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BACTERIOLOGICAL PROFILE OF *STAPHYLOCOCCUS AUREUS* WITH EICR (ERYTHROMYCIN INDUCED CLINDAMYCIN RESISTANT) AND MRSA (METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS)* IN TERTIARY CARE HOSPITAL, RAJKOT

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INTRODUCTION

Staphylococcus aureus (S. aureus) remains a significant human pathogen responsible for a wide spectrum of clinical manifestations, ranging from minor skin and soft tissue infections to life-threatening conditions such as pneumonia, endocarditis, and septicemia.(3) In the environment of tertiary care hospitals, the management of staphylococcal infections is increasingly complicated by the emergence of multidrug-resistant strains, which contribute to higher morbidity, mortality, and healthcare costs.(1)Globally, The resistance to antimicrobial agents among Staphylococcus aureus is an increasing and it is a leading cause of nosocomial and community-acquired infections.(10) A major challenge in contemporary clinical practice is the rising prevalence of Methicillin-Resistant Staphylococcus aureus (MRSA). MRSA is considered a severe global threat because it is resistant to nearly all beta-lactam antibiotics, leaving clinicians with limited therapeutic options(3). This resistance has led to a renewed interest in the Macrolide-Lincosamide-Streptogramin B (MLSB) family of antibiotics, particularly clindamycin, due to its excellent tissue penetration, low cost, and ability to inhibit bacterial toxin production.(1)(5)However, the clinical utility of clindamycin is often compromised by various resistance mechanisms. Resistance to MLSB antibiotics is primarily mediated by erm (erythromycin ribosome methylase) genes, which modify the ribosomal target site.(4)This resistance can be expressed in two ways: Constitutive (cMLSB): The bacteria are resistant to both erythromycin and clindamycin in vitro tests and Inducible (iMLSB or EICR): Inducible MLSB phenotype showing resistance to Erythromycin with a flattened zone of inhibition around Clindamycin, forming a D-shaped zone of inhibition.(7)(2) The phenomenon of Erythromycin-Induced Clindamycin Resistance (EICR) is particularly dangerous because if these isolates are misidentified as clindamycin-susceptible, treatment can result in clinical failure as resistant mutants emerge during therapy.(4)Since standard susceptibility methods do not detect this inducible phenotype, the use of the D-zone test (disk approximation test) is essential for accurate reporting.(6) Given the regional variations in the prevalence of MRSA and EICR, periodic monitoring of the bacteriological profile and resistance patterns is vital for formulating effective empirical treatment protocols in tertiary care settings. This study aims to determine the prevalence of MRSA and different clindamycin resistance phenotypes among S. aureus isolates at our institution.

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AIM AND OBJECTIVES

- To study the resistance pattern of *Staphylococcus aureus* among various clinical isolates.
- To identify Methicillin Resistance *Staphylococcus aureus* (MRSA).
- To identify Inducible Clindamycin Resistance in *Staphylococcus aureus* (EICR).

MATERIALS AND METHODS

This Retrospective study was conducted in the Department of Microbiology at PDU Medical College,Rajkot a period of 6 months from July 2024 to December 2024.*S. aureus* isolates was identified by conventional methods such as colony morphology on culture medium, Gram staining, catalase activity, slide& tube coagulase tests and Mannitol fermentation test. Antibiotic susceptibility testing were performed by Kirby-Bauer disc diffusion method and interpretation was done according to current CLSI guidelines. Ethical clearance was obtained from the Institutional Ethics Committee (IEC) prior to the commencement of the study.

Sample Collection and Identification

A total 391clinical specimen were obtained which include pus, wound swabsand blood. The isolates were identified as S. aureus based on:

- Colony morphology:

  Nutrient agar- Showed 1 to 3 mm diameter,circular, smooth, low convex,glistening densely opaque colonies with golden yellow pigmentation.colonies were surrounded by a narrow zone of beta hemolysis. MacConkey agar: Colonies were pink and smaller in size.

