Original Research Article

Prevalence of MRSA and Antimicrobial Susceptibility *Staphylococcus aureus* in Clinical Samples, in NCR region

Abstract

Background: Infections caused by Staphylococci are frequently linked to indwelling medical equipment. These are extremely difficult to treat with antibiotics. In India, the prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) varies from 30 to 70%, resulting in high mortality, increased economic burden, and high treatment failure in tertiary care hospitals. Rapid and reliable identification of MRSA is critical for infection management and avoiding the needless use of antibiotics.

Material and Method: This prospective study was carried out in the Department of Microbiology, Santosh Medical College, Ghaziabad, from the 1st of August 2020 to the 31st of January 2021. MRSA isolates were screened and confirmed using standard methods recommended by the Clinical and Laboratory Standards Institute (CLSI). Methicillin resistance in *Staphylococcus aureus* strains was evaluated using oxacillin/cefoxitin. The Kirby-Bauer disc diffusion technique was used to assess the antibiotic susceptibility pattern of all MRSA strains.

Result: In this investigation, MRSA was identified in 29.4% of the 384 *Staphylococcus aureus* strains. When compared to females, men outnumbered females. Cefoxitin detects a greater amount of MRSA than oxacillin. In this investigation, the majority of MRSA was found in pus samples.

Conclusion: MRSA prevalence is known to vary depending on geographical region, hospital type, investigated population, and technique of detection used. Given the clinical implications of MRSA infection and its fast transmission capability, MRSA strains must be monitored on a regular basis.

Keywords

MRSA, MSSA Prevalance, Cefoxitin disc, Oxacillin disc; phenotypic method

Introduction

Staphylococcus aureus is a multilateral bacterial pathogen capable of causing a wide range of infections in humans and animals, ranging from mild skin infections to severe systemic diseases such as pneumonia, and has been recognised as a significant cause of human disease for more than 100 years [1]. The classifications Staphylococcus aeruginosa is commonly found in human skin or nasal colonisation. [2]. It is one of the top three major pathogens responsible for community and hospital acquired infections, causing diseases ranging from minor skin and soft tissue infections to life-threatening systemic infections that can be toxin or non-toxin mediated, resulting in high morbidity and mortality worldwide [3,4]. Staphylococci Infections are frequently linked to indwelling medical equipment. These are extremely difficult to treat with antibiotics. Penicillin and its derivatives, particularly methicillin, have been used to treat *S. aureus* infections [5]. Certain strains of *S. aureus*, however, acquired resistance and were known as methicillin resistant *Staphylococcus aureus* (MRSA).

Most medicines used to treat infections are resistant to some hospital-acquired strains. Glycopeptides are the only antibiotics left to treat drug-resistant *Staphylococcus aureus* infections [2, 6, 7]. With the exception of Vancomycin, MRSA isolates are typically resistant to other anti-staphylococcal drugs (Clindamycin, Erythromycin, Tetracycline, and occasionally Gentamicin and Trimethoprim/Sulphomethoxazole) [8]. The resistance to methicillin is caused by the mecA gene, which codes for the penicillin-binding protein (PBP 2A). Recently, a novel methicillin resistance mechanism gene, mecC, was discovered in *S. aureus* and reported MRSA isolates containing mecC gene from humans and animals highlighted the public health risk of mecC-positive MRSA isolates as it has been found in human cases and livestock [9, 10, 11, 12].

Methicillin-resistant other -lactam agents, such as Cephalosporins, appear to be sensitive to *Staphylococcus aureus* in vitro; nevertheless, they are clinically ineffective [3]. Because MRSAs are resistant to all -lactam antibiotics, treatment choices are severely restricted. In India, the prevalence of MRSA varies from 30 to 70% [13, 14]. MRSA infections must be treated and prevented through laboratory diagnostics and susceptibility testing. As a result, techniques used to identify MRSA in clinical samples must be very sensitive and specific, and the results must be accessible in a timely manner. Various techniques for fast detection of methicillin-resistant staphylococci have emerged, however the best method remains debatable [15].

