"Prevalence and Phenotypic Characterisation of Methicillin-Resistant*Staphylococcus aureus* Isolated from Respiratory Tract Infections."

Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of respiratory tract infections and poses a significant challenge to clinical management due to its resistance to multiple antimicrobial agents. The present study was conducted to determine the prevalence and phenotypic characteristics of MRSA isolated from respiratory clinical samples. A total of 200 respiratory specimens, including throat swabs, sputum samples, tracheal aspirates, and bronchoalveolar lavage (BAL) samples, were collected from patients clinically suspected of respiratory tract infections.

All samples were processed using standard microbiological techniques. Isolation of *Staphylococcus aureus* was performed on Mannitol Salt Agar and Blood Agar, followed by phenotypic identification using Gram staining, catalase test, and coagulase test. Phenotypic detection of methicillin resistance was carried out using the cefoxitin disc diffusion method.

Throat swabs constituted the majority of samples, followed by sputum, tracheal aspirates, and BAL samples. The highest prevalence of MRSA was observed among individuals aged 16–30 years, with nearly equal distribution between male and female patients. On Mannitol Salt Agar, a large proportion of isolates produced yellow colonies indicative of mannitol fermentation. Phenotypic testing confirmed the presence of *Staphylococcus aureus*, and cefoxitin resistance identified a substantial number of isolates as MRSA.

The findings of this study demonstrate a considerable prevalence of MRSA among respiratory tract infections and highlight the importance of routine phenotypic screening for early detection. Continuous surveillance and implementation of effective infection control measures are essential to reduce the burden of MRSA and improve patient outcomes.

Keywords: Methicillin-resistant *Staphylococcus aureus*; MRSA; Respiratory tract infections.

1. Inroduction

Staphylococcusaureus often referred to as "staph" is a commonly occurring bacterium which normally resides on the skin or in the nose of healthy person **(Rybak*et a*l.2005-25).***S. aureus*** may cause certain skin infection, soft tissue infection such as boils & also cause severe infection, pneumonia, sepsis & abscesses **(Rasmussen *et al.* Future Microbiology 2011).***S. aureus*** continues to be a harmful pathogen for both the hospital associated as well as community acquired infections. A report from the years 2010, shows that the community – acquired *S. aureus* infections are increases at a higher rate **(Joshi et al. 2013).**Several mechanisms for the development of MRSA have been reported among these productions of a unique penicillin -binding protein (PBP) that has low affinity for beta -lactam antibiotics & whose effects are determined by several structural genes (e.g., mecR1&mecl) **(Methicillin-resistant staphylococci genetics & mechanism of resistance,2010)**, production of penicillinase enzyme are most important ones. **(Fruit *et al*.,2012).**MRSA spreads more readily than other strains once established.*S. aureus* colonizes the nares of 28–32% of the US population. MRSA nasal colonization rates range from 0.9% to 1.5% in the United States. Elevated risk of colonization mirrors risk of infection as noted above: athletes, those in prisons, military recruits, children, persons in urban, underserved areas, individuals with an indigenous background, pet owners, livestock workers, individuals with prior MRSA infection, individuals with HIV or cystic fibrosis and individuals with frequent health- care contact are all at increased risk of MRSA colonization **(Lekkerkerk*et al*.2016).**

In some country's MRSA makeup to 75% of all *S. aureus* isolates in hospitals. **(Kesha et al. 2003:9)**.Therefore, studies on the prevalence & drug susceptibility patterns of *S. aureus* are of the highest priorities.After the introduction of penicillin in medicine in the early 1940's, penicillin resistant *S. aureus* strains have emerged. The percentage of penicillin-resistant strains has risen to 75-95% with the highest rate. The most of these strains were found among the hospital strains **(Topley&Wilson's,2017).**Methicillin-resistant *Staphylococcus aureus* (MRSA) was first discovered by the British scientists in the early 1960's and it is now regarded as a major hospital-acquired pathogen. MRSA is resistant to many antibiotics such as methicillin, amoxicillin, penicillin, and oxacillin**(Batabyal et al.,2012).**MRSA is endemic in most hospitals in Asia, and some Asian countries have among the highest MRSA prevalence in the world. However, most available data are from high-income countries (for example, Japan, South Korea and Singapore), with limited information from other nations. Although there is country-to-country variability, MRSA accounts for up to 50% of *S. aureus* bloodstream infections in parts of Asia **(Chen&Huang.,2014).**

MRSA control interventions have been widely implemented across health-care facilities. These interventions aim to limit the emergence of MRSA by facilitating judicious use of antimicrobial agents (including introducing restrictions on their prescription), control the reservoir of patients who are carriers, prevent MRSA transmission between patients and prevent the development of infection in carriers. Several measures are usually required to successfully prevent transmission and infection with MRSA **(Calfee *et al*.2015)** Decolonization, an important control intervention for which there is growing evidence, is discussed in the Management section.

2. Materials and Methods

2.1 Study Design and Study Population

The present study was carried out in the Department of Microbiology using respiratory clinical samples collected from patients with suspected respiratory tract infections. A total of 200 respiratory specimens, including sputum, throat swabs, tracheal aspirates, and Broncho alveolar lavage samples, were analyzed for the isolation and characterization of methicillin-resistant *Staphylococcus aureus* (MRSA). Standard microbiological culture media such as Blood agar, Mannitol Salt Agar (MSA), and Mueller-Hinton agar were used for isolation, identification, and antimicrobial susceptibility testing of *S. aureus*.Biochemical reagents

required for Gram staining, catalase test, and coagulase test were employed for phenotypic confirmation of isolates.

Antimicrobial susceptibility testing was performed using cefoxitin (30 µg) discs for phenotypic detection of methicillin resistance and selected antibiotics including vancomycin, linezolid, ciprofloxacin, erythromycin, and tetracycline for evaluation of antibiotic effectiveness.

