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Original Research Article

Prevalence of infections due to Methicillin Resistant Staphylococcus aureus (MRSA) and the phenotypic and genotypic characterization on the clinical MRSA isolates

Rajalakshmi Kesavan[1]*, Asha A[1], Sumetha Suga D[1], Ananthi B[1]

[1]Dept. of Microbiology, ACS Medical College and Hospital, Chennai, Tamil Nadu, India

Abstract

Introduction: A superbug is a type of bacteria that has evolved resistance to antibiotics that are used to treat common and severe infections. One such bacteria is Methicillin Resistant Staphylococcus aureus (MRSA). Since the number of nosocomial infections caused by MRSA which are resistant to all β-lactam antibiotics is on the rise globally, detecting them is crucial for therapeutic reasons. Hence clinical microbiologists should find a way to identify Staphylococcus aureus isolates as MRSA and report their findings correctly and quickly.

Aim and Objective: 1. To detect the prevalence of MRSA strains from the various clinical specimens; 2. To study the antibiotic susceptibility pattern of MRSA isolates by disc diffusion method, 3.To detect MRSA by Phenotypic and Genotypic methods.

Materials and Methods: A cross-sectional study was carried at ACS Medical College and Hospital in Chennai for a period of one year from September 2022 to October 2023. A total of 100 non-duplicate MRSA isolates from various clinical samples were collected and specific phenotypic tests for screening and confirmation of MRSA were done as per CLSI guidelines (32nd and 33rd edition). Specific genotypic tests for detection of mecA gene will be done by Real Time Polymerase Chain Reaction (RT- PCR).

Result: Male predominance was higher in this study of about 55% with majority of 39% were between 41-60 years. Outpatient and inpatient scenarios were used to classify MRSA isolates. The inpatient group had the highest number of MRSA isolates (94%). Among the 94 inpatients, 28(29.8%) remained longer than 2 weeks, whereas 66(70.2%) stayed for less than 2 weeks. Nearly majority of the MRSA isolates (98%) developed bluish-green coloured colonies on CHROMagar. mecA gene was detected in all 50 samples by RT-PCR method. Among 100 MRSA isolates, highest resistance was observed in Azithromycin (79%) followed by Ciprofloxacin (78%) and Erythromycin (64%). All 100 isolates were susceptible for Vancomycin and Linezolid. Among 33 Clindamycin resistant MRSA isolates, 60.6% isolates showed inducible clindamycin resistance and 39.4% showed constitutive resistance.

Conclusion: The high prevalence of methicillin-resistant Staphylococcus aureus (MRSA) infections in India highlights the urgent need to implement surveillance and prevention measures in healthcare facilities and the general public to curb the infection's spread. Thanks to its high sensitivity, specificity, ease of interpretation, and rapid results, CHROMagar is an effective diagnostic tool for Methicillin resistance. A number of previous studies have demonstrated that the gold standard method for finding mecA gene is Real-Time Polymerase Chain Reaction (PCR).

Keywords: Methicillin-Resistant *S. aureus* (MRSA), MRSA detection, Multi-drug resistant MRSA and Cefoxitin disc diffusion.

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1. Introduction

Staphylococcus aureus, Gram-positive, coagulase-positive pathogen is a member of the Staphylococcaceae family and is commonly found as a commensal in the nasal and digestive tracts of healthy people as well as on skin, skin glands, and other mucous membranes.[1]

Since *S. aureus* has become resistant to the majority of antimicrobial drugs, it has lead to source of potentially fatal infections in both the community and healthcare facilities. Possible contributors to the development of MRSA include prolonged hospitalization, irrational use of antibiotics, and a lack of public knowledge. The transmission of Methicillin-Resistant Staphylococcus aureus (MRSA) isolates occurs via a vector who are infected and colonized individuals in

*Corresponding author: Rajalakshmi Kesavan
Email: rathi.k7@gmail.com

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healthcare facilities and healthcare workers play an important role in the transmission of MRSA even further.[2]

Staphylococcus aureus resistant to methicillin were first reported in October 1960 in Guildford, also in Surrey. Methicillin Resistant Staphylococcus aureus (MRSA) emerged in the U.S. in the early 1960‘s with the first significant outbreak in 1968.[3] India is now experiencing an epidemic of Methicillin Resistant Staphylococcus aureus. In western India, the prevalence of MRSA is 25% and it is 50% in southern India.[4]