- Gram Staining: Observation of Gram-positive cocci in clusters.
- Biochemical Tests: Positive results for catalase, slide/tube coagulase, and mannitol fermentation tests.
- Antibiotic Susceptibility Testing (AST)

All isolates were subjected to antimicrobial susceptibility testing using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (MHA), following the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

Detection of MRSA: Cefoxitin (30 µg) discs were used. Isolates with a zone of inhibition ≤ 21 mm were reported as Methicillin-Resistant (CLSI, 2024).

Detection of EICR (The D-Zone Test): To detect inducible clindamycin resistance, an Erythromycin (15 µg) disc and a Clindamycin (20µg) disc were placed manually at a distance of 15 mm (edge to edge) on an MHA plate inoculated with the test organism.

Interpretation of D-Test Phenotypes

After incubation at 37°C for 18–24 hours, the following patterns were recorded:

MS Phenotype: Resistance to Erythromycin but Susceptibility to Clindamycin (circular zone).

iMLSB Phenotype (D-test positive): Resistance to Erythromycin with a flattened zone of inhibition around Clindamycin, forming a "D" shape.

cMLSB Phenotype: Resistance to both Erythromycin and Clindamycin.

RESULT

Table 1. Distribution of S. aureus in Clinical Samples

Sample type	Total Number of clinical samples	Total number of Positive clinical samples	Percentage (%) (n=152)
Pus	221	84	55.26%
Wound swab	52	26	17.11%
Blood	118	42	27.63%
Total	391	152	100%

Table 2. Prevalence of MRSA vs. MSSA

Type of isolates	Number	Percentage (%)
MRSA (Methicillin-Resistant)	112	73.68%
MSSA (Methicillin-Sensitive)	40	26.32%
Total	152	100%

Table 3. Antibiotic susceptibility pattern of Methicillin resistant Staphylococcus aureus isolates

Antibiotics	Sensitive (%)	Resistant (%)
Penicillin	0 %	100 %
Cefoxitin	0 %	100 %
Gentamycin	65 %	35 %
Tetracycline	94 %	6 %
Ciprofloxacin	78 %	22 %
Levofloxacin	72 %	28 %
Cotrimoxazole	25 %	75 %
Chloramphenicol	100 %	0 %
Linezolid	100 %	0 %
Vancomycin	100 %	0 %

Table 4. Correlation between Methicillin resistance and Clindamycin resistance

Phenotype	MRSA (n=112)	MSSA (n=40)	Total (n=152)
E=S, C=S	15.18%(17)	20%(8)	16.45%(25)
E=R, C=R (D-ZONE Positive) iMLSB	37.5%(42)	15% (6)	31.57% (48)
E=R, C=R (D-ZONE Negative) MS	19.65%(22)	25%(10)	21.05%(32)
E=R, C=R cMLSB	27.67%(31)	40%(16)	30.92%(47)

DISCUSSION

The current study analyzed 391 clinical samples, of which 152 (38.87%) were positive for Staphylococcus aureus. This isolation rate highlights the significant burden of staphylococcal infections within our tertiary care setting.

In the present study, Staphylococcus aureus was isolated from 38.87% (152/391) of clinical samples. The highest recovery was from Pus samples (55.26%), followed by Blood (27.63%) and Wound swabs (17.11%). The high isolation rate from pus and wound swabs (totaling over 72%) reinforces the standing of S. aureus as the leading cause of pyogenic infections and skin and soft tissue infections (SSTIs) in hospital settings.

A critical finding of this study is the high prevalence of Methicillin-Resistant Staphylococcus aureus (MRSA), which accounted for 73.68% (112/152) of the isolates.This rate is significantly higher than many national averages, suggesting a high selection pressure of antibiotics or a robust presence of resistant clones within the tertiary care environment.