2.2 Collection and Distribution of Respiratory Clinical Samples

A total of 200 respiratory clinical samples were collected from patients with clinically suspected respiratory tract infections attending the Department of Microbiology and its associated hospitals during the study period. The respiratory specimens comprised sputum, throat swabs, tracheal aspirates, and Broncho alveolar lavage (BAL) samples, which were obtained based on clinical indications and standard hospital protocols. Patients of different age groups and both genders were included in the study to ensure representative sampling.

All specimens were collected aseptically by trained healthcare personnel in accordance with established biosafety and infection control guidelines to minimize the risk of contamination and ensure the safety of both patients and laboratory personnel (**Wayne, 2015**).

Immediately after collection, the samples were transported to the microbiology laboratory without delay to preserve the viability of the microorganisms. When immediate processing was not possible, samples were stored temporarily at 4 °C and processed within the recommended time frame.

Prior to initiation of the study, ethical approval for the collection and use of clinical samples was obtained from the Institutional Ethics Committee.All procedures involving human samples were conducted in compliance with ethical standards and institutional guidelines.

2.3 Primary Isolation and Culture Characteristics

2.3.1 Growth on Mannitol Salt Agar

All respiratory clinical samples were inoculated onto Mannitol Salt Agar (MSA) plates and incubated at 37 °C for 24 hours. After incubation, plates were examined for colony morphology, including colony color, size, pigmentation, and zone formation. Yellow colonies with surrounding yellow zones indicated mannitol fermentation, characteristic of Staphylococcus aureus, whereas pink or colorless colonies suggested non-mannitol-fermenting organisms.

2.3.2 Growth on Blood Agar

Simultaneously, samples were inoculated onto Blood Agar plates and incubated at 37 °C for 24–48 hours. Colonies were observed for size, shape, texture, pigmentation, and hemolytic activity. Colonies appearing milky white to creamy with or without hemolysis were selected for further identification.

2.4 Phenotypic Identification of *Staphylococcus aureus*

2.4.1 Gram Staining

Gram staining was performed to differentiate bacterial isolates based on differences in cell wall characteristics, following the standard method originally described by Gram and later modified by Hucker (**Beveridge, 2001**).A thin smear of each bacterial culture was prepared on a clean glass slide, air-dried, and heat-fixed. The smear was flooded with crystal violet for 1 minute and gently rinsed with distilled water. Gram's iodine was then applied as a mordant for 1 minute, followed by rinsing with water.

Decolorization was carried out using 95% ethanol for 15–20 seconds until the runoff appeared clear, and the reaction was immediately stopped by washing with distilled water. The smear was subsequently counterstained with safranin for 30–60 seconds, rinsed with water, and air-dried (**Cheesbrough 2005)**.

Bacterial cells that retained the crystal violet–iodine complex appeared purple and were identified as Gram-positive, whereas cells that were decolorized by ethanol and subsequently stained with safranin appeared pink to red and were identified as Gram-negative bacteria (**Tortora*et al.*, 2019**).

2.4.2 Catalase Test

The catalase test was carried out to differentiate *Staphylococcus* species from catalase-negative organisms. A clean glass slide was taken, and a drop of freshly prepared 3% hydrogen peroxide was placed on its surface. Using a sterile wooden stick or platinum loop, a small portion of bacterial growth from a pure culture was transferred onto the hydrogen peroxide drop. The reaction was observed immediately for the formation of oxygen bubbles. The appearance of immediate and vigorous effervescence was considered a **positive catalase reaction**, supporting the identification of the isolates as members of the genus *Staphylococcus* (**Cheesbrough 2005**).

2.4.3 Coagulase Test

Coagulase testing was performed using both slide and tube methods to confirm *Staphylococcus aureus*. For the slide coagulase test, a smooth bacterial suspension was prepared in normal saline on a clean glass slide, followed by the addition of plasma and gentle mixing. The slide was rocked for 10–15 seconds and observed for visible clumping, indicating a positive reaction. For the tube coagulase test, approximately 0.5 mL of plasma was inoculated with the test organism and incubated at 37 °C. The tube was examined after 4 hours and, if negative, re-examined after 24 hours for clot formation. The presence of a stable clot confirmed a**positive tube coagulase test**, thereby confirming the isolates as *Staphylococcus aureus* (**Cheesbrough 2005**).

2.5 Confirmed *S. aureus* isolates using the cefoxitin

Confirmed *S. aureus* isolates were subsequently screened for methicillin resistance using the cefoxitin (30 µg) disc diffusion method, recommended as a reliable surrogate marker for mecA-mediated resistance. Testing was performed on Mueller–Hinton agar, with the bacterial inoculum standardized to 0.5 McFarland turbidity standard. Plates were incubated at 35–37 °C for 18–24 hours, and zones of inhibition were measured in millimeters. Interpretation of results was carried out in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines, where isolates exhibiting a zone diameter of ≤21 mm were classified as methicillin-resistant *Staphylococcus aureus* (MRSA), while those showing a zone diameter of ≥22 mm were considered methicillin-sensitive *Staphylococcus aureus* (MSSA) (**Rai *et al.*, 2023**).

Cefoxitin was preferred over Oxacillin due to its enhanced ability to induce expression of the mecA gene, thereby improving the accuracy of phenotypic MRSA detection (**Skov*et al.*, 2006**). All phenotypically confirmed MRSA isolates were preserved on appropriate media and stored under recommended conditions for subsequent molecular analysis and antimicrobial susceptibility testing.

2.6 Quality Control and Interpretation Criteria

Quality control procedures were strictly followed throughout the study. Standard laboratory protocols were used for culture, biochemical testing, and antimicrobial susceptibility testing. Interpretation of phenotypic tests and antibiotic susceptibility results was carried out

according to established microbiological criteria and Clinical and Laboratory Standards Institute (CLSI) guidelines. Reference strains were used as controls where applicable to ensure accuracy and reproducibility of results.

