

In vitro antibiogram pattern of *Staphylococcus aureus* isolated from wound infection and molecular analysis of *mecA* gene and restriction sites in methicillin resistant *Staphylococcus aureus*

V. Hemamalini, V. Kavitha¹,
Sridhar Ramachandran²

Department of Plant Biology and
Plant Biotechnology, Quaid-e-Millath
Government Arts College (W),
¹Department of Microbiology, Valliammal
College for Women, ²Department of
Biology, A.B.S.V.M., Thiruvallur,
Tamil Nadu, India

J. Adv. Pharm. Technol. Res.

ABSTRACT

Staphylococcus aureus is a common nosocomial pathogen with property to develop resistance to antimicrobial agents. But in the modern era, drug resistance had been developed by microbes due to its continuous usage of antibiotics. This study was carried out to evaluate antibiotic resistant pattern of methicillin resistant *Staphylococcus aureus* (MRSA) using molecular genotyping. In view of the present problem, the study has been conducted to detect the molecular genotyping of *mecA* gene from MRSA and confirmation of its restriction sites using *EcoRI* and *BamHI*. The pus samples were swabbed out, and clinical strains were isolated using standard microbiological procedures. Then the strains were subjected to *in vitro* antibiotic susceptibility assay and identified MRSA. Further molecular genotyping of *mecA* gene was determined by polymerase chain reaction technique. The percentage analysis was done. The clinical strains were isolated from the wound infected patients. A total of 60 samples were collected, of 60 samples, 40 (66.7%) were showed positive to strains of *S. aureus*. The *in vitro* antibiotic susceptibility assay was carried to find the drug sensitive and resistant patterns. Further methicillin resistant strains (35%) of *S. aureus* were screened and subjected to molecular genotyping of *mecA* gene and was confirmed by restriction digestion. Overall, 70% of plasmids show positive for the presence of *mecA* gene, although all strains have restriction sites. Hence, the present study revealed that the early detection of antibiotic resistant character using molecular genotyping will help the infected patient to cure short period and will reduce the development of multidrug resistance.

Key words: Antibiogram, *in vitro*, *mecA* gene, methicillin resistant *Staphylococcus aureus*, *Staphylococcus aureus*

Address for correspondence:

Dr. V. Hemamalini,
Department of Plant Biology and Plant Biotechnology,
Quaid-e-Millath Government Arts College (W),
Chennai - 600 002, Tamil Nadu, India.
E-mail: hemaraju97@yahoo.com

Access this article online

Quick Response Code:



Website:

www.japtr.org

DOI:

10.4103/2231-4040.165019

INTRODUCTION

Staphylococcus aureus caused a wide range of major and minor infection in man and animals and is characterized by its ability to clot blood plasma by the action of the

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Hemamalini V, Kavitha V, Ramachandran S. *In vitro* antibiogram pattern of *Staphylococcus aureus* isolated from wound infection and molecular analysis of *mecA* gene and restriction sites in methicillin resistant *Staphylococcus aureus*. *J Adv Pharm Technol Res* 2015;6:170-5.

enzyme coagulase. The emerging drug resistant strain of *S. aureus* associated with risk factors such as drug resistant in Intensive Care Unit, changes in blood cells etc., in children.^[1]

Methicillin resistant *Staphylococcus aureus* (MRSA) is present account for 3–5% of all isolates of *S. aureus*. The recent research carried out by Abbas *et al.*^[2] was reported that hospital acquired-MRAS and community acquired-MRSA were more prevalent at a tertiary care hospital. The detailed molecular mechanism with respect to MRSA resistant genes and their regulation was propounded by Sangappa and Thiagarajan.^[3]

The present study is undertaken to investigate the antibiotic resistant pattern of MRSA and determination of the presence of *mecA* gene and amplification of *mecA* gene by polymerase chain reaction (PCR) and analysis of its restriction sites.

MATERIALS AND METHODS

Collection of specimens

The wound swabs were collected from patients admitted in Sundaram Medical Foundation, Chennai, Tamil Nadu. The samples were collected aseptically with ice box and transported to the laboratory immediately, stored in refrigerator, and the analysis was carried out within 2 h of times.