Diagnosing MRSA might be difficult due to the diversity of methicillin resistance. The mecA gene is extremely conserved among Staphylococcal species, making polymerase chain reaction (PCR) detection of this gene the "gold standard" for identifying methicillin-resistant Staphylococci.[5]

Since many MRSA strains are resistant to most of the drugs, the only group that is susceptible are glycopeptides like vancomycin. Knowing the present microbiological profile and the incidence of MRSA is crucial for choosing the right empirical treatment for these illnesses.[6]

Various techniques which are employed in the laboratory to identify MRSA, includes disc diffusion with oxacillin or cefoxitin, MIC measurement by dilution in agar or broth, and the oxacillin screen agar technique. There are several ways to enhance MRSA detection faster. Many consider polymerase chain reaction to be the gold standard, as it is fast and exhibits a high level of accuracy and precision.[7]

It is critical to quickly diagnose Methicillin Resistant Staphylococcus aureus infections and determine their sensitivity to antimicrobial drugs so that the right treatment and control measures may be begun. When it comes to treating, controlling, and preventing MRSA infections, the identification and testing of antibiotic susceptibility takes the lead.[8]

Therefore, the purpose of this study was to compare various phenotypic and genotypic approaches to diagnose MRSA infection and to determine the prevalence of MRSA strains in different specimens. In addition, it intends to use disc diffusion methods to ascertain the antibiotic susceptibility pattern of MRSA isolates from tertiary care hospitals, enabling patients to receive the best effective treatment.

2. Materials and Methods

2.1. Study design

A one year (September 2022 to October 2023) of cross-sectional study was conducted at ACS Medical College and Hospital in Chennai, India.

2.2. Study population

A total of 100 non-duplicate MRSA isolates from clinical samples were taken into the study.

2.3. Exclusion criteria

Isolates from nasal swabs and stool samples were excluded.

2.4. Ethical clearance

Approval was obtained from the Institutional ethics committee before the commencement of the study.

2.6. Statistical analysis

Using Statistical Package for Social Sciences (SPSS) and Epi – info softwares.

2.7. Proforma

The proforma was filled out with the following details: name, age, sex, ward, clinical diagnosis, risk factors, surgical history, length of hospital stay, and other pertinent values for this study.

2.8. Bacterial isolation by phenotypic method

The Microbiology Department's Laboratory received several clinical specimens like sputum, pus, blood, wound, and tissue for further analysis. Their data were entered and culture onto MacConkey and Blood agar plate and incubated at 37Celsius for 18-24 hrs. Colony morphology and Gram stain both pointed to growing pathogens. Suspected Staphylococci were proceeded to slide and tube coagulase, biochemical reactions and mannitol fermentation. The organism was further cultured on CHROMagar plate which allowed them to grow into colonies with a greenish yellow colour after incubating at 37 C for 18-24 hours. Hicroma Rapid MRSA Agar Plate was purchased from The HiMedia Laboratories in Thane, India and it’s constituents were Phenol Red indicator, a proprietary chromogenic mix (6.5 g/liter), agar (15 g/liter), peptones (40 g/liter) and sodium chloride (8 g/liter).

2.9. Antibiotic susceptibility testing

The antibiotic susceptibility testing of all Staphylococcal isolates was assessed using the Kirby-Bauer disk diffusion technique. Mueller Hinton agar was used with the following drug discs (HiMedia Laboratories, Mumbai, India): Penicillin (P) 10U, Cefoxitin (CX) 30µg, Vancomycin (VA) 30µg, Gentamicin (GEN) 10µg, Erythromycin (E) 15µg, Tetracycline (TE) 30µg, Ciprofloxacin (CIP) 5µg, Clindamycin (CD) 2µg, Linezolid (LZ) 30µg, Trimethoprim-sulfamethaxole (COT) 25µg, Ciprofloxain (CIP) 5µg. The data were analyzed according to the parameters set out by the Clinical and Laboratory Standard Institute (CLSI M100-Ed34)[9] where CX inhibition zones of <21 mm and susceptible zone of >22mm for *S. aureus*. A control strain of ATCC *S.aureus* 25923 was used along with the test organism.