3. Result and Discussion

3.1 Distribution of Respiratory Samples

A total of 200 respiratory clinical specimens were analyzed during the course of the present study. The specimens comprised throat swabs, sputum, tracheal aspirates, and bronchoalveolar lavage (BAL) samples. These samples were collected from patients of different age groups and both genders who presented with clinical manifestations suggestive of respiratory tract infections at the participating healthcare facilities. The distribution of respiratory samples according to specimen type is presented in Table.3 Among the total specimens analyzed, throat swab samples were the most frequently collected, accounting for 108 samples (54%), followed by sputum samples with 51 specimens (25.5%). Tracheal aspirate samples constituted 25 specimens (12.5%), while BAL (bronchoalveolar lavage) samples were the least frequent, comprising 16 specimens (8%) of the total.

Table.3 Distribution of Respiratory Clinical Samples.

Sample Type	Number of Samples	Percentage
Throat Swab	108	54%
Sputum	51	25.5%
Tracheal Aspirate	25	12.5%
BAL (bronchoalveolar lavage)	16	8%
Total No. of sample	**200**	**100%**

Table.4Age-wise Distribution of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Patients (n = 200)

S.no	Age group	Total number	Percentage
1	16 to 30	115	57.5%
2	30 to 40	36	18%
3	40 to 50	35	17.5%
4	50 and above	14	7%
5	Total No.	200	100%

The age-wise distribution of Methicillin-Resistant *Staphylococcus aureus* (MRSA) patients shows that the highest proportion of cases was observed in the 16–30 years' age group, accounting for 57.5% of the total study population. This was followed by the 30–40 years' age group (18%) and the 40–50 years' age group (17.5%). The lowest proportion of MRSA cases was found among patients aged 50 years and above (7%).

Table. 5Gender-wise Distribution of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Patients (n = 200).

S.NO	**Gender**	**total**	**%**
1	Male	98	49
2	Female	102	51
3	Total no.	200	100%

Gender-wise analysis showed a nearly equal distribution of MRSA cases, with females accounting for 51% and males 49% of the study population.

Our study found that the main pathogens isolated from sputum samples and BALF were roughly the same. However, the sensitivities of the main pathogens to commonly used antibiotics were different. The sensitivity of BALF isolates to the most commonly used antibiotics was higher compared with the sensitivity of sputum isolates, especially after quality control of sputum samples **(Peng et.at.,2020**).

3.2 Isolation of *Staphylococcus aureus*

Respiratory clinical samples were cultured on Mannitol Salt Agar (MSA) and Blood Agar (BA) and incubated at 37 °C for 24–48 hours. Distinct colonies were observed based on size, color, texture, and hemolytic activity. On MSA, presumptive *Staphylococcus aureus* isolates produced yellow colonies with surrounding yellow zones, indicating mannitol fermentation, while non-fermenters appeared pink or colorless. Mannitol fermentation is a characteristic feature of *S. aureus* and is widely used as a differential criterion for its presumptive identification **(Cheesbrough, 2010; Forbes et al., 2016).** On Blood Agar, colonies were milky to white, smooth, and circular, with some isolates exhibiting clear hemolytic zones, which reflects the production of hemolysins and is commonly associated with pathogenic staphylococci **(Koneman et al., 2017).**

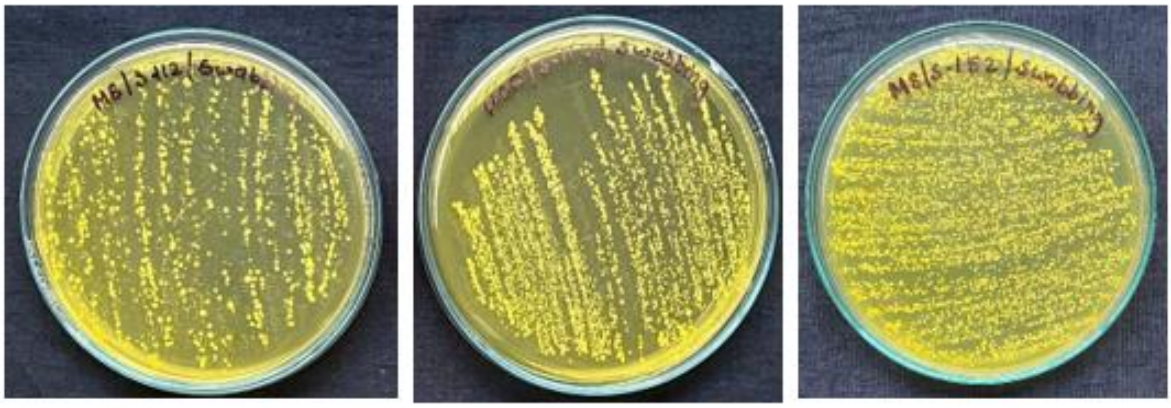

Fig.1 Representative images of randomly selected MSA culture plates showing characteristic Staphylococcus aureus colonies after Mannitol Salt Agar confirm mannitol fermentation.

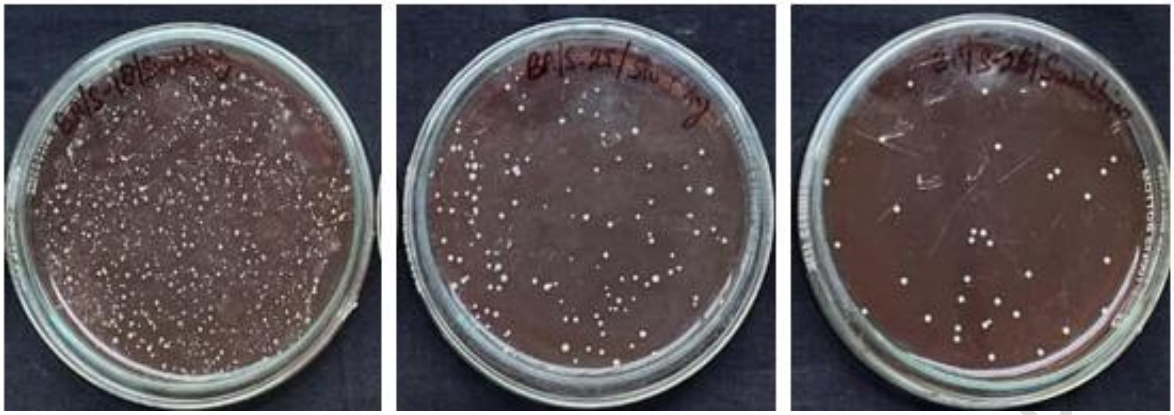

Fig.2 Representative images of randomly selected BA culture plates showing a large white pigment colony, characteristic *Staphylococcus aureus* colonies on Blood Agar after incubation at 37 °C.

