e-ISSN: 0975-9506, p-ISSN: 2961-6093

Available online on www.ijpqa.com doi: 10.25258/ijpqa.16.3.9

International Journal of Pharmaceutical Quality Assurance 2025; 16(3); 55-60

Original Research Article

Antibiotic Resistance Profile and Molecular Confirmation of MRSA isolates by PCR from Clinical Samples at Tertiary Care Hospital in India

Versha Rajput[1], Kailash Jatav[2], Naveen Kumar Patidar[3]

[1]PhD Scholar, Department of Microbiology, Index Medical College Hospital & Research Centre, Indore, Madhya Pradesh, India

[2]Assistant Professor, Department of Microbiology, Amaltas Institute of Medical Sciences, Dewas, Department of Microbiology

[3]PhD scholar, Department of Microbiology, Index Medical College Hospital & Research Centre, Indore, Madhya Pradesh, India

Received: 02-01-2025 / Revised: 23-01-2025 / Accepted: 26-02-2025

Corresponding Author: Dr. Versha Rajput

Conflict of interest: Nil

Abstract:

Background: Antimicrobial resistance is a significant global issue causing millions of deaths, long-term impairments, and increased medical expenditures. The investigation reveals a direct relationship between the widespread use of antimicrobials in human and veterinary medicine and the proliferation of resistant bacteria strains.

Aim & Objective: Antibiotic Resistance Profile and Molecular Confirmation of MRSA isolates by PCR from Clinical Samples at Tertiary Care Hospital in India.

Material & Methods: In this study total 750 samples were included, out of which 358 samples were found positive for Staph aureus. Samples were cultured on Blood agar & MacConkey agar and bacterial identification along with antibiotic susceptibility testing. PCR also done for the confirmation of Positive MRSA.

Result: In this study total 750 samples were included, out of which 358 samples were found positive for Staph aureus, after that antibiotic sensitivity testing was done for 358 Staph aureus isolates. Out of a total of 358 positive samples, 200 were identified as MRSA and confirmed by the detection of the MecA gene.

Conclusion: Emergence of MRSA and VRSA infections is a growing problem in our hospital. Our study gives an outline of antibiotics susceptibility patterns of Staph aureus isolates which will help in formulating the local antibiotic policy for the hospital and to start the appropriate empirical antibiotic treatment before the culture and sensitivity are available.

Keywords: MRSA- Methicillin resistance staphylococcus aureus, PCR-Polymerase Chain Reaction.

Introduction

Throughout history, infectious diseases have shaped humanity, creating a higher burden on health and the economy [1]. Over centuries, inspecting the pathogenicity mechanism of microorganisms in correlation with disease causing aided in developing antimicrobial drugs that reduce the spread of infectious diseases. Antimicrobial resistance (AMR) is a critical and emerging global health threat that poses challenges to effectively preventing and treating infections caused by bacteria, viruses, parasites, and fungi [2].

Therefore, it is crucial to induce a higher spread of antimicrobial-resistant pathogens that restrain the ability to treat infectious diseases, particularly the global spread of multi- and pan-resistant bacteria known as superbugs, including methicillin-resistant Staphylococcus aureus (MRSA) [3]. Staphylococcus aureus (S. aureus) is a Gram-positive bacteria that often colonizes the human skin, mucous membranes, nose, and other areas without causing symptoms and is considered part of the normal flora [4]. Approximately 30% of the human population is colonized by S. aureus [4]. Over the past few decades, S. aureus has emerged with a new form resistant to a wide range of beta-lactam antibiotics, including penicillin, methicillin, amoxicillin, nafcillin, oxacillin, and cephalosporins known as MRSA. The ability of MRSA to produce a protein called penicillin-binding protein (PBP2a) led to its resistance to multiple beta-lactam antibiotics; the protein allows the bacteria to escape the antibiotic inhibitory effect [5]. This means that healthcare providers are facing a new challenge when treating MRSA clinical manifestations.

