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MOLECULAR DETECTION OF THE *mecA* GENE AND ANTIMICROBIAL RESISTANCE PATTERN OF METHICILLIN-RESISTANT *Staphylococcus aureus* ISOLATED FROM RESPIRATORY SAMPLES

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of respiratory tract infections and poses a serious challenge due to its multidrug-resistant nature. The present study was undertaken to detect the presence of the *mecA* gene and to evaluate the antimicrobial resistance pattern of MRSA isolated from respiratory clinical samples. A total of respiratory specimens were processed for the isolation of *Staphylococcus aureus* using standard microbiological techniques. Phenotypic identification was carried out based on colony morphology, Gram staining, catalase test, and coagulase test. Methicillin resistance was initially screened by the cefoxitin disc diffusion method. Molecular confirmation of MRSA was performed by polymerase chain reaction (PCR) targeting the *mecA* gene.

The study findings demonstrated that a significant proportion of phenotypically identified MRSA isolates were confirmed to carry the *mecA* gene, establishing the genetic basis of methicillin resistance. Antimicrobial susceptibility testing revealed a high level of resistance to commonly used antibiotics, indicating a multidrug-resistant pattern among MRSA isolates. However, most isolates remained susceptible to glycopeptides and oxazolidinones, highlighting their effectiveness as treatment options.

The study emphasises the importance of molecular techniques alongside conventional phenotypic methods for accurate detection of MRSA. Continuous monitoring of antimicrobial resistance patterns and the implementation of appropriate infection control strategies are essential to prevent the spread of MRSA in healthcare settings and to ensure effective management of respiratory tract infections.

***Keywords*:** Methicillin-Resistant *Staphylococcus aureus* (MRSA); *mecA* gene; Respiratory tract infections.

Introduction

Respiratory tract infections represent a major clinical concern and are frequently associated with a wide range of bacterial pathogens. Among these, *Staphylococcus aureus* is an important organism responsible for both community-acquired and hospital-acquired respiratory infections. The organism commonly colonises the upper respiratory tract and can cause severe lower respiratory tract infections, particularly in hospitalized and immunocompromised patients (Cheesbrough, 2005).

The increasing occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a significant public health problem due to its resistance to β-lactam antibiotics and its association with multidrug resistance. Methicillin resistance in *S. aureus* is primarily mediated by the *mecA* gene, which encodes an altered penicillin-binding protein (PBP2a) that exhibits reduced affinity for β-lactam antibiotics, thereby rendering standard antimicrobial therapy ineffective (Murakami *et al*., 1991). The presence of MRSA in respiratory infections often leads to prolonged hospitalization, increased treatment costs, and limited therapeutic options. Although daptomycin

is the recommended treatment for bacteraemia, resistance develops through changes in the membrane. According to a 2014 study, 2% of MRSA isolates had daptomycin resistance, which was linked to mprF mutations and made treatment more difficult. Fifth-generation cephalosporins like cefazoline bind PBP2a, but mecA mutations are making them more resistant.

Routine laboratory detection of MRSA commonly relies on phenotypic methods such as growth on selective media, coagulase testing, and cefoxitin disc diffusion testing. Cefoxitin resistance has been widely accepted as a reliable phenotypic marker for the detection of methicillin resistance in *S. aureus* isolates (Smyth *et al*., 2005). However, phenotypic methods alone may sometimes yield ambiguous results, highlighting the need for molecular confirmation.

Polymerase chain reaction (PCR)–based detection of the *mecA* gene is considered the most definitive method for confirming methicillin resistance in *S. aureus*. Molecular techniques offer high sensitivity and specificity and are particularly useful in confirming MRSA isolates identified by phenotypic screening methods (Bhattacharya *et al*., 2008; Murakami *et al*., 1991). Therefore, molecular confirmation plays a crucial role in the accurate diagnosis and epidemiological surveillance of MRSA.

In addition to methicillin resistance, MRSA isolates frequently exhibit resistance to multiple classes of antibiotics, posing serious challenges for clinical management. Variations in antimicrobial susceptibility patterns among MRSA isolates recovered from different respiratory specimens have been reported, emphasising the importance of continuous monitoring of resistance trends (Peng *et al*., 2020). Knowledge of antimicrobial resistance patterns is essential for guiding appropriate empirical therapy and implementing effective infection control strategies.

Based on these considerations, the present study was undertaken to molecularly detect the presence of the *mecA* gene among phenotypically confirmed MRSA isolates obtained from respiratory clinical samples and to evaluate their antimicrobial resistance patterns. The findings of this study aim to provide valuable insights into the molecular confirmation and resistance characteristics of MRSA associated with respiratory tract infections.