Wound swabs

A sterile dry cotton swab was pressed and rolled over the infected tissue, and the swab was inserted in the alkaline peptone water. The swab was pushed down one-third into the alkaline peptone water, and the cap was screwed.

Isolation and biochemical screening of *Staphylococcus aureus*

The specimen was inoculated on nutrient agar and incubated at 37°C overnight. The biochemical tests such as catalase, oxidase, coagulase, and sugar fermentation were done for further confirmation.^[4]

In vitro antibiotic susceptibility test by disc diffusion assay

The pure clinical isolates of *S. aureus* were Gram-stained before the test. The *S. aureus* cultures were inoculated in peptone water and incubated at 37°C to get turbidity equal. A sterile cotton swab was dipped into the inoculation, and the excess was removed by pressing the swab to the sides of the tube. The entire agar surface was swabbed. The inoculation was allowed to dry for 15 min. Methicillin and other antibiotic discs such as ampicillin, chloramphenicol, gentamycin, tetracycline, and erythromycin were applied on the medium. The plates were incubated at 37°C and examined after 18–24 h.

Plasmid isolation by alkaline lysis method

The pure culture of *S. aureus* was inoculated into LB broth with ampicillin and incubated overnight at 37°C. To this, 10 ml of well-growth culture was transferred into a centrifuge tube. The cells were pelleted at 10,000 rpm for 5 min in a refrigerated centrifuge, and the supernatant was discarded. The pellet was resuspended in 150 µl of solution A and was incubated in ice for 30–40 min. Added 250 µl of solution B into the centrifuge tube and vortex it and then incubated on ice for 10 min. Similarly, 150 µl of solution C was added and incubated in ice for 60 min. Centrifugation was done at 6000 rpm for 10 min. The supernatant was transferred into a fresh microfuge tube. Added equal amount of ice cold 95% ethanol and kept it in –20°C for overnight. The solution D was added after 24 h and kept for 10 min. Then centrifugation was done at 6000 rpm for 10 min. The supernatant was removed, and the pellet was dried. The dried plasmid DNA was dissolved in 50 µl of Tris buffer. Loading dye was mixed with 20 µl of sample and run in agarose gel electrophoresis.

Agarose gel electrophoresis

Tris-acetate-ethylenediaminetetraacetic acid (TAE) is most commonly used buffer, but it gets easily exhausted during extended or high voltage electrophoresis. Agarose gel electrophoresis is carried out in a horizontal submarine electrophoresis unit. Prepare 1 L of tank buffer using ×100 TAE buffer (take 10 ml of × 1 TAE buffer and then add 990 ml of double distilled water). For the preparation of gel electrophoresis, dissolve by heating 1.0 g of agarose in 100 ml of × 1 TAE buffer, before pouring into the gel cast add ethidium bromide. Load the gel with 15 µl of sample and 5 µl of gel loading dye. Electrophoresis is carried for 1 h depending upon the length of the gel, until the gel loading dye migrates more than half the length of gel. At the end of the electrophoresis, visualize the plasmid DNA under UV transilluminator (Biorad, USA).

RESULTS

The pus samples were collected from 60 wound patients for the isolation of MRSA. Of the 60 pus samples, 40 (66.7%) samples showed positive result for *S. aureus*. Of 40 isolated strains, 14 are MRSA.

The organisms were identified from the morphological, cultural, and biochemical testing. The *S. aureus* was isolated in nutrient agar and blood agar. In nutrient agar, the colony shows circular, convex, shiny, opaque, smooth colonies with slightly golden yellow pigment. In blood agar, beta-hemolytic white colonies were observed. *S. aureus* was confirmed using selective media like Mannitol Salt Agar. *S. aureus* strains are detected by the sugar fermentation test. Further, *S. aureus* was confirmed

from catalase, oxidase, coagulase, and various biochemical characterization test like indole production test, methyl red test, Voges–Proskauer test, citrate utilization test, urease production test, and Nitrate reduction test. The results are shown in Table 1.

The *in vitro* antibiotic sensitivity patterns of the *S. aureus* toward the antibiotics like ampicillin, penicillin, chloramphenicol, tetracycline, erythromycin, and methicillin. The organisms showed resistant toward methicillin are called as MRSA [Figure 1]. The sensitivity was determined from comparing the results with zone size interpretative chart provided by Hi-Media Laboratories Ltd. The results are shown in Table 2.

