http://jmscr.igmpublication.org/home/
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v9i7.25

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Original Article

Prevalence and Antibiotic Susceptibility Pattern of Methicillin Resistant *Staphylococcus aureus* isolated from Skin and Soft tissue infections in a Rural Teaching Hospital

Authors
Swathikrishna C[1], Merin Tom Yogiaveedu[2], Dr Shareen George[3], Dr Anna Mathew[4] Mr Prakash Ramasami[5]
[1]MBBS student, MOSC Medical College Kolenchery
[2]MBBS student, MOSC Medical College Kolenchery
[3]Additional Professor, Microbiology, MOSC Medical College Kolenchery
[4]Professor, Pharmacology, MOSC Medical College Kolenchery
[5]Consultant Biostatistician
*Corresponding Author
Dr Anna Mathew
Professor of Pharmacology, Mepurathu, Mannoor, Keezhillam, Ernakulam, Kerala 682311, India

Abstract
Title of study: *Prevalence and Antibiotic Susceptibility Pattern of Methicillin Resistant Staphylococcus aureus isolated from Skin and Soft tissue infections in a Rural Teaching Hospital*
Background: *Skin and soft tissue infections (SSTI) are ubiquitous and Staphylococcus aureus is almost the universal cause of furuncles, carbuncles and skin abscesses worldwide. Strains resistant to beta-lactam antibiotics, termed Methicillin Resistant Staphylococcus aureus (MRSA), are now causing community-acquired infections. Clinically it is important to distinguish between MRSA and Methicillin sensitive Staphylococcus aureus (MSSA) because their treatments are different.*
Objectives: *To find the prevalence and antibiotic susceptibility pattern of MRSA and MSSA isolated from SSTIs coming to this tertiary care hospital.*
Methodology: *This cross-sectional study, conducted in the microbiology department, on pus-samples received from skin and soft tissue infections during the study period had a sample size of of 53 specimens. After obtaining Institutional Scientific Review Board and Ethics committee approval, isolates of Staphylococcus aureus from SSTI were selected by consecutive sampling, cultured, tested and antibiotic susceptibility pattern was obtained by automated and disc diffusion methods*
Results: *The prevalence of MRSA was 45.3% and MSSA was 54.7%. Out of 53 samples Only 6(11.3%) of the 53 isolates were sensitive to Ampicillin and Penicillin. All MRSA were sensitive to Vancomycin and Linezolid.*
Conclusion: *About half of Staphylococcus aureus isolated were MRSA. The significant emergence of multi drug resistant Staphylococcus aureus calls for judicious antimicrobial treatment based on Antibiotic Sensitivity Test. Vancomycin and linezolid shows 100% sensitivity, hence are the drugs of choice for treating MRSA. Robust antimicrobial stewardship is advised.*
Keywords: *MRSA, SSTI, antibiotic susceptibility, Vancomycin, Linezolid.*

Background

Skin and soft tissue infections (SSTI) are ubiquitous and the most identified agent responsible is *Staphylococcus aureus*.[1] *Staphylococcus aureus* has a high pathogenic potential causing a wide range of infections. SSTI reflect inflammatory microbial invasion of the epidermis, dermis and subcutaneous tissues.[2]Skin is colonized with an indigenous microbial flora, consisting of several species of *Staphylococci*, *Corynebacteria, Propionibacterium* and *yeast*. Although *Staphylococcus aureus* is a commensal, in 80% of healthy individuals, it is the universal cause of furuncles, carbuncles and skin abscesses.[3] Inadequately treated staphylococcal SSTI may progress to severe muscle and bone infections and may disseminate to the lungs or heart valves (endocarditis). Treatment of early infections includes incision and drainage the lesion and using beta-lactam antimicrobial drugs.[4]

Stains resistant to beta-lactam antimicrobial drugs termed Methicillin Resistant *Staphylococcus aureus* (MRSA),were recognized since the 1960s as Healthcare Associated (HA) pathogens.

However, more recently, it has been recognized increasingly in community settings globally.[5]Any strain of *Staphylococcus aureus* developing resistance against a beta-lactam antibiotic (methicillin or cloxacillin) through chromosomal changes (in the MecA gene) is termed MRSA. Strains sensitive to these antibiotics are called Methicillin Sensitive *Staphylococcus aureus* (MSSA). Since the 1990s, the increasing incidence of community-acquired MRSA, (CA-MRSA), has been causing concern.[6, 7]

Incision and drainage remain the most important measure for treating patients with *S.aureus* infections and earlier many cases did not require systemic antibiotic therapy, in addition.[8]However with the advent of CA-MRSA antibiotic stewardship, it is crucial to bacterial ecology and future public health that the antibiotic is rationally chosen following in vitro antibiotic susceptibility testing (AST) of every MRSA isolate.[9]

We asked the research question, "How common is MRSA in isolates from SSTI and what is the antibiotic susceptibility profile?" Our objective was to find the prevalence and antibiotic susceptibility pattern of MRSA and MSSA isolated from SSTI in this institution. The AST was also done manually by the Kirby Bauer disc diffusion method to test if the results were in agreement with the automated AST. This will confirm that it is possible to perform accurate and reliable AST in low resource situations enabling rational antibiotic use.