Identifying MRSA and methicillin-sensitive Staphylococcus aureus (MSSA) ranges from

various cultures to molecular methods. These molecular methods include end-point polymerase chain reaction (PCR), and various molecular automated detection systems showed sensitivity to clinical specimens that ranged from 69.2 to 100% and specificity from 64.5 to 100% with a turnaround time that fluctuated from 1 h to 3 h [6]. The most significant barrier when considering molecular assays is the cost. Thus, designing a reasonable strategy for rapidly screening MRSA directly from clinical samples is crucial

Material and Methods

According to inclusion and exclusion criteria of the study total 358 positive Staph aureus samples were selected for this study. Sample of all the patients in the hospital were collected with all the proper safety measurements and subjected to the bacterial culture, their identification and antibiotic susceptibility testing.

Study Design: This was a cross-sectional study, entitled Antibiotic resistance profile and molecular confirmation of MRSA isolates by PCR from clinical samples at tertiary care hospital in India was conducted prospectively on clinical isolates of Staphylococcus aureus.

Study Duration: The study was conducted in the Microbiology Laboratory, Index Medical College Hospital & Research Centre from December 2023 to December 2024.

Sample size: A total of 358 Staphylococcus aureus clinical specimens were processed.

Clinical Specimens - In this study, a total of 358 clinically significant, non-repetitive and consecutive clinical isolates of Staphylococcus aureus were isolated from various clinical specimens.

Collection of samples:

Wound and pus samples: Specimens were collected aseptically in an autoclaved container. If scab was present over the wound, it was peeled off and a swab was well soaked in wound material. Two swabs were collected, one for smear and another for culture. The swabs were collected in a sterile leak-proof container & transported within two hours to the lab. The nutrient broth was used as the transport medium.

Urine sample: Midstream urine or clean-catch specimen was collected after washing the genital area in a sterile container. Then it was transported within 2 hours to the laboratory after the collection to avoid undue multiplication of possible contamination.

Diabetic foot swab: Specimens were collected from Diabetic foot had been cleansed (using sterile saline and gauze) and debrided (removal of foreign material, calluses, necrotic tissue, and undermined wound edges). No antimicrobial agent (e.g., alcohol or iodine) or antiseptic was introduced into the wound before specimen collection.[8]

Blood sample: Like urine, blood is also a rich nutrient medium for the growth of microorganisms. Blood was collected in cases where blood-borne or systemic infections were suspected. Intravenous blood was collected by using a sterile disposable syringe. Then the blood sample was transferred into a tube containing an anticoagulant. The sample was immediately transported to the laboratory. In case of delay, the sample was inoculated in Brain heart infusion broth (BHIB), after which it was used to carry out further tests.[9]

Throat swab: With a sterile cotton swab, both of the tonsillar arches and the posterior nasopharynx were swabbed without touching the sides of the mouth. The swab was taken in a sterile container for processing.[9]

Sputum sample: 5 ml (about 1 teaspoon) of the sputum sample was collected in a sterile container with an airtight lid. The specimen must be from the lungs. The specimen was transported within two hours to the laboratory and processed.[10]

Processing of samples: Methodology used for identification of Staphylococcus aureus and mecA gene [11].

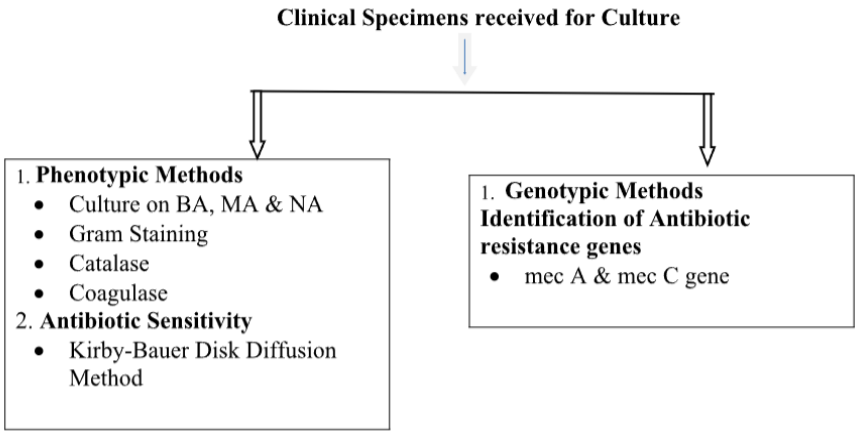


Figure 1: Yellow colour colonies present in Blood agar

Antibiotic discs used for the detection of (MRSA) – [8]

