns of MRSA isolates to anti-Staphylococcal drugs in recent years have been compared in [Table/Fig-1]. There was no change in place of isolation of MRSA during these years. Although there was a minor year wise variation in number of cases, patients who were admitted in general surgery and orthopaedic wards were the main source of MRSA at our hospital. MRSA isolates showed highest resistance to cotrimoxazole, ciprofloxacin and gentamicin as compared to the resistance which they showed to amikacin, netilmicin and chloramphenicol in both inpatients and outpatients. High prevalence of ciprofloxacin resistance among outpatients as compared to inpatients was statistically significant (a two tailed p value of 0.0159). Although tetracycline resistance decreased and ciprofloxacin, amikacin, and netilmicin resistance showed increases in recent years, no statistically significant difference was found between MRSA isolates which were recovered during 2004-2009 and 2010-2011. All other drugs showed marginal increases in resistance. All MRSA isolates were sensitive to vancomycin, linezolid, mupirocin and rifampicin.

Out of 102 cases, 81 patients (47 inpatients and 34 outpatients) had infections with multi-resistant MRSA (MORSA) (they were resistant to ≥ 3 non beta lactam antibiotics). A majority of MORSA strains had resistance to 3-5 drugs. Furthermore, a multiple non beta lactam antibiotic resistance was seen more in MRSA isolates of 2010-2011 as compared to 2004-2009 isolates [Table/Fig-2]. All strains were sensitive to vancomycin by both disc diffusion and HiComb strip methods. [Table/Fig-3] However, they showed a slight increase in MIC of vancomycin in recent years [Table/Fig-4].

Antibiotics	2004-2009 n=40	2010-2011 n=62	p–value
Ciprofloxacin	28 (70%)	50 (80.6%)	0.239169
Tetracycline	20 (50%)	30 (48.3%)	1.000000
Gentamicin	25 (62.5%)	41 (66.1%)	0.832279
Amikacin	7 (17.5%)	20 (32.2%)	0.113130
Netilmicin	1 (2.5%)	6 (9.6%)	0.241063
Cotrimoxazole	31 (77.5%)	53 (85.4%)	0.425385
Chloramphenicol	4 (10%)	9 (14.5%)	0.560303

[Table/Fig-1]: Resistance pattern of MRSA isolates to anti-staphylococcal drugs in past & recent years



[Table/Fig-2]: Year wise distribution of multi drug resistance in MRSA strains

	0.001 µg/ml	0.01 µg/ml	0.05 µg/ml	0.1 µg/ml	1 µg/ml	2 µg/ml
OPD (n=39)	1	21	9	3	5	0
IPD (n=63)	5	14	27	12	2	3

[Table/Fig-3]: Vancomycin MIC of MRSA strains by HiComb strip

	0.001 µg/ml	0.01 µg/ml	0.05 µg/ml	0.1 µg/ml	1 µg/ml	2 µg/ml
2004-2009 (n=40)	2 (5%)	16 (40%)	13 (32.5%)	8 (20%)	0	1 (2.5%)
2010-2011 (n=62)	4 (6.4%)	19 (30.6%)	23 (37.1%)	7 (11.3%)	7 (11.3%)	2 (3.2%)

[Table/Fig-4]: Changes in Vancomycin MIC of MRSA strains in past & recent years

DISCUSSION

MRSA is a major cause of nosocomial outbreaks and serious infections, which causes increased mortality and morbidity. Skin and soft tissue infections, wound infections, burns, and ulcers, pressure sores, lower respiratory and urinary tract infections, septicaemia and infections associated with invasive devices are most frequently reported [3]. Hospital workers with dermatitis or with inadequate hand washing or asepsis, burns patients and long term care facility patients are the main sources of MRSA in hospitals. In some countries with high prevalences of MRSA, after

admission screening, potential MRSA carriers are isolated in single rooms or they are nursed together (cohort) in a room or a bay by maintaining contact precautions, until their screening reports turn out to be negative. In case of positive screening results, isolation measures are employed, along with decolonisation regimens. The entry of visitors, especially children and persons with compromised immunity, should be restricted. In present study, we found a higher proportion of MRSA cases among surgical patients. This may be related to the poor environmental cleaning, operation theatre surveillance and infection control measures of hospitals in Indian setup. According to one study, 80% MRSA isolates were isolated from surgical units, due to higher numbers of post–operative wound infections [6]. Not only infections, even asymptomatic colonisations were also reported to be significantly high in surgical (18%) and orthopaedic (34%), patients as compared to medical unit (1%) patients [11]. Consistent with suppurative nature of Staphylococcal infections, we found that highest number of MRSA isolates was obtained from pus. Although some investigators have reported no significant correlation with gender in MRSA infection [12], in Southern India, it was found more frequently in male patients [13], which was also observed in our study.