Materials and Methods

Selection of Phenotypically Confirmed MRSA Isolates

The present study included phenotypically confirmed methicillin-resistant *Staphylococcus aureus* (MRSA) isolates obtained from respiratory clinical samples. Initial identification of *S. aureus* was carried out using standard microbiological techniques including culture characteristics, Gram staining, catalase test, and coagulase test. Phenotypic detection of methicillin resistance was performed using the cefoxitin disc diffusion method, and isolates showing resistance to cefoxitin were considered presumptive MRSA, as described previously (Cheesbrough, 2005; Smyth *et al*., 2005).

DNA Extraction

Genomic DNA was extracted from phenotypically confirmed methicillin-resistant *Staphylococcus aureus* (MRSA) isolates obtained from respiratory clinical samples using the boiling lysis method. Each confirmed isolate was inoculated into nutrient broth and incubated overnight at 37 °C to achieve optimal bacterial growth. Following incubation, the cultures were centrifuged to pellet the bacterial cells, and the supernatant was discarded. The obtained cell pellet was resuspended in sterile distilled water and thoroughly mixed to ensure uniform suspension. The suspension was then subjected to heat treatment by boiling at 95–100 °C for 10 minutes to facilitate cell lysis and release of genomic DNA.

Immediately after boiling, the tubes were cooled on ice to prevent DNA degradation and subsequently centrifuged at high speed to separate cellular debris. The clear supernatant containing genomic DNA was carefully collected without disturbing the pellet and transferred to sterile microcentrifuge tubes. The extracted DNA was used as a template for downstream molecular applications, including polymerase chain reaction (PCR) amplification. The quality and integrity of the genomic DNA were assessed by agarose gel electrophoresis. All DNA samples were stored at −20 °C until further molecular analysis. This rapid and cost-effective method yielded DNA of sufficient quality for PCR-based detection of resistance-associated genes in MRSA isolates (Murakami *et al.*, 1991).

Gene Amplification by Polymerase Chain Reaction (PCR)

Molecular confirmation of methicillin resistance was carried out by amplification of the mecA gene using polymerase chain reaction (PCR). Genomic DNA extracted from phenotypically confirmed MRSA isolates was used as template DNA. PCR amplification was performed in a reaction mixture containing template DNA, mecA-specific forward and reverse primers, dNTPs, $MgCl_2$, Taq DNA polymerase, and

PCR buffer prepared according to standard molecular protocols.

Amplification was performed in a thermal cycler under optimised cycling conditions. The PCR program included initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 45 seconds. Final extension was carried out at 72 °C for 7 minutes, followed by holding at 4 °C.

Appropriate positive controls (known mecA-positive S. aureus strain) and negative controls (PCR reaction mixture without template DNA) were included in each PCR run to ensure the specificity and reliability of amplification.

Table 1 : Primers Used for Amplification of the mecA Gene

Target Gene	Primer	Sequence (5′–3′)	Amplicon Size
mecA	Forward	AAA ATC GAT GGT AAA GGT TGG C	**533 bp**
	Reverse	AGT TCT GCA GTA CCG GAT TTG C	

Table 2 : PCR Cycling Conditions for mecA Gene Amplification.

Step	Temperature (°C)	Time	Number of Cycles
Initial denaturation	94 °C	5 minutes	1
Denaturation	94 °C	30 seconds	
Annealing	55 °C	30 seconds	35
Extension	72 °C	45 seconds	
Final extension	72 °C	7 minutes	1
Hold	4 °C	∞	—

Appropriate positive controls (known mecA-positive *S. aureus* strain) and negative controls (reaction mixture without template DNA) were included in each PCR run to ensure the specificity and reliability of amplification. The amplified PCR products were analysed by agarose gel electrophoresis, and the presence of a 533 bp amplicon was considered confirmatory for the presence of the mecA gene.

Agarose Gel Electrophoresis

PCR products of the *mecA* gene were analysed by agarose gel electrophoresis. A 1.5% agarose gel was prepared in Tris–acetate–EDTA (TAE) buffer, stained with ethidium bromide or an alternative nucleic acid dye, and allowed to solidify. PCR products mixed with loading dye were electrophoresed alongside a 100 bp DNA ladder at 80–100 V in TAE buffer. The gel was visualised under UV transillumination and documented using a gel documentation system. The presence of a distinct 533 bp band confirmed successful amplification of the *mecA* gene and molecular confirmation of MRSA isolates (Bhattacharya *et al*., 2008; Murakami *et al*., 1991).