A total of 280 bacterial isolates (MS1–MS200 series entries) were analyzed for colony morphology on Mannitol Salt Agar (MSA) and evaluated based on colony colour, size, zone formation, and pigmentation. The colony colour distribution showed that yellow colonies were most frequent (140 isolates, 50.0%), followed by pink colonies (92 isolates, 32.9%) and colourless colonies (48 isolates, 17.1%). The predominance of yellow colonies suggests a high proportion of mannitol-fermenting staphylococci, particularly *S. aureus*, as reported in earlier studies using MSA as a selective-differential medium **(Winn et al., 2014)**. In terms of growth characteristics, 232 isolates (82.9%) showed zone formation, while 48 isolates (17.1%) did not, indicating that most isolates were metabolically active on the medium. Zone formation on MSA is associated with acid production due to carbohydrate fermentation, reflecting active metabolic processes **(Cappuccino & Sherman, 2018)**.

Colony size analysis revealed that large colonies (170 isolates, 60.7%) were more prevalent than small colonies (110 isolates, 39.3%), suggesting better adaptation to the high-salt environment of MSA. This observation is consistent with the known salt tolerance of staphylococci, which enables robust growth under selective conditions that inhibit many competing bacterial species **(Jawetz et al., 2019).** Based on typical colony morphology observed on both media, presumptive *Staphylococcus aureus* isolates were selected for further biochemical and confirmatory identification tests.

The results observations A total of 200 BA-series culture entries were analyzed for colony morphology on culture media. Among these, 153 isolates (76.5%) showed growth, while 47 entries (23.5%) showed no growth or no colonies, indicating variability in bacterial viability under the culture conditions. Such variation may be influenced by factors including sample quality, bacterial load, and prior antimicrobial exposure **(Tille, 2021).** Among growth-positive isolates, colony colour distribution showed white colonies as the most predominant (47.7%), followed by mucoid (31.4%), milky (11.8%), and colourless colonies (9.1%). Mucoid and milky colonies were frequently associated with active growth and strong metabolic expression, which may reflect capsular material production and enhanced virulence potential **(Lowy, 1998).**

Zone formation was observed in 64.1% of isolates, while 35.9% showed no zone formation, indicating varying levels of metabolic activity. Hemolytic activity on Blood Agar is a useful indicator of pathogenic potential, as hemolysins contribute to tissue damage and immune evasion in *Staphylococcus aureus* infections **(Otto, 2014)**. Colony size analysis revealed that small colonies (62.7%) were more frequent than large colonies (37.3%), suggesting slower or limited growth in a majority of isolates. Additionally, 73.2% of isolates were pigmented, indicating active physiological processes and metabolic diversity, as staphylococcal pigmentation (e.g., staphyloxanthin) is linked to oxidative stress resistance and enhanced survival within the host **(Clauditz et al., 2006)**. These findings are representative in table form and support the phenotypic diversity observed among respiratory bacterial isolates.

3.3 Identification of *Staphylococcus aureus*

3.3.1 Microscopic Identification by Gram Staining

The majority of the isolates appeared as Gram-positive cocci arranged in grape-like clusters, which is a typical microscopic characteristic of Staphylococcus species. These cells retained the crystal violet stain and appeared purple under the microscope, confirming their Gram-positive nature. A wet mount preparation of each colony was examined using compound microscope. Staphylococcus Gram positive bacterium (coccus) appears in pairs, or grapelike clusters **(Bremer et al., 2004).**

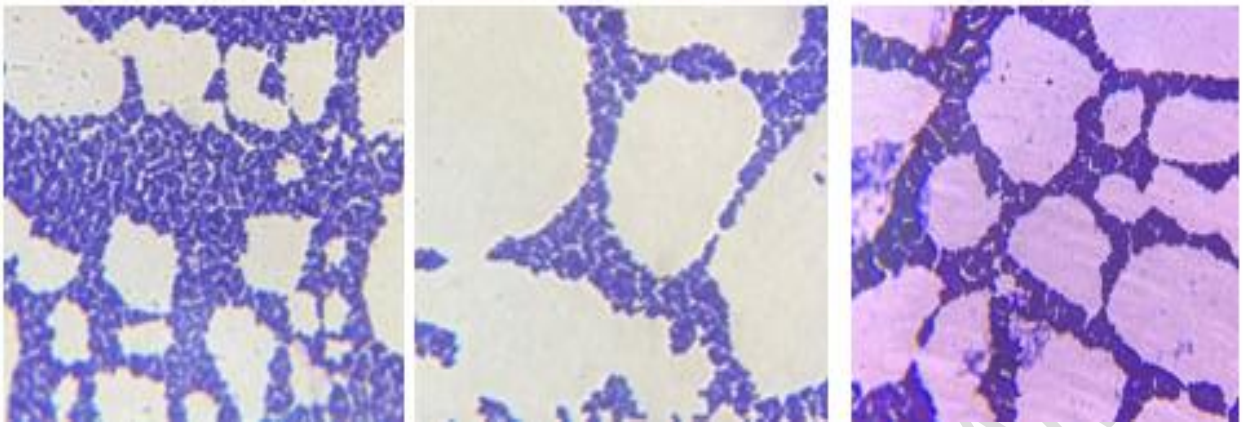

Fig.3 Microscopic images of MRSA isolate (MS176-A, MS122-A and MS22-A) from Gram-stained bacterial samples show Gram-positive characteristics, with spherical (cocci) cells retaining the purple crystal violet stain.

