



RESEARCH ARTICLE

STUDY OF ANTIBIOTIC SUSCEPTIBILITY PATTERN OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS AT TERTIARY CARE HOSPITAL IN SOUTH INDIA

Venkatesh V N^{1*}, Shwetha D C², Ravish Kumar M³, Sudhindra K S⁴, Srinivasa settee⁵¹Professor & Head, Department of Microbiology, Basaveshwara Medical College Hospital, Chitradurga, India²Assistant Professor, Department of Microbiology, Basaveshwara Medical College Hospital, Chitradurga, India³Assistant Professor, Department of Microbiology, Basaveshwara Medical College Hospital, Chitradurga, India⁴Associate Professor, Department of Microbiology, Basaveshwara Medical College Hospital, Chitradurga, India⁵Tutor, Department of Microbiology, Basaveshwara Medical College Hospital, Chitradurga, India

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ABSTRACT

Aim: Methicillin resistant staphylococcus aureus (MRSA) has become a serious problem worldwide because of its multidrug resistance to commonly used drugs. Present study was undertaken to know the antibiotic susceptibility patterns in MRSA isolates.

Method: A total of 2000 clinical samples submitted to Microbiology Laboratory between June 2013 and December 2013 were analysed. Among these a total of 172 staphylococcus aureus (*S.aureus*) were isolated and included in the study. All the isolates were identified as *Staphylococcus aureus* and MRSA detection was done by using 1µg of oxacillin disc. Antimicrobial susceptibility testing was done by Kirby-Bauer's disc diffusion method by using a panel of nine drugs like ampicillin, azithromycin, clindamycin, amikacin, gentamicin, levofloxacin, doxycycline, linezolid and teicoplanin.

Result: Out of 2000 samples processed, 172 turned out to be staphylococcus aureus. Among 172, 62 (36.04%) were Methicillin sensitive *S.aureus* (MSSA) and 110 (63.95%) MRSA. The prevalence of MRSA in our study was 63.95%. None of the MRSA isolates showed susceptibility to Ampicillin. They also exhibited resistance to other commonly used drugs like Azithromycin (63.64%), Gentamicin (54.55%), Clindamycin (50.90%), Amikacin (40%), Levofloxacin (16.36%), Doxycycline (5.46%), Linezolid (02.72%) and Teicoplanin (01.82%)

Conclusion: Injudicious and empirical use of antibiotics will lead to drug resistance. Every clinical Laboratory should carry out detection method for MRSA to understand new and emerging resistance trends in these strains.

Key words: Antimicrobial susceptibility, Methicillin resistant staphylococcus aureus (MRSA), *Staphylococcus aureus*.

INTRODUCTION:

Nosocomial infections accounts for morbidity and mortality of millions of patients annually worldwide. *Staphylococcus aureus* especially Methicillin Resistant *Staphylococcus aureus* (MRSA) is relatively ubiquitous and is the cause of many community, endemic and epidemic nosocomial colonization and infections¹. MRSA is of concern not only because of its resistance to methicillin but also because it is generally resistant to many other chemotherapeutic agents. Staphylococcal cassette chromosome *mec* (*SCCmec*) is a genomic island

of unknown origin containing the antibiotic resistance gene *mecA*. *mecA* is responsible for resistance to methicillin and other β -lactam antibiotics².

The knowledge of prevalence of MRSA and their antibiotic sensitivity pattern in any environment becomes necessary for selection of appropriate treatment for these patients. Hence, accurate detection of MRSA is an important prerequisite for appropriate therapy and epidemiological assessment of nosocomial infections caused by this strains^{3,4}. Currently the treatment options for MRSA infections are limited to very few and expensive drugs like Teicoplanin, Vancomycin and Linezolid. Thus,

control of MRSA is essential to curtail the introduction and spread of infection⁵.

The present study was undertaken to know the antibiotic susceptibility pattern of MRSA isolates and also to know the options available to us in the treatment of such cases.

MATERIALS AND METHODS:

A prospective study was conducted in Basaveshwara Medical College Hospital and Research Center. The duration of the study was 6 months from June 2013 to December 2013. The study was done after obtaining the Institutional Ethical clearance (IEC).

A total of 2000 clinical specimens received in microbiology laboratory were processed according to the standard procedure. The different samples received were pus, urine, sputum, blood, high vaginal swab, seminal fluid, pleural fluid etc. 172 Staphylococcus aureus were isolated from these specimens. The isolates were confirmed as Staphylococcus aureus by colony morphology, Gram's staining from colony, catalase test, positive slide and tube coagulase test, mannitol fermentation and urea hydrolysis⁶.

