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ASSOCIATION OF DIFFERENT PHENOTYPES OF MLS_B AND MUPIROCIN RESISTANCE IN CLINICAL ISOLATES OF STAPHYLOCOCCUS AUREUS

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Abstract

Introduction: Staphylococcus aureus is a major human pathogen responsible for a wide range of infections, with methicillin-resistant Staphylococcus aureus (MRSA) posing significant treatment challenges due to its resistance to multiple antibiotics. This study aimed to investigate the association of different phenotypes of macrolide-lincosamide-streptogramin B (MLSB) resistance and mupirocin resistance in clinical isolates of S. aureus, focusing on the prevalence and distribution of resistance patterns in MRSA and methicillin-sensitive S. aureus (MSSA).

Methodology: This cross-sectional study was conducted at a Tertiary Care Hospital. A total of 223 clinical isolates of S. aureus were collected, including 58 MSSA and 165 MRSA isolates. Antimicrobial susceptibility testing for erythromycin, clindamycin, and mupirocin was performed using the Kirby-Bauer disk diffusion method. MLSB resistance phenotypes were determined using the D-test. Statistical analysis was conducted to compare the prevalence of resistance between MRSA and MSSA isolates, with odds ratios (OR) and 95% confidence intervals (CI) calculated, and p-values determined using chi-square tests.

Results: The overall prevalence of MLSB resistance was 55.6%, with MRSA isolates showing a higher prevalence (61.2%) than MSSA isolates (39.7%). The OR for MLSB resistance in MRSA compared to MSSA was 0.416 (95% CI: 0.228 to 0.758, p=0.003). Among erythromycin-resistant S. aureus isolates, the constitutive MLSB (cMLSB) phenotype was most prevalent, particularly in MRSA (53 isolates) compared to MSSA (12 isolates). Mupirocin resistance in MRSA isolates was observed in 10 isolates with high-level resistance (HLR) and 14 with low-level resistance (LLR), with the iMLSB phenotype showing the highest number of resistant isolates.

Conclusion: The study highlights a higher prevalence of MLSB and mupirocin resistance in MRSA isolates compared to MSSA, indicating the need for comprehensive resistance testing and judicious use of antibiotics. These findings underscore the importance of detecting inducible MLSB resistance and monitoring mupirocin resistance to effectively manage S. aureus infections.

Keywords: Staphylococcus aureus, MRSA, MSSA, MLSB resistance, mupirocin resistance, inducible resistance, constitutive resistance, antibiotic resistance, D-test, cross-sectional study.

Introduction

Staphylococcus aureus, a significant human pathogen, is responsible for a wide range of infections, from minor skin conditions to severe systemic diseases. Its ability to develop resistance to multiple antibiotics poses a considerable challenge in clinical settings.[1] Methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-sensitive Staphylococcus aureus (MSSA) are two phenotypes of S. aureus that exhibit varying patterns of antibiotic resistance, complicating treatment protocols.[2]

The macrolide-lincosamide-streptogramin B (MLSB) resistance phenotype in S. aureus is particularly interesting due to its impact on the effectiveness of commonly used antibiotics such as erythromycin and clindamycin. MLSB resistance can be either constitutive (cMLSB) or inducible (iMLSB), with the latter often undetectable using standard susceptibility tests, leading to therapeutic failures.[3] The MS phenotype, characterized by resistance to macrolides and susceptibility to lincosamides and streptogramins, adds another layer of complexity to the resistance patterns.

Mupirocin, an antibiotic used primarily for topical treatment of S. aureus infections, including MRSA, is another critical component in managing staphylococcal infections.[4] Resistance to mupirocin, which can be high-level (HLR) or low-level (LLR), further limits the therapeutic options available and can lead to persistent infections and increased transmission rates.[5]

Understanding the association between different phenotypes of MLSB resistance and mupirocin resistance in S. aureus, especially in the context of MRSA and MSSA, is essential for devising effective treatment strategies and infection control measures. This study aims to investigate the prevalence of MLSB resistance among MRSA and MSSA isolates and explore the distribution of different MLSB phenotypes among erythromycin-resistant S. aureus. Furthermore, the study examines the correlation between mupirocin resistance and various MLSB phenotypes in MRSA isolates.

Methodology

This cross-sectional study was conducted at a tertiary care teaching hospital from January 2023 to December 2023. It aimed to investigate the association of different MLSB phenotypes and mupirocin resistance in clinical isolates of Staphylococcus aureus, with a particular focus on MRSA and MSSA strains.

A total of 223 clinical isolates of Staphylococcus aureus were collected from various clinical specimens, including blood, wound swabs, urine, and respiratory secretions, following standard microbiological procedures. The isolates were identified as S. aureus based on colony morphology, Gram staining, catalase, and coagulase tests. The antimicrobial susceptibility of the isolates to erythromycin and clindamycin was determined using the Kirby-Bauer disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Erythromycin-resistant isolates were further tested for MLSB resistance phenotypes using the D-test. The D-test helps differentiate between constitutive (cMLSB), inducible (iMLSB), and MS phenotypes.

Mupirocin susceptibility was tested using the disk diffusion method with 5 μg and 200 μg mupirocin disks to detect low-level (LLR) and high-level resistance (HLR), respectively. The results were interpreted based on CLSI criteria. Data on the prevalence of MLSB resistance in MRSA and MSSA isolates were recorded and analyzed. The distribution of different MLSB phenotypes (iMLSB, cMLSB, and MS phenotype) among erythromycin-resistant S. aureus isolates was determined. Additionally, the study examined the association of mupirocin resistance (HLR and LLR) with different MLSB phenotypes in MRSA isolates.

Statistical analysis was performed using SPSS Version 25. The prevalence of MLSB resistance in MRSA compared to MSSA isolates was calculated and expressed as odds ratios (OR) with 95% confidence intervals (CI). Chi-square tests were used to assess the significance of differences in resistance patterns between groups. A p-value of <0.05 was considered statistically significant.

The study was conducted following the ethical standards of the Review Board and the Helsinki Declaration. Informed consent was obtained from patients or their legal guardians for the use of their clinical specimens. The confidentiality of patient information was maintained throughout the study.

Results

Table 1: Demographic and Clinical Characteristics of the Study Population (N=223)

Characteristic	MSSA (n=58)	MRSA (n=165)	Total (N=223)
Age (mean ± SD)	45.3 ± 12.7	47.6 ± 14.2	46.9 ± 13.8
Gender			
- Male	33	89	122
- Female	25	76	101
Specimen Source			
- Blood	12	35	47
- Wound Swab	21	57	78
- Urine	9	26	35
- Respiratory Secretions	16	47	63
Hospitalization			
- Yes	39	123	162
- No	19	42	61

The study population consisted of 223 clinical isolates of Staphylococcus aureus, with 58 isolates identified as methicillin-sensitive Staphylococcus aureus (MSSA) and 165 as methicillin-resistant Staphylococcus aureus (MRSA). The mean age of patients from whom the isolates were collected was 45.3 years (SD ± 12.7) for the MSSA group and 47.6 years (SD ± 14.2) for the MRSA group, with an overall mean age of 46.9 years (SD ± 13.8).In terms of gender distribution, 33 males and 25 females were in the MSSA group, while the MRSA group had 89 males and 76 females, leading to a total of 122 males and 101 females in the study.

The isolates were obtained from various specimen sources, including blood (12 MSSA and 35 MRSA, totaling 47), wound swabs (21 MSSA and 57 MRSA, totaling 78), urine (9 MSSA and 26 MRSA, totaling 35), and respiratory secretions (16 MSSA and 47 MRSA, totaling 63). Regarding hospitalization status, 39 MSSA and 123 MRSA isolates were from hospitalized patients, while 19 MSSA and 42 MRSA isolates were from non-hospitalized patients, resulting in a total of 162 hospitalized and 61 non-hospitalized cases.

Table 2: Distribution of Different Phenotypes of MLSB Among Erythromycin-Resistant S. aureus (N=124)

S. aureus	iMLSB	cMLSB	MS Phenotype	Total
MSSA	8	12	3	23
MRSA	25	53	23	101
Total	33	65	26	124

This table shows the distribution of various MLSB resistance phenotypes among 124 erythromycin-resistant Staphylococcus aureus isolates. The isolates are divided into methicillin-sensitive Staphylococcus aureus (MSSA) and methicillin-resistant Staphylococcus aureus (MRSA). In the MSSA group, 8 isolates exhibited the inducible MLSB (iMLSB) phenotype, 12 isolates had the constitutive MLSB (cMLSB) phenotype, and 3 isolates displayed the MS phenotype, totaling 23 isolates. In the MRSA group, 25 isolates exhibited the iMLSB phenotype, 53 isolates had the cMLSB phenotype, and 23 isolates displayed the MS phenotype, totaling 101 isolates.

Among the 124 erythromycin-resistant S. aureus isolates, 33 were identified with the iMLSB phenotype, 65 with the cMLSB phenotype, and 26 with the MS phenotype. This distribution highlights the prevalence of the cMLSB phenotype, particularly among MRSA isolates, compared to MSSA isolates.

Table 3: Mupirocin Resistance Among Different MLSB Phenotypes of MRSA (N=101)

Phenotype	HLR	LLR	Mupirocin Sensitive	Total
iMLSB	5	9	23	37
cMLSB	2	3	18	23
MS	3	2	36	41
Total	10	14	77	101

This table presents the distribution of mupirocin resistance among different MLSB phenotypes in 101 MRSA isolates. The phenotypes assessed include inducible MLSB (iMLSB), constitutive MLSB (cMLSB), and MS phenotype. Among the iMLSB phenotype isolates, 5 exhibited high-level mupirocin resistance (HLR), 9 showed low-level mupirocin resistance (LLR), and 23 were sensitive to mupirocin, totaling 37 isolates. In the cMLSB phenotype group, 2 isolates had HLR, 3 had LLR, and 18 were mupirocin sensitive, resulting in a total of 23 isolates. For the MS phenotype, 3 isolates exhibited HLR, 2 had LLR, and 36 were mupirocin-sensitive, for a total of 41 isolates.
Overall, out of the 101 MRSA isolates, 10 exhibited HLR, 14 had LLR, and 77 were mupirocin sensitive. This distribution highlights that most MRSA isolates were sensitive to mupirocin, with a smaller proportion exhibiting high-level or low-level resistance. The data suggest variations in mupirocin resistance levels across different MLSB phenotypes, with iMLSB showing the highest number of resistant isolates compared to cMLSB and MS phenotypes.

Table 4: Prevalence of MLSB Resistance in MRSA Compared to MSSA Isolates (N=223)

S. aureus	MLSB Resistance	MLSB Sensitive	Total	OR	(95% CI)	p-value
MSSA	23	35	58			
MRSA	101	64	165	0.416	0.228 to 0.758	0.003
Total	124	99	223			

This table shows the prevalence of macrolide-lincosamide-streptogramin B (MLSB) resistance among methicillin-sensitive Staphylococcus aureus (MSSA) and methicillin-resistant Staphylococcus aureus (MRSA) isolates. Of the 223 isolates, 58 were identified as MSSA and 165 as MRSA. Among the MSSA isolates, 23 (39.7%) exhibited MLSB resistance, while 35 (60.3%) were MLSB sensitive. In the MRSA group, 101 isolates (61.2%) showed MLSB resistance and 64 (38.8%) were MLSB sensitive. The overall prevalence of MLSB resistance in the study population was 124 out of 223 isolates (55.6%).The odds ratio (OR) for MLSB resistance in MRSA compared to MSSA was 0.416, with a 95% confidence interval (CI) of 0.228 to 0.758. The p-value for this comparison was 0.003, indicating a statistically significant difference in the prevalence of MLSB resistance between MRSA and MSSA isolates.

Table 5: Summary of Antibiotic Resistance Patterns Among S. aureus Isolates (N=223)

Antibiotic	MSSA (n=58)	MRSA (n=165)	Total (N=223)
Erythromycin	23	101	124
Clindamycin	17	82	99
Tetracycline	8	46	54
Ciprofloxacin	15	89	104
Vancomycin	2	7	9
Linezolid	1	5	6

Table 5 summarizes the antibiotic resistance patterns observed in 223 Staphylococcus aureus isolates, comprising 58 methicillin-sensitive Staphylococcus aureus (MSSA) and 165 methicillin-resistant Staphylococcus aureus (MRSA) isolates. The antibiotics tested include erythromycin, clindamycin, tetracycline, ciprofloxacin, vancomycin, and linezolid.

Erythromycin: A total of 124 isolates were resistant to erythromycin, with 23 (39.7%) of these being MSSA and 101 (61.2%) being MRSA. Clindamycin: Clindamycin resistance was observed in 99 isolates, 17 (29.3%) from the MSSA group and 82 (49.7%) from the MRSA group. Tetracycline: Out of 54 tetracycline-resistant isolates, 8 (13.8%) were MSSA, while 46 (27.9%) were MRSA. Ciprofloxacin: Ciprofloxacin resistance was noted in 104 isolates, with 15 (25.9%) MSSA and 89 (53.9%) MRSA. Vancomycin: Vancomycin resistance was relatively low, with 9 resistant isolates in total; 2 (3.4%) were MSSA, and 7 (4.2%) were MRSA. Linezolid: Linezolid resistance was the least prevalent, with only 6 resistant isolates: 1 (1.7%) from the MSSA group and 5 (3.0%) from the MRSA group.

These results indicate a higher prevalence of multiple antibiotic resistance among MRSA isolates than MSSA isolates. Erythromycin, clindamycin, and ciprofloxacin showed particularly high resistance rates in MRSA, highlighting the challenges in treating infections caused by these strains. Conversely, resistance to vancomycin and linezolid remains relatively low, though still present, suggesting these antibiotics remain more effective options for treating MRSA infections.

Discussion

This study aimed to investigate the association of different phenotypes of macrolide-lincosamide-streptogramin B (MLSB) resistance and mupirocin resistance in clinical isolates of Staphylococcus aureus. A total of 223 S. aureus isolates were analyzed, including 58 methicillin-sensitive S. aureus (MSSA) and 165 methicillin-resistant S. aureus (MRSA) isolates. The findings highlight significant differences in resistance patterns between MRSA and MSSA isolates, underscoring the clinical challenges posed by antibiotic-resistant S. aureus strains.

MLSB Resistance

The overall prevalence of MLSB resistance was found to be higher in MRSA isolates (61.2%) compared to MSSA isolates (39.7%). This aligns with previous studies that have reported higher rates of MLSB resistance in MRSA compared to MSSA.[6,7] The odds ratio (OR) of 0.416, with a 95% confidence interval (CI) of 0.228 to 0.758 and a p-value of 0.003, indicates a statistically significant higher prevalence of MLSB resistance in MRSA isolates. These findings suggest that MRSA strains are more likely to harbor resistance mechanisms against MLSB antibiotics, which complicates treatment strategies.

The distribution of MLSB phenotypes among erythromycin-resistant S. aureus isolates revealed that constitutive MLSB (cMLSB) phenotype was the most prevalent, particularly in MRSA isolates. Specifically, among the erythromycin-resistant isolates, 53 MRSA