The observed Gram-positive reaction is attributed to the presence of a thick peptidoglycan layer in the bacterial cell wall, which retains the crystal violet–iodine complex during the decolorization step of Gram staining. This structural characteristic is a defining feature of Staphylococcus species and aids in their differentiation from Gram-negative bacteria **(Cheesbrough, 2005)**. The grape-like clustering pattern results from division occurring in multiple planes without complete separation of daughter cells, a morphological feature commonly used for preliminary identification of Staphylococcus aureus in clinical microbiology laboratories **(Tortora et al., 2019)**. The combined observations from Gram staining and wet mount examination provided an essential preliminary basis for further biochemical and confirmatory identification of Staphylococcus species **(Forbes et al., 2007).**

3.3.2 Microscopic Identification by Catalase test

A small amount of bacterial colony was placed on a clean glass slide and a drop of 3% hydrogen peroxide (H_2O_2) was added. The immediate formation of oxygen bubbles indicated a positive catalase reaction, while the absence of bubble formation indicated a negative reaction. The catalase test is an important biochemical method used to distinguish Staphylococcus species (catalase positive) from Streptococcus species (catalase negative).

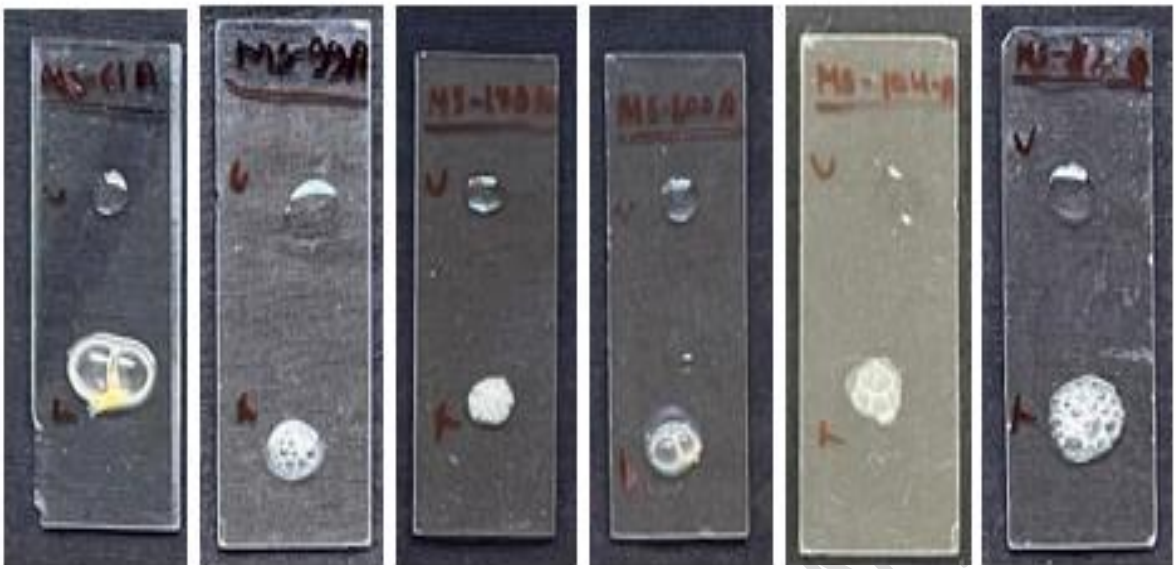

Fig.4 Randomly arranged slides showing catalase test results with visible bubble formation indicating positive catalase activity in different bacterial isolates.

Catalase-positive organisms possess the catalase enzyme, which decomposes hydrogen peroxide into water and oxygen, thereby protecting the bacterial cells from oxidative damage caused by reactive oxygen species generated during aerobic metabolism. This enzymatic activity enables *Staphylococcus* species to survive in oxygen-rich environments and contributes to their pathogenic potential **(Forbes et al., 2016)**. In contrast, catalase-negative organisms such as *Streptococcus* species lack this protective mechanism and do not produce visible oxygen bubbles upon exposure to hydrogen peroxide **(Cheesbrough, 2010).**

The catalase test is widely regarded as a rapid, simple, and reliable screening method in clinical microbiology laboratories for the preliminary identification of Gram-positive cocci. When used in conjunction with Gram staining and colony morphology, it provides a critical first step in the differentiation and accurate identification of staphylococcal isolates prior to further biochemical or molecular characterization **(Koneman et al., 2017).**

3.3.3 Microscopic Identification by Coagulase Test

Out of the total isolates tested, 160 isolates were coagulase-positive, as indicated by clot formation in the plasma, confirming their identification as Staphylococcus aureus. And 40 isolates showed a negative coagulase reaction, indicating the absence of the coagulase enzyme and suggesting that these isolates may belong to coagulase-negative staphylococci.

Only coagulase-positive isolate was considered for further confirmatory identification and analysis, as they represent the pathogenic Staphylococcus aureus strains.

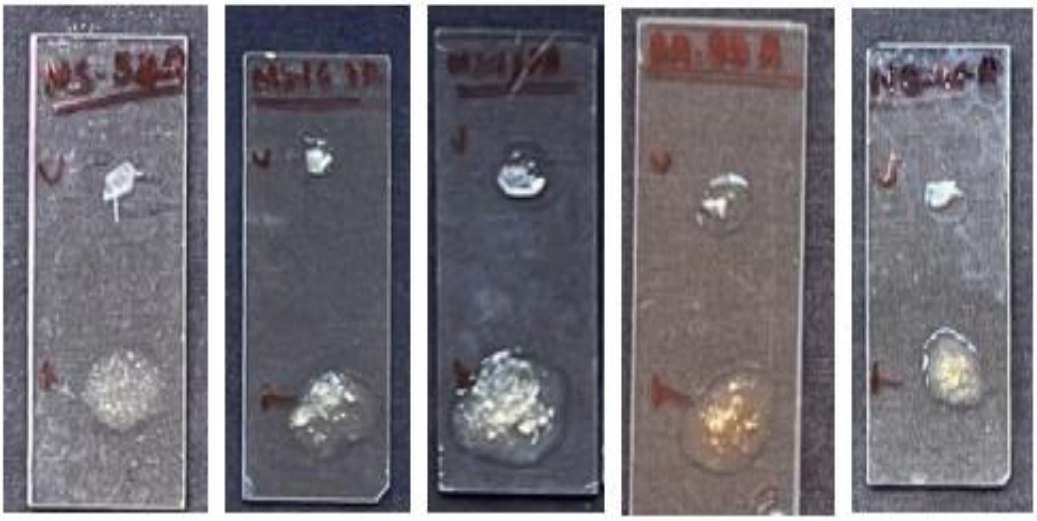

Fig.5 Randomly arranged slides showing slide coagulase positive results with visible agglutination patterns of different bacterial isolates.