Only 26.32% of the isolates were Methicillin-Sensitive (MSSA), indicating that MRSA is no longer an occasional finding but the dominant phenotype in our facility.

The susceptibility profile of MRSA isolates (Table 3) presents a challenging therapeutic landscape:As expected for MRSA, there was 100% resistance to Penicillin and Cefoxitin.A notably high resistance rate was observed for Cotrimoxazole (75%), limiting its utility as an oral alternative for MRSA infections. Resistance to Ciprofloxacin (22%) and Levofloxacin (28%) was relatively moderate but signifies an increasing trend toward multi-drug resistance.

Fortunately, several antibiotics maintained excellent efficacy against these MRSA strains with100% Sensitivity. Chloramphenicol, Linezolid, and Vancomycin all showed 0% resistance. Vancomycin & Linezolid are remain the "gold standard" and the most reliable reserve drugs for treating serious MRSA infections in our hospital.

Gentamycin (65% sensitive) and Tetracycline (94% sensitive) still show promising activity, which may be useful in combination therapies or for less severe infections.

The most clinically significant aspect of this study is the correlation between methicillin resistance and iMLSB (inducible Macrolide-Lincosamide-Streptogramin B resistance), commonly referred to as EICR.

* Prevalence of iMLSB: Out of 152 isolates, 31.57% (48/152) were D-test positive, indicating inducible clindamycin resistance.

* MRSA vs. MSSA Correlation: There was a notably higher frequency of the iMLSB phenotype in MRSA (37.5%) compared to MSSA (15%). This suggests that methicillin-resistant strains are more likely to harbor the erm genes responsible for inducible resistance.

* Constitutive Resistance (cMLSB): the MSSA group showed a higher percentage of cMLSB (40%) compared to the MRSA group (27.67%).

The discovery that 37.5% of MRSA isolates possess inducible clindamycin resistance is a critical warning for clinicians. If the D-test is not performed, these isolates would be reported as "Clindamycin Sensitive" by routine disk diffusion, potentially leading to therapeutic failure when used in a clinical setting.

Only 15.18% of MRSA isolates were truly sensitive to both Erythromycin and Clindamycin. This underscores the fact that Clindamycin should not be used for MRSA infections in our hospital without a negative D-test result.

The isolation rate of S. aureus in your study was 38.87%, with a staggering 73.68% identified as MRSA. This is notably higher than the national Indian average reported by the ICMR Antimicrobial Resistance Surveillance Network, which typically ranges between 30% and 50%. This study findings more closely with studies from high-burden tertiary centers, such as Murugan et al. [8](2022), who reported MRSA rates exceeding 60% in similar hospital settings. The high resistance to Cotrimoxazole (75%) in your MRSA isolates further suggests a multi-drug resistant (MDR) profile common in hospital-acquired strains.

This study found that iMLSB was significantly higher in MRSA (37.5%) than in MSSA (15%).This trend is mirrored in a study by Dey et al. [9](2021), where iMLSB was found in 33.3% of MRSA isolates compared to only 12% in MSSA. The higher prevalence in MRSA suggests that the erm genes are often co-located with methicillin-resistance determinants on mobile genetic elements.

CONCLUSION

This study underscores a critical epidemiological challenge in our tertiary care hospital, characterized by an exceptionally high prevalence of MRSA (73.68%). Furthermore, the significant burden of inducible clindamycin resistance (31.57%), particularly within MRSA isolates (37.5%), highlights a severe limitation in therapeutic options for routine skin and soft tissue infections.

* Mandatory Screening: Routine D-testing must be implemented for all S. aureus isolates demonstrating resistance to Erythromycin to prevent clinical failure associated with masked clindamycin resistance.

* Empiric Therapy: Given the high MRSA rate, empirical treatment protocols should be re-evaluated. Vancomycin and Linezolid remain the most reliable options, displaying 100% sensitivity, but their usage must be governed by strict antimicrobial stewardship to prevent the emergence of further resistance.

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