ABSTRACT
Introduction: Antimicrobial resistance is on the rise, posing a global threat to the success of modern surgical procedures. A common and notorious superbug causing community and hospital-acquired infections is Methicillin-resistant Staphylococcus aureus (MRSA). Its potential for easy and rapid spread leads to increased mortality in hospitals. Clindamycin, a relatively inexpensive and effective drug, is empirically prescribed for the treatment of MRSA infections. Due to the risk of misidentifying clindamycin resistance as susceptibility, leading to treatment failure, its judicious use after D-test is advocated.

Aim: To isolate Staphylococcus aureus (S. aureus) from various clinical specimens and to identify MRSA using cefoxitin disc (30 µg), further determining the incidence of inducible clindamycin resistance among MRSA by the disc diffusion method (D-test).

Materials and Methods: The present cross-sectional study was conducted in the Department of Microbiology, Government Mohan Kumaramangalam Medical College Hospital (GMMKCH), Salem, Tamil Nadu, India, from June 2023 to August 2023. Study was conducted using 185 S. aureus isolates collected from samples received from patients of all genders treated at the present study hospital. Various clinical samples such as pus, sputum, blood, urine and fluids collected from patients treated at the study Institute Salem during the study period were included.

RESULTS: Among 185 S. aureus isolates, 130 (70.27%) were Methicillin-sensitive S. aureus (MSSA) and 55 (29.7%) were MRSA. Inducible clindamycin resistance was observed in 22 (40%) isolates, while 17 (30.9%) isolates showed constitutive resistance, 9 (16.36%) showed the MS phenotype, and the remaining 7 (12.72%) showed the susceptible phenotype. Among the clindamycin resistance patterns in MRSA, inducible clindamycin resistance was reported predominantly.

Conclusion: The majority of S. aureus was isolated from pus samples, highlighting its importance as a pyogenic microorganism. The current study records a high rate of MRSA resistance among S. aureus isolates. The present study reports a higher rate of inducible clindamycin resistance among MRSA isolates when compared to other phenotypes of clindamycin resistance. Therefore, routine D-testing must be implemented to identify inducible resistance to clindamycin in MRSA to avoid treatment failure.

INTRODUCTION
Staphylococcus aureus is a facultative anaerobe that produces toxins and causes pyogenic skin infections. Bacteremia due to S. aureus can occur from the spread of local infection to systems. It can also occur spontaneously in patients suffering from diabetes mellitus, renal and liver diseases [1]. The emergence of MRSA has complicated the treatment of patients due to their therapeutic resistance to current antibacterial drugs. MRSA increases the duration of hospitalisation by causing disseminated infections such as osteomyelitis, endocarditis and pneumonia [2]. MRSA is a global threat causing both nosocomial and community-acquired infections. MRSA harbouring the mecA gene enables methicillin resistance [3]. Cefibroprile, cefaroline, dalbavancin, iclaprim and delafloxacin are recent new antimicrobials against MRSA [4]. Clindamycin is still a drug of choice in both community and hospital-acquired MRSA. Macrolide, Lincosamide, Streptogramin B (MLSBI) antimicrobials are used for treating S. aureus infections. MLSB resistance is the resistance conferred to Macrolides, lincosamides and group B streptogramins (MLSBI). Clindamycin is a congener of lincosamine. The enzyme Ribosomal Ribonucleic Acid (rRNA) methylase (RM) expressed by S. aureus is encoded by the erm gene and exhibits a clindamycin-resistant phenotype (inducible or constitutive) [5]. Constitutive resistance (cMLSBI) in MRSA possesses resistance to erythromycin and clindamycin both in-vitro and in-vivo. Inducible resistance (iMLSBI) isolates possess resistance to clindamycin in-vivo, but in-vitro, they appear sensitive along with erythromycin resistance. So, when clindamycin is taken in-vivo, resistance to clindamycin results in treatment failure, which is troublesome for the physician and the patient. Therefore, it is necessary to perform the D-test to avoid treatment failure [5]. Erythromycin induces and activates methylase, expressing inducible clindamycin resistance [6]. Epidemiological information is absolutely necessary for medical personnel for relevant treatment against microbial threats. Precise characterisation of drug resistance patterns of MRSA is needed to devise medical institution protocols. The present study investigated the incidence of inducible clindamycin resistance (induced by erythromycin) in MRSA prevailing in Salem, Tamil Nadu, India. This kind of surveillance is necessary, as it varies according to patient group, bacterial strains and geographic area. As such, there is no available data on inducible clindamycin resistance from Government Mohan Kumaramangalam Medical College Hospital, Salem, so far. Moreover, the study will be an initiative, paving the pathway for the Institution to explore new techniques that provide a solution to antimicrobial resistance. Hence, the aim of the present study was...
to isolate Staphylococcus aureus from various clinical specimens and to identify MRSA using the cefoxitin disc (30 µg) and further determining the incidence of inducible clindamycin resistance among MRSA by disc diffusion method (D-test).

