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Prevalence of inducible clindamycin resistance amongst methicillin resistant and sensitive strains of Staphylococcus aureus from a tertiary care hospital, West Bengal, India.

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Abstract

Background: Staphylococcus aureus is a prevalent pathogen causing both nosocomial and community-acquired infections worldwide. The emergence of methicillin-resistant Staphylococcus aureus (MRSA) due to the acquisition of mecA and mecC genes poses a significant clinical challenge. The injudicious use of clindamycin for treating MRSA has led to the development of clindamycin resistance. This study aimed to determine the prevalence of inducible clindamycin resistance (iMLSB resistance phenotype) in Staphylococcus aureus isolates, employing the D test according to CLSI guidelines, particularly focusing on erythromycin-resistant strains.

Methodology: A total of 147 Staphylococcus aureus isolates were subjected to antibiotic susceptibility testing using the Kirby Bauer disc diffusion method. The D test was employed to identify inducible clindamycin resistance.

Results: The study revealed that 34% of isolates exhibited inducible clindamycin resistance, 40% demonstrated constitutive resistance, and the remaining 26% exhibited the MS phenotype. Notably, inducible clindamycin resistance was more prevalent in MRSA (40%) compared to MSSA (22%).

Conclusion: The findings underscore the importance of incorporating the D test as a mandatory procedure in standard disc diffusion testing to accurately identify inducible clindamycin resistance. This knowledge is crucial for guiding appropriate antibiotic therapy in the face of increasing resistance patterns.

Keywords

Inducible Clindamycin Resistance, Methicillin-Resistant Staphylococcus Aureus, Meticillin-Sensitive Staphylococcus Aureus, D-Test, Kirby Disc Diffusion Method.

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Introduction

Staphylococcus aureus, commonly referred to as S. aureus, stands as a globally recognized pathogen responsible for a spectrum of nosocomial and healthcare-associated infections, encompassing skin and soft tissue infections, abscesses, pneumonia, osteomyelitis, endocarditis, sepsis, and arthritis[1]. The emergence of methicillin-resistant strains, denoted as MRSA[2], has become a critical concern, driven by the acquisition of the highly transmissible mecA and mecC genes. The prevalence of MRSA worldwide is notably substantial, with a pooled global prevalence of 14.69%[3], and in India, it ranges from 40% to 70% among S. aureus isolates[4].

The primary mode of MRSA transmission involves contact, particularly within healthcare settings, and community spread occurs through contact with infected wounds and shared personal items[5]. Notably, healthcare-associated MRSA (HA-MRSA)[6] transmission frequently occurs through cross-infection by healthcare providers, with surgical site infections being a common outcome.

Clindamycin, a crucial antibiotic in the treatment of MRSA infections, is susceptible to resistance development, leading to therapeutic challenges. The mechanisms of clindamycin resistance involve genes such as msrA, erm(A), and erm(C), contributing to constitutive MLSB (cMLSB)[7] and inducible MLSB (iMLSB) phenotypes. The empirical use of clindamycin without prior determination of these resistance mechanisms has contributed to the alarming rise in clindamycin resistance[8-10].

Hence, this study was undertaken to assess the prevalence of iMLSB and related resistance genes among Staphylococcus aureus isolates in our institution. Recognizing and understanding the resistance patterns is vital for optimizing treatment strategies and curbing the spread of resistant strains. The identification of various clindamycin resistance phenotypes, including iMLSB, cMLSB, and MS, will contribute valuable insights to guide empirical antibiotic use and enhance the efficacy of MRSA infection management.

Methodology

Study Design

This study was designed as a prospective observational study conducted over a one-year period, from January to December 2023.

Setting

The study was conducted at the Department of Microbiology, Calcutta National Medical College. Clinical samples were collected from patients with informed consent. The laboratory procedures followed the guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI), specifically the M100 performance standard for antimicrobial susceptibility testing 33rd edition 2023.

Participants

The study included 147 non-repeated isolates of S. aureus obtained from clinical specimens, such as pus, blood, and wound swabs. The samples were collected from both male and female patients, spanning all age groups. The participants attended the Outpatient Department (OPD) and Inpatient Department (IPD) of CNMC, Kolkata.

Isolation and Identification

On sheep blood agar and MacConkey agar, specimens were inoculated and aerobically incubated for 24 hours at 37 °C. Identification of S. aureus was carried out based on colony morphology, Gram stain, catalase test, and coagulase test. Colonies ranging from cream to golden yellow with or without haemolysis were considered for further analysis[11].

