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Infections by *Staphylococcus aureus*: a rising threat and a major clinical challenge! Prevalence and Antibiogram of *S.aureus* isolated from various clinical samples received in a tertiary care hospital in Central India, with special reference to MRSA

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Abstract

Introduction: *MRSA infections have been increasing in Indian hospital ICU's and wards many of which are resistant to antibiotic treatment.*

Aim: *To study the prevalence of MRSA and antibiotic resistance pattern in Staphylococcus aureus from various clinical samples in tertiary care hospital, in central India*

Materials & Methods: *A total of 2660 samples received in the Microbiology Laboratory over a period of 3 months. 1320 were from in-patients department. Out of these 772 samples were culture positive. Among these, 215 were Staphylococcus aureus, and included in the study, while all others were excluded. Standard biochemical tests were used for identification of S.aureus. The MRSA strains were identified by using Cefoxitin 30µgdisc on Muller Hinton agar and antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion and zones of inhibition interpreted as per CLSI 2017 guidelines.*

Results: *Out of the total of 772 culture positive samples, Staphylococcus aureus was isolated in 215 samples. A total of 90 isolates (41%) were from blood, 80 (37%) from pus and wound swab, 32(14.9%) from ear discharge, 8 (3.7%) from respiratory and 5 (2.3%) were from CSF. Most isolates were resistant to Penicillin and Ampicillin (96.7% & 94.9% respectively), while 62% isolates were resistant to Ciprofloxacin, 70% to Erythromycin, 54% to Clindamycin, and 60% to Gentamycin. All the isolates were sensitive to Teicoplanin i.e no isolates were found to be resistant and 99 % isolates were sensitive to Linezolid. Among the 215S.aureus isolates, 115 (53%) were MRSA.*

Conclusion: *This study documents drug resistant patterns in S.aureus and the high prevalence of MRSA strains among hospital patients. It emphasises the need for prompt and effective measures required to limit the spread drug resistant pathogens and MRSA strains by use ofappropriate antibiotic selection and effective infection control practices.*

Keywords: *Methicillin Resistance, Staphylococcus aureus, Teicoplanin, Linezolid, Drug Resistance.*

Introduction

Staphylococcus aureus remains one of the commonest human bacterial pathogen implicated to cause wide range of infections like the skin and soft tissue infections, meningitis, endovascular infections, pneumonia, tonsillitis, pharyngitis, septic arthritis, endocarditis, enterocolitis, osteomyelitis, toxic shock syndrome, sepsis, etc. Due to inappropriate and indiscriminate use of antibiotics, the drug resistant *S.aureus* strains, like

the MRSA, are increasing throughout the world, posing a challenge to the clinicians. If not treated properly such infections can invariably have fatal outcomes[1],[2].

Methicillin resistant *Staphylococcus aureus* (MRSA) prevalence is also rising throughout the world, with the overall prevalence ranging from 23.3% to 73%. MRSA are very common isolates from patients suffering from bacteremia, respiratory and skin infections.[3] Recent data from the Centres for Disease Control and Prevention showed that 59.5% of all the health care associated *Staphylococcus aureus* infections in the united states are caused by MRSA [2][4].

MRSA infections have been increasing in India. Hospitals across India have a considerable rate of MRSA infections in their ICU s and wards, many of which are resistant to multiple antibiotics. The incidence of MRSA varies according to the region, 25% in western part of India6 to 50% in South India.[5],[6]

Material and Methods

This study was conducted in the Department of Microbiology, Mahatma Gandhi Memorial Medical College, Indore. A total of 2660 samples received in the Microbiology Laboratory over a period of 3 months from the various clinical departments in Maharaja Yashwanthrao (MY) Hospital.

Inclusion and Exclusion criteria: A total of 1320 specimens from IPD were included. Out of these 772 were culture positive. Among these, 215 were *Staphylococcus aureus*, which were included in the study, while all others organisms were excluded.