To perform the D-test, discs containing 15µg of Erythromycin and 2µg of Clindamycin were placed on an MHA plate at a distance of 15 mm from edge to edge. After overnight incubation, a "D" shape (flattening of the zone towards the Clindamycin disc) suggested inducible Clindamycin resistance.

2.10. Molecular study

DNA was extracted from all MRSA isolates using the HELINI Purefast Bacterial DNA micro spin prep kit by Helini Biomolecules in Chennai, India. The existence of mecA gene was detected by HELINI Custom antibiotic gene assay Real-time polymerase chain reaction (PCR) kit. Each PCR tube contained a 10-µl master mix, 5 primer probe mix and 10-µl purified DNA. PCR conditions were initial annealing at 95°C for 15 min, denaturation at 95°C for 20 sec, annealing at 65°C for 20 sec, extension at 72°C for 20 s for 35 cycles.

3. Results

The study was conducted at ACS Medical College and Hospital at the Department of Microbiology for a period of 12 months. A total of 100 MRSA isolates from a various clinical samples were involved in this study.

Among the 100 isolates, 55% were found to be males and 45% were found to be females (**Table 1**). Majority of them were in the 41-60 age group (39%) followed by 21- 40 years (28%) and more than 60 years (18%) (**Table 2**). In the inpatient group, 94% of the MRSA isolates were found (**Table 3,4**).

Out of 100 MRSA isolates 98 (98%) produced greenish yellow colonies (positive) by CHROMagar and only 2 (2%) of the isolates did not produce greenish yellow colonies which is negative (**Table 5**). MRSA was found to be 100% positive by Cefoxitin Disc Diffusion method and 98% positive by CHROMagar method (**Table 6**). mecA gene was detected in all 50 (100%) isolates (**Table 8**).

Among 100 MRSA isolates, highest resistance was observed in Azithromycin (79%), Ciprofloxacin (78%) and Erythromycin (64%). All 100 isolates were susceptible for Vancomycin and Linezolid. Highest Susceptibility was observed in Tetracycline (89%) followed by Co-trimoxazole (88%) and Gentamycin (78%). Clindamycin resistance among MRSA isolates were 33 (33%) (**Table 7**). Among 33 Clindamycin resistant MRSA isolates, 20 (60.6%) isolates showed inducible clindamycin resistance and 13 (39.4%) showed constitutive resistance.

Table 1: Gender wise distribution of MRSA isolated in patients (n=100)

Gender	Number of isolates(n=100)	Percentage (%)
Male	55	55.0
Female	45	45.0
Total	100	100.0

Table 2: Age wise distribution of MRSA isolated in patients: (n=100)

Age category	Number of isolates (n=100)	Percentage (%)
< 20 years	15	15.0
41-60	39	39.0
21-40	28	28.0
> 60 years	18	18.0
Total	100	100.0

Table 3: Categorization of MRSA isolates among inpatient and outpatient basis (n=100)

Category of the samples	Number of isolates(n=100)	Percentage (%)
IP	94	94.0
OP	6	6.0
Total	100	100.0

Table 4: Identification of MRSA isolates by CHROMagar method (n=100)

Greenish Yellow Colonies	Number of isolates(n=100)	Percentage (%)
Positive	98	98
Negative	2	2
Total	100	100

Table 5: Comparison of cefoxitin disc diffusion and CHROMagar method in detection of MRSA isolates (n=100)

Total Number of isolates (n=100)	Methods	Positive isolates	
		n	%
100	Cefoxitin DD	100	100
100	CHROMAgar	98	98

Table 6: Antimicrobial Susceptibility pattern of MRSA isolates (n=100)

Drugs	MRSA					
	Susceptibility		Intermediate		Resistance	
	n	%	n	%	n	%
Erythromycin (E)	34	34	2	2	64	64
Clindamycin (CD)	67	67	0	0	33	33
Cefoxitin (CX)	0	0	0	0	100	100
Tetracycline (TE)	89	89	0	0	11	11
Vancomycin (VA)	100	100	0	0	0	0
Penicillin (P)	0	0	0	0	100	100
Linezolid (LZ)	100	100	0	0	0	0
Ciprofloxacin (CIP)	22	22	0	0	78	78
Gentamycin (GEN)	78	78	1	1	21	21
Co-trimoxazole (COT)	88	88	0	0	12	12

Azithromycin (AZM)	21	21	0	0	79	79

Table 7: Results of mec A gene detection by PCR (n=50)

Number of Isolates (n=50)	Percentage (%)
50	100

Table 8: Clindamycin resistance in MRSA isolates (n=33)

Total (n=33)	Inducible resistance		Constitutive resistance	
	n	%	n	%
33	20	60.6	13	39.4

4. Discussion

One of the leading cause of nosocomial infection is due to Methicillin Resistant Staphylococcus aureus (MRSA) pathogen which causes a great deal of illness and death. The specificity and sensitivity of various methods used to identify MRSA differ. For clinical laboratories operating with limited resources, improved detection technologies with cost effectiveness are essential.