Antibiotic Susceptibility Test

The antibiotic susceptibility test was performed on Mueller Hinton Agar plates using Kirby Bauer's disc diffusion method. The following antibiotics and their respective disc concentrations were used: ampicillin (10µg), cotrimoxazole (25µg), ciprofloxacin (5µg), vancomycin (30µg), linezolid (30µg), doxycycline (30µg), ceftriaxone (30µg), and gentamycin (10µg). Methicillin resistance was determined using cefoxitin, and inducible clindamycin resistance was identified using erythromycin and clindamycin discs[7].

Statistical Methods

In this study, descriptive statistics were employed to summarize the demographic characteristics of the study population, including the prevalence of MRSA across various age groups and genders. Inferential statistics, such as confidence intervals and hypothesis testing, were likely used to make broader inferences about the population based on the observed data. The chi-square test was applied to analyze categorical data, examining the distribution of MRSA isolates. Trend analysis was conducted to identify significant patterns in MRSA isolates across different age groups. Antibiotic susceptibility analysis, involved statistical comparisons to determine significant differences in sensitivity patterns among MRSA isolates. Additionally, potential association tests were performed to assess relationships between variables, such as age groups and MRSA prevalence.

Results

In our study, out of 215 processed samples, 147 demonstrated positive growth of S. aureus, with 100 (68%) identified as MRSA. Notably, the 21-40 age group exhibited the highest rate of MRSA isolation.

Table 1: Distribution of MRSA vs MSSA and Demographic data for MRSA isolates.

Variables		N(%)
Gender (n=100)	Male	65(65)
	Female	35(35)
Age Groups (n=100)	0-20 years	23(23)
	21-40 years	36(36)
	41-60 years	25(25)
	61-80 years	16(16)
Distribution of Isolates (n=147)	MRSA	100(68)
	MSSA	47(32)
Type of Sample (n=100)	Blood	37(37)
	Pus Swab	36(36)
	Wound Swab	27(27)

Antimicrobial sensitivity tests conducted among MRSA isolates revealed resistance to Amoxicillin/clavulanic acid and ceftriaxone, while demonstrating high sensitivity to vancomycin and linezolid. The comprehensive results of antibiotic susceptibility testing are presented in table 2.

Table 2: Antibiotic Susceptibility Test for Methicillin-Resistant Staphylococcus Aureus Isolates.

Antibiotic Used	Sensitive (%)	Resistant (%)
Vancomycin	100	00
Levofloxacin	72	28
Linezolid	100	00
Co-trimoxazole	20	80
Amoxycillin/Clavulanic Acid	00	100
Ciprofloxacin	06	94
Gentamycin	74	26
Cefoxitin	00	100
Doxycycline	38	62
Ceftriaxone	00	100

According to table 3, the distribution of iMLSB, cMLSB among MRSA, MSSA, and total isolates indicated a higher rate of inducible clindamycin resistance in MRSA compared to MSSA.

Table 3: Susceptibility Pattern of Clindamycin and Erythromycin among the Isolates.

Susceptibility Pattern (phenotype)	MRSA (%) (N=100)	MSSA (%) (N=47)	Total (%) (N=147)
E=R, C=S (D test +ve) = iMLSB	40	22	34
E=R, C=S (D test -ve) = MS	24	27	26
E=R, C=R cMLSB	36	51	40

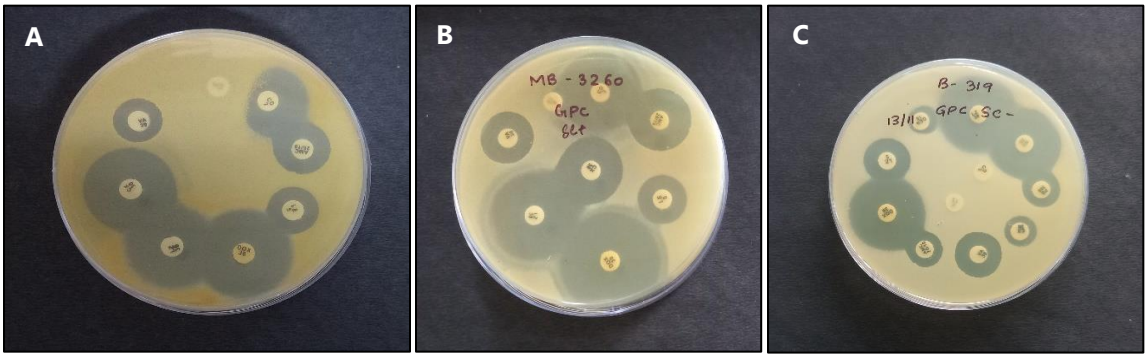

Figure 1: Disc Diffusion Test for Inducible Clindamycin Resistance.