S. No.	Antibiotic	Disc Content	Interpretative Criteria		
			R (mm)	I (mm)	S (mm)
1.	Cefoxitin	30mcg	14	15-17	18
2.	Oxacillin	1mcg	19	19-20	22

Result: In this study total 750 samples were included, out of which 358 samples were found positive for Staph aureus, after that antibiotic sensitivity testing was done for 358 Staph aureus isolates. Out of a total of 358 positive samples, 200 were identified as MRSA and confirmed by the detection of the MecA gene. The age group wise distribution of Staph aureus:-

Table 1: Age group wise distribution of Staph aureus

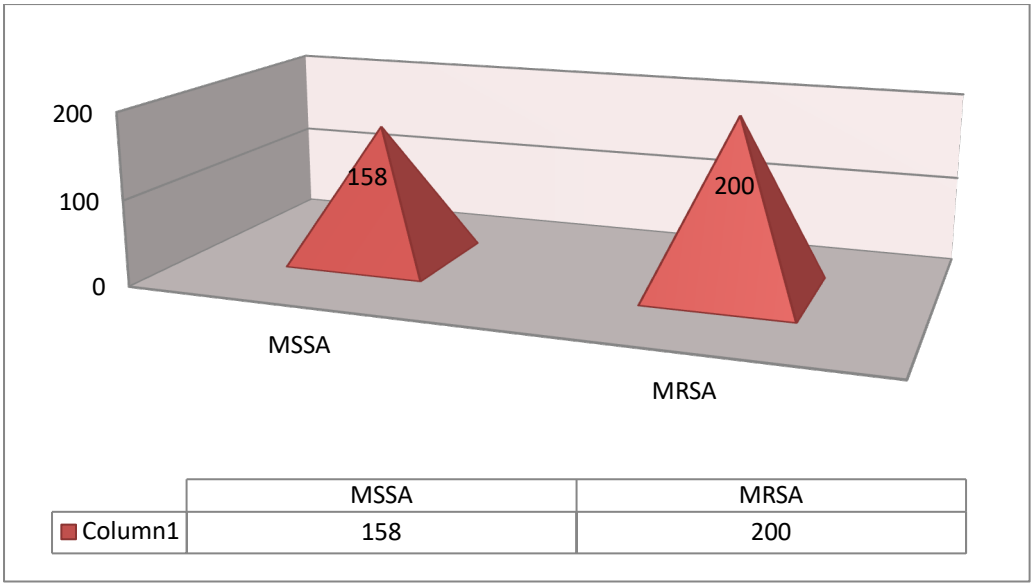
S.No.	Age Group	Staph aureus	Total Samples
1	20-40	79 (22.2%)	358
2	41-60	189 (52.7%)	358
3	61-80	90 (25.1%)	358

Among 358 Staph aureus isolated, 79 (22.2%) belonged to (20-40) age group followed by 189 (52.7%) from (41-60) age group, 90 (25.1%) from (61-80) age group. The maximum number of Staph aureus were obtained from (41-60) years of age group.

Detection of MRSA by Phenotypic Method-

Table 2: Show MSSA and MRSA in positive Staph aureus

S.No.	Methods	MRSA	MSSA	Total
1	**Cefoxitin Disc Diffusion**	**200 (55.8%)**	**158(44.1%)**	**358**
2	**Oxacillin Disc Diffusion**	**172 (48.0%)**	**186 (51.9)**	**358**