Study duration was three months, from September 2017 to December 2017. Isolation and identification of *S.aureus* was done as per standard biochemical tests described subsequently in detail. The MRSA strains were identified by using Cefoxitin 30µg disc on Muller Hinton agar and antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion and zones of inhibition interpreted as per CLSI 2017 guidelines.[7]

The strains were isolated and identified from clinical specimens received at the diagnostic microbiology laboratory. Specimens includes pus, urine and fluids. The specimens were collected using all aseptic precautions and transported to the laboratory immediately for processing.

Specimen processing and Identification of *S.aureus*

Direct examination: For all the specimens (except blood)received in the microbiology laboratory, smears were prepared and stained with Gram stain. They were microscopically examined to determine the presence and type of cells along with the number of microorganisms and their relative morphology.[2]

The specimens (except blood) were plated onto culture media 5% sheep blood agar and MacConkey's agar immediately after transporting them to the laboratory and were incubated at 37°C for 24 hours at a carbon dioxide concentration of 5-10%. Blood was inoculated inthe automated blood culture bottles (BacT Alert) and incubated in the system before subculturing them onto the respective media after flagging positive by the same. After inoculation, the plates were examined for growth and identified by standard microbiological techniques.[2][7]

Antimicrobial susceptibility testing and detection of MRSA

Antimicrobial susceptibility testing was done by Kirby- Bauer method to determine the sensitivity pattern and interpretation was done according to Clinical Laboratory Standards Institute (CLSI) guidelines 2017.[7]

Antibiotics tested included Penicillin(10mcg) Ampicillin (10mcg),Ciprofloxacin (5mcg), Clindamycin (2mcg), Co-trimoxazole (1.25/23.75 mcg), Erythromycin (15mcg), Gentamycin (10mg), Linezolid (30mcg) and Teicoplain (30mcg). After 18-24 hours incubation, the sensitivity plates were observed and the diameter of the inhibitory zone was measured. The zone size around each antimicrobial disc was interpreted as sensitive, intermediate or resistant

according to CLSI 2017 interpretative criteria.[7] The data of each isolate was subsequently entered in WHONET and data analysis was done using SPSS-19.

MRSA detection: Methicillin resistance was detected using Cefoxitin Disk (30mcg) by Disk Diffusion test. *Staphylococcus aureus* ATCC 25923 was used as the standard control strain.[7]

Table 1.0 Specimen wise distribution

Specimen	Frequency	Percent
Blood	90	41.9%
CSF	5	2.3%
Pus and Wound swab	80	37.2%
Ear swab	32	14.9%
Respiratory samples	8	3.7%
Total	215	100.0

Results

Out of the total of 772 culture positive samples, 215 *Staphylococcus aureus were* isolated. A total of 90 isolates (41%) were from blood, 80 (37%) from pus and wound swab, 32(14.9%) from ear discharge, 8 (3.7%) from respiratory and 5 (2.3%) were from CSF from various clinical departments.(Table 1, 2)

In our study out of 215 *S.aureus*, MRSA and MSSA were 115 (53%) 100(47%) respectively. Specimen and department wise distribution of MRSA and MSSA is given in table 3 and 4.

Table 2.0 Distribution of samples based on Source (Clinical department in the hospital)

	Frequency	Percent
Medicine	53	24.7
Surgery	77	35.8
Pediatrics	47	21.9
Orthopedics	11	5.1
ENT	27	12.6
Total	215	100.0