Antibiotic Susceptibility Test

Antimicrobial susceptibility testing was conducted to evaluate the effectiveness of selected antibiotics against polymerase chain reaction (PCR)–confirmed methicillin-resistant *Staphylococcus aureus* (MRSA) isolates. A total of 25 MRSA isolates were analysed for their susceptibility to five commonly prescribed antibiotics, namely vancomycin, linezolid, ciprofloxacin, erythromycin, and tetracycline. Antibacterial activity was quantitatively assessed by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using the broth microdilution method, in accordance with the Clinical and Laboratory Standards Institute (Rai *et al.,* 2023) guidelines. The broth microdilution technique allowed precise measurement of antibiotic concentrations required to inhibit visible bacterial growth and to achieve bactericidal effects. MIC values were used to evaluate the inhibitory potential of each antibiotic, while MBC values provided insight into their bactericidal activity. This approach facilitated a comparative assessment of antimicrobial efficacy and contributed to a better understanding of the therapeutic potential of the tested antibiotics against MRSA infections.

Result and Discussion

Detection by Molecular Analysis

It is anticipated that approximately 25 isolate us of 100 will show methicillin-resistant phenotypically and will be further subject to molecular confirmation for the presence of the *mecA gene* in the tested isolates. The *mecA* gene, as the key mechanism of methicillin resistance in staphylococcal species, enabled the development of rapid molecular tests to distinguish between colonies of MRSA and colonies of methicillin-susceptible *S. aureus* (MSSA), first by DNA Extraction and then by PCR (Tenover *et al*., 2022).

Several molecular methods have been developed to detect the mecA gene in MRSA clinical isolates. However, genotypic tests involving mecA gene detection by PCR, which is considered to be the reference, are not practical for routine use in clinical laboratories. The main advantage of the PCR method over routine culture methods is shorter turnaround time, which allows rapid implementation of infection-control practices. (Baddour *et al.,* 2007). Among these, PCR-based detection of *mecA* is considered the gold-

standard method for confirming methicillin resistance due to its high sensitivity and specificity (Skov *et al*., 2003; Oliveira and de Lencastre, 2002). However, despite its diagnostic accuracy, routine implementation of molecular methods in all clinical laboratories remains limited because of higher costs, technical complexity, and the need for specialised infrastructure.

Nevertheless, the major advantage of PCR-based mecA detection over conventional phenotypic methods lies in its rapid turnaround time, which allows early and accurate identification of MRSA and facilitates timely initiation of infection-control measures and appropriate antimicrobial therapy. Rapid molecular confirmation is particularly important in hospital settings, where delayed detection of MRSA may contribute to nosocomial transmission and therapeutic failure (Brown and Walpole, 2001; Stefani *et al*., 2012).

DNA Extraction

Efficient bacterial lysis is essential for the extraction of genomic DNA. Gram-positive bacteria are more resistant to lysis due to the thick peptidoglycan layer present in their cell wall. Traditional DNA extraction methods commonly employ enzymatic digestion followed by detergent-based lysis and organic extraction. In the phenol–chloroform method, bacterial cells are initially treated with lysozyme, which hydrolyzes the peptidoglycan layer, weakening the cell wall and rendering it susceptible to osmotic shock. Subsequent treatment with sodium dodecyl sulfate (SDS) disrupts residual membranes and denatures proteins, including nucleases. Chelating agents such as EDTA and citrate further inhibit nuclease activity by sequestering divalent cations. Protein contaminants are dissociated from DNA using perchlorate ions, facilitating purification of high-quality bacterial genomic DNA. Chloroform–isoamyl alcohol is used to denature and precipitate proteins by lowering the dielectric constant of the aqueous phase.

A total of 25 bacterial samples were processed, and the DNA concentrations obtained ranged from 25.56 ng/µl to 436.46 ng/µl, indicating substantial variability in DNA yield among the isolates. The mean DNA concentration was calculated to be 135.70 ng/µl, while the median value was 101.33 ng/µl, suggesting a moderately skewed distribution influenced by a few high-yield samples. The results show that the efficiency of DNA extraction was evaluated by measuring DNA concentration (ng/µl) for each sample. The observed variation in DNA concentration across samples may be attributed to several factors, including differences in bacterial cell wall composition, growth phase of the cultures, efficiency of cell lysis, and DNA recovery during purification steps. Samples such as MS-122A (436.46 ng/µl) and MS-98A (426.02 ng/µl) exhibited exceptionally high DNA yields, which may indicate highly efficient lysis and recovery, or possible co-extraction of contaminants such as RNA or proteins. Conversely, samples like MS-48A (25.56 ng/µl) and MS-192A (26.02 ng/µl) showed relatively low DNA concentrations, potentially due to incomplete cell lysis or DNA loss during extraction.