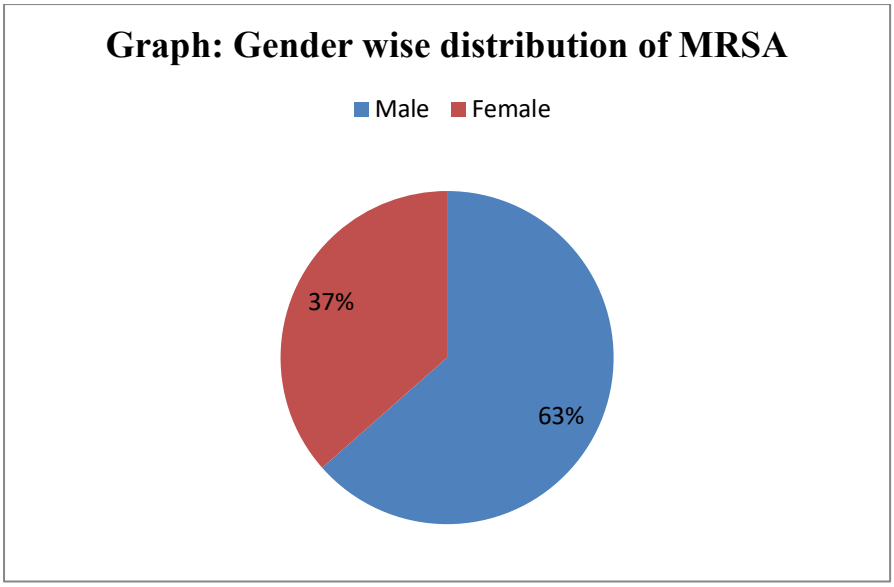
Graph 2: Show MSSA and MRSA in positive Staph aureus samples

Out of 358 Staph aureus were isolated, 200 (55.8%) were Methicillin Sensitive Staph aureus (MRSA) and 158 (44.1%) were Methicillin Resistance Staph aureus (MSSA).

Gender wise distribution of MRSA isolates

Table 3: Show Gender wise isolated of MRSA

S.No.	Methods	MRSA	Female	Male
1	Cefoxitin Disc Diffusion	200	73 (36.5.2 %)	127 (63.5 %)



Graph 3: Gender wise distribution of MRSA isolates

Out of the total 200 MRSA isolates, 127 (63.5 %) and 73 (36.5.2 %) of MRSA belong to males and females respectively.

Table 4: Detection of Mec A gene among MRSA isolates by PCR:-

Phenotypic methods for Detection of MRSA	Number of MRSA isolates by Phenotypic methods	Genotypic method (PCR for mecA gene) for Detection of MRSA	
		mecA positive	mecA negative
Cefoxitin Disc Diffusion	200	197	03
Oxacillin Disc Diffusion	200	189	12

Methicillin-resistant Staphylococcus aureus (MRSA) detection is crucial for infection control and treatment decisions. Various phenotypic methods, such as Cefoxitin and Oxacillin disc diffusion, are commonly employed to identify MRSA isolates. Additionally, the genotypic method, specifically PCR targeting the mecA gene, is considered the gold standard for MRSA confirmation. In the present study, the Cefoxitin disc diffusion method detected 200 MRSA isolates, out of which 197 were confirmed as mecA positive by PCR, while only three were mecA negative. This indicates a high sensitivity and specificity of Cefoxitin disc diffusion for MRSA detection, aligning closely with molecular confirmation. Conversely, the Oxacillin disc diffusion method identified 200 isolates as MRSA, but only 189 were mecA positive, and 12 were mecA negative. The discrepancy suggests that Oxacillin disc diffusion may have lower sensitivity and specificity compared to Cefoxitin disc diffusion, potentially leading to false-positive results. This could be attributed to hetero resistance or other resistance mechanisms that do not involve the mecA gene.

Discussion

Staph aureus are gram positive cocci bacteria. Staph aureus is one of the most common pathogenic organisms causing skin or soft tissue abscesses. In addition to form pus it is also responsible for the Toxic Shock Syndrome, Pneumonia, Exfoliative Skin disease and Nosocomial infections.[9] Staph aureus is one of the most repeatedly isolated pathogens in both community and hospital practice. Methicillin Resistant Staph aureus (MRSA) is a specific strain of the Staphylococcus aureus which is resistant to Methicillin. Outbreak of Methicillin resistant staph aureus (MRSA) is a major emerging concern in many hospitals.[10]