The purpose of this study was to investigate the antibiotic susceptibility of *S. aureus* in a Tertiary Care Hospital in Ghaziabad, as well as the current status of methicillin resistance *S. aureus* in our hospital setting.

Material and Methods

This study was carried out in the Department of Microbiology at Santosh Medical College, Ghaziabad over the time span of six months, from August 1st, 2020 to January 31st, 2021. A total of 384 clinical isolates of *Staphylococcus aureus* were isolated from diverse clinical specimens such as pus, wound or vaginal swabs, blood, pleural fluid, urine, Throat Swab, and so on from different wards including surgery, obstetrics and Gynaecology, medicine, orthopaedics and ICU of Santosh Hospital. This study was ethically approved by the institutional ethical committee of Santosh Medical College, Ghaziabad. Patient consent was obtained from each participant. The chi-square test was applied for the calculation of data by using statistical software of social science (SPSS version 23.0).

Inclusion criteria

All *Staphylococcus aureus* strains isolated from various clinical specimens, were included in the study.

Exclusion criteria

Clinical specimen's yielding growth of Gram positive cocci other than *Staphylococcus aureus* and all gram negative bacteria were excluded.

Methodology

Bacterial identification and antimicrobial susceptibility testing

Clinical specimens were inoculated on 5 percent sheep blood agar, MacConkey's agar, and CLED agar (Only for Urine), incubated at 37°C for 24 hours, and bacterial growth was observed. Standard techniques for identifying *Staphylococcus aureus* were used, including colony morphology, Gram's stain, catalase test, and coagulate test. *S. aureus* was identified in 100 different isolates. They were evaluated for methicillin resistance using the Kirby- Bauer disc diffusion technique, which included oxacillin and cefoxitin. The isolates were considered methicillin-resistant if the zone of inhibition was 10 mm or less. Isolates obtained using cefoxitin disc diffusion that had an inhibition zone diameter of 19 mm were categorised as methicillin resistant, whereas isolates that had an inhibition zone diameter of >20mm were classed as methicillin susceptible.

The other antibiotics were also put to the test. Linozoline, Teicoplanin, Gentamycin, Tetracycline, Erythromycin, Clindamycin, Ciprofloxacin, Vancomycin, Cotrimoxazole,

Amoxyclave, and Rifampicin are some of them. The collected data was then recorded and evaluated using proper statistical procedures.

Results:

A total no. of 384 *staphylococcus aureus* strains were found, in which both Methicillin resistant *staphylococcus aureus* (MRSA) as well as Methicillin-sensitive *staphylococcus aureus* (MSSA) were identified. The characteristics of *S. aureus* include golden yellow colour colonies on Nutrient agar, lactose fermentation on MacConkey agar, gram positive cocci arranged in clusters seen in gram staining and positive catalase test, tube coagulase and mannitol fermentation test (Table-1 & 2).

Table 1: Detection and identification of colony of *S. aureus*

Identification media	Testing feature
Nutrient Agar	Colonies are 2-4mm in diameter, circular, smooth, convex, opaque and easily Emulsifiable and most of the strains produce golden yellow pigment.
Blood Agar	Colonies are 2-4mm in diameter, circular, smooth, convex, opaque and easily Emulsifiable and a beta type of hemolysis is seen.
MacConkey's Agar	Colonies are very small and pink due to lactose fermentation.
In liquid media	Uniform turbidity is produce.

Table 2: Biochemical characteristics of *S. aureus*

S. No.	Biochemical Test	Reaction (+/-)
1	Catalase	+
2	Oxidase	+
3	Slide coagulase	+
4	Tube coagulase	+
5	Mannitol fermentation	+
6	NADase	+

In total 384 samples there were total 223 male sample and 161 samples from females. Out 223 samples from there 65 samples were of MRSA and rest 158 were of MSSA. Similarly in 161 female's samples there were 48 samples of MRSA and remaining 113 of MSSA. Most number (61.71%) of *staphylococcus aureus* were found in 20 to 50 years of age group. Out of which highest number of cases were found from 31 to 40 years (85) of age followed by 21 to 30 years (81) and 41 to 50 years (71). (Table-3)

Table – 3. Sex wise distribution of MRSA.