**MATERIALS AND METHODS**

The present cross-sectional study was conducted in the Department of Microbiology, GMKMCH, Salem, Tamil Nadu, India, from June 2023 to August 2023. The study commenced after clearance from the Institutional Ethical Committee of GMKMCH Salem, Tamil Nadu, India (Ref. No. GMKMCH/H/114/IEC/2023 dated 14.06.2023) and informed consent was obtained.

**Sample size calculation:** The sample size was calculated using the following formula: 
\[ n = \frac{Z^2 \times (p \times q)}{\varepsilon^2} \] 
In the present study, \( n = 1.96^2 \times (0.4 \times 0.6) / 0.07^2 = 188.16 \), where \( n = \) sample size, \( Z = 1.96 \) for 95% Confidence Interval (CI), \( p = \) prevalence of the study was taken from the previous study, 40% [7,8], \( q = 1-p \), \( \varepsilon = \) margin of error, 7%. Hence, a total of 185 S. aureus isolates were taken.

**Inclusion criteria:** Various clinical samples such as pus, sputum, blood, urine and fluids collected from patients treated in the hospital during the study period were included.

**Exclusion criteria:** The bacteria other than S. aureus were excluded from the study.

**Study Procedure**

Conventional media such as nutrient agar, blood agar and MacConkey agar were used to culture S. aureus isolates. Gram staining and routine biochemical tests such as mannan fermentation, indole and catalase were performed. Slide and tube coagulase tests were primarily used to confirm the presence of S. aureus. The control stains for S. aureus MRSA were ATCC-43300, and for S. aureus MSSA, it was ATCC-25923.

A suspension of S. aureus with 0.5 McFarland standard turbidity was prepared. The Mueller Hinton agar plate was inoculated with S. aureus using the lawn culture method. The disc diffusion technique, following the Kirby Bauer method, was used to test antibiotic discs such as cefoxitin (30 µg), ofloxacin (5 µg), vancomycin (30 µg), co-trimoxazole (25 µg), penicillin (10 µg), doxycycline (30 µg) and linezolid (30 µg). Drug sensitivity results were recorded based on the 2023 CLSI guidelines [9]. For cefoxitin (30 µg) disc testing, zones of 21 mm or less were considered indicative of MRSA.

The detection of inducible and constitutive clindamycin resistance was determined by performing the D-test. A clindamycin disc (2 µg) was placed 15-20 mm apart from an Erythromycin (15 µg) disc on a Mueller Hinton agar plate inoculated with MRSA suspension. The plates were then incubated at 37°C for 18 hours. The following clindamycin resistance phenotypes were observed and explained:

Inducible clindamycin resistance was labelled as D-test positive due to the D-shape susceptible zone of clindamycin (≥21 mm) with flattening adjacent to erythromycin, while erythromycin showed a resistance zone of ≤13 mm. Constitutive clindamycin resistance isolates exhibited erythromycin resistance with a zone of ≤13 mm and clindamycin resistance with a zone of ≤14 mm, displaying circular inhibition around the discs. The MS phenotype was designated for isolates manifesting an erythromycin resistance zone of ≤13 mm and a clindamycin susceptible zone of ≥21 mm. The MS phenotype was considered D-test negative due to a circular susceptible zone around clindamycin. Isolates with an erythromycin zone of ≥23 mm and a clindamycin zone of ≥21 mm were considered susceptible (S phenotype) [10].