The plasmid profile was done by alkaline lysis method [Figure 2] and was carried out to study the level of sensitivity pattern between the antibiotic resistant strains of MRSA and determining the presence of *mecA* gene by PCR [Figure 3] and analyzing of its restriction sites using *EcoRI* and *BamHI* [Figures 4 and 5].

Table 1: The biochemical test for the identification of isolated *Staphylococcus aureus*

Test	Result
Preliminary test	
Gram's staining	+
Motility	+
Catalase	+
Oxidase	+
Coagulase	+
Biochemical test	
Indole	–
Mr	+
Vp	+
Citrate	–
Urease	+
Nitrate	+
Sugar fermentation	
Sucrose	+
Mannitol	+
Maltose	+

(+): Positive, (-): Negative, Mr: Methyl red, Vp: Voges-Proskauer

DISCUSSION

In the recent years, there has been more report on the prevalence and drug resistant of *S. aureus*. As these bacteria are increasingly gaining resistance to commonly used antibacterial agents, there is immediate need for finding authentic detection of drug resistant strains for controlling risk factors due to infection. Therefore, the present study has made an attempt in exploring molecular detection of *mecA* gene from MRSA from wound infected patients and confirmation of its restriction sites using *EcoRI* and *BamHI*.

Qureshi *et al.*^[5] had screened the clinical isolates of *S. aureus* using a set of primers specific for *mecA* gene. The researchers reported that 76.44% were identified as MRSA and 19.6% as MSSA. The *mecA* gene was found positive in 89 (MRSA and MSSA) isolates (87.22%), 20 of 98 isolates that were categorized as MSSA by disc diffusion method, 11 showed amplification of *mecA* gene by PCR. Sasirekha *et al.*^[6] had evaluated different conventional phenotypic methods in detecting MRSA and determined the prevalence of biofilm producer among MRSA. A total of 153 clinical isolates of *S. aureus* were subjected to antibiotic susceptibility test by disc diffusion. Of 153 isolates, 42 (57.7%) were found to be methicillin resistant, and drug resistance patterns of MRSA



Figure 1: *Staphylococcus aureus* showing methicillin resistant

Table 2: *In vitro* antibiotic sensitivity pattern of *Staphylococcus aureus*

Antibiotics	Diameter of zone (mm)													
	I ₁	I ₂	I ₃	I ₄	I ₅	I ₆	I ₇	I ₈	I ₉	I ₁₀	I ₁₁	I ₁₂	I ₁₃	I ₁₄
Methicillin	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Erythromycin	R	R	I	R	R	R	R	R	I	I	I	I	I	I
Gentamycin	S	I	I	R	R	R	R	R	S	I	R	I	R	R
Chloramphenicol	R	R	R	R	I	R	R	R	I	I	I	I	I	I
Ampicillin	R	R	R	R	R	R	R	I	I	I	I	I	I	I
Tetracyclin	R	R	R	R	R	R	R	R	I	I	I	I	I	I

R: Resistance, S: Sensitive, I: Intermediate, I₁ to I₁₄ -Strains

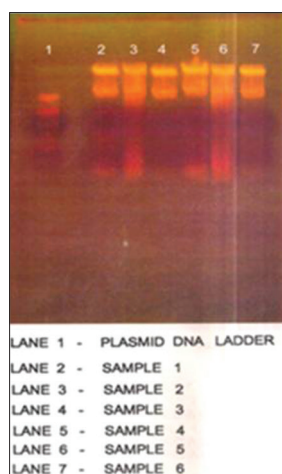


Figure 2: Extraction of plasmid DNA from methicillin resistant *Staphylococcus aureus*