Methodology

Permission to conduct this cross-sectional study on Staphylococcal isolates from SSTIs was obtained from the Medical Superintendent and approval from the Institutional Review Board and Ethics committee. As the study was done on samples discarded by the microbiology department waiver of informed consent was approved by the Ethics committee.

The isolates *of Staphylococcus aureus* from samples of pus from SSTI, sent for culture and sensitivity to the microbiology laboratory, were consecutively included in the study. Repeated samples from same patient were excluded. Organisms were isolated and identified by the principal investigator and AST for *Staphylococcus aureus* was performed by the disc diffusion method. The findings were compared with the automated results obtained from the Vitek 2 (Biomerioux) in the microbiology laboratory.

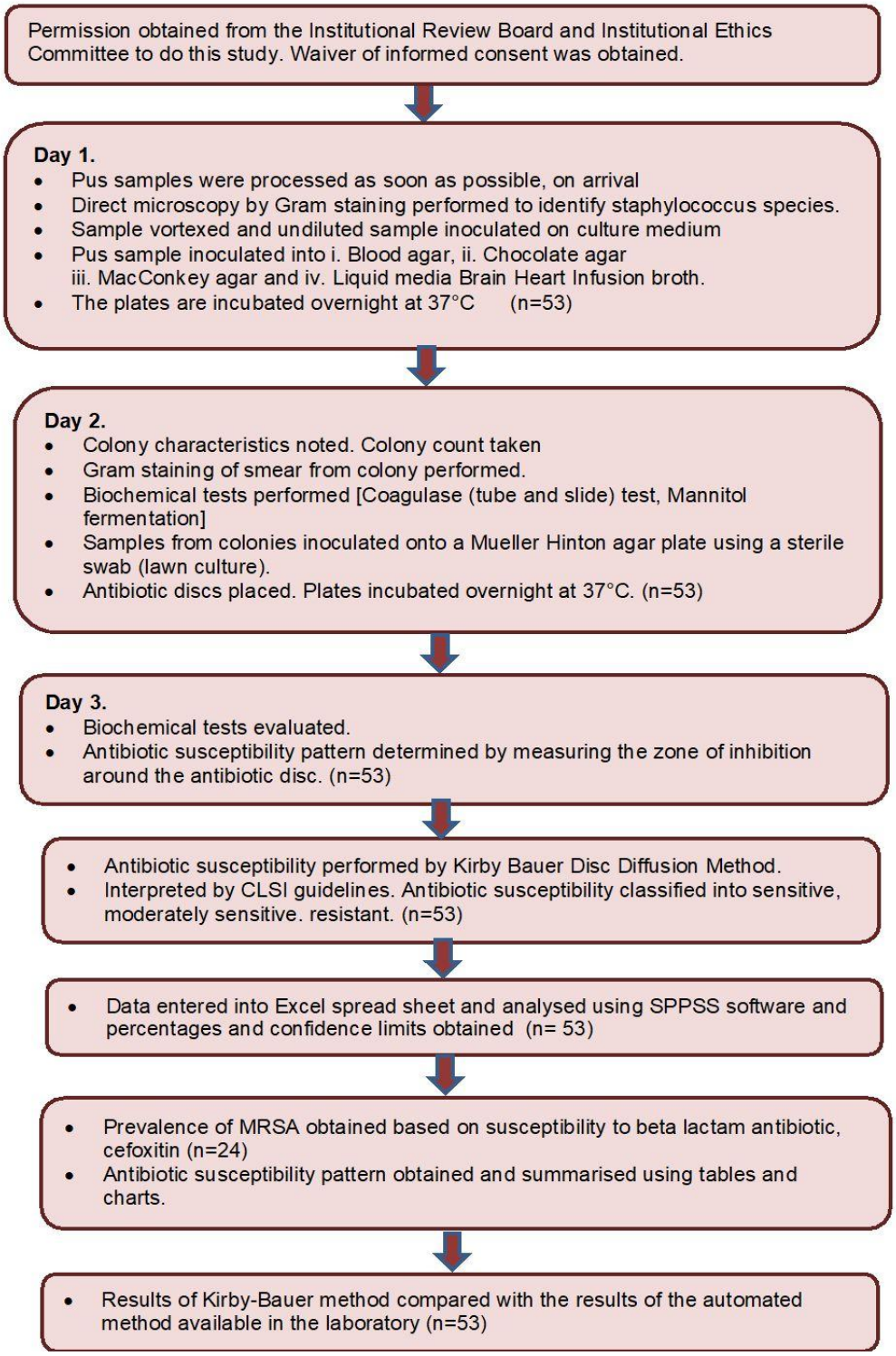
AST was performed using the Kirby-Bauer disc diffusion method. A lawn culture of the bacterial suspension is made by spreading the entire surface of Mueller-Hinton agar using a sterile cotton swab dipped in the suspension. After 5-10 minutes, antibiotic impregnated filter paper discs of definite concentration are applied on the surface of the medium using a sterile forceps. After incubation at 37˚C for 18-24 hours, results are read by measuring the zone diameter in millimetres using a ruler and are interpreted using standard