The results analysis of identification of *Staphylococcus aureus*a total of 200 clinical samples was processed, from which 240 bacterial isolates were obtained and categorized into MS and BA groups. All isolates were subjected to Gram staining, morphological examination, and biochemical tests including catalase and coagulase tests presented in Table 4.3.1- Gram Staining Results, out of 240 isolates, 201 (83.8%) were Gram-positive, while 39 (16.2%) were Gram-negative. In the MS group, 154 out of 167 isolates (92.2%) were Gram-positive, whereas only 13 (7.8%) were Gram-negative. In contrast, the BA group showed 47 Gram-positive isolates (64.4%) and 26 Gram-negative isolates (35.6%), indicating a higher diversity of organisms compared to MS samples. Biochemical Test Result, Catalase testing showed that the majority of isolates were positive. In MS isolates, 156/167 (93.4%) were catalase positive, while 11 (6.6%) were negative. In BA isolates, 64/73 (87.7%) were catalase positive and 9 (12.3%) were negative. Coagulase testing revealed that 160 MS isolates (95.8%) were positive, while 7 (4.2%) were negative. In BA isolates, only 40 (54.8%) were coagulase positive and 33 (45.2%) were negative**.**

Out of the total tested isolates, 196 isolates showed catalase-positive results, indicated by rapid bubble formation after the addition of hydrogen peroxide, confirming their association with Staphylococcus species. In contrast, 14 isolates showed negative catalase reaction,

indicating the absence of catalase enzyme and suggesting that these isolates may belong to other bacterial genera. Therefore, only catalase-positive isolates were considered for further biochemical and confirmatory identification of Staphylococcus aureus.

The results obtained in this catalase test bacteria form gas bubbles around the colony. The formation of gas bubbles in the colony indicates that the bacteria are positive. Bacteria produce catalase enzymes that can break down H2O2 into H2O and O2. Hydrogen peroxide is formed during aerobic metabolism, so aerobic microorganisms describe the material. The results of the catalase test are presented in **(Khusna et al., 2019).**

The pathogenicity of coagulase positive staphylococci is related to the production of many virulence factors including toxins, and enzymes from which coagulase enzyme was consider as the most important one. Coagulase production was described as one of the most reliable criteria for the identification of pathogenic Staphylococcus species. Staphylococci producing coagulase are usually pathogenic **(Hasan *et. al.*, 2014).**

Similar observations have been reported in earlier studies, where Mannitol Salt Agar yielded a higher proportion of Gram-positive, catalase-positive, and coagulase-positive isolates compared to Blood Agar, highlighting its selective advantage for isolating *Staphylococcus aureus* from mixed clinical samples **(Cheesbrough, 2005; Smyth et al., 2005)**. The present findings further support the use of conventional phenotypic and biochemical tests as reliable preliminary tools for the identification of pathogenic *Staphylococcus aureus* prior to antimicrobial susceptibility testing and molecular confirmation.

3.4 Detection of Methicillin Resistance

All *Staphylococcus aureus* isolates confirmed by cultural characteristics, Gram staining, catalase test, and coagulase test were further screened for methicillin resistance using the cefoxitin (30 µg) disc diffusion method in accordance with CLSI guidelines. Only those isolates that were positive in all three confirmatory tests **(**Gram-positive, catalase-positive, and coagulase-positive) were selected for antimicrobial susceptibility testing.