(a) Erythromycin-resistant and clindamycin-sensitive staphylococcal isolate shows D-shaped zone of inhibition around clindamycin - inducible MLSB phenotype. (b) Erythromycin-resistant and clindamycin-sensitive staphylococcal isolate with a sensitive zone of inhibition around clindamycin - MS phenotype. (c) Staphylococcal isolate resistant to both erythromycin and clindamycin - constitutive MLSB phenotype.

Discussion

The examination of clindamycin resistance phenotypes among erythromycin-resistant S. aureus isolates, as determined by the D test, yielded notably significant results in our study. The overall prevalence of iMLSB was 34%, with 40% observed in MRSA and 22% in MSSA. The higher occurrence of iMLSB in MRSA compared to MSSA suggests that clindamycin therapy may be more efficacious for MSSA infections than for MRSA. Comparable findings were observed in other Indian studies, including Odisha (22%), Kashmir Valley (5.2%), Assam (7%), Chennai (15.2%), and Central India (14.8%). Our study's results closely aligned with global statistics, although variations in prevalence across regions could be attributed to differences in study populations, antibiotic usage, sample sizes, and infection control policies. The prevalence of constitutive clindamycin resistance (cMLSB) in our study was 40%, comprising 36% MRSA and 51% MSSA.

The overall prevalence of cMLSB exceeded that of iMLSB, highlighting the transition of many iMLSB strains to the cMLSB phenotype during continued treatment. The total MS phenotype was 26%, slightly higher than observed in some studies but consistent with others[10]. The D test, a routine laboratory tool, guides clinicians in the judicious use of clindamycin, particularly in skin and soft tissue infections, as recommended by CLSI[12,13]. The discussion emphasizes the critical threat posed by MRSA in causing pneumonia, septicemia, and various infections, underscoring the importance of

hospital infection control programs prioritizing MRSA containment. To comprehend the molecular epidemiology and dissemination of MRSA strains[14], various molecular typing techniques have been developed, requiring selectivity, standardization, repeatability, affordability, and accessibility[15].

Infection control strategies, including patient isolation and decolonization therapy, along with protective measures for visitors and healthcare personnel, are vital to halt further MRSA spread. Stringent adherence to hand hygiene practices, routine cleaning, and proper disinfection of hospital supplies, rooms, surfaces, and equipment are crucial components in reducing the risk of healthcare-associated MRSA infections[16-19]. The study acknowledges its limitations, such as a representation of the hospital population rather than the entire state, a modest sample size, and the inability to conduct genotyping due to resource constraints. Nevertheless, the study underscores the importance of routine D-test inclusion and methicillin resistance testing in susceptibility assessments for effective S. aureus management. Continuous surveillance for inducible clindamycin resistance is crucial to prevent treatment failures, with clinicians urged to consider alternative therapies like Vancomycin and Linezolid in cases of inducible clindamycin resistance.

Limitations

This study, while providing valuable insights, has several limitations. Firstly, the determination of prevalence is confined to our hospital population, potentially limiting its generalizability to the broader state population. The study's reliance on a relatively smaller sample size introduces a degree of bias, and caution should be exercised in extrapolating findings to larger populations. Additionally, due to resource constraints and limited funding, genotyping of S. aureus was not feasible, which could have offered a more comprehensive understanding of strain diversity. Moreover, the study did not assess the Minimum Inhibitory Concentration (MIC) of the antibiotics used, which could have provided important information on the drugs' effectiveness.

Conclusion

The observed high percentages of Methicillin-Resistant Staphylococcus aureus (MRSA) and inducible clindamycin resistance (iMLSB) phenotypes among erythromycin-resistant S. aureus underscore the critical need for the routine inclusion of the D-test and methicillin resistance testing in susceptibility assessments. These measures are essential for the effective management of S. aureus infections. The geographical location, drug usage patterns, and infection trends play pivotal roles in influencing the incidence of Macrolide-Lincosamide-Streptogramin B (MLSB) resistance. To avert treatment failures, continuous surveillance for iMLSB resistance using the D-test is imperative, especially for S. aureus isolates resistant to erythromycin. Clinicians must be cognizant of the existence of in vitro inducible clindamycin resistance. In cases presenting such resistance, alternative therapies, such as Vancomycin and Linezolid, should be contemplated to ensure optimal patient outcomes. This study provides a foundation for future research endeavors aimed at addressing these limitations and advancing our understanding of antimicrobial resistance dynamics in S. aureus infections.

Conflicts of Interest

The authors affirm that they have no conflicts of interest related to this publication.