interpretative charts as per CLSI (Clinical Laboratory Standards Institute) guidelines.[11]

The sample size was calculated using the prevalence of MRSA of 31.1% isolates reported by K Rajaduraipandi et al.[10]The sample size for a confidence interval of 95% and a precision of 12.5% was found to be 53 samples. The proportions and confidence limits of the various pathogens isolated and their antibiotic susceptibility patterns were obtained and the results summarized using tables, charts and graphs.

Figure 1 details the process of data collection, sample preparation, AST and analysis in the Study Flow Diagram.

Figure 1. Study Flow diagram



Results

Of the 53 isolates of *Staphylococcus aureus* received in the laboratory, during the study period, 24(45.3%) were resistant and 29(54.7%) were sensitive to cefoxitin, which is used as the beta-lactam antibiotic for identifying MRSA. Thus the prevalence of MRSA in SSTIs presenting to this tertiary hospital was found to be 45.3%.Fourteen samples were received from hospital wards while 39 were from the out-patient services. A history of recent antibiotic use was elicited in 19(35.8%) patients.

Most of the patients 23(43.4%) were over 50 years of age, while 15(28.3%) were in the age group 18 to 49 years and 15(28.3%) were below the age of 18 years. The mean age of patients from whom samples were received was found to be 40.6(SD 28.04) years. Twenty-eight (52.8%) isolates were from male patients and 25(47.2%) were from female patients. The prevalence of MRSA was 13(46.4%) of the 28 specimens from male patients and 11(44%)in isolates from 25 female patients.

Table 1 shows the baseline characteristics of the patients from whom samples were received and Figure 1 illustrates the prevalence of MRSA and MSSA in this study

.

Table 1: Baseline Characteristics of Patients with Skin and Soft Tissue Infections

Characteristic		MRSA (n=24)	MSSA (n=29)	Total (n=53)
Age group	<18 years	8 (53.3 %)	7 (46.7%)	15
	18-50 years	8 (53.3 %)	7 (46.7%)	15
	>50 years	8 (34.8 %)	15 (65.2%)	23
Gender	Male	13 (46.4%)	15 (53.6%)	28
	Female	11 (44.0%)	14 (53.6%)	25
Sample received from	Hospital wards	5 (35.7%)	9 (64.3%)	14
	Out-patient service	19 (48.7%)	20 (51.3%)	39
History of recent antibiotic use	Yes	10 (52.6%)	9 (47.4%)	19
	No	14 (41.2%)	20 (58.8%)	34