Figure 1.0 Age wise distribution

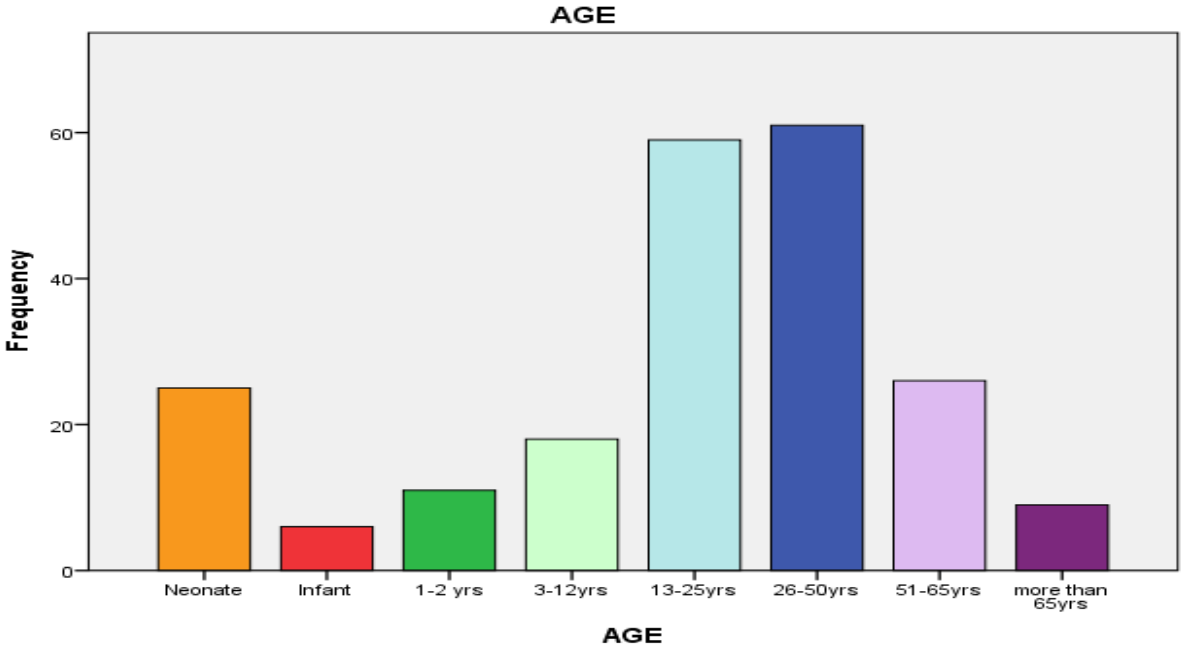


Table 3.0 Specimen wise distribution of MRSA & MSSA

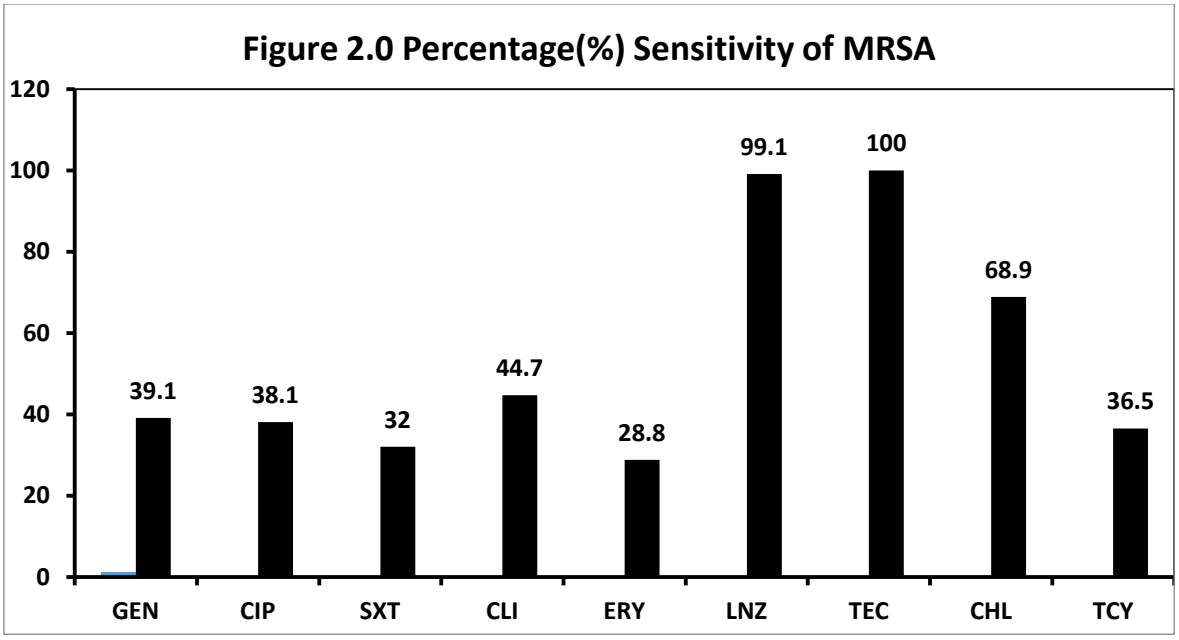
MRSA_SCREEN		SPECIMEN					Total
		Blood	CSF	Pus and Wound swab	Ear Swab	Respiratory samples	
	MRSA	47	3	44	18	3	115
	MSSA	43	2	36	14	5	100
Total		90	5	80	32	8	215