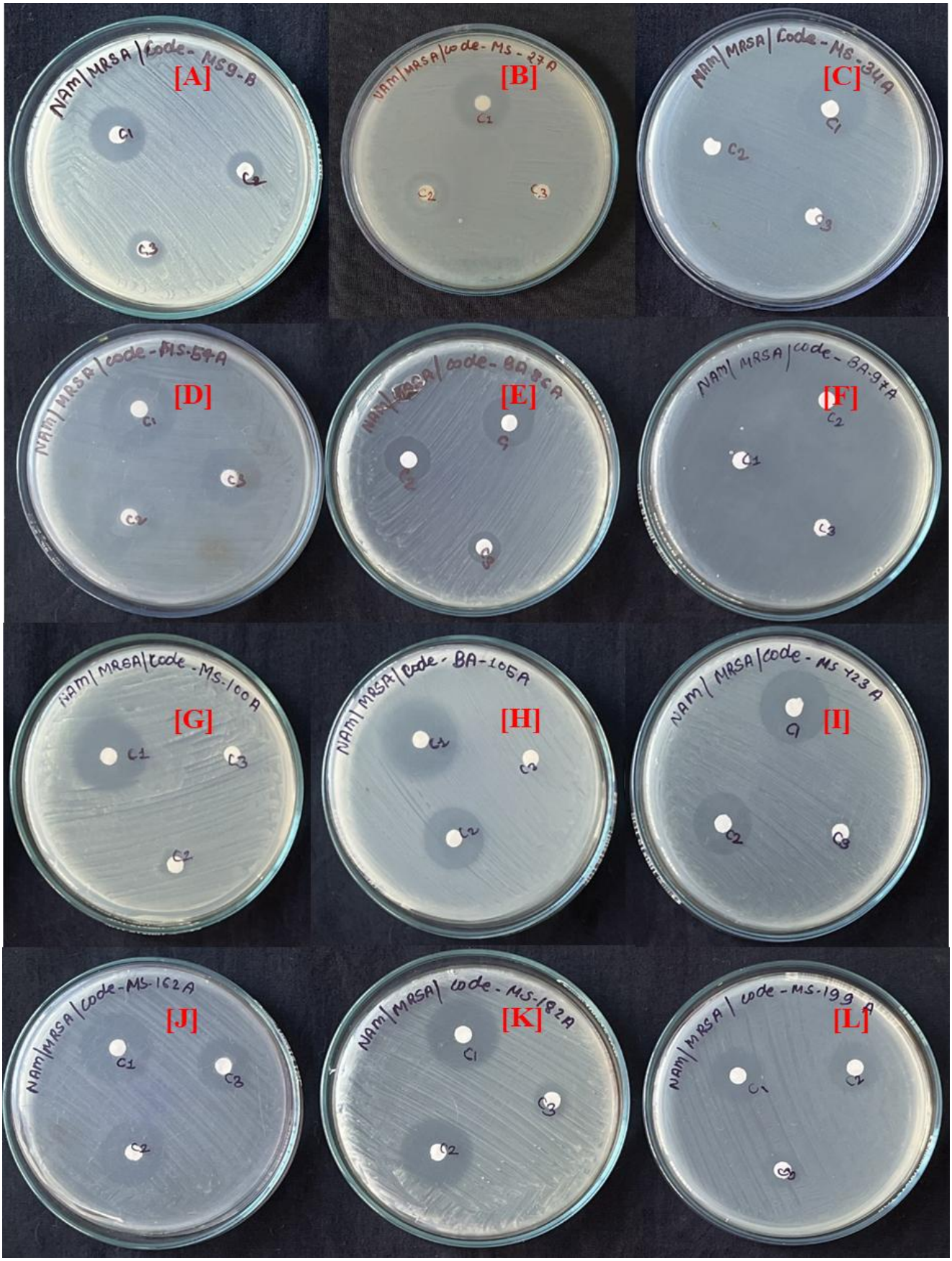

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Fig.6 Representative images of randomly selected cefoxitin-sensitive plates showing MSSA isolates of Staphylococcus aureus, exhibiting clear zones of inhibition around cefoxitin discs as per Clinical and Laboratory Standards Institute (CLSI) interpretative criteria.

A total of 158confirmed*Staphylococcus aureus*isolates obtained from Mannitol Salt Agar and Blood Agar were subjected to cefoxitin susceptibility testing. Among these, 25 isolates (15.82%) exhibited resistance to cefoxitin with a zone of inhibition ≤21 mm and were therefore identified as methicillin-resistant*Staphylococcus aureus*(MRSA**)**. The remaining 133 isolates (84.18%) showed sensitivity to cefoxitin with a zone of inhibition ≥22 mm and were categorized as methicillin-sensitive *Staphylococcus aureus*(MSSA)**.** The greater reliability of tests with cefoxitin disks confirmed earlier studies which showed that cefoxitin disk tests, without modification to conditions to improve expression of resistance, are as reliable or more reliable than oxacillin disk tests for the detection of methicillin resistance in *S. aureus***(Boutiba-Ben Boubaker*et.al*.,2004).**

3.5 Isolate of Methicillin-Resistant *Staphylococcus aureus*

A total of 25 bacterial isolates were recovered from different respiratory clinical specimens during the study. The isolates were cultured on Blood Agar (BA) and MacConkey (MSA)mediawere further characterized based on colony morphology, Gram staining, and biochemical reactions including catalase and coagulase tests**.**

The majority of isolates were obtained on MS medium**,** while a smaller proportion grew onBA medium**,** indicating differential growth patterns on selective and enriched media. On culture examination, two distinct colony morphologies were observed. The predominant morphology consisted of large, smooth yellow colonies with surrounding zones, which was observed in most isolates. A smaller number of isolates showed milky white or small white colonies withzone formation, particularly in specimens S-3, S-22, and S-98. All isolates were found to be Gram-positivecocci on Gram staining, indicating organisms with a thick peptidoglycan cell wall structure. Biochemical characterization revealed that all isolates were catalase positive**,** confirming the presence of the catalase enzyme. Furthermore, all isolates were also coagulase positive, which is a key virulence-associated characteristic used for the identification of *Staphylococcus aureus*.