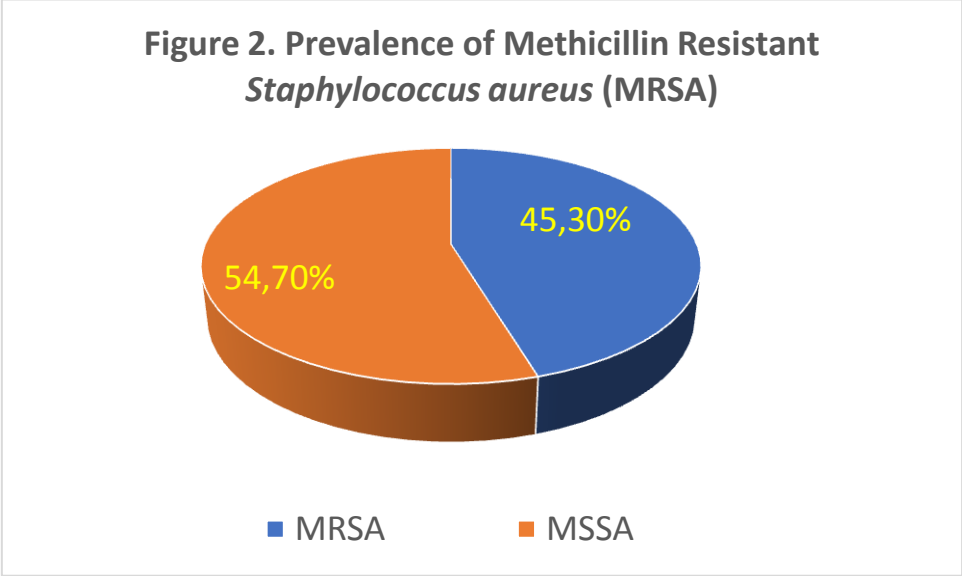


Figure 2 Prevalence of Methicillin Resistant *Staphyloccocus aureus*

Legend: The prevalence of methicillin resistance in *Staphylococcus aureus* in the isolates received from patients coming to this rurally located tertiary care hospital is 45.3%.

Table 2 shows the antibiotic susceptibility and resistance of the *Staphylococcus aureus* identified in our laboratory.

Table 2 Antibiotic Susceptibility of *Staphylococcus aureus Isolated from SSTI**

Anti-microbial Agents	MRSA** n=24		MSSA*** n=29		Total n=53		p value
	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	
Cefoxitin	24(100%)	0	0	29(100%)	24(45.3)	29(54.7)	0.000
Penicillin	24(100%)	0	23(79.3%)	6(20.6%)	47(88.7%)	6(11.3%)	0.021
Ampicillin	24(100%)	0	23(48.9%)	6(20.6%)	47(88.7%)	6(11.3%)	0.021
Eryrthromycin	17(70.8%)	7(29.2%)	10(34.5%)	19(65.5%)	26(49.1%)	27(50.9%)	0.003
Gentamicin	4(16.7%)	20(83.3%)	0	29(100%)	4(7.5%)	49(92.5%)	0.036
Cotrimoxazole	10(41.7%)	14(58.3%)	5(17.2%)	24(82.8%)	15(28.3%)	38(71.7%)	0.063
Cephalexin	14(58.3%)	10(41.7%)	0	29(100%)	14(26.4%)	39(73.6%)	0.000
Vancomycin Linezolid	All MRSA are sensitive						

*SSTI = Skin and soft tissue Infections
**MRSA = Methicillin Resistant Staphylococcal Infections
*** MSSA = Methicillin Sensitive Staphylococcal Infection

Discussion

Cefoxitin resistance is used as the basis of determining MRSA once *Staphylococcus aureus* is identified. In this study 24 isolates of *Staphylococcus aureus* were resistant to cefoxitin making the prevalence of MRSA 45.3%. A study conducted in 2004 in emergency department in 11 US cities found that MRSA was isolated from 59% of patients with skin and soft tissue infections.[12] This shows that the prevalence in this rural setting is less than in the more developed countries.

The prevalence of MRSA has been steadily rising in our country. Panda et al in 2014, contrasted their prevalence rate of MRSA (30.66%) with the prevalence rate of 24% reported by Pulimood et al in 1996.[7, 9]Our study has shown a prevalence of 45.3% in 2017 while Vidhani et al has reported 51.6%in 2001.This implies that the incidence of infection by MRSA isolates has shown a rising trend in the last two decades.[9, 13]

Most of the isolates were received from the hospital wards 39 (73.58%), of which MRSA were 19(48.7%) and MSSA were 20(51.3%). The remaining 14 isolates were received from the out-patient service, among which 5(35.7%) were MRSA while 9(64.3%) were MSSA.

Out of 19 cases who had taken prior antibiotic treatment, 10(52.6%) were found to be MRSA while only 14(41.2%) of the 20 who had not taken antibiotics recently had MRSA.

In a South Indian study, Rajduraipandi et al found most clinical MRSA strains (99.6%) were resistant to penicillin, 93.6% to ampicillin, and 63.2% towards gentamicin, co-trimoxazole, cephalexin, erythromycin, andcephotaxime.[10] In our study, resistance was noticed for penicillin and ampicillin in 47(88.7%) isolates, followed by erythromycin in 27(50.9%) isolates. Out of 53 isolates 15(28.3%) were resistant to cotrimoxazole, 4(7.5%) were resistant to gentamicin and 14(26.4%) were resistant to cephalexin.