Table 4.0 Department wise distribution of MRSA & MSSA

	Department					Total
	Medicine	Surgery	Pediatrics	Orthopedics	ENT	
MRSA	29	39	26	6	15	115
MSSA	24	38	21	5	12	100
Total	53	77	47	11	27	215

Table 5.0 Drug Susceptibility and Resistance pattern in MRSA isolates

Code	Antibiotic name	%R	%S
PEN	Penicillin G	100	00
AMP	Ampicillin	100	00
GEN	Gentamicin	60.9	39.1
CIP	Ciprofloxacin	61.9	38.1
SXT	Trimethoprim/Sulfamethoxazole	67.5	32
CLI	Clindamycin	54.4	44.7
ERY	Erythromycin	69.8	28.8
LNZ	Linezolid	0.9	99.1
TEC	Teicoplanin	0	100
CHL	Chloramphenicol	31.1	68.9
TCY	Tetracycline	63.5	36.5



Discussion

Staphylococcus aureus, is one of the commonest nosocomial pathogen reported worldwide. It is responsible for causing skin and soft tissue infections in both community and hospitalized patients[8]. In the last few decades, the alarming trend of increasing drug resistance of *Staphylococcus aureus*, particularly MRSA, has posed serious threat to the patients and posed a daunting challenge to treating clinicians, public health workers and healthcare professionals responsible for hospital infection control[2][8]

Methicillin-resistant *Staphylococcus aureus* (MRSA) first emerged as a serious infectious threat in the late 1960s as the bacterium developed resistance to methicillin.[9][10] Life threatening sepsis, endocarditis and osteomyelitis caused by methicillin resistant *Staphylococcus aureus* (MRSA) have been reported from several geographical regions worldwide. MRSA has been considered one of the most important nosocomial pathogen of late[11][12]. MRSA infections in hospitals have imposed a high burden on healthcare resources as well as significant

morbidity and mortality. It is not surprising then, that MRSA has been the focus of intense scientific and political interest around the world and has frequently been labelled as a superbug in the popular media [11][12][13].

The prolonged hospital stay, indiscriminate use of antibiotics, lack of awareness, receipt of the hospital etc are predisposing factors of MRSA emergence[4][15]. Hence, early detection of MRSA and effective antibiotic policy in referral hospitals are of paramount importance from the hospital epidemiological point. Therefore, the knowledge of prevalence of MRSA and their current antimicrobial profile become necessary in the selection of appropriate empirical treatment of these infections. The present study has been carried out with an aim to know the antibiotic sensitivity pattern of Staphylococcal isolates with special reference to MRSA. The present study indicates the prevalence and antibiotic susceptibility patterns of various *Staphylococcus aureus* isolates identified from clinical specimens such as blood, pus, ear swab, respiratory secretions and CSF[7].

The age group of 26-50 years showed highest isolation of *S.aureus* and MRSA. The highest prevalence of MRSA in the middle aged population. Studies have found that this could be due to indiscriminate or prolonged use of antibiotics, self medication, increased travel and mobility in addition to busy lifestyles with reduced attention to healthcare due to hectic work schedules.[15]