In general, DNA isolation is a multi-step procedure involving cell lysis by treatment with lytic enzymes and/or detergents, DNA extraction with organic solvents, and DNA recovery by alcohol precipitation. Therefore, a rapid, easy-to-use, efficient, and cost-effective method for bacterial DNA isolation is necessary (Ahmed *et al*., 2014).

The quality and quantity of extracted DNA are critical determinants of downstream molecular applications such as PCR amplification, sequencing, and genotypic characterisation of resistance genes. Several studies have demonstrated that variations in DNA yield do not necessarily compromise PCR efficiency, provided the DNA is free from inhibitory contaminants (Wilson, 1997). Moreover, phenol–chloroform extraction, despite being labour-intensive, is considered a reliable method for obtaining high-molecular-weight DNA suitable for molecular diagnostics, particularly in resource-limited laboratory settings (Sambrook & Russell, 2001). The successful extraction of amplifiable genomic DNA in the present study further validates the suitability of this method for molecular detection of resistance-associated genes such as *mecA* in Gram-positive pathogens.

Gene Amplification by PCR

PCR amplification of bacterial genomic DNA was carried out to evaluate the presence and integrity of the mecA gene, a key genetic determinant responsible for methicillin resistance in *Staphylococcus* species. The mecA gene encodes an altered penicillin-binding protein (PBP2a), which has low affinity for β-lactam antibiotics, thereby conferring resistance.

The amplified PCR products were resolved using agarose gel electrophoresis, and the resulting banding patterns are presented in Figures 1 (a), (b), and (c). The PCR amplicons of bacterial genomic DNA were analyzed by agarose gel electrophoresis (1.5%) containing ethidium bromide and subsequently visualized under UV transillumination. A 100 kb DNA ladder was used as a molecular size marker to determine the size of amplified fragments. The

expected amplicon size for the mecA gene (~533 bp, depending on primer design) was used as a reference for confirming positive amplification.

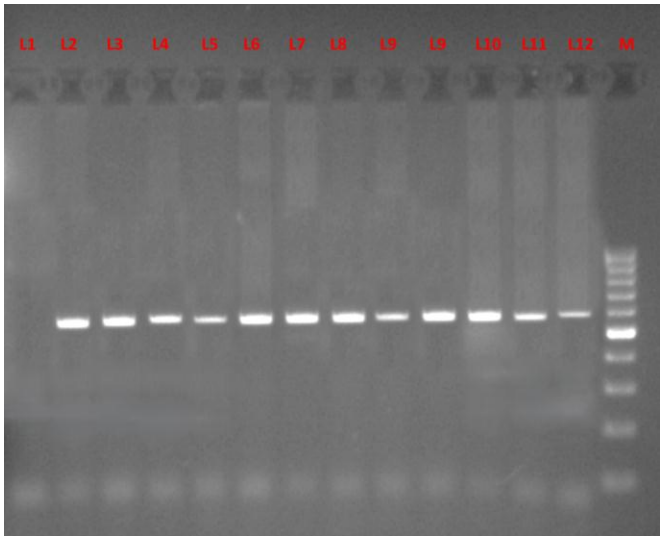

Fig. 1 (a): Lanes 2–12 (Samples), Lane M (100 kb marker), L1: BA-3A, L2: MS-5A, L3: MS-17A, L4: BA-22A, L5: MS-22A, L6: MS-29A, L7: MS-31A, L8: MS-38A, L9: MS-45A, L10: MS-48A, L11: MS-51A, L12: MS-63A.

In Figure 1 (a), represents the PCR amplification of the *mecA* gene in selected isolates. Lanes 2–12 correspond to the tested samples, while lane M represents the 100 kb DNA marker. Lane-wise sample identification includes L1: BA-3A, L2: MS-5A, L3: MS-17A, L4: BA-22A, L5: MS-22A, L6: MS-29A, L7: MS-31A, L8: MS-38A, L9: MS-45A, L10: MS-48A, L11: MS-51A, and L12: MS-63A. In Figure 1(a), distinct bands corresponding to the expected *mecA* gene size were observed in lanes 2 to 7, confirming successful amplification in these isolates. Similar findings have been reported in earlier studies where the presence of *mecA*-specific bands was used as a definitive marker for methicillin resistance (Oliveira and de Lencastre, 2002). Although most samples showed clear amplification, faint bands observed in a few lanes may be attributed to lower template DNA concentration or reduced amplification efficiency, which is commonly observed in PCR-based assays (Chambers, 1997). Nevertheless, the presence of the specific *mecA* gene band confirms the methicillin-resistant nature of these isolates.