**STATISTICAL ANALYSIS**

The data was descriptive and percentages were calculated for all the numerical data obtained for the comparison of data. The data was analysed using SPSS software version 21.0, employing appropriate statistical tests such as the Chi-square test. A p-value ≤0.005 was considered statistically significant. In situations where the Chi-square test could not be used, Fisher’s-exact test was employed for categorical data.

**RESULTS**

Out of 185 S. aureus samples, 55 were MRSA and 130 were MSSA. About 185 S. aureus were isolated from source such as pus, blood, sputum and urine. Pus constituted the major sample source, with 155 (83.7%) isolates, followed by blood with 18 (9.7%) isolates, urine with 10 (5.4%) isolates and sputum with 2 (1.08%) isolates. MRSA were predominantly isolated from pus samples, accounting for 44 (23.7%) isolates, with the remainder isolated from urine, 5 (2.7%) isolates, blood, 5 (2.7%) isolates and sputum, 1 (0.5%) isolate, as shown in [Table/Fig-1].

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total no. of MRSA, n (%)</th>
<th>Total no. of MSSA, n (%)</th>
<th>Total no. of Staphylococcus aureus isolates (N=185), n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus</td>
<td>44 (23.7)</td>
<td>111 (60)</td>
<td>155 (83.7)</td>
</tr>
<tr>
<td>Blood</td>
<td>5 (2.7)</td>
<td>13 (7)</td>
<td>18 (9.7)</td>
</tr>
<tr>
<td>Urine</td>
<td>5 (2.7)</td>
<td>5 (2.7)</td>
<td>10 (5.4)</td>
</tr>
<tr>
<td>Sputum</td>
<td>1 (0.5)</td>
<td>1 (0.5)</td>
<td>2 (1.08)</td>
</tr>
</tbody>
</table>

[Table/Fig-1]: Distribution of MRSA and MSSA in different samples.

Out of 55 MRSA cases, 31 (56.4%) were males, and 24 (43.6%) were females, as summarised in [Table/Fig-3].

<table>
<thead>
<tr>
<th>Sex</th>
<th>MRSA (N=55) n (%)</th>
<th>MSSA (N=130) n (%)</th>
<th>Total Staphylococcus aureus isolates (N=185), n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>31 (56.4)</td>
<td>79 (60.7)</td>
<td>110 (59.4)</td>
</tr>
<tr>
<td>Female</td>
<td>24 (43.6)</td>
<td>51 (39.2)</td>
<td>75 (40.5)</td>
</tr>
</tbody>
</table>

[Table/Fig-3]: Gender distribution of Staphylococcus aureus (S. aureus) isolates.

The MRSA were isolated mainly from orthopaedic and surgical wards. Complete resistance to penicillin was observed in MRSA, while their susceptibility to vancomycin and linezolid was 100.9%. Moderate resistance was observed for ofloxacin in 28 (50.9%) isolates, doxycycline in 21 (38.18%) isolates, and co-trimoxazole in 24 (43.63%) isolates. MSSA showed 100% susceptibility to linezolid and vancomycin, while their susceptibility to doxycycline, ofloxacin, and co-trimoxazole was 119 (91.53%), 115 (88.46%) and 109 (83.84%), respectively. The highest percentage of resistance for MSSA was noticed in penicillin, with 129 (99.23%) cases showing resistance. When compared to MSSA, antibiotic resistance in MRSA was at higher levels in the present study, as shown in [Table/Fig-4].