Out of 358 Staph aureus were isolated, 200 (55.8%) were Methicillin Sensitive Staph aureus (MRSA) and 158 (44.1%) were Methicillin Resistance Staph aureus (MSSA). MRSA were detected by their sensitivity to Cefoxitin/Oxacillin as performed according to CLSI guidelines [8]. In our study it was observed that 200 (55.8%) Staph aureus isolated were MRSA. Prevalence of MRSA in previous study by Harshan K H et al [23], Banker N et al [1] and Devi U et al [3] were isolated 29.7%, 28.8% and 26.9%. [11,12,13] Few studies from India has reported slightly higher resistance rate of MRSA as compared to our study such as (60.9%) by Kumar A et al.[14] in 2018 from Jamshedpur, (52%) by Khara R R et al in 2016 from Gujarat. [14, 15]

Infections caused by Methicillin Resistance Staph aureus have been associated with high morbidity and mortality rate. In India MRSA is one of the common cause of hospital acquired infection and (30%) to (76.5%) MRSA based on antibiotic susceptibility test [16]. In the present study, the Cefoxitin disc diffusion method detected 200 MRSA isolates, out of which 197 were confirmed as mecA positive by PCR, while only three were mecA negative. This indicates a high sensitivity and specificity of Cefoxitin disc diffusion for MRSA detection, aligning closely with molecular confirmation. Similar results were reported by Swenson et al., [17] Skov et al., [18] and Nair D et al.[19]. The high resistance against the most commonly used antibiotics have been found because of the overuse of antibiotics without any bacterial infections which may cause drugs resistance. There are many mechanisms that cause bacteria to start resistance:-Capsule formation, genes mutations, enzyme production.

Conclusion

In India prevalence rate of MRSA from different studies has been in the range of 26% to 87%. In our study this has been 54.2% which is accordance with the other studies.[13,14] The continuous observation of MRSA will also be helpful to identify the varying trends of antibiotics susceptibility pattern, in improving hospital antimicrobial strategy for selecting a suitable antibiotic to alternate the use of powerful antibiotic like Vancomycin.

Present study findings favor for the utilization of Cefoxitin disc diffusion technique among all different phenotypic methods. Cefoxitin disc diffusion technique has greater sensitivity when compared with available methods of phenotypic identification of MRSA and also its reports are quite equivalent to the end results of PCR method. It should be followed for clinical diagnostic laboratory owing to its simple procedure, need of less technical expertise, also is less costly when compared with other techniques.[15]

Vancomycin is the most effective drug against infections caused by MRSA and to preserve its value for the life threatening infections, we should avoid the use of Vancomycin as first treatment. Linezolid, Doxycyclin and Teicoplanin also show low resistance to MRSA so they can also play a significant role in the treatment of Staphylococcal infections.

References

1. Palavecino EL. Rapid Methods for Detection of MRSA in Clinical Specimens. In: Ji Y, editor. Methicillin-Resistant Staphylococcus Aureus (MRSA) Protocols: Cutting-Edge Technologies and Advancements [Internet]. New York, NY: Springer US; 2020 [cited 2025 Jan 31]. p. 29–45. Available from: https://doi.org/10.1007/978-1-4939-9849-4_2.