Most isolates were resistant to Penicillin and Ampicillin (96.7% & 94.9% respectively), while 62% isolates were resistant to Ciprofloxacin, 70% to Erythromycin, 54% to Clindamycin, and 60% to Gentamycin. All the isolates were sensitive to Teicoplanin i.e. no isolates were found to be resistant and 99 % isolates were sensitive to Linezolid. A total of 115 MRSA strains were identified in our study. The prevalence of MRSA obtained in our study is 53%,which is higher than those from other studies across other regions of India done by Debnath et al(30%) and 46% by Dominic et al respectively, Goyal et al(32.6%), Pai et al(29.1%), Tripathi (33.33%).[1,2,9,15,16,17] However, studies by Surpur et al(54.91%)15 and Savitha et al (62.14%)16 have reported a high prevalence of MRSA.[18][19] The earlier reported incidence of MRSA varies from 25 per cent in western part of India to around 50 percent in South India. [1],[6] Our study documents a clear increase in prevalence in MRSA. The variations seen between different studies including ours could probably be influenced by a variety of factors such as the limited sample size, duration of study, demography of the region, infection control and antibiotic prescribing practices.

Among the 115 isolates of MRSA, none of the isolates were sensitive to Penicillin G and Ampicillin (i.e. all isolates were resistant). Sensitivity to other drugs were variable. (table 5 and Figure 2). Sensitivity to Fluroquinoles (ciprofloxacin) was 38%, while sensitivity to Trimethoprim-sulphomethoxazol (Cotrimoxazole) was 32%, Tetracyclines 36.5%, Chloramphenicol 68.9%, Clindamycin 44.7%, Erythromycin 28.8% and Gentamicin 39.1%(Table 5.0). Most isolates (99%) were resistant to Linezolid, while none of the isolates were found resistant to Glycopeptides like Teicoplanin. As majority of the strains tested against Linezolid, and Teicoplanin and showed 100% sensitivity. These findings correlate well with studies done by Surpur et al, Goyal et al, INSAR groupand Tripathiwhich also showed similar drugsusceptibility pattern.[1,6,17,20]. Strains of Methicillin resistant *Staphylococcus aureus* when isolated from clinical specimens can create therapeutic difficulties since they are known to be multi-drug resistant and the alternatives available are also limited.

Therefore, this study indicates that S.aureus is a very common nosocomial pathogen, as shown by other studies done across India.[1,6,17,20,21]. The drugs once known as reserve drugs like the Glycopeptides (e.g. Teicoplanin, Vancomycin) and Linezolid now appear to be the only effective therapeutic options available for treating MRSA infections right now. Glycopeptides must be

reserved only for MRSA and must be avoided for the treatment of MSSA bacteremia and endocarditis[20] De-escalation of Teicoplanin or Vancomycin to beta lactams should be encouraged in all cases of MSSA. During empirical therapy for suspected cases of MRSA infection, it is imperative that treating doctors deescalate to beta lactams or other narrow spectrum drugs which are sensitive, once the culture sensitivity results reveal an MSSA isolate. Preservation of Glycopeptides and Linezolid for use only in MRSA cases should be encouraged.[22] Therefore, this study highlights the fact that MRSA continues to be a major problem in healthcare institutions. There is an alarming rate of increase in reports of MRSA throughout the country. Increased prevalence of MRSA is a global phenomenon and to make things worse, the availability of newer and effective antibiotic options is restricted with only few drugs available to treat them. Low level resistance even to Vancomycin is also emerging at present.5 Judicious use and strict antibiotic stewardship program is the need of the hour.