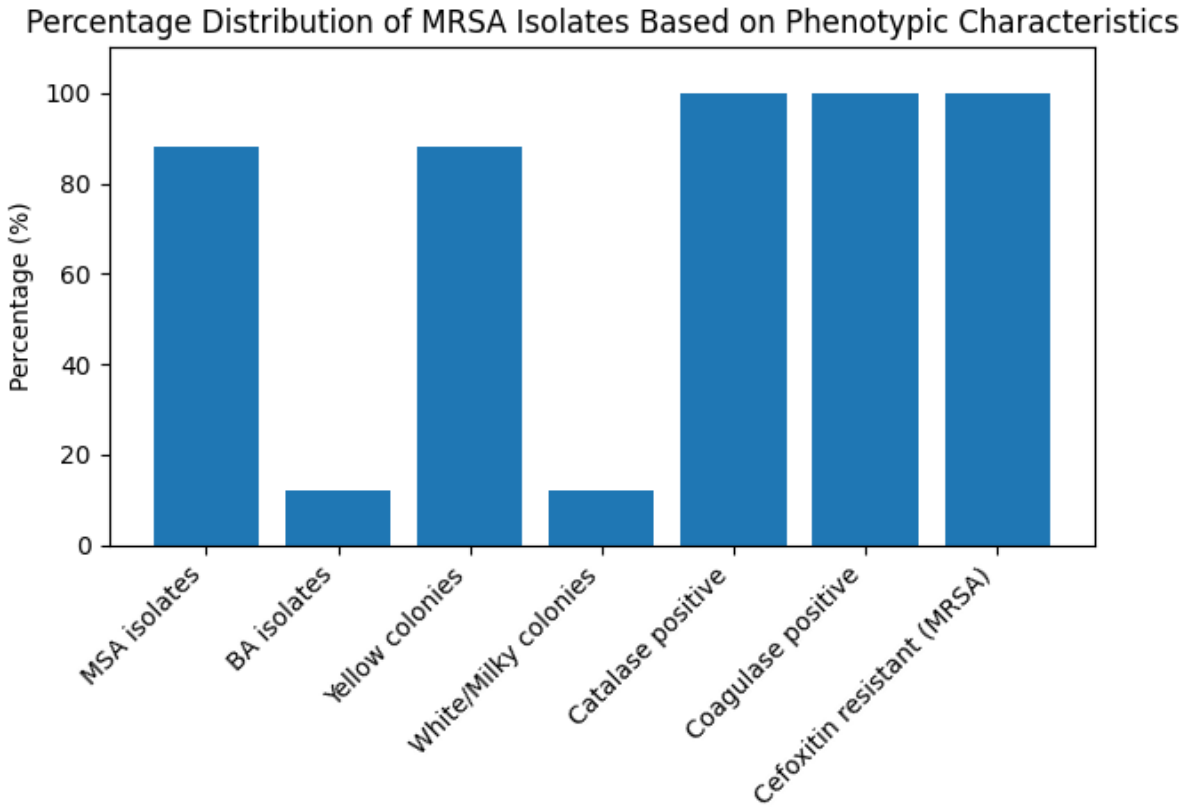

Chart. 1 Percentage distribution of MRSA isolates based on phenotypic characteristics

In the present study, phenotypic characterization of *Staphylococcus aureus* isolates revealed that 88% of MRSA isolates were recovered on Mannitol Salt Agar (MSA**)**, while only 12%were isolated on Blood Agar (BA**)**. The high recovery rate on MSA highlights its effectiveness as a selective and differential medium for the isolation of MRSA from respiratory specimens. Similar observations have been reported by **Smyth et al. (2005)**, who demonstrated that MSA enhances the detection of mannitol-fermenting *S. aureus* and facilitates early presumptive identification of MRSA.

Colony morphology analysis showed that 88% of isolates produced yellow pigmentedcolonies, indicating mannitol fermentation, whereas 12% showed white or milky colonies. This finding is consistent with reports by **Cheesbrough (2005) and Tortora et al. (2019),** who described yellow pigmentation on MSA as a characteristic feature of *S. aureus*, while non-pigmented colonies are often associated with other staphylococcal species.

Biochemical characterization revealed that 100% of isolates were catalase-positive andcoagulase-positive, confirming their identity as *Staphylococcus aureus*. Comparable results were reported by **Louie et al. (2000),** who documented universal catalase and

coagulase positivity among confirmed MRSA isolates. Furthermore, all isolates (100%) exhibitedcefoxitin resistance, indicating phenotypic methicillin resistance. Cefoxitin resistance has been widely accepted as a reliable surrogate marker for mecA-mediated methicillin resistance, as recommended by CLSI and supported by previous studies **(Murakami et al., 1991; Bhattacharya et al., 2008).**

Despite the low sample numbers, this study does begin to fill gaps and expand our understanding of the epi demiology of S. aureus by providing data on clinical isolates of S. aureus from other parts of the country as previous studies in Kenya have been limited to four healthcare institutions within close geographic proximity. The isolates in this study, collected over a 3-year period (2015 to Aug 2018), uncover patterns of distribution of different strain types that are interesting and will be explored further as part of the ongoing surveillance to examine whether the observed S. aureus distribution patterns hold and other patterns emerge over time with more isolates

When compared with other Indian and international studies, the findings of the present study demonstrate a higher proportion of confirmed MRSA among phenotypically screened isolates, emphasizing the increasing burden of MRSA in respiratory tract infections. Studies from India have reported MRSA prevalence ranging from 30–50% among *S. aureus* isolates from clinical samples, with similar resistance patterns to β-lactam antibiotics **(Indian Network forSurveillance of Antimicrobial Resistance, 2013)**. The 100% cefoxitin resistance observed in this study may reflect selective sampling of clinically significant isolates and increased antibiotic pressure in hospital settings.

Overall, the percentage bar chart-based analysis clearly demonstrates the dominance of mannitol-fermenting, biochemically confirmed, and cefoxitin-resistant *Staphylococcus aureus* isolates, reinforcing the reliability of conventional phenotypic methods for preliminary MRSA detection prior to molecular confirmation.

4. Conclusion

The present study highlights the significant prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) among respiratory tract infections and underscores its growing importance as a major clinical pathogen. A diverse range of respiratory specimens, including throat swabs, sputum, tracheal aspirates, and bronchoalveolar lavage samples, were

analyzed, enabling comprehensive evaluation of both upper and lower respiratory tract infections.

Phenotypic characterization using selective and differential media, along with standard biochemical tests such as Gram staining, catalase, and coagulase testing, proved effective for the identification of *Staphylococcus aureus*. The predominance of mannitol-fermenting yellow colonies on Mannitol Salt Agar further supported the reliability of conventional microbiological methods in routine diagnostics. The application of the cefoxitin disc diffusion method allowed efficient phenotypic detection of methicillin resistance, facilitating the identification of presumptive MRSA isolates.

The distribution of MRSA cases across different age groups and genders revealed that infections were not restricted to a specific demographic, indicating widespread circulation of MRSA in the community and hospital settings. The nearly equal gender distribution and higher prevalence among younger age groups emphasize the need for continued surveillance across all population categories.

Overall, the findings of this study demonstrate that MRSA is a prevalent pathogen in respiratory tract infections and reinforce the importance of early detection through phenotypic screening methods. Routine monitoring of MRSA prevalence and strict adherence to infection control practices are essential to limit its spread and to guide appropriate antimicrobial therapy. The study further supports the integration of conventional phenotypic methods with confirmatory approaches for effective diagnosis and management of MRSA-associated respiratory infections.

Acknowledgment

The authors would like to sincerely acknowledge the support and facilities provided by the CytoGene Research and Development, Lucknow. We are grateful for their assistance in carrying out the experiments and providing the necessary resources, which greatly contributed to the successful completion of this study.

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