Age	MRSA (N=113)		MSSA (N=271)		Total (n=384)
	Male (65)	Female (48)	Male (158)	Female (113)	
≤10	6	4	13	7	30 (7.8)
11- 20 years	2	2	18	17	39 (10.2)

21- 30 years	17	13	27	24	81 (21.1)
31- 40 years	12	10	32	31	85 (22.1)
41- 50 years	14	11	29	17	71 (18.5)
51- 60 years	8	6	21	9	44 (11.5)
>61 years	6	2	18	8	34 (8.9)

Table – 4. Distribution of MRSA in various clinical samples Total n. 384

S. No	Samples	MRSA	MSSA	Chi-square	p-value
1.	Blood	18(29.5%)	43(71.4%)	0.489	0.974
2.	Urine	30(28.8%)	74(71.1%)		
3.	Sputum	4(21%)	15(78.9%)		
4.	Pus	39(31.45%)	85(68%)		
5.	Pleural fluid	3(23.07%)	10(76.92%)		
6.	Wound swab	10(26.3%)	28(73.68%)		
7.	Vaginal swab	4(28.5%)	10(71.4%)		
8.	CSF	1(100%)	0		
9.	Throat swab	4(40%)	6(60%)		

In this study incidence of MRSA from clinical sample *S. aureus* were more in pus 39(31.45%) followed blood 18(29.5%), urine 30 (28.8%), Sputum 4 (21%), pleural fluid 3 (33.3%), wound swab 10 (26.3%), Vaginal swab 4 (28.5%), CSF 1 (100%), Throat swab 4 (40%). (Table-4)

Table-5. Antibiotics resistance pattern from clinical specimens (Total n. 384)

Antibiotics	n (%) (MRSA)	n (%) (MSSA)
Linezolid	30 (7.8)	354 (92.2)
Tiecoplan	59 (15.4)	325 (84.6)
Gentamycin	112 (29.2)	272 (70.8)
Tetracyclin	144 (37.5)	240 (62.5)
Erythromycin	265 (69.0)	119 (31.0)
Clindamycin	135 (35.2)	249 (64.8)
Ciprofloxcin	141 (36.7)	243 (63.3)
Cefoxitin	113 (29.4)	271 (70.6)
Oxacillin	99 (25.8)	285 (74.2)
Clotrimozole	228 (59.4)	156 (40.6)
Amoxyclave	103 (26.8)	281 (73.2)
Vancomycin	102 (26.6)	282 (73.4)
Refampicin	102 (26.6)	282 (73.4)

The antibiotic sensitivity pattern of *S. aureus*. The majority of isolates MRSA from cefoxitin 113 (29.4%) and disc diffusion oxacillin 99 (25.8%). However, we observed a high incidence of resistance to other antibiotics such as Erythromycin 265 (69.0%), followed by Clotrimozole 228 (59.4%), Tetracyclin 144 (37.5), Vancomycin 102 (26.6%) and Refampicin 102 (26.6%). We also observed highly sensitivity to the Linezolid 354 (92.2%) followed by Tiecoplanin 325 (84.6%), Gentamycin 272 (70.8%), Clindamycin 249 (64.8%) and Amoxyclave 281 (73.2%). (Table-5)

Discussion:

MRSA has been linked to considerable morbidity and death, and it is a serious public health concern across the world. Data on MRSA transmission patterns remain poor in underdeveloped countries like as India. Antibiotics produced against *S. aureus* have three targets: cell envelope, ribosomes, and nucleic acids. Methicillin belongs to the beta lactamase class, which attacks the cell envelope. Methicillin resistance develops through the acquisition of genes that are less sensitive to antibiotic action [16]. In this investigation, 113 of the 384 *S. aureus* isolates were MRSA. MRSA was determined to be prevalent at our hospital at 29.4%, according to our research. Other investigations have found a significant incidence of MRSA in various regions of the nation, such as 32% in a study by Bilal Ahmad et al [17] similar to this study. Another research conducted by Karem H. Alzoubi in Jordan found that the total prevalence of MRSA was 34% [18]. In support to the above findings, Rajaduraipandi et al. also found 31.1% MRSA strains in their investigation, [19]. Table-6 shows the prevalence of MRSA in different regions of India in previous years.