Among MSSA isolates, the susceptible phenotype predominated, comprising 97 (74.61%) of the total, followed by the inducible clindamycin resistance phenotype with 15 (11.53%) isolates, constitutive clindamycin resistance with 11 (8.46%) isolates, and the MS phenotype with 7 (5.38%) isolates. The present study reports a higher rate of inducible clindamycin resistance among MRSA isolates, with 22 (40%) isolates, followed by constitutive clindamycin resistance with 17 (30.9%) isolates, the MS phenotype with 9 (16.36%) isolates, and the susceptible phenotype with 7 (12.72%) isolates, as summarised in [Table/Fig-5].
When comparing MRSA and MSSA, clindamycin resistance is significantly higher in MRSA 39 (70.9%), than in MSSA 26 (20%). Additionally, clindamycin sensitivity is higher in MSSA, with 104 (80%), than in MRSA, with 16 (29.09%), as shown in Table/Fig-6.

The comparison between the inducible clindamycin resistance and constitutive clindamycin resistance phenotypes in MRSA and MSSA isolates did not reveal any significant association (p-value=0.91), as depicted in Table/Fig-7.

### DISCUSSION

Clindamycin belongs to the MLSB group of antibiotics used against MRSA [7]. It is an alternative drug in penicillin allergy with excellent tissue penetration [12]. This drug can be given orally as outpatient therapy, due to its outstanding activity against infections [10]. Clindamycin reduces toxin production [6]. This drug can be given orally as outpatient therapy, due to its outstanding activity against infections [10]. Clindamycin reduces toxin production [6].

Staphylococcus aureus was mainly isolated from pus samples (151, 83.7%), followed by blood, 18 (9.7%), urine, 10 (5.4%) and sputum, 2 (1.08%). This aligns with Tyagi S et al., study, which recorded the highest number of S.aureus in pus samples, 778 (63.1%), followed by blood, urine, and respiratory fluids with 369 (29.9%), 59 (4.8%) and 14 (1.2%) isolates, respectively [1].

Among the 185 S.aureus isolates, 130 (70.27%) were MSSA and 55 (29.7%) were MRSA in the current analysis. This is consistent with Ghosh S et al., who revealed that, out of the 46 S.aureus isolates tested, 11 (23.9%) were methicillin-resistant and 35 (76.1%) were methicillin-sensitive [11].

The present study showed an increased number of MRSA from pus samples, with 44 (23.7%) cases, followed by 5 (2.7%) in blood samples, 5 (2.7%) in urine samples and 1 (0.5%) in sputum samples. This coincides with the research of Archana GJ et al., which reported MRSA in pus exudates of 22 (73.3%) isolates, 6 (20%) isolates in blood samples, and the least in sputum, with 2 (6.7%) isolates [3]. On the other hand, Chaudhary BL et al., documented 288 MRSA isolates, mainly from blood, with the highest being 101 (35.07%), followed by pus with 97 (33.68%), and the least from body fluids, with 2 (0.69%) [12]. According to Sasirekha B et al., out of 42 MRSA isolates, 32 (76.19%) were from male patients and 10 (23.80%) were from female patients, showing male preponderance [5].

A greater number of MRSA cases were seen in Orthopaedic and Surgical ward patients, due to postoperative wound infections, which correlated with the study of Khan AS et al., [13]. This is because operations for fractures lead to prolonged hospitalisation. Factors such as preoperative and postoperative care, overcrowding of attendants in the ward, theatre sterility, type of surgery and infection control measures followed in hospitals contribute to serious infections in the surgical area. Microbial contamination of the wound from the surroundings is a feasible cause [13], Tyagi A et al., documented that excessive usage of antibiotics in surgical wards and intensive medical care units leads to a high intensity of resistant pathogens [14].

Compared to MSSA, antibiotic resistance to MRSA was at higher levels in the present study. Ghosh S et al., revealed full sensitivity of S.aureus to both linezolid and vancomycin, similar to the present study [11]. In concordance with the study of Perumal PG et al., MRSA isolates were 100% resistant to penicillin, while clomoxazol showed less resistance [15]. Likewise, this coincided with a study by Alfekey AE et al., which stated that MRSA isolates had the highest resistance rate for penicillin (100%), followed by doxycycline (57.2%), with linezolid showing the highest sensitivity (92%) [16]. The majority of MRSA were sensitive to ofloxacin (70.4%) and tetracycline (83.6%), as per the study of Kishik RM et al., [17].