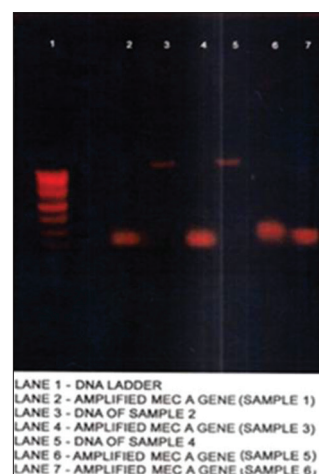


Figure 3: Polymerase chain reaction amplification of *mecA* gene

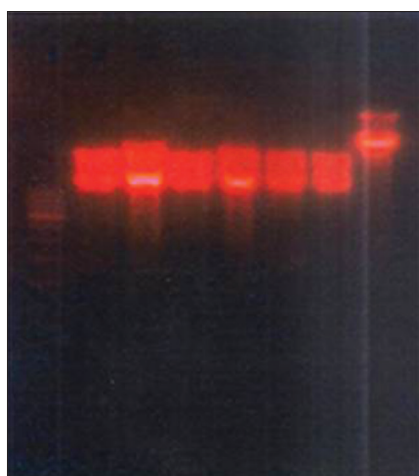


Figure 4: Restriction analysis of plasmid DNA by *EcoRI*

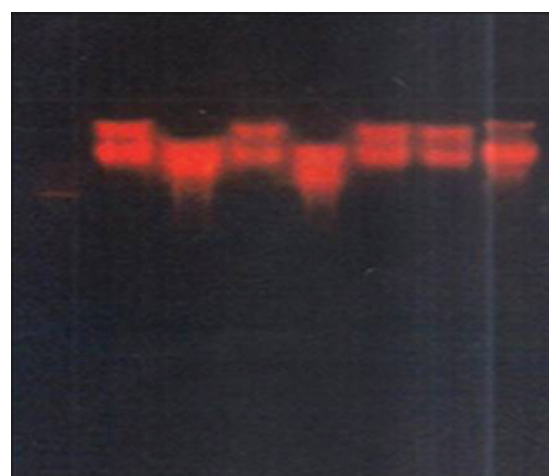


Figure 5: Restriction analysis of plasmid DNA by *BamHI*

isolates were performed. The another study conducted by Kaur *et al.*^[7] had estimated the frequency of Pantone-Valentine leukocidin (PVL) positive *S. aureus* in Belgaum, South India. The clinical isolates were identified, phenotypically characterized as methicillin sensitive and methicillin resistant strains of *S. aureus* by disc diffusion method by employing oxacillin disc (1 µg) and genetically by multiplex PCR for *mecA* and *femB* genes. The researchers were confirmed 38.6% as MRSA by PCR for *mecA* and also showed high percentage of PVL positive MRSA and MSSA, respectively.

Hussain *et al.*^[8] had screened the antimicrobial susceptibility pattern of *S. aureus*. They were isolated 80 strains, in which 53 (66.25%) strains were found to be methicillin resistant while the rest of the 27 (33.75%) strains were methicillin-susceptible. In contrast to the present study, we had isolated 66.7% of *S. aureus*, of which 35% of clinical strains were showed resistance to methicillin.

The plasmid DNA was extracted by alkaline lysis method and bands observed on agarose gel electrophoresis revealed

that there is no remarkable change in the molecular level between MRSA strains. The isolated plasmid DNA was used as template in the PCR study. The protocol developed by Merlino *et al.*^[9] was followed in the present study for amplification of plasmid DNA. Amplification was performed using specific primers *mecA* gene forward primer (5'-AAAATCGATGGTAAAGTTGGC-3') and *mecA* reverse primer (5'-AGTTCTGCAGTACCGGATTGTC-3').

Irayif *et al.*^[10] had identified MRSA by a rapid PCR technique. The researchers were collected blood sample from the wound infected patients. The bacterial genomic DNA was isolated and amplified using the forward primer, 5'CTGCTATCCACCCTCAAACAG3' and the reverse primer, 5'CACCTTGTCGTAACCTGAATC3', respectively. Further, the plasmid isolation was done by alkaline lysis method, and confirmation of the clones was subjected to restriction digestion using *BamHI* and *EcoRI*. Another study conducted by Khan *et al.*^[11] had amplified *mecA* and *nuc* genes in multidrug resistant *S. aureus* strains from hospital personnel. The *mecA* gene was amplified using primer 5'AGTTGTAGTTGTCGGGTTT-3' and

5'AGTGGAAACGAAGGTATCATC-3'. The result revealed that of 17 clinical isolates, 7 (41.18%) strains were showed the present of *mecA* gene.