This study analysed antibiotic susceptibility pattern of 102 isolates against a panel of non-beta lactam antibiotics and found three major developments in antibiotic resistance over seven years. Firstly, increased resistance to ciprofloxacin in outpatients was statistically significant. Secondly, except tetracycline, all other drugs, specifically, ciprofloxacin, amikacin and netilmicin, showed a greater increase in resistance from 2004-2009 to 2010-2011. Last, but not the least, we found a slow emergence of a reduced susceptibility to vancomycin, as was indicated by an increase in its MIC in recent years. This may reflect slow development of bacterial antimicrobial tolerance in response to increasing use of antibiotics in recent years, especially fluoroquinolones, in outpatients. There are evidences of a significant decrease in fluoroquinolone-resistant MRSA over years, on reduction of fluoroquinolone use [14]. As per recent studies, the resistance pattern of MRSA in India has shown a variable pattern. There are inadequate countrywide studies which have been done on changes in antibiotic resistance on a long term basis in India. Verma et al., has reported a rapid increase in MRSA prevalence, from 12% to 80.89%, over seven years, in a tertiary care centre at Indore [8]. As per current Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group's report, the prevalence of MRSA varies from 22% to 68% in Indian hospitals, which is clearly higher than previous estimates [9]. According to INSAR report, 79.3%, 70.8%, 58.3%, 55.6% and 46.6% MRSA isolates showed resistance to ciprofloxacin, erythromycin, gentamicin, co-trimoxazole and clindamycin respectively. Thind et al*.,* found only 12.5% isolates to be resistant to tetracycline and 37% isolates to be resistant to cotrimoxazole, while they were fully sensitive to chloramphenicol, ciprofloxacin, gentamicin, amikacin, netilmicin and rifampicin [15]. In contrast to this, Anupurba et al*.,* reported a higher resistance of 84.1%, 47.5%, 89.7% and 60.5% against ciprofloxacin, netilmicin, gentamicin and amikacin respectively [16]. The resistance which was detected in other studies was intermediate of these two reports [11, 17-19].

All studies reported universal sensitivity of MRSA to vancomycin, linezolid and mupirocin. However, from northern India, Deep et al., had reported linezolid resistance in 9% MRSA [20]. Reports on reduced vancomycin sensitivities with borderline MICs are not uncommon nowadays [21, 22]. Although, we found that all MRSA strains had MICs in sensitive range, [Table/Fig-3] more number of MRSA isolates from 2010-2011 showed higher MICs for vancomycin as compared to 2004-2009 isolates [Table/Fig-4]. This indicated emergence of a decreased sensitivity to vancomycin, which could develop into a low level resistance in future.

MRSA strains with three or more non-beta lactam drug resistances have been described as multi-resistant oxacillin resistant *S. aureus* (MORSA) and they have been correlated with treatment failure [23]. We found that 79% MRSA isolates were multi drug resistant. Furthermore, multi-drug resistant strains were more common in inpatients (58%) than in outpatients (42%) and their proportion has increased from 34.5% to 65.4% in 2010-2011 as compared to that in 2004-2009. This finding was similar to a that of a study which was done in Northern India, where out of 115 MRSA, 73% strains had shown multi-resistance [24]. Among other reports, Shrestha et al*.,* had reported 97% MORSA [23]. However, their sample size was less (65 out of 67 strains). In another study which was done in 2006, multi-drug resistance was observed among 63.6% MRSA isolates which were obtained from clinical samples and among 23% MRSA isolates which were obtained from carriers. These results indicated a slowly emerging resistance of MRSA strains to several non-beta lactam drugs in different parts of India [25].

In conclusion, our study showed the changing patterns of antimicrobial resistance of MRSA strains in our hospital, which were consistent with the findings of studies which were done in other parts of the country. MRSA had displayed an increase in resistance to most of the antibiotics except tetracycline, along with a substantial increase in multi-resistant MRSA strains over a period of seven years. Although all MRSA strains showed in vitro sensitivities to vancomycin and had MICs in sensitive range, isolates which were recovered during 2010 and 2011 showed marginal increases in MICs as compared to those of older isolates. Resistance to co-trimoxazole, ciprofloxacin and gentamicin was high in both recent and old MRSA isolates and therefore, these drugs are not suitable for empirical therapy of suspected Staphylococcal infections. We conclude that *S. aureus* is a pervasive pathogen in our hospital and in community settings with constantly changing trends in virulence, resistance and epidemiology and thus, monitoring of clinical and microbiological parameters is necessary, for modifying our existing infection control measures and treatment options accordingly.