For all 172 isolates antimicrobial susceptibility testing was performed on Mueller-Hinton agar plate (Hi-Media) with commercially available discs (Hi-Media) by Kirby-Bauer's disc diffusion method after matching the inoculum with 0.5 MacFarland standard. The panel of antimicrobial agents used were Amikacin (30µg), Gentamicin (10µg), Ampicillin / sulbactam (10/10µg), Ampicillin (10µg), Azithromycin (15µg), Doxycycline (30µg), Levofloxacin (5µg), Clindamycin (2µg), Teicoplanin (30µg) and Linezolid (30µg). All the plates were interpreted after incubation at 37°C for 24 hours. The zone diameter was

measured around each disc and was interpreted based on recent CLSI guidelines.

Detection of methicillin resistance: Staphylococcus aureus were tested for methicillin resistance by using 1µg oxacillin disc. Agar plates were incubated at 35° C and read at 48 hours of incubation. Organisms deemed resistant when zone of inhibition was less than 13mm. S.aureus ATCC 25923 and ATCC 43300 were used as oxacillin sensitive and resistant controls respectively. The susceptibility pattern of MSSA isolates are not dealt in this study.

RESULTS:

A total of 2000 clinical specimens were collected from various sources. 172 staphylococcus aureus were isolated among the total samples screened. Out of these 172 isolates, 62(36.04%) were MSSA and 110(63.95%) were MRSA. The prevalence of MRSA in our study was 63.95%. The most common sample from which MRSA isolated was pus 86(78.18%) followed by urine 16(14.54%), Blood 02(1.81%), Pleural fluid 02(1.81%), high vaginal swab 2(1.81%), Sputum 02(1.81%) as shown in table I.

None of the MRSA isolates were sensitive to Ampicillin (100%). The next most common antibiotics to which MRSA isolates showed resistance were Azithromycin (63.64%), Gentamicin (54.55%), followed by Clindamycin (50.90%), Amikacin (40%), Levofloxacin (16.36%), Doxycycline(5.46%), Linezolid (02.72%) and Teicoplanin (01.82%) which is shown in table II.

The antimicrobial agents which showed maximum susceptibility to MRSA were Linezolid (95.46%) followed by Teicoplanin (92.73%) and Doxycycline (83.64%). The MRSA isolates showed maximum intermediate sensitive to Gentamicin (21.81%) and least for Linezolid (1.81%).

Table 1: Staphylococcus aureus isolates from various clinical specimens

Sample	MRSA	MSSA
Pus	86	45
Urine	16	13
Blood	02	02
Pleural fluid	02	01
High vaginal swab	02	Nil
Sputum	02	01
Total	110	62

Table 2: Antibiotic susceptibility pattern of MRSA isolates (n=110)

Antibiotics	Susceptible Number (%)	Intermediate Number (%)	Resistant Number (%)
Ampicillin	Nil	Nil	110(100)
Azithromycin	28(25.46)	12(10.90)	70(63.64)
Gentamicin	26(23.64)	24(21.81)	60(54.55)
Erythromycin	32(29.08)	Nil	78(70.92)
Clindamycin	48(43.64)	06(05.46)	56(50.90)
Amikacin	66(60.00)	Nil	44(40.00)
Levofloxacin	84(76.36)	08(07.28)	18(16.36)
Linezolid	105(95.46)	02(01.81)	03(02.72)
Teicoplanin	102(92.73)	06(05.45)	02(01.82)
Doxycycline	92(83.64)	12(10.90)	06(5.46)

DISCUSSION:

MRSA is a global health problem with the prevalence rate ranging from 2% in the Netherland and Switzerland to 70% in Japan and Hong Kong^{7,8}. In our study the Prevalence of MRSA was found to be 63.95% which is comparable to the study by Safdar et al (65%)⁹ and high compared to the study by Pai V et al, Saikia L et al and Joshi S et al which reported prevalence of 29.1%, 34.7% and 41% respectively^{10,11,12}. This variation might be because of several factors like efficacy of infection control practices, healthcare facilities and antibiotic usage that vary from hospital to hospital¹³.