Figure.1 (b) illustrates PCR products of additional isolates. Lanes 1–7 represent samples MS-74A, MS-83A, MS-98A, MS-112A, MS-119A, MS-122A, and MS-138A, respectively, with lane M serving as the 100 kb DNA marker. Clear and well-defined bands at the expected mecA gene size were detected in the majority of samples, indicating successful amplification and the presence of the resistance gene. These observations are consistent with previous reports demonstrating reliable detection of the mecA gene using PCR in clinical isolates of *Staphylococcus aureus* (McClure *et al*., 2006; CLSI, 2022). Minor variations in band intensity were observed, which may reflect differences in DNA purity, concentration, or PCR amplification efficiency, as also reported by Murakami *et al*. (1991). Overall, these results further support the widespread presence of the mecA gene among the tested isolates.

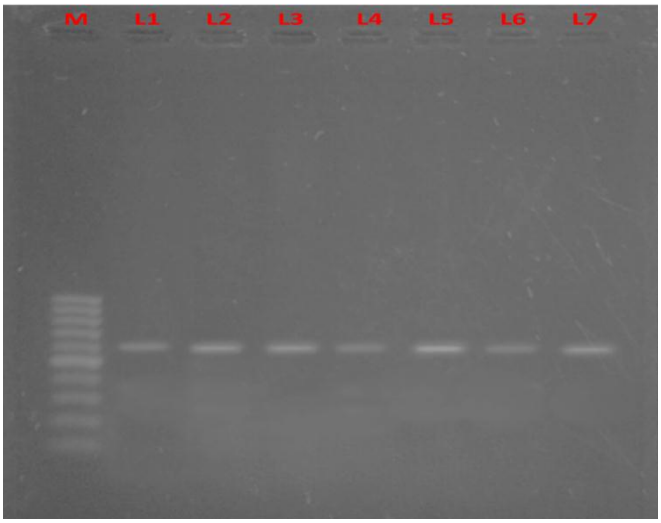

Fig.1 (b) : Lanes 1–7 (Samples), Lane M (100 kb marker), L1: MS-74A, L2: MS-83A, L3: MS-98A, L4: MS-112A, L5: MS-119A, L6: MS-122A, L7: MS-138A.

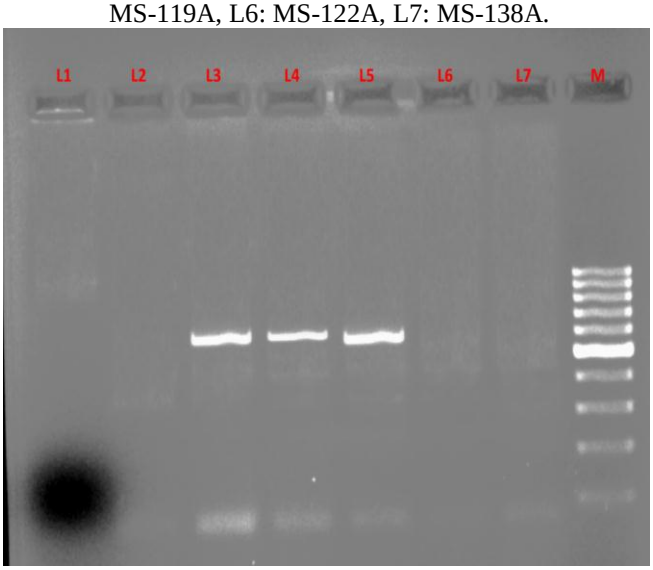

Fig. 1 (c) : Lanes 2–7 (Samples), Lane M (100 kb marker), L2: MS-143A, L3: MS-152A, L4: MS-176A, L5: MS-186A, L6: MS-192A, L7: MS-198A**.**

The results presented In Figure.1 (c), presents PCR amplification results of the remaining isolates. Lanes 2–7 correspond to MS-143A, MS-152A, MS-176A, MS-186A, MS-192A, and MS-198A, respectively, while lane M denotes the molecular weight marker. The appearance of visible bands at the expected amplicon size confirms successful PCR

amplification of the mecA gene in these samples as well. Similar amplification patterns have been reported in earlier molecular studies of MRSA isolates (Oliveira and de Lencastre, 2002). Some isolates exhibited comparatively faint bands, which may be due to lower DNA yield or partial amplification; however, the detection of specific bands clearly indicates the presence of the mecA gene, supporting their classification as methicillin-resistant strains (Chambers, 1997).