Though vancomycin resistant strains have been reported in USA and Japan we have found that all the MRSA strains in this study were sensitive to vancomycin and linezolid.[14]

The manual Kirby Bauer method is a reliable and accurate method of performing Antibiotic Sensitivity Test (AST) in low resource situations. In our study there was agreement in the results obtained by the Kirby Bauer method and automated laboratory antibiotic susceptibility testing.

Conclusion

Over 45% of the isolated *Staphylococcal* samples were MRSA. Due to the significant emergence of multi drug resistant *Staphylococcus aureus,* it is essential that treatment is based on AST. Vancomycin and Linezolid showed 100% sensitivity, hence can be used as the drug of choice for treating MRSA. Robust antimicrobial

stewardship and strengthened infection control measures are required to prevent spread and reduce emergence of resistance.Periodic testing to provide an antibiogram to the clinicians will help in optimizing treatment and reducing the risk of the emergence of more resistant strains.

Sources of support: MOSC Medical College, Kolenchery.

Conflict of Interest: Nil

Acknowledgements

We are grateful to the Dean, Management and Research department of MOSC Medical College for the encouraging medical students to undertake research projects and for all the support and encouragement received to do this research. The Microbiology department of the MOSC Medical College has been very helpful in supervising procedures and ensuring all processes followed standard guidelines. The research department ensured the study was done following all the good practices of good biomedical research methodology.

References

1. Centres for Disease Control and Prevention. Methicillin-resistant *Staphylococcus aureus* infections in correctional facilities. 2001-2003. MMWR Morb Mortal Wkly Rep 2003;52:992-96.
2. Dryden MS. Complicated skin and soft tissue infections. J Antimicrob Chemother 2010; 65 Suppl 3:iii35–44.
3. Alvarez-Uria G, Reddy R. Prevalence and Antibiotic Susceptibility of Community-Associated Methicillin-Resistant *Staphylococcus aureus* in a Rural Area of India. Dermatology. 2012: 2012:ID 248951:5.
4. Liu C, Bayer A,Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ. Clinical Practice Guidelines by the Infectious Diseases Society of America for the Treatment of Methicillin-Resistant Staphylococcus aureus Infections in Adults and Children. IDSA Guidelines. Clinical Practice Guidelines. Clin Infect Dis. 2011 Feb 1;52(3):e18-55.
5. Boucher HW, Corey GR. Epidemiology of methicillin-resistant Staphylococcus aureus. Clin Infect Dis 2008;46(Suppl 5):S344–9.
6. Charlebois ED, Bangsberg DR, Moss NJ,Moore MR, Moss AR, Chambers HF, Perdreau-Remington F. Population-based community prevalence of methicillin-resistant *Staphylococcus aureus* in the urban poor of San Francisco. Clin. Infect. Dis. 2002;34:425–433.
7. Panda A, Agnihotri M, Rahule A. Prevalence and Antibiotic Susceptibility Patterns of Methicillin Resistant Staphylococcus Aureus.JCont Med A Dent September-December 2014;2:3:55-58.
8. Hsiao C-B, Dryja D, Abbatessa L, Patel PH. Staphylococcus aureus Antimicrobial Susceptibility of Abscess Samples from Adults and Children in Western New York State, 2003 to 2006. Journal of Clinical Microbiology, May 2010;48:5:1753–1757.
9. Pulimood TB, Lalitha MK, Jesudason MV, Pandian R, Selwyn J, John TJ.The spectrum of antimicrobial resistance among methicillin resistant *Staphylococcus aureus* (MRSA) in a tertiary care centre in India.Indian J Med Res. 1996 Apr;103:212-5.
10. Rajaduraipandi, Mani KR, Panneerselvam K, Mani M, Bhaskar M, Manikandan P. Prevalence and antimicrobial susceptibility pattern of Methicillin Resistant Staphylococcus Aureus. Indian Journal of Medical Microbiology, (2006) 24 (1):34-8.
11. Clinical Laboratory Standards Institute (CLSI) Guidelines. Performance standards for antimicrobial Disk Susceptibility Testing; Twenty-Fifth Informational Supplement - M100-S25. January 2015.
12. Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, Talan DA. for the Emergency ID Net Study

Group. Methicillin resistant *staphylococcus aureus* infections among patients in the emergency department. N Engl J Med. 2006:355;666-74.

13. Vidhani S. Mehndiratta PL, Mathur MD. Study of Methicillin – resistant Staphylococcus aureus (MRSA) Isolates from high risk patients. Indian J Med Microbiol. 2001;19:13-6.
14. David MZ, Daum RS. Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev. 2010; 23(3):616-687. doi:10.1128/CMR.00081-09.