REFERENCES

[1] Whitby M, McLaws ML, Berry G. Risk of death from methicillin-resistant *Staphylococcus aureus* bacteraemia: a meta-analysis. *Med J Aust*. 2001;175: 264-7.
[2] Shanson DC, Kensit JC, Duke R. Outbreak of hospital infection with a strain of *Staphylococcus aureus* resistant to gentamicin and methicillin. *Lancet*. 1976;2:1347-8.
[3] Pantosti A, Venditti M. What is MRSA? *Eur Respir J*. 2009;34:1190-6.
[4] Pavillard R, Harvey K, Douglas D, Hewstone A, Andrew J, Collopy B, et al. Epidemic of hospital-acquired infection due to methicillin-resistant *Staphylococcus aureus* in major Victorian hospitals. *Med J Aust*. 1982;1:451-4.
[5] Simor AE, Louie L, Watt C, Gravel D, Mulvey MR, Campbell J, et al. Antimicrobial susceptibilities of health care-associated and community-associated strains of methicillin-resistant *Staphylococcus aureus* from hospitalized patients in Canada, 1995 to 2008. *Antimicrob Agents Chemother*. 2010;54:2265-8.
[6] Srinivasan S, Sheela D, Mathew R, Bazroy J, Kanungo R. Risk factors and associated problems in the management of infections with methicillin resistant *Staphylococcus aureus*. *Indian J Med Microbiol*. 2006;24:182-5.
[7] Kumar S, Joseph NM, Easow JM, Singh R, Umadevi S, Pramodhini S, et al. Prevalence and current antibiogram of staphylococci isolated from various clinical specimens in a tertiary care hospital in Pondicherry. *The Internet J Microbiol*. 2012;10?.
[8] Verma S, Joshi S, Chitnis V, Hemwani N, Chitnis D. Growing problem of methicillin resistant staphylococci – Indian scenario. *Indian J Med Sci*. 2000;54:535-40.
[9] Indian Network for Surveillance of Antimicrobial Resistance group, India. Methicillin resistant *Staphylococcus aureus* (MRSA) in India: Prevalence and susceptibility pattern. *Indian J Med Res*. 2013;137:363-9.
[10] CLSI. 2010. Performance standards for antimicrobial susceptibility testing. CLSI approved standard M100-S20. CLSI, Wayne, PA.
[11] Sarma JB, Ahmed GU. Characterisation of methicillin resistant S. aureus strains and risk factors for acquisition in a teaching hospital in northeast India. *Indian J Med Microbiol*. 2010;28:127-9.
[12] Ghaznavi-Rad E, Nor Shamsudin M, Sekawi Z, Khoon LY, Aziz MN, Hamat RA, et al. Predominance and emergence of clones of hospital-acquired methicillin-resistant *Staphylococcus aureus* in Malaysia. *J Clin Microbiol*. 2010;48:867-72.
[13] Mathanraj S, Sujatha S, Sivasangeetha K, Parija SC. Screening for methicillin-resistant *Staphylococcus aureus* carriers among patients and health care workers of a tertiary care hospital in south India. *Indian J Med Microbiol*. 2009;27:62-4.

[14] Lafaurie M, Porcher R, Donay JL, Touratier S, Molina JM. Reduction of fluoroquinolone use is associated with a decrease in methicillin-resistant *Staphylococcus aureus* and fluoroquinolone-resistant Pseudomonas aeruginosa isolation rates: a 10 year study. *J Antimicrob Chemother*. 2012;67:1010-5.
[15] Thind P, Prakash SK, Wadhwa A, Garg VK, Pati B. Bacteriological profile of community-acquired pyodermas with special reference to methicillin resistant *Staphylococcus aureus*. *Indian J Dermatol Venereol Leprol*. 2010;76:572-4.
[16] Anupurba S, Sen MR, Nath G, Sharma BM, Gulati AK, Mohapatra TM. Prevalence of methicillin resistant *Staphylococcus aureus* in a tertiary referral hospital in eastern Uttar Pradesh. *Indian J Med Microbiol*. 2003;21:49-51.
[17] Vidhani S, Mehndiratta PL, Mathur MD. Study of methicillin resistant *S. aureus* (MRSA) isolates from high risk patients. *Indian J Med Microbiol*. 2001;9:13-6.
[18] Shenoy MS, Bhat GK, Kishore A, Hassan MK. Significance of MRSA strains in community associated skin and soft tissue infections. *Indian J Med Microbiol*. 2010;28:152-4.
[19] Saikia L, Nath R, Choudhury B, Sarkar M. Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* in Assam. *Indian J Crit Care Med*. 2009;13:156-8.
[20] Deep A, Goel N, Sikka R, Chaudhary U, Yadav S, Gupta A. Quinpristin dalfopristin resistance in gram Positive bacteria: Experience from a tertiary care referral center in North India. *J Infect Dis Antimicrob Agents*. 2008;25:117-21.
[21] Menezes GA, Harish BN, Sujatha S, Vinothini K, Parija SC. Emergence of vancomycin-intermediate Staphylococcus species in southern India. *Methods*. 2006;911-2?.
[22] Abdel Hady W, Bayer AS, Seidl K, Nast CC, Kiedrowski MR, Horswill AR, et al. Reduced Vancomycin Susceptibility in an in vitro Catheter-Related Biofilm Model Correlates with Poor Therapeutic Outcomes in Experimental Endocarditis due to Methicillin-Resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother.* 2013.
[23] Shrestha B, Pokhrel BM, Mohapatra TM. Phenotypic characterization of nosocomial isolates of *Staphylococcus aureus* with reference to MRSA. *J Infect Dev Ctries*. 2009;3:554-60.
[24] Arora S, Devi P, Arora U, Devi B. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in a tertiary care hospital in northern India. *J Lab Physicians*. 2010;2:78-81.
[25] Rajaduraipandi K, Mani KR, Panneerselvam K, Mani M, Bhaskar M, Manikandan P. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus*: a multicentre study. *Indian J Med Microbiol.* 2006;24:34-8.

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