Males accounted for 55% of the MRSA isolates in this study, with females accounting for the remaining 45%. Males accounted for 63.2% of MRSA isolates and females for 35.4%, according to a Malaysia research by Sit et al.[10] Alternatively, a research carried out at hospitals in Doon Valley by Sachin et al. revealed that men made up just 39.13% of the MRSA isolates, while females constituted 60.86%.[11] In their analysis of MRSA isolates by Buzaid et al, discovered no statistically significant difference between males (31.8%) and females (30.4%).[12]

In this study, a significant portion of the MRSA isolates (39%) falls within the age category of 41-60 years and a smaller percentage (28%), however, belonged to the age group of 21-40 years. Similarly, a study conducted by Terry Ali et al. found that 60% of MRSA isolates belonged to the 51-60 years old age category, which is greater than the percentage we found.[13] In contrast, a study by N. Giri et al conducted among healthcare workers in tertiary care hospitals in Nepal showed that 47.8% of the samples were from the 25-35 years of age group followed by 34.1% from under 25 years of age group.[14]

In this study, we have majority of the MRSA isolates belong to inpatient with 94% followed by outpatient with 6%. Similarly study done by Soe P E et al in Myanmar and Mathanraj et al in JIPMER isolated more number of MRSA from inpatients with 97%.[10]

A more recent development and rapid identification of MRSA isolates is chromogenic media. Although the results may be simply understood by looking at the colored colonies, this approach does need a significant period of incubation in the event where no growth takes place. In this study, a phenotypic approach called CHROMagar was used to identify 98% of the MRSA isolates after 24 hours of incubation. The remaining 2% did not show any results even after 48 hours. Almost Similar findings has been noted in the study done by Taguchi H et al and Van Hoecke F et al with 100% identification.[15] Contrast this with the results of the study by Hernandez DR et al., in which CHROMagar incorrectly labeled 15% of MSSA isolates as MRSA.[16]

In the current study, due to financial constraints, PCR could only be done on 50 MRSA isolates. In order to determine if any of the fifty isolates had the mecA gene, a multiplex PCR was employed. The study confirmed a 100% detection rate by finding the mecA gene in all isolates. In contrast, a 2020 research by G Vieira et al. found that 69.2% of strains were identified as MRSA when the mecA gene was used in multiplex PCR.[17]

5. Conclusion

This study implements the prevalence of MRSA isolates in various clinical samples. It currently accounts for at least 30% of all severe infections. Since MRSA may colonize in humans for several months to even years and has a higher risk of cross infection, it is important to take precautions. *Staphylococcus aureus*, which are resistant to Methicillin, is causing an upsurge in illness and death all across the world. There is much more to the story than what the current study reveals. To combat the growing threat of antibiotic resistance, more research is required in the upcoming years. Due to the ongoing growth of multi-drug resistance and the need for new, powerful, expensive anti-microbials, further research on improved treatment plans and affordable alternative antibiotics is urgently needed. In order to eradicate Methicillin Resistant Staphylococcus aureus (MRSA) from healthcare facilities and the general public, it is necessary to raise public awareness through educational campaigns promoting good hygiene practices and to implement adequate barrier measures to stop the spread of the infection.

6. Limitations

Nasal swab screening of Health care workers was not included in the study. Because of financial constraints, we were unable to do other phenotypic methods such as latex agglutination test or use the broth microdilution technique to determine the oxacillin Minimum Inhibitory Concentration (MIC). Genotypic identification was also restricted to only mecA gene due to cost constraints.

7. Ethical Committee Approval

Ethics approval was obtained from ACS Medical College and Hospital Ethics committee. Project number (No.583/2022/IEC/ACSMCH)

8. Conflict of Interest

The authors declare no competing interests.

9. Source of Funding

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