The detection of the mecA gene by PCR is considered the gold standard for confirmation of methicillin resistance in *Staphylococcus aureus*, as the gene encodes penicillin-binding protein 2a (PBP2a), which confers resistance to β-lactam antibiotics (Murakami *et al*., 1991; Chambers, 1997). PCR-based detection provides high sensitivity and specificity and is widely used for molecular confirmation of MRSA in research and reference laboratories (CLSI, 2022).

Electrophoretic Analysis

The electrophoretic profiles of the PCR products are presented in Figures 1 (a), (b), and 1 (c). The presence of a distinct band at 533 bp was considered indicative of the presence of the *mecA* gene in the tested isolates. Clear and well-defined bands corresponding to the expected 533 bp amplicon were observed in the majority of the samples, confirming successful amplification and indicating the presence of the *mecA* gene in those isolates. Similar observations have been reported by Murakami *et al*. (1991), who demonstrated that amplification of a 533 bp fragment is a reliable marker for *mecA*-mediated methicillin resistance in *Staphylococcus aureus*.

Samples such as MS-98A and MS-122A, which exhibited higher genomic DNA concentrations, showed strong and intense bands, reflecting efficient amplification. This correlation between DNA concentration and band intensity has also been documented in earlier PCR-based detection studies of MRSA (Chambers, 1997). In contrast, some isolates, including MS-48A and MS-192A, showed comparatively faint bands, which may be attributed to their lower DNA concentrations. Despite this, the presence of visible bands at the expected size confirms that these samples also tested positive for the *mecA* gene, as faint amplification products can still represent true positives when specific primers are used (Oliveira and de Lencastre, 2002).

A few samples may show reduced band intensity or absence of bands, suggesting either low template concentration, partial amplification, or absence of the target gene. Such variability in PCR amplification efficiency has been previously reported and is influenced by factors such as DNA purity, inhibitor presence, and primer-template binding efficiency (McClure *et al*., 2006). Overall, the electrophoretic analysis demonstrates that the majority of the bacterial isolates harbour the *mecA* gene, as evidenced by the presence of the 533 bp PCR product, while variations in band intensity are primarily associated with differences in genomic DNA concentration and amplification efficiency.

Pulsed-field gel electrophoresis (PFGE), due to its great discriminatory power and high degree of specimen typeability, is accepted as the gold standard for molecular typing of *Staphylococcus aureus* isolates. It has been successfully used to study the epidemiology of *S. aureus* nosocomial infections and methicillin resistance. PFGE is, however, time-consuming and labour-intensive, limiting its routine use in diagnostic laboratories (Tenover *et al*., 1995). In this study, PFGE exhibited superiority as a technique for analysing the epidemiology of *S. aureus* strains.

In the present investigation, when three MRSA strains were subjected to PFGE analysis, three distinct pulsotypes or PFGE patterns were observed. The presence of different pulsotypes suggests genetic diversity among the MRSA isolates, indicating possible variation in strain origin or transmission pathways. Upon interpretation of PFGE banding patterns, these findings are in agreement with previous studies reporting heterogeneous PFGE profiles among clinical MRSA isolates (Khan *et al*., 2010), highlighting the usefulness of PFGE in epidemiological surveillance and molecular characterization of MRSA.

Evaluation of Antibiotic Effectiveness

All 25 PCR-confirmed MRSA isolates will be tested for susceptibility against such commonly used antibiotic such as *Vancomycin, Linezolid, Ciprofloxacin, Erythromycin, Clindamycin, Tetracycline, Gentamicin, Levofloxacin, Azithromycin* and *Amoxicillin-Clavulanic acid.* Each test will be performed in triplicate, and average values will be considered. The antibiotic susceptibility pattern of the test isolate was determined using the disk diffusion method. The zone of inhibition (mm) was measured for three replicates, and the mean along with standard deviation (S.D.) was calculated to ensure accuracy and reproducibility. The results demonstrated variation in the effectiveness of different antibiotics against the test organism.