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References

1. Prabhu K, Rao S, Rao V. Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. J Lab Physicians. 2011;3(01):025-027.
2. Goudarzi M, Razeghi M, Chirani AS, Fazeli M, Tayebi Z, Pouriran R. Characteristics of methicillin-resistant Staphylococcus aureus carrying the toxic shock syndrome toxin gene: high prevalence of clonal complex 22 strains and the emergence of new spa types t223 and t605 in Iran. New microbes new infect. 2020;36:100695.
3. Hasanpour AH, Sepidarkish M, Mollalo A, Ardekani A, Almukhtar M, Mechaal A, Hosseini SR, Bayani M, Javanian M, Rostami A. The global prevalence of methicillin-resistant Staphylococcus aureus colonization in residents of elderly care centers: a systematic review and meta-analysis. Antimicrob Resist Infect Control. 2023;12(1):4.
4. Singhal T, Rodrigues C, Soman R, Wattal C, Swaminathan S, Nambi S, Talwar D, Singh RK, Todi S. Treatment of MRSA infections in India: clinical insights from a Delphi analysis. Indian J Med Microbiol. 2022;40(1):35-45.
5. Pantosti A, Venditti M. What is MRSA? Eur Respir J. 2009;34(5):1190-1196.
6. Methicillin-resistant Staphylococcus aureus (MRSA). Centers for Disease Control and Prevention. Available at: https://www.cdc.gov/mrsa/community/index.html
7. Assefa M. Inducible clindamycin-resistant Staphylococcus aureus strains in Africa: a systematic review. Int. J. Microbiol. 2022:1835603
8. Adhikari RP, Shrestha S, Barakoti A, Amatya R. Inducible clindamycin and methicillin resistant Staphylococcus aureus in a tertiary care hospital, Kathmandu, Nepal. BMC Infect Dis. 2017;17(1):1-5.
9. Chavez-Bueno S, Bozdogan B, Katz K, Bowlware KL, Cushion N, Cavuoti D, Ahmad N, McCracken Jr GH, Appelbaum PC. Inducible clindamycin resistance and molecular epidemiologic trends of pediatric community-acquired methicillin-resistant Staphylococcus aureus in Dallas, Texas. Antimicrob Agents Chemother. 2005;49(6):2283-2288.
10. Bala R, Kaur N, Gupta N, Chauhan J, Garg R, Kumar H, Saini AK. Detection of Inducible Resistance to Clindamycin among Methicillin Resistant and Sensitive strains of staphylococcus aureusfrom India. J Pure Appl Microbiol. 2021;15(4):1957-1962.
11. Timsina R, Shrestha U, Singh A, Timalsina B. Inducible clindamycin resistance and erm genes in Staphylococcus aureus in school children in Kathmandu, Nepal. Future Sci OA. 2020;7(1):FSO361.
12. Patel M, Waites KB, Moser SA, Cloud GA, Hoesley CJ. Prevalence of inducible clindamycin resistance among community-and hospital-associated Staphylococcus aureus isolates. J Clin Microbiol. 2006;44(7):2481-2484.
13. Lall M, Sahni AK. Prevalence of inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. Med J Armed Forces India. 2014;70(1):43-47.
14. Woods CR. Macrolide-inducible resistance to clindamycin and the D-test. J Pediatr Infect Dis. 2009;28(12):1115-1118.
15. Ostojić M, Hukić M. Genotypic and phenotypic characteristics of Methicillin-resistant Staphylococcus aureus (MRSA) strains, isolated on three different geography locations. Bosn J Basic Med Sci. 2015;15(3):48.
16. Akhter S, Nazir A, Karnain O, Rouf M. Prevalence of Constitutive and Inducible Clindamycin Resistance among Methicillin-Resistant Staphylococcus aureus Isolates in a Tertiary Care Hospital, Kashmir Valley. JMID. 2022;10(3):104-113.
17. Navidinia M. Detection of inducible clindamycin resistance (MLSBi) among methicillin-resistant Staphylococcus aureus (MRSA) isolated from health care providers. AAB. 2015;6(1):91-96.
18. Thapa D, Pyakurel S, Thapa S, Lamsal S, Chaudhari M, Adhikari N, Shrestha D. Staphylococcus aureus with inducible clindamycin resistance and methicillin resistance in a tertiary hospital in Nepal. Trop Med Health. 2021;49(1):1-7.
19. Mama M, Aklilu A, Misgna K, Tadesse M, Alemayehu E. Methicillin-and inducible clindamycin-resistant Staphylococcus aureus among patients with wound infection attending Arba Minch Hospital, South Ethiopia. Int J Microbiol. 2019: 2965490.