2. Nair D, Shashindran N, Kumar A, Vinodh V, Biswas L, Biswas R. Comparison of Phenotypic MRSA Detection Methods with PCR for mecA Gene in the Background of Emergence of Oxacillin-Susceptible MRSA. Microb Drug Resist. 2021 Sep; 27(9):1190–4.
3. Adeyemi FM, Oyedara OO, Yusuf-Omoloye NA, Ajigbewu OH, Ndaji OL, Adegbite-Badmus MK, et al. Guardians of resistance and virulence: detection of mec, femA, Van, pvl, hlg and spa genes in methicillin and vancomycin-resistant Staphylococcus aureus from clinical and food samples in Southwestern Nigeria. BMC Microbiol. 2024 Nov 26; 24(1):498.
4. Mizusawa M, Carroll KC. Recent updates in the development of molecular assays for the rapid identification and susceptibility testing of MRSA. Expert Rev Mol Diagn. 2023 Aug 3; 23(8):679–99.
5. Madhavan A, Sachu A, Balakrishnan A, Vasudevan A, Balakrishnan S, Vasudevapanicker J. Comparison of PCR and phenotypic methods for the detection of methicillin resistant Staphylococcus aureus. Iran J Microbiol. 2021 Feb; 13(1):31–6.
6. Xing SY, Wei LQ, Abushelaibi A, Lai KS, Lim SHE, Maran S. Current molecular approach for diagnosis of MRSA: a meta-narrative review. Drug Target Insights. 2022 Dec 31; 16:88–96.
7. Systematic Review of the Clinical Utility of Methicillin-Resistant Staphylococcus aureus (MRSA) Nasal Screening for MRSA Pneumonia - Melanie N. Smith, Amy L. Brotherton, Katherine Lusardi, Carrie A. Tan, Drayton A. Hammond, 2019 [Internet]. [Cited 2025 Jan 31]. Available from: https://journals.sagepub.com/doi/abs/10.1177/1060028018823027.
8. Patel J B, Patel R, Satlin M, Mazzulli T, Weinstein M P, Richter S S et al. Performance Standards for Antimicrobial Susceptibility Testing. Clin Lab Stand Inst.2017; 1(37):132-33.
9. Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research | Nature Reviews Microbiology [Internet]. [Cited 2025 Jan 31]. Available from: https://www.nature.com/articles/s41579-018-0147-4.
10. Vogkou CT, Vlachogiannis NI, Palaiodimos L, Kousoulis AA. The causative agents in infective endocarditis: a systematic review comprising 33,214 cases. Eur J Clin Microbiol Infect Dis. 2016 Aug 1; 35(8):1227–45.
11. Harshan K H, Chavan K D. Prevalence and Susceptibility Pattern of Methicillin Resistant Staphylococcus aureus (MRSA) in Pus Samples at a Tertiary Care Hospital in Trivandrum.2015;4(11):718-23.
12. Banker N, Wankhade A, Bhramana R B, Hathiwala R, Chandi D H. Bacteriological profile of PUS/WOUND SWAB and Antimicrobial susceptibility of staphylococcus aureus from pus and wound swab of Indoor Patients of Tertiary Care Hospital, Drug, Chhattisgarh India. Int J Innov Res Med Sci.2018; 3(4):2455-737.
13. Devi U. Methicillin Resistant Staphylococcus aureus in Pus Samples. Int J Med Sci and Clin Inv.2017; 4(12):3374-76.
14. Kumar A, Kumar A. Prevalence of Methicillin Resistant Staphylococcus Aureus (MRSA) In A Secondary Care Hospital In Northeastern Part of India. Arch Inf Dis Therp.2018;2(1):1-2
15. Khara R R, Lakhani S J, Panjwani D. Methicillin resistant Staphylococcus aureus (MRSA) and Vancomycin resistant Staphylococcus aureus (VRSA) from a rural tertiary care and teaching hospital in Vadodara district, Gujarat. Int Arc Integ Med.2016; 3(7):187-95.
16. S R, V P, D'Souza AO, Vinod R. Comparison of Phenotypic and Genotypic Characterization Methods for the Detection of Methicillin-Resistant Staphylococcus Aureus. Cureus. 14(3):e23396.
17. Swenson JMWong B, Simor AE, Thomson RB, Ferraro MJ, Hardy DJ, Hindler J, Jorgensen J, Reller LB, Traczewski M, McDougal LK, Patel JB2010.Multicenter Study To Determine Disk Diffusion and Broth Microdilution Criteria for Prediction of High- and Low-Level Mupirocin Resistance in Staphylococcus aureus. J Clin Microbiol48:.https://doi.org/10.1128/jcm.00340-10
18. R.L. Skov, K.S. Jensen. Community-associated meticillin-resistant Staphylococcus aureus as a cause of hospital-acquired infections,Journal of Hospital Infection. 2009 73(4), 364-370, ISSN 0195-6701,https://doi.org/10.1016/j.jhin.2009.07.004.
19. Nair D, Shashindran N et al. Comparison of Phenotypic MRSA Detection Methods with PCR for mecA Gene in the Background of Emergence of Oxacillin-Susceptible MRSA.2021, 27(9), 1190-94, https://doi.org/ 10.1089/mdr.2020.0361.