MRSA isolates also exhibited resistance to other Antimicrobial agents like Azithromycin, Clindamycin. In our study none of the MRSA isolates found sensitive to Ampicillin. This finding is also seen in study by Vidhani S et al¹⁴. Susceptibility of MRSA for Erythromycin and clindamycin were 29.08%% and 43.64% only. Susceptibility for Linezolid was 95.46% which is comparable to the study by Rajaduraipandi K et al, where MRSA strains showed 96.6% sensitivity to Linezolid¹⁵. Higher percentage of resistance noted to many antibiotics in the present study could be due to the irrational antibiotic usage, easy availability at the drug store without prescription and injudicious use in hospitals.

Higher percentage of Intermediate sensitivity was noted against antimicrobial agents like Gentamicin, Azitromycin, doxycycline, Levofloxacin, Clindamycin and Teicoplanin. However in a multicenter study by Rajaduraipandi K et al, higher percentage of Intermediate sensitivity was noted

against Erythromycin, Ofloxacin, Amikacin, Ciprofloxacin and Cephataxime¹⁵.

CONCLUSION:

Injudicious, empirical use of antibiotics will lead to drug resistance. MRSA strains show resistant to other commonly used anti staphylococcal drugs. Every clinical Laboratory should routinely carry out MRSA detection which is important because of the ability of the pathogens to acquire resistance to newer classes of antimicrobial agents. Susceptibility pattern helps in understanding the new and emerging resistance trends and appropriate use of antimicrobial agents to treat infections with such organisms.

Limitations of the study:

MecA gene detection by PCR technique, which is considered the gold standard method for MRSA detection was not done in our study due to economic constraints and susceptibility testing for vancomycin was not done which is the main stay of treatment for MRSA infections.

REFERENCES:

1. Mansouri S, Khaleghi M. Antibacterial resistance pattern and frequency of Methicillin resistant staphylococcus aureus. *Iran J Med Sci* 1997;22:93.
2. K Jayatilleke, P Bandara. Antibiotic sensitivity pattern of Staphylococcus aureus in a tertiary care hospital of Sri Lanka. *Sri Lanka Journal of Infectious Diseases* 2012;2:13-17
3. Majumder D, Sarma Bordoloi JN, Phukan AC, Mahanta J. Antimicrobial susceptibility pattern

- among Methicillin resistant Staphylococcus isolates in Assam. Indian J Med Microbiol 2001;19:138-40.
4. 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.
 5. Siddiqui F, Madahiah-bint-e-Masood, Noor-us-sabq, Samad A, Quayyum M, Qazilbash AA. AntibioGram sensitivity pattern of Methicillin resistant Staphylococcus aureus isolates from pus sample. Pak J Biol Sci 2002;5:491-3.
 6. Baird D. Staphylococci: Cluster-forming Gram-positive cocci In: Collee JG, Fraser AG, Marmion BP, Simmons A, Editors. Mackie & McCartney Practical Medical Microbiology, 14th ed. (Churchill Livingstone, New York) 1996:245-61.
 7. Fluitac, Wielders CL, Verhoef JF, Schmitz J. Epidemiology and susceptibility of 3,051 Staphylococcus aureus isolates from 25 university hospitals participating in the European SENTRY study. J Clin Microbiol 2001;39:3727-32.
 8. Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN et al. Survey of infections due to Staphylococcus species. Frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe and the Western Pacific region for the SENTRY antimicrobial surveillance program, 1997-1999. Clin Infect Dis 2001;32:114-32.
 9. Safdar N, Narans L, Gordon B, Maki DG. Comparison of culture screening methods for detection of nasal carriage of methicillin-resistant *Staphylococcus aureus*: a prospective study comparing methods. J Clin Microbiol 2003;41:3163-6.
 10. Pai V, Rao V, Rao SP. Prevalence & antimicrobial susceptibility pattern of Methicillin-resistant Staphylococcus aureus (MRSA) isolates at a tertiary care hospital in Mangalore, South India. J Lab Physician 2010;2(2):82-4
 11. 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(3):156-58.
 12. Joshi S, Ray P, Manchand V, Bajaj J, Chitnis DS, Gautam et al. Methicillin Resistant Staphylococcus aureus (MRSA) in India: Prevalence and susceptibility pattern. Indian J Med Res 2013;137:363-69
 13. 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(2):78-81
 14. Vidhani S, Mehnadiratta PL, Mathur MD. Study of Methicillin resistant Staphylococcus aureus isolates from high risk patients. IJ Med Microbiol 2001;19:13-16.
 15. Rajadurai Pandi K, Mani KR, Panneerselvam K, Mani M, Bhaskar M, Manikandan XP. Prevalence and antimicrobial susceptibility pattern of Methicillin resistant Staphylococcus aureus. A multicentre study. Ind J Med Microbiol 2006;24:34-38.