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References

1. Goyal A, Diwakar MK, Bhooshan S, Goyal,S, Agrawal A. Prevalence and Antimicrobial Susceptibility Pattern of Methicillin-resistant *Staphylococcus aureus*[MRSA] Isolates in Tertiary Care Hospital in Agra, North India- A systematic annual review. IOSR- Journal of Dental and Medical Sciences 2013; 11(6):80-84.
2. Hannath AR , Dominic RM. Prevalence and antimicrobial susceptibility pattern of clinical isolates of Methicillin-resistant Staphylococcus aureus in a tertiary care hospital in Mangalore Journal of International Medicine and Dentistry 2016; 3(3): 134-139.
3. Palavecino E. Clinical, epidemiological, and laboratory aspects of methicillin-resistant Staphylococcus aureus (MRSA) infections. Methods in Molecular Biology. 391:1 1, 2007.
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. Batabyal B, Kundu G, Biswas S, Methicillin Resistant Staphylococcus aureus: A Brief Review, International Research Journal of Biological Sciences, 1(7), 2011, 65-71.
6. Gopalakrishnan R, Sureshkumar D. Changing trends in antimicrobial susceptibility and hospital acquired infections over an 8 year period in a tertiary care hospital in relation to introduction of an infection control programme. J Assoc Physicians India 2010;58:25-31.
7. Clinical and Laboratory Standards Institute [CLSI]. Performance for Antimicrobial disk Susceptibility Testing-Approved Standard. 27th information supplement. Wayne, PA. M100-S27; 2017.
8. Chambers HF. The changing epidemiology of Staphylococcus *aureus*. Emerg Infect Dis 2001; 7: 178-182.
9. Debnath A, Chikkaswamy BK. Antibiogram and susceptibility pattern of methicillin-resistant *Staphylococcus aureus* collected from various clinical samples inBengaluru. Asian Journal of Pharmaceuticaland Clinical Research 2015; 8(6): 260-264.
10. Washer P, Joffe H. The hospital superbug social representations of MRSA. Soc Sci Med. 2006 Oct;63(8):2141-52.

11. Song JH, Hsueh PR, Chung DR, Ko KS, Kang CI, Peck KR, Yeom JS, Kim SW, Chang HH, Kim YS, Jung SI. Spread of methicillin-resistant Staphylococcus aureus between the community and the hospitals in Asian countries: an ANSORP study. Journal of antimicrobial chemotherapy. 2011 Feb 20;66(5):1061-9.
12. DeLeo FR, Chambers HF. Reemergence of antibiotic-resistant *Staphylococcus aureus* in the genomics era. The Journal of Clinical Investigation. 2009;119(9):2464-2474.
13. Easton PM, Marwick CA, Williams FL, Stringer K, McCowan C, Davey P, Nathwani D. A survey on public knowledge and perceptions of methicillin-resistant Staphylococcus aureus. Journal of Antimicrobial Chemotherapy. 2008 Nov 4;63(1):209-14.
14. Rupali SM, Akshay RK, Nitin AA, Sarika PK. Prevalence of Methicillin Resistant Staphylococcus aureus in tertiary care hospital, Central India. Int. J. Curr. Microbiol. App. Sci 3 (10). 2014:582-6.
15. VanRijen MM, Kluytmans-van den Bergh MF, Verkade EJ, Ten Ham PB, Feingold BJ, Kluytmans JA. CAM Study Group. Lifestyle-associated risk factors for community-acquired methicillin-resistant Staphylococcus aureus carriage in the Netherlands: an exploratory hospital-based case-control study. PLoS One. 2013 Jun 19;8(6):e65594.
16. Pai V, Rao VI, Rao SP. Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* [MRSA] isolates at a tertiary carehospital in Mangalore, South India. J LabPhysicians 2010; 2(2): 82-84.
17. Tripathi A. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* in Central India. Med Pulse- International Medical Journal2015; 2(1): 45.
18. Surpur RR, Patil VM, Rao A, Hegadi S,Kalpana. Prevalence of Methicillin resistance *Staphylococcus aureus* and antibiotic susceptibility pattern among patients admittedat Navodaya Medical College, Hospital And Research Center, Raichur. International Journal of Recent Trends in Science And Technology 2013; 9(1): 152-154.
19. Savitha P, Swetha K and Beena PM. Methicillin resistant *Staphylococcus aureus* and their antibiotic resistance pattern amongclinical samples in a tertiary care hospital inrural South India. Asian J Adv Basic Sci 2015;4(1):89-92.
20. Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, India. Methicillin resistant *Staphylococcus aureus* (MRSA) in India: Prevalence and susceptibility pattern. Indian J Med Res 2013;137: 363-369.
21. Sumathi B G, Vijay CR, Kumaraswamy, Shaffiulla M,A Comprehensive Overview of *Staphylococcus aureus* Isolates from Cancer Patients. Journal of Medical Science and Clinical Research 2017;5(12):32185-93.
22. Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, et al. Clinical practice guidelines by the infectious disease society of America for the treatment of methicillin resistant *Staphylococcus aureus* infections in adults and children. Clin Infect Dis 2011; 52: e18-e55