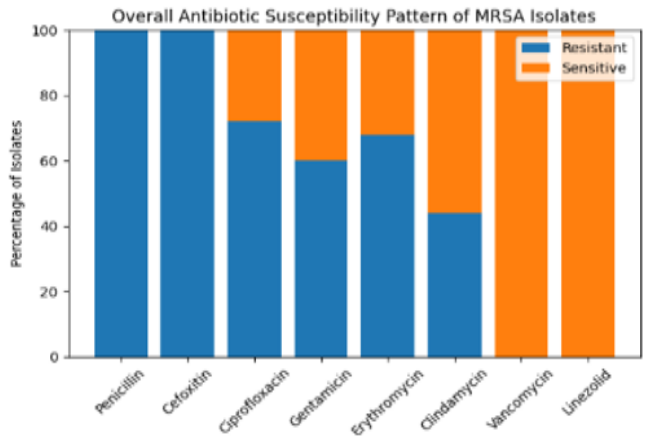

Chart 1 : Overall antibiotic susceptibility pattern of MRSA isolates

The results indicate strong antibacterial susceptibility of the isolate to several antibiotics. The highest zones of inhibition were observed with Vancomycin (VN) (~30.33 mm) and Tetracycline (TC) (~30.33 mm), indicating excellent antibacterial activity. Ciprofloxacin (CP) (~29.17 mm) and Amoxicillin–Clavulanic acid (ACA) (~27.67 mm) also exhibited very high effectiveness, followed by Gentamicin (GM) (~25.33 mm) and Levofloxacin (LF) (~24.50 mm), which showed strong inhibitory effects. Azithromycin (AM) (~17.83 mm) and Erythromycin (EM) (~17.33 mm) demonstrated moderate antibacterial activity. Clindamycin (CM) (~11.83 mm) showed low to moderate effectiveness, while Linezolid (LZ) (~8.00 mm) exhibited minimal activity, indicating reduced susceptibility of the isolate to this antibiotic.

The present study demonstrates that Methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated from a variety of respiratory clinical samples, predominantly throat swabs, followed by sputum, tracheal aspirates, and Bronchoalveolar lavage (BAL) specimens. The higher frequency of MRSA in throat swabs may reflect colonisation of the upper respiratory tract, whereas its presence in sputum, tracheal aspirates, and BAL samples indicates its involvement in lower respiratory tract infections such as pneumonia (Turner *et al*., 2019; Lakhundi & Zhang, 2018).

The detection of MRSA in tracheal aspirates and BAL samples is particularly significant, as these specimens are typically obtained from critically ill or mechanically ventilated patients. This finding suggests a strong association of MRSA with hospital-acquired infections, especially ventilator-associated pneumonia (VAP), where *Staphylococcus aureus* remains a leading pathogen (Koulenti *et al*., 2019; WHO, 2023). The presence of MRSA in sputum samples further supports its role in both community-acquired and hospital-associated respiratory infections (Lee *et al*., 2023).

Recent studies (2022–2024) have reported that respiratory specimens are among the most common sources of MRSA isolation. In India and other developing regions, approximately 20–40% of *Staphylococcus aureus* isolates from respiratory samples are methicillin-resistant, with higher rates observed in intensive care units due to increased antibiotic pressure and prolonged hospitalisation (Kaur *et al*., 2022; Ahmed *et al*., 2023). Similarly, global surveillance data indicate a substantial burden of MRSA in lower respiratory tract infections, particularly in ventilated patients (WHO, 2023; Lee *et al*., 2023).

The predominance of throat swab isolates in the present dataset may also indicate asymptomatic carriage, which is a well-recognised factor in MRSA transmission. Colonised individuals serve as reservoirs for the spread of MRSA within healthcare settings, thereby contributing to both community and nosocomial infections (Turner *et al*., 2019).

Overall, the incidence pattern observed in this study highlights the widespread distribution of MRSA across both upper and lower respiratory tract samples. These findings are consistent with recent literature and emphasise the increasing burden of MRSA in respiratory infections. Continuous surveillance, strict infection control practices, and antimicrobial stewardship programs are essential to limit the spread of MRSA in clinical settings (WHO, 2023; Lee *et al*., 2023).

The antibiotic susceptibility pattern of Methicillin-resistant *Staphylococcus aureus* (MRSA) in the present study reflects the characteristic multidrug-resistant nature of these isolates. MRSA strains are inherently resistant to all β-lactam antibiotics, including amoxicillin-clavulanic acid, due to the presence of the mecA gene, which encodes the altered penicillin-binding protein (PBP2a) (Lakhundi & Zhang, 2018). Therefore, high levels of resistance to amoxicillin-clavulanic acid (A10) are expected and consistent with global findings.

Among the antibiotics tested, vancomycin (A1) and linezolid (A2) remain highly effective against MRSA isolates and are considered drugs of choice for the treatment of serious MRSA infections. Recent studies have reported near-universal susceptibility of MRSA to these agents, although occasional emergence of vancomycin-intermediate strains has been documented (Turner *et al*., 2019; Lee *et al*., 2023).