For molecular genotyping, the gene of interest is *mecA* gene that is responsible for the resistance to the drug called methicillin and codes for penicillin-binding protein 2A.^[12] But in the recent year, apart from studying *mecA* gene, the researchers^[13,14] were found a new mechanism of MRSA for screening *mecC* gene for genotypic confirmation of MRSA.

The PCR was performed in thermal cycler (Eppendorf Company, USA) using PCR conditions. The final PCR products were resolved in 1% agarose using TE buffer.

The results clearly indicated that 4 strains among 6 carried *mecA* gene. The resistance for the remaining 2 strains may have been encoded by different gene, such as *femA* gene.^[15]

The difference in strains may also be analyzed by screening for restriction sites using restriction enzymes. Hence, screening for restriction sites were carried out by employing *EcoRI* and *BamHI* restriction enzymes. The results in our study showed that the presence of restriction sites for both the enzymes in all the positive strains.

CONCLUSIONS

S. aureus is a common nosocomial pathogen with property to develop a resistant to antimicrobial agents. In the present study, isolation of clinical strains from wound sample, microbiological characterization of *S. aureus*, Plasmid isolation, *mecA* gene amplification by PCR and restriction digestion pattern of the MRSA were carried out. Among 60 samples from the infected patients, 40 (66.7%) samples were showed positive for *S. aureus*. From this, 14 (35%) were resistant to the antibiotic-methicillin, so called MRSA. All the MRSA isolates have the same molecular weight plasmid. The plasmid DNA on restriction digestion with *EcoRI* and *BamHI* showed banding pattern that confirms the presence of restriction sites. Dialyzed plasmid DNA was amplified for the confirmation of the presence of *mecA* gene. Seventy percentage of plasmids show positive for the presence of *mecA* gene.

Several research works^[16-23] had been conducted in India with reference to the molecular detection of *mecA* gene from MRSA. But no studies have been reported in India by detecting *mecA* gene using *mecA* gene forward primer (5'-AAAATCGATGGTAAAGGTTGGC-3') and *mecA* gene reverse primer (5'-AGTTCTGCAGTACCGGATTTGC-3'), which was prescribed and developed by Merlino *et al.*^[9] To the best of our knowledge, this constitutes the first report in India on the molecular detection of *mecA* gene from *S. aureus*. In addition, 70% of MRSA plasmids confirm the presence of *mecA* gene although all MRSA strains have