The present analysis detects a predominance of the susceptible phenotype in 97 (74.61%) isolates of MSSA. Similar to the current study, Prabhud K et al., documented higher percentages of erythromycin and clindamycin-sensitive MSSA isolates at 106 (81.64%), followed by 8 (6.15%) constitutive phenotype,
Inducible and constitutive clindamycin resistance were reported in a higher percentage with MRSA than MSSA in the current research. Similarly, Panwala T et al. also documented constitutive clindamycin resistance in MRSA as in 19 (15.44%) isolates and in MSSA as 16 (14.67%) isolates. They also reported inducible clindamycin resistance in MRSA as 73 (59.34%) isolates and in MSSA as 14 (12.84%) isolates [19].

In contrast to the present study by Bala et al., which reported a higher rate of 98 (51.6%) isolates with constitutive clindamycin resistance, followed by 42 (22.1%) isolates with inducible clindamycin resistance, 6 (3.1%) with the MS phenotype, and 44 (23.1%) with the S phenotype, Banik et al. also reported an increased incidence of the constitutive resistance phenotype [6,20].

Contrary to the present analysis, Sasirekha B et al., documented a higher rate of susceptible phenotype in 26 (16.99%) MRSA isolates, with constitutive resistance at 5.22% and inducible clindamycin resistance at 0.65% [5]. Mallamgunta S et al., showed the highest percentage of the MS phenotype at 25 (40%) in S. aureus [21]. Furthermore, Regmi RS et al., reported a 21 (60%) incidence of the MS phenotype in MRSA isolates [22].

Variations in the prevalence of various phenotypes of clindamycin resistance in different studies could be due to the excessive use of antimicrobials on an empirical basis for common infections, variations in the study population, and differences in bacterial sensitivity in different regions. Strict enforcement of rules for dispensing antibiotics with a prescription is the need of the hour. Infection control measures must be strictly followed, and operation theatre surveillance should be regularly monitored to prevent the spread of clindamycin-resistant MRSA in hospitals.

The incidence rate of MRSA, as per the World Health Organisation (WHO), surpasses 20% in all zones and is even higher in some countries, reaching up to 80% [23]. In the present study, the incidence rate of tracked MRSA was 29.7%. This discrepancy in resistance patterns in different geographical areas emphasises the importance of scrutinising pertinent resistance data locally.

In summary, out of 185 S. aureus isolates, 55 (29.7%) were MRSA, with most of them isolated from pus samples. Inducible clindamycin resistance was the highest reported phenotype, as shown by 22 (40%) isolates in the present study. Novel drugs like glycopeptide-cephalosporin antibiotic (TD-1792), tedizolid, radezolid, eravacycline, omadacycline, zaborfloxacin, nemonoxacin, retapamin and lefamulin are some of the drugs under clinical trial against MRSA [23]. Until their approval, routine D-testing is advocated. Considering the high incidence of inducible clindamycin resistance, the current study recommends the use of clindamycin only after the D-test in routine drug susceptibility testing. Institutional upgradation is needed in detecting erm genes, which helps in the rapid diagnosis of inducible clindamycin resistance. Quorum sensing inhibitors, lectin inhibition, iron chelation, phage therapy, nanoparticles, are some of the future therapeutic strategies under research in the battle against MRSA [24].

**Limitation(s)**

The agar dilution method and broth microdilution method are not routinely performed in the institutional set-up.

**CONCLUSION(S)**

Most of the S. aureus isolated from pus samples, which concludes that it is an important pyogenic microorganism. The current study indicates a high-rate of MRSA resistance among S. aureus isolates. The present study reports a higher rate of inducible clindamycin resistance among MRSA isolates when compared to other phenotypes of clindamycin resistance. Vancomycin and Linezolid showed maximum sensitivity with respect to MRSA, and can be used as promising drugs for the treatment of severe infections associated with the same. The present study highlighted the value of the simple “D” test, which must be incorporated into routine disc diffusion tests for identifying inducible clindamycin resistance, preventing misidentification of clindamycin resistance as susceptibility, and thus avoiding treatment failure.

**Acknowledgement**

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