Table-6 The prevalence of MRSA in different regions of India in previous years

Study_Region	**No. of isolates (*S. aureus*)**	**MRSA (%)**	**Year**
[20]Shah S et al_Mumbai	1562	526 (33.67%)	2021
[21]Lohan K et al_Haryana	240	81 (33.75%)	2021
[22]Nazar A_Greter Noida	158	59 (37.34%)	2019
[23]Kiranjeet Kaur et al_Punjab	162	83 (51.23%)	2019
[24]Chatterjee et al_Karnataka	551	284 (51.54%)	2018
[25]Kulshrestha et al_Rajsthan	161	82 (50.93%)	2017
[26]Choudhary et al_Asam	724	311 (42.95%)	2016
[27]Jana H et al_West Bengal	122	23 (18.50%)	2015
[28]Poddar N et al_Odhisa	529	190 (35.91%)	2015
[29]Kulkarni S et al_Pune	1217	856 (70.33%)	2014

In the inpatient setting, a compromised immune system is one of the major risk factors for MRSA. Those most at risk for infection were infants, the elderly, the chronically ill, burn survivors, steroid users, diabetic patients.[30] In this investigation, the pus sample had the greatest number of MRSA cases (31.45%) followed blood (29.5%), urine (28.8%), Sputum (21%), pleural fluid (33.3%), wound swab (26.3%), Vaginal swab (28.5%), CSF (100%), Throat swab (40%). According to Goel A et al, the highest prevalence was detected in pus samples (66.03%), followed by urine (11.45%), and blood and tips (9.16%) similar to this study.[31] MRSA isolates resistant to three or more types of antibiotics were discovered in this investigation. As showed in the table-5, MRSA presented with highly resistant

(Erythromycin-69.0) to lowest resistant (Linozolid-7.8). Another research also found that 44.4 percent of MRSA isolates were resistant to cefotaxime, 40.7% to gentamicin, 86.4% to ciprofloxacin, 40.7 percent to clindamycin, 66.7% to erythromycin, and 49.4% to ofloxacin [32].

Conclusion:

Finally, it may be stated that the routine monitoring of MRSA's antimicrobial susceptibility pattern and the establishment of a clear antimicrobial policy may be beneficial in reducing the incidence of these infections in hospitals. MRSA prevalence is known to vary depending on geographical region, hospital type, investigated population, and technique of detection used. Due to the prevalence of MRSA, people infected with MRSA must visit the clinic; therefore, it is important for health care providers to identify potential MRSA skin infections. Because MRSA infection can mimic other lesions, appropriate precautions and clinical suspicion are warranted. Furthermore, given the clinical implications of MRSA infection and its fast transmission capability, MRSA strains must be monitored on a regular basis.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

1. Lowy FD. Staphylococcus aureus infection. New Engl Jour Med. 1998;339:520-32.
2. Dilnessa T, Bitew A. Prevalence and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus isolated from clinical samples at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia. BMC Infect Dis. 2016;16(1).
3. Harkins, C.P.; Pichon, B.; Doumith, M.; Parkhill, J.; Westh, H.; Tomasz, A.; De Lencastre, H.; Bentley, S.D.; Kearns, A.M.; Holden, M.T.G. Methicillin-resistant

Staphylococcus aureus emerged long before the introduction of methicillin into clinical practice. Genome Biol. 2017, 18, 1–11.

4. Elmer K, Washington W, Staphen A, Gary P, editors. Color Atlas & Textbook of Diagnostic Microbiology, 6th edition. Wolters Kluwer; 2006: 643–648.
5. Rayner C, Munckhof WJ (2005). Antibiotics currently used in the treatment of infections caused by *Staphylococcus aureus*. Intern. Med. J. 35 (2): 3-16.
6. Rahima T, Nafissa B AD. Prevalence of methicillinresistant Staphylococcus aureus and/or intermediate susceptibility to vancomycin isolated from private laboratories in Annaba" Algeria. J Chem Pharm Res. 2015;7(5):780–6.
7. Jayshree VK, Kumar A YS. Prevalence of Methicillin Resistant Staphylococcus aureus (MRSA) at tertiary care hospital of Mathura, India. Progress Res. 2016;11((Spl VIII)):5495–8.
8. Susan LK. Concepts in Antimicrobial therapy; TB of Diagnostic Microbiology: 3rd edition: WB Saunders. Editors; Mahon C.R, Manuselis G:2007 pg 82.
9. Wielders, C. L. C. A. C. Fluit, S. Brisse, J. Verhoef, and F. J. Schmitz (2002). mecA Gene Is Widely Disseminated in *Staphylococcus aureus* Population. J. Clin. Microbiol. 40(11): 3970-3975
10. Paterson GK, Larsen AR, Robb A, Edwards GE, Pennycott TW, Foster G (2012). The newly described mecA homologue, mecALGA251, is present in methicillin-resistant *Staphylococcus aureus* isolates from a diverse range of host species. J. Antimicrob. Chemother. 67(12): 2809-2813.
11. Porrero CM , Mentaberre G, Sánchez S, Fernández- Llario P, Gómez-Barrero S, Navarro-Gonzalez N, Serrano E, Casas-Díaz E, Marco I, Fernández- Garayzabal J, Mateos A, Vidal D, Lavín S, Domínguez L. (2013). Methicillin resistant *Staphylococcus aureus* (MRSA) carriage in different free-living wild animal species in Spain. Vet. J. 198(1): 127-130
12. Harrison EM, Paterson GK, Holden MTG, Morgan FJE, Larsen AR, Petersen A, et al. A Staphylococcus xylosus Isolate with a New mecC Allotype. Antimicrob. Agents Chemother. 2013;57(3):4-8.
13. Verma S, Joshi S, Chitnis V, Hemavani N, Chitnis D. Growing problems of Methicillin Resistant Staphylococci-Indian Scenario. *Indian J Med Sci* 2000; 54:535-40.

14. Bratzler DW, Hunt DR. The surgical infection prevention and surgical care improvement projects: national initiatives to improve outcomes for patients having surgery. *Clin Infect Dis* 2006; 43: 322-330.
15. Leung ECM, Lee MKP, Lai RWM. Admission Screening of Methicillin- Resistant Staphylococcus aureus with Rapid Molecular Detection in Intensive Care Unit: A Three-Year Single-Centre Experience in Hong Kong. ISRN Microbiol [Internet]. 2013;1–5. Available from: http://dx.doi.org/10.1155/2013/140294
16. Lim D, Strynadka NCJ. Structural basis for the β lactam resistance of PBP2a from methicillin-resistant Staphylococcus aureus. Nat Struct Biol . 2002;9(11):870
17. Mir BA, Srikanth. Prevalence and antimicrobial susceptibility of Methicillin Resistant *Staphylococcus aureus* and Coagulase-negative staphylococci in a tertiary care hospital. Asian J Pharm Clin Res. 2013; 6(3):231-4.
18. Alzoubi KH, Hayajneh WA, Ayoub AM, Al-Safi SA, A-Azzam SI, Mhaidat NM. Prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) at a Tertiary Hospital in North Jordan. Jordan Journal of Pharmaceutical Sciences. 2010; 3(1):37-43.
19. K. Rajaduraipandi, KR Mani, K. Panneerselvam, M. Mani, M. Bhaskar, P. Manikandan. Prevalence and Antimicrobial Susceptibility pattern of Methicillin Resistant *Staphylococcus aureus*: A Multicentre Study. Indian J Medi Microbiol. 2016; 24(1):34-8.
20. Shah S, Rampal R, Thakkar P, Poojary S, Ladi S. The Prevalence and Antimicrobial Susceptibility Pattern of Gram-Positive Pathogens: Three-Year Study at a Private Tertiary Care Hospital in Mumbai, India. Journal of Laboratory Physicians. 2021 Jul 2.
21. Lohan K, Sangwan J, Mane P, Lathwal S. Prevalence pattern of MRSA from a rural medical college of North India: A cause of concern. J Family Med Prim Care. 2021 Feb;10(2):752-757
22. Nazar A, Imran Y, Rastogi V, Singhal P. Prevalence and Antibiotic Susceptibility Pattern of Methicillin Resistant *Staphylococcus aureus* (MRSA) at a Tertiary Care Hospital from Northern India. Int J Curr Microbiol App Sci.2019;8(09):2-8
23. Kaur K, Gill AK, Kaur M. Methicillin Resistance, Vancomycin Intermediate and Vancomycin Resistance *Staphylococcus aureus* Prevalence in a Tertiary Care Hospital of Punjab, India.NJLM. 2019;8(3):MO01-MO03

24. Chatterjee A, Rai S, Guddattu V, Mukhopadhyay C, Saravu K. Is methicillin-resistant *Staphylococcus Aureus* infection associated with higher mortality and morbidity in hospitalized patients? A cohort study of 551 patients from South Western India. Risk management and healthcare policy. 2018;11:243.
25. Kulshrestha A, Anamika V, Mrithunjay K, Himanshu V, Manish K, Dalal AS. A prospective study on the prevalence and antibiotic sensitivity pattern of methicillin resistant *Staphylococcus aureus* isolated from various clinical specimen at a tertiary care post graduate teaching institute. Int J Curr Microbiol App Sci. 2017;6(3):1859-69.
26. Choudhury D, Chakravarty P. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* in Silchar Medical College and Hospital, Assam, India. Int J Basic Clin Pharmacol 2016;5:2174-7.
27. Jana H, Roy T, Dey R, Dey JB, Ghosh A, Mondal KC. Prevalence and antimicrobial susceptibility patterns of different clinical isolates of HA MRSA and CA MRSA in in a tertiary care rural hospital, Bankura, West Bengal, India. Sch J Appl Med Sci. 2015;3(2):944-8.
28. Poddar N, Pattnaik D, Panigrahi K, Pathi B, Lenka PR, Mohanty S, et al. Prevalence and Antimicrobial Susceptibility Pattern of Methicillin-resistant *Staphylococcus aureus* (MRSA) Isolates and Inducible Clindamycin resistance in *Staphylococcus aureus*: study at a Tertiary Care Hospital in KIMS, Bhubaneswar, India. Int J Adv Res Biol Sci. 2015;2(1):9-15.
29. Kulkarni S, Khare A, Charan Kaur D. Prevalence of methicillin resistant *staphylococcus aureus*-A study in a tertiary care rural hospital. *Indian J Basic Appl Med Res.* 2014;3:414–21
30. Green BN, Johnson CD, Egan JT, Rosenthal M, Griffith EA, Evans MW. Methicillin-resistant *Staphylococcus aureus*: an overview for manual therapists. J Chiropr Med. 2012 Mar;11(1):64-76.
31. Goyal A, Diwakar MK, Bhooshan S, Goyal S, Agrawal A. Prevalence and Antimicrobial Susceptibility Pattern of Methicillin-resistant *Staphylococcus aureus* [MRSA] isolates at a Tertiary Care Hospital in Agra, North India – A systemic annual review. IOSR Journal of Dental and Medical Sciences. 2013;11:80-84. 10.9790/0853-1168084.

32. Preeja PP, Kumar SH, Shetty V. Prevalence and Characterization of Methicillin-Resistant *Staphylococcus aureus* from Community- and Hospital-Associated Infections: A Tertiary Care Center Study. Antibiotics 2021, 10, 197.