restriction sites. Henceforth, early detection of MRSA could reduce morbidity and mortality and also reduce the development of multidrug antibiotic resistance.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Kapil A. Risk factors associated with MRSA infection in children. *Indian Pediatr* 2015;52:22-4.
- Abbas A, Nirwan PS, Srivastava P. Prevalence and antibiogram of hospital acquired-methicillin resistant *Staphylococcus aureus* and community acquired-methicillin resistant *Staphylococcus aureus* at a tertiary care hospital National Institute of Medical Sciences. *Community Acquir Infect* 2015;2:13-5.
- Sangappa M, Thiagarajan P. Methicillin resistant *Staphylococcus aureus*: Resistance genes and their regulation. *Int J Pharm Pharm Sci* 2012;4:658-67.
- Versalovic J, Carroll KC, Funke G, Jørgensen JH, Landry ML. *Manual of Clinical Microbiology*. 10th ed. Washington: ASM Press; 2007.
- Qureshi A, Ingle R, Musaddiq M, Ali Y, Khan Z. Status and distribution of *mecA* gene in hospitalized patient's MRSA isolates. *Biosci Discov* 2012;3:52-7.
- Sasirekha B, Usha MS, Amrutha AJ, Ankit S, Brinda N, Divya R. Evaluation and comparison of different phenotypic tests to detect methicillin resistant *Staphylococcus aureus* and their biofilm production. *Int J PharmTech Res* 2012;4:532-41.
- Kaur H, Purwar S, Saini A, Kaur H, Karadesai SG, Kholkute SD, *et al.* Status of Methicillin resistant *Staphylococcus aureus* infections and evaluation of PVL producing strains in Belgaum, South India. *J Krishna Inst Med Sci Univ* 2012;1:43-51.
- Hussain JH, Thakur A, Mishra B, Dogra V, Jaggi T. Antimicrobial susceptibility pattern of methicillin-resistant strains of *Staphylococcus aureus* in a super specialty hospital. *Int J Health Allied Sci* 2015;4:69-72.
- Merlino J, Watson J, Rose B, Beard-Pegler M, Gottlieb T, Bradbury R, *et al.* Detection and expression of methicillin/oxacillin resistance in multidrug-resistant and non-multidrug-resistant *Staphylococcus aureus* in Central Sydney, Australia. *J Antimicrob Chemother* 2002;49:793-801.
- Irayyif SM, Senthil Kumar R, Malla S. Identification of methicillin resistant *Staphylococcus aureus* by a rapid polymerase chain reaction technique. *Asian J Pharm Clin Res* 2014;7:16-9.
- Khan AU, Sultan A, Tyagi A, Zahoor S, Akram M, Kaur S. Amplification of *mecA* gene in multi-drug resistant *Staphylococcus aureus* strains from hospital personnel. *J Infect Dev Ctries* 2007;1:289-95.
- Wielders CL, Fluit AC, Brisse S, Verhoef J, Schmitz FJ. *mecA* gene is widely disseminated in *Staphylococcus aureus* population. *J Clin Microbiol* 2002;40:3970-5.
- Walther B, Wieler LH, Vincze S, Antão EM, Brandenburg A, Stamm I, *et al.* MRSA variant in companion animals. *Emerg Infect Dis* 2012;18:2017-20.
- Paterson GK, Morgan FJ, Harrison EM, Peacock SJ, Parkhill J, Zadoks RN, *et al.* Prevalence and properties of *mecC* methicillin-resistant *Staphylococcus aureus* (MRSA) in bovine bulk tank milk in Great Britain. *J Antimicrob Chemother* 2014;69:598-602.

15. Sakoulas G, Gold HS, Venkataraman L, DeGirolami PC, Eliopoulos GM, Qian Q. Methicillin-resistant *Staphylococcus aureus*: Comparison of susceptibility testing methods and analysis of *mecA*-positive susceptible strains. *J Clin Microbiol* 2001;39:3946-51.
16. Nadig S, Namburi P, Raghunath D, Arakere G. Genotyping of methicillin-resistant *Staphylococcus aureus* Isolates from Indian hospitals. *Curr Sci* 2006;91:1364-9.
17. Khan AU, Sultan A, Tyagi A, Zahoor S, Akram M, Kaur S, *et al.* Amplification of *mecA* gene in multi-drug resistant *Staphylococcus aureus* strains from hospital personnel. *J Infect Dev Ctries* 2007;1:289-95.
18. Mohanasoundaram KM, Lalitha MK. Comparison of phenotypic versus genotypic methods in the detection of methicillin resistance in *Staphylococcus aureus*. *Indian J Med Res* 2008;127:78-84.
19. Bennimath VD, Gavimath CC, Kalburgi PB, Kelmani C. Amplification and sequencing of *mecA* gene from methicillin resistance *Staphylococcus aureus*. *Int J Adv Biotechnol Res* 2011;2:310-4.
20. Sasikala S, Ramganes S, Sundararaj T. Drug resistance of *Staphylococcus aureus* in sinusitis patients. *Int J Biosci* 2011;1:63-71.
21. Pramodhini S, Thenmozhivalli PR, Selvi R, Dillirani V, Vasumathi A, Agatha D. Comparison of various phenotypic methods and *mecA* based PCR for the detection of MRSA. *J Clin Diagn Res* 2011;5:1359-62.
22. Sajith Khan AK, Shetty PJ, Lakshmi Sarayu Y, Chidambaram A, Anganathan R. Detection of *mecA* genes of methicillin-resistant *Staphylococcus aureus* by polymerase chain reaction. *Int J Health Rehabil Sci* 2012;1:64-8.
23. Pillai MM, Latha R, Sarkar G. Detection of methicillin resistance in *Staphylococcus aureus* by polymerase chain reaction and conventional methods: A comparative study. *J Lab Physicians* 2012;4:83-8.