In contrast, antibiotics such as ciprofloxacin (A3) and levofloxacin (A8), belonging to the fluoroquinolone class, often show high resistance rates among MRSA isolates due to chromosomal mutations and efflux mechanisms (Foster, 2017). Similarly, macrolides like erythromycin (A4) and azithromycin (A9) frequently exhibit high resistance, primarily mediated by methylation of ribosomal target sites (erm genes) (Kaur *et al*., 2022).

Clindamycin (A5) shows variable sensitivity among MRSA isolates, with inducible resistance (MLS_B phenotype) being commonly reported; therefore, D-test confirmation is recommended before therapeutic use (Leclercq, 2002; Patel *et al*., 2023). Tetracycline (A6) and gentamicin (A7) generally demonstrate moderate to variable effectiveness depending on regional antibiotic usage and resistance trends (Ahmed *et al*., 2023).

Overall, the observed antibiotic sensitivity pattern highlights the limited therapeutic options available for MRSA infections and underscores the importance of continuous antimicrobial surveillance and stewardship programs to prevent further emergence and spread of resistant strains (WHO, 2023).

The detection of the mecA gene in the present study aligns with recent findings that identify mecA as the primary genetic determinant responsible for methicillin resistance in clinical *Staphylococcus aureus* isolates. The mecA gene encodes the altered penicillin-binding protein (PBP2a), which reduces the efficacy of β-lactam antibiotics and contributes significantly to multidrug resistance patterns observed in hospital settings (Lakhundi & Zhang, 2018; Turner *et al*., 2019).

Recent studies (2022–2024) have consistently reported a high prevalence of mecA among MRSA isolates derived from clinical samples such as pus, blood, urine, and wound swabs. A study conducted in North India reported that nearly all phenotypically confirmed MRSA isolates carried the mecA gene, demonstrating a strong correlation between mecA presence and methicillin resistance (Kaur *et al*., 2022). Similarly, studies from South Asia have reported mecA positivity ranging from 80% to 95% among MRSA isolates, emphasizing its widespread occurrence in clinical settings (Ahmed *et al*., 2023).

Globally, the prevalence of mecA-positive strains varies depending on geographical location, healthcare practices, and antibiotic usage patterns, with reported rates ranging from 30% to over 90% (Lee *et al*., 2023). Despite these variations, mecA remains the most reliable molecular marker for MRSA detection and is widely used in diagnostic microbiology.

In addition, emerging studies have reported the presence of mec gene variants such as mecC, along with the coexistence of other virulence and biofilm-associated genes, which may enhance bacterial persistence and pathogenicity in clinical environments (Becker *et al*., 2023). These findings highlight the evolving genetic diversity of methicillin-resistant strains.

Overall, the findings of the present study are consistent with contemporary research, confirming that the mecA gene is widely distributed among clinical isolates and plays a crucial role in antimicrobial resistance. Continuous surveillance and molecular characterisation are essential for effective infection control and management of MRSA-associated infections.

Conclusion

The present study provides a comprehensive evaluation of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from respiratory clinical samples through a combination of phenotypic, biochemical, and molecular approaches. The isolation and identification of *S. aureus* based on colony morphology on Mannitol Salt Agar and Blood Agar, Gram staining, and catalase testing established a reliable preliminary screening framework for presumptive MRSA isolates.

Phenotypic antimicrobial susceptibility testing revealed a considerable proportion of isolates exhibiting resistance to methicillin, highlighting the growing burden of antimicrobial resistance among respiratory pathogens. Molecular confirmation through PCR-based detection of the mecA gene, which encodes the altered penicillin-binding protein PBP2a, conclusively validated methicillin resistance in the majority of phenotypically resistant isolates. The consistent amplification of the expected 533 bp mecA gene fragment confirms PCR as a highly sensitive and specific method for MRSA detection.

Electrophoretic analysis demonstrated variability in band intensity among isolates, largely attributable to differences in genomic DNA concentration and amplification efficiency rather than the absence of the target gene. High-DNA-yield samples showed strong amplification, while low-yield samples produced faint but detectable bands, emphasising the importance of efficient DNA extraction for molecular diagnostics.

Overall, the study underscores the clinical significance of MRSA in respiratory infections and

highlights the limitations of relying solely on phenotypic methods for accurate detection. The integration of molecular diagnostics with routine microbiological techniques enables early and precise identification of MRSA, facilitating timely therapeutic decisions and effective infection control strategies. These findings reinforce the need for continuous surveillance of MRSA prevalence and resistance patterns, along with the adoption of molecular tools in routine clinical laboratories to curb the spread of multidrug-resistant *S. aureus*.

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