Prevalence of Methicillin Resistant Staphylococcus aureus in patients admitted in a Tertiary Care Hospital of North India

Ankur Kumar[*], Sumit Kumar, Umar Farooq, Rehana Begum, Surbhi Kansal, Anusha Venkatesan
Department of Microbiology, Teerthanker Mahaveer Medical College and Research Centre, Moradabad, U.P., India

Article Info

Abstract

Introduction: Methicillin resistant Staphylococcus aureus (MRSA) is endemic in India. In the present era of antibiotic resistance, the emergence of multi-drugs resistant organism is becoming more common. The present study is performed in patients admitted in hospital in various department of hospital to study the infection rate of MRSA from these units. Objectives: The aim of this study was to isolate Staphylococcus aureus from various samples of patients admitted in tertiary care hospital, & to study the prevalence rate of MRSA in the hospital. Material and method: The study was conducted in TMMC & RC hospital from January 2013 to Sepetember2013. A total of one hundred Staphylococcus aureus isolates from clinical samples were subjected to MRSA screening using Kirby-Bauer methods with cefoxitin 30μg discs per CLSI guidelines. Results: There were 225 different samples collected and all the samples were inoculated on Blood agar medium than incubated. After overnight incubation 100 Staphylococcus aureus were isolated, among which 26 were MRSA and 74 were MSSA has been isolated.The susceptibility pattern of antibiotics showed that all MRSA isolates were significantly less sensitivity to antibiotics as compared to MSSA. None of the MRSA strain showed resistance towards Vancomycin & Linezolid. Conclusion: The emergence of MRSA and other antibiotic resistant organisms reflects widespread ubiquitous over-use of antibiotics on a global scale. Control measures are required to prevent spread and reduce emergence of resistance.

1. Introduction

MRSA is not only present but also growing throughout the world. Its prevalence ranges from 23.3% to 73%. Across the globe, it was found to be the most common cause of bacteraemia, respiratory, and skin infections [1]. Recent data from the Centers for Disease Control and Prevention showed that 59.5% of all healthcare–associated S. aureus infections in the United States are caused by MRSA [2, 3]. MRSA infections have been increasing in India [4]. Hospitals in India have a high burden of MRSA infections in their ICUs and wards, many of which are resistant to antibiotic treatment, according to the Global Antibiotic Resistance Partnership (GARP)-India Working Group and the Centre for Disease Dynamics, Economics & Policy (CDDEP). The 2011 GARP report, Situation Analysis: Antibiotic Use and Resistance in India also states that a large proportion of these hospital acquired infections (HAI) are preventable with increased infection control measures [5].

MRSA infections are more difficult to treat than ordinary staphylococcal infections. This is because the strains of staph known as MRSA do not respond well to many common antibiotics used to kill bacteria [6]. Methicillin was first introduced in the 1960s to treat S. aureus infections. Soon after The first reports of methicillin resistance in S. aureus appeared in 1961 in UK. MRSA has since becomes resistant to a wide range of antimicrobial agents [7]. The methicillin resistance gene (mecA) encodes a methicillin-resistant penicillin-binding protein (PBP2a)

Corresponding Author,
E-mail address: Meet2ankur143@gmail.com

that is not present in methicillin-susceptible S. aureus (MSSA) strains. MecA is carried on a mobile genetic element called a staphylococcal cassette chromosome (SCC) and codes the low affinity 78 kda penicillin binding protein (PBP2a) or (PBP2) that posses both transglycolase & transpeptidase enzyme [8]. The beta lactam antibiotics normally binds to PBPs in the cell wall, which result in the disruption of synthesis of the peptidoglycan layer and death of bacterium but B lactam can not bind to low affinity PBP2 which cause synthesis of the pepdoglycan layer & cell wall synthesis are able to continue[9].condition Methicillin-resistant Staphylococcus aureus (MRSA) is a major healthcare-associated (HA MRSA) as well as a community-associated (CA-MRSA) infection causing a wide range of diseases, including skin, soft-tissue, muscular, respiratory, bone, joint, and endovascular diseases; in addition to life threatening conditions including endocarditis, osteomyelitis, toxic-shock syndrome, pneumonia, food poisoning bacteremia, Meningitis, sepsis, Contagion with MRSA is capable of causing serious infections in young and healthy individuals, though severe infections commonly develop in hospitalized

patients, especially in those with immune Compromising diseases, MRSA can colonize the skin and various body parts of an individual The risk factors known to predispose a patient for hospital- associated (HA)MRSA comprise operative procedures, treatment intensive care or trauma units, implanted foreign material, insertion of intravascular catheters or urine catheters, and damaged skin therefore Methicillin-resistant staphylococcus aureus is an important cause of nosocomial infections worldwide[10,11].

The aim of this study was to isolate Staphylococcus aureus from various samples of patients admitted in hospital and to detect MRSA by several methods.
1) To study the prevalence rate of MRSA in our hospital.
2) To study the susceptibility pattern of MRSA isolates.
3) To do a comparative evaluation of antibiotics susceptibility profile of MRSA & MSSA.

2. Materials and Methods

The study was conducted in Department of Microbiology, Teerthanker Mahaveer Medical College and research Centre from January 2013 to Sepetember2013. A total of one hundred S. aureus isolates from clinical samples were subjected to MRSA screening using conventional microbiological methods. Specimens included pus, sputum, throat swab, genital specimen (high vaginal, semen, and urethral discharge), urine, devices (urinary catheter), blood, and body fluids. Samples were transported to the laboratory within two hours and processed immediately or refrigerated at 4 0C – 8 0C as soon as possible. All samples were cultured on the blood agar & Mannitol salt agar media & incubated at 370C for 18 to 24 hours.

Identification of Staphylococcus aureus from clinical samples:

On a blood agar plate S. aureus typically forms large pin head $1 – 2$ μm in diameter, circular, raised, opaque & golden yellow pigment colonies accompanied by a surrounding haemolytic zone. In Gram's staining, they are Gram positive cocci (GPC), spherical $1 - 2$μm in size & arrange in grape like clusters [12, 13].

Table: 1. Colony Characteristics of S. Aureus on Different Media

Medium	Colony Appearance
Blood agar	Creamy Golden yellow with α, β hemolysis
Mannitol salt agar	Yellow colonies
DNAse agar	Clearance around bacterial line

All isolates were confirm by manniton fermentation, DNAse test, and mandatory biochemical reaction; Catalase test, Coagulase test (slide & tube coagulase test) as per CLSI guidelines.

Detection of Methicillin resistance Staphylococcus aureus:

The positive isolated samples were subjected to cefoxitin disc diffusion test using a 30 μg disc. A 0.5 Mc Farland standard suspension of the isolate was made and lawn culture done on MHA (Muller Hinton Agar) plate, after that plates were incubated at 37˚c for 18 hrs. and zone of inhibition was measured. An inhibition zone diameter of ≤21mm was reported as Cefoxitin resistant indicating Methicillin resistant and ≥ 22 mm was considered as Cefoxitin sensitive indicating Methicillin sensitive as per CLSI guidelines [12, 14].

3. Results

There were 225 different samples were collected among the patients admitted in Teerthanker Mahaveer Medical College and Research Centre attached with multi specialty hospital, Moradabad, Uttar Pradesh, India. All the samples were inoculated on Blood agar as well as Mannitol salt agar medium than incubated. After overnight incubation 100 organisms were isolated, among which 29 were MRSA and 71 were MSSA has been isolated. Table [1.2]

Age and sex Distribution of S. aureus positive cases

Out of 100 S. aureus isolates, 55(55%) were from male patients out of which 17(30.9%) belonged to (21-30) age group followed by 13(26.6%) from (41-50) age group, 8(14.5%) from (31-40) age group, 5(9.1%) from (51-60), 5(9.1%) from(61-70), 4(7.3%) from (0-10) and 3(5.5%) from (11-20) age group. however 45(45%) of total S. aureus isolates were from female patients, out of which 13(28.7%) from (31-40) age group followed by 12(26.7%) from (21-30) age group, 7(15.6%) from (11-20) age group, 5(11.1%) from (0-10),4(8.9%) from(41-50) age group,2(4.5%)from (51-60) age group and 2(4.5%) from 61-70) in each age group. Table [1.3]

Prevalence of MRSA isolates from different clinical samples.

Out of 29 MRSA isolates, the maximum isolation of MRSA were from pus 12(20.68%) followed by urine 11(30.58%), nasal swab 2(100%) and blood3 (66.66%). Table [4]

Prevalence of S. aureus & MRSA isolates in different departments

Out of 100 S. aureus isolates, MRSA stains were 29(29%) of which the maximum number of strains were obtained from Surgery 8(20.51%) followed by Gynaecology 5(26.31%), 5 from Paediatrics (45.5%), 4 from ENT(50%), 3 from Medicine (14.28%), 3 from Orthopaedics (50%), and 1from dermatology(14.28%).Table[1.5]

Table: 2. MSSA Isolated

Total samples	225
S. aureus	100
MSSA	71
MRSA	29

Table: 3. Age wise and sex wise Distribution of *S. aureus* Positive Cases

S. No.	Age group	Total Number	Male	Female
1	0-10	9	4	5
2	11-20	10	3	7
3	21-30	29	17	12
4	31-40	21	8	13
5	41-50	17	13	4
6	51-60	7	5	2
7	61-70	7	5	2
	Total	100	55	45

Table: 4. Prevalence of MRSA Isolates from Different Clinical Sample

Clinical samples	No. of *S. aureus* (n=100)	No. of MRSA (n=2)	% of MRSA
Pus	56	12	21.42
Urine	36	11	30.55
Sputum	2	1	50
Nasal swab	2	2	100.00
Blood	5	3	60.00
Total	100	29	NA

Table: 5. Prevalence of *S. aureus* & MRSA Isolates in Different Departments

S. N o.	Department	Total No. of *S. aureus*	No of MRSA	Prevalence of MRSA (%)
1	ENT	8	4	50
2	Surgery	39	10	25.64
3	Medicine	7	3	42.85
4	Gynaecology	19	5	26.31
5	TB chest	3	0	0
6	Orthopaedics	6	3	50
7	Dermatology	7	1	14.28
8	Paediatrics	11	3	18.12
	Total	100	29	29

Comparative evaluation of antibiotics susceptibility profile of MRSA and MSSA

The susceptibility pattern of antibiotics showed that all MRSA isolates were significantly less sensitivity to antibiotics as compared to MSSA. The value were statistically significant as P-value was <0.05 for every antibiotics. Vancomycin was 100% sensitive in case of both MRSA and MSSA. Out of 29 MRSA isolates were sensitive 23(88.5%) to Linezolid, 18(69.2%) to Amikacin, 16(61.5%) to Clindamycin, 14(53.8%) to Ciprofloxacin, 14(53.8%) to Pristinamycin, 11(42.3%) to Gentamycin,10(38.5%) to Erythromycin, 8(30.8%) to Ceftazidime and 4(15.4) to penicillin. Whereas out of 71 MSSA isolates were sensitive 70(94.6%) to linezolid, 62(89.2%) to Ceftazidime, 62(83.8%) to Clindamiycin,60(81.1%) to Amikacin, 55(74.3%) to Pristinamycin, 53(71.7%) to erythromycin, 40(54.1%) to Ciprofloxacin, 36(48.66%) to Gentamycin and 21(28.4) to Penicillin.

According to the analyzed data, the prevalence of antibiotic resistance among
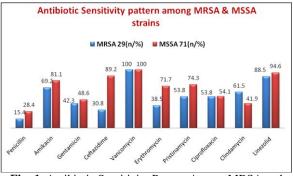
MRSA was much higher than that among MSSA.Fig[1.1]

Fig: 1. Antibiotic Sensitivity Pattern Among MRSA and MSSA Strains

4. Discussion

In this study, we isolated 29 (29%) MRSA out of 100 *Staphylococcus aureus* isolates from different clinical specimens from patients admitted in different department of TMMC & RC attached with multi speciality hospital from the duration of February 2013 to January 2014. Out of 29 MRSA isolates, 16 were from Male and 13 were from Female. The distribution of MRSA isolates was higher in male than female, and male female ratio was (1:8).

Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, a multi hospital based study in various part of India shown that the overall MRSA prevalence in India was 42 per cent in 2008 and 40 per cent in 2009[15]. The prevalence of MRSA in a study from Chennai 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. Around 60 per cent of the *S. aureus* isolates were from skin and soft tissue infections (SSTI). The prevalence of MRSA varies between regions and between hospitals in the same region as seen in a study from Delhi where the MRSA prevalence in nosocomial infection varied from 7.5 to 41.3 per cent between three tertiary care teaching hospitals. In this study, the MRSA isolation varied between different hospitals [15].

In the study of Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, from north India, the prevalence of MRSA was 46 per cent and MRSA isolates were found to be more resistant to other antibiotics than MSSA. Significant difference was observed in case of erythromycin, ciprofloxacin, gentamycin and Amikacin[15].

Anila A. Mathew has reported a prevalence rate of MRSA of about 34% in clinical specimen & Prevalence rate of MRSA in Eastern U.P. and AIIMS in New Delhi, was 54.85% and 44% respectively[16,17]. The prevalence rate of MRSA was found to be 29% in our study, which is in accordance with the findings of Anbumani N et al from Chennai (31%)[18]

In a study at Aligarh in India, it was shown that 35.1% of *S. aureus* and 22.5% of Coagulase-negative staphylococcal isolates were resistant to methicillin. Highest percentage of MRSA (35.5%) was found in pus specimens (*n*=151). it was same as compare to present study. In case of both methicillin-resistant as well as methicillin-sensitive *Staphylococcal* isolates, zero

resistance was found to Vancomycin [19, 20]. The present study shown that the Out of 29 MRSA isolates, the maximum isolation of MRSA were from pus 12(20.68%) followed by urine 11(30.58%), nasal swab 2(100%) and blood 3(66.66%). Majority of MRSA strains were obtained from ENT ward (50%), followed by (50%) orthopaedics, medicine (42%), gynaecology 5(26.31%), surgery (25.64), paediatrics (18.12), dermatology 1(14.28%) and TB chest (0%).

Mehta [21] AA et al from Mumbai (31.8%) where as few studies from India has reported high prevalence rate of MRSA as compared to this study such as 46% by Arora[22] S et al from Amritsar, 48.72% by Deepa[23] S et al from Mysore, South India, 51.6% by Vidhani[24] S et al from New Delhi, 54.85% by Anupurba S et al from Banaras Hindu University[25].

In our study the Prevalence rate of *S. aureus* was 44% and Prevalence rate of MRSA was 29% at a tertiary care hospital in north India. This variation in prevalence as compare with other previous studies may be because of several factors like healthcare facilities available in the particular hospital, implementation and monitoring of infection control committee, rationale antibiotic usage which varies from hospital to hospital.

5. Conclusion

In conclusion, this study demonstrates that MRSA is a major problem in India. More number of MRSA isolates was multidrug resistant as compared with the MSSA isolates. Glycopeptides and linezolid continue to remain the mainstay for the treatment of MRSA infections.

The present study showed that a high level prevalence of MRSA strains resistance against widely used antimicrobial agents. The regular surveillance of MRSA

will also be useful for selecting an appropriate antibiotic, to know the changing trends of antibiotic susceptibility pattern, for developing hospital antibiotic policy and for limiting the use of powerful antibiotics like Vancomycin as initial treatment and save it for the treatment.

High prevalence of MRSA in hospital setting indicating need of good control measures such as proper hand hygiene and treating patient, surveillance cultures and monitoring of susceptibility patterns of MRSA may also help in arresting the spread of infections in this part of India.

In India prevalence and incidence of MRSA from different studies have been in the range of 6.9% to 87%. In our study this has been 29% which is accordance with the other studies; however a larger study is needed to confirm our findings.

The emergence of MRSA and other antibiotic resistant organisms reflects widespread ubiquitous over-use of antibiotics on a global scale. MRSA and other resistant bacteria have been unwittingly and selectively favoured by our society's over-reliance on and overuse of antibiotic drugs.

The control efforts should continue but the responsibility of controlling HAIs rests majorly on Infection control professionals and Health care workers while patients and relatives could also be carried along in the process of preventing these infections.

References

[1] D. J. Diekema, M. A. Pfaller, F. J. Schmitz, Bell J. J Smayevsky, R. N. Jones, M. Beach, SENTRY Participants Group: 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. Clinical Infection Disease 2001, 32 (Suppl 2):114-132.

[2] Palavecino E. Clinical, epidemiological, and laboratory aspects of methicillin-resistant Staphylococcus aureus (MRSA) infections. Methods in Molecular Biology. 391:1 1, 2007

[3] Centre for Disease Control and Prevention, "MRSA: Methicillin-resistant Staphylococcus aureus in Healthcare Settings, 2007

[4] B. Batabyal, G. K. R. Kundu, S. Biswas, Methicillin-Resistant Staphylococcus Aureus: A Brief Review, International Research Journal of Biological Sciences, 1(7), 2011, 65-71

[5] Global Antibiotic Resistance Partnership (GARP)-India Working Group and the Centre for Disease Dynamics, Economics & Policy (CDDEP), 2011

[6] M. C. Enright, The evolution of a resistant pathogen--the case of MRSA. Curr. Opin. Pharmacol 3:47, 2003, 4-9

[7] K. Hiramatsu, H. Hanaki, T. Ino, K. Yabuta, T. Oguri, F. C. Tenover. Methicillin-resistant Staphylococcus aureus clinical strain with reduced vancomycin susceptibility. J. Antimicrob. Chemother 40:135-6, 1997

[8] R. H. Deurenberg, E. E. Stobberingh, The evolution of Staphylococcus aureus. Infect Genet Evol, 2008

[9] H. F. Chambers, F. R. Deleo, Waves of resistance: Staphylococcus aureus in the antibiotic era. Nat. Rev. Microbiol 7, 2009. 629-41. http.icmr.nic.in/2011/ September/0905.pdf

[10] D.H. Tambekar, D.V. Dhanorkar, S. R. Gulhane, M. N. Dudhane, prevalence and antimicrobial susceptibility pattern of methicillin resistant staphylococcus aureus from healthcare and community associated sources, Afr. J. Infect. Diseases www.africanethnomedicines.net

[11] S. Vandecasteele, J. Boelaert, A. De Vriese, Staphylococcus aureus infections in hemodialysis: what a nephrologist should know. Clin J Am Soc Nephrol, 4(8), 2009, 1388-1400.

[12] J. Gerald collee, Andrew G Fraser, Barrie P. Marmion, Anthony Simmons. Identification & Isolation of S. aureus from clinical samples, Mackie & McCartney Practical Medical Microbiology, 14th edition, 2007, 249

[13] Bailey & Scott's, Diagnostic Microbiology, 20th edition, chapter 13, 222

[14] Clinical and Laboratory Standards Insititute, Performance Standards for Antimicrobial Susceptibility testing; 22nd information supplement, M100-S22 Wayne, PA; 2012.

[15] Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, India, Methicillin resistant Staphylococcus aureus (MRSA) in India: Prevalence & susceptibility pattern, Indian J Med Res 137, 2013, 363-369

[16] A. Tyagi, A. Kapil, P. Singh, Incidence of Methicillin Resistant Staphylococcus aureus (MRSA) in Pus Samples at a tertiary Care Hospital, AIIMS, New Delhi., Journal of Indian Academy of Clinical Medicine. 9, 2008, 33-35. 6

[17] N. Anbumani, J. Kalyani, M. Mallika, Prevalence of Methicillin-resistant Staphylococcus aureus in Tertiary refferal hospital in Chennai, South India. Indian J for the practising doctor, 3, 2006

[18] S. Anupurba, M. R. Sen, G. Nath, B. M. Sharma, A. K. Gulati, T. M. Mohapatra, Prevalence of methicillin resistant staphylococcus aureus in a tertiary referral hospital in eastern Uttar Pradesh. Indian J Med Microbiol, 21, 2003, 49-51

[19] S. S. Tahnkiwale, S. Roy, S. V. Jalgaonkar, Methicillin resistance among isolates of Staphylococcus aureus: Antibiotic sensitivity pattern & phage typing. Indian J Med Sci, 56, 2002, 330-4

[20] K. Rajaduraipandi, K. R. Mani, K. Panneerselvam, M. Mani, M. Bhaskar, P. Manikandan, Prevalence and Antimicrobial Susceptibility pattern of Methicillin resistant Staphylococcus aureus: A Multicentre study. Indian Journal of Medical Microbiology 24, 2006, 34-38

[21] A. A. Mehta, C. C. Rodrigues, R. R Kumar, A. A. Rattan, H. H. Sridhar, V. V. Mattoo, V. V. Ginde, A pilot programme of MRSA surveillance in India. (MRSA Surveillance Study Group). J Postgrad Med 42, 1996, 1-3.

[22] S. Arora, P. Devi, U. Arora, B. Devi, Prevalence of Methicillin-resistant S. aureus (MRSA) in a tertiary care hospital in Northern India. J Lab Physicians 2, 2010, 78-8

[23] S Deepa, B. Amruta Kumari, D. Venkatesha, Increasing Trends of Methicillin Resistant Coagulase Negative Staphylococcus in Neonatal Septicaemia - A Study in a Tertiary Care Hospital, Mysore, South India.J of Health Sci., 9(4), 2010

[24] S. Vidhani, P. L. Mehndiratta, M. D. Mathur, Study of methicillin resistant S. aureus (MRSA) isolates from high risk patients. Indian J Med Microbiol, 19, 2001, 13-6

[25] S. Anupurba, M. R. Sen, G. Nath, B. M. Sharma, A. K. Gulati, T. M. Mohapatra, Prevalence of methicillin resistant staphylococcus aureus in a tertiary referral hospital in eastern Uttar Pradesh. Indian J Med Microbiol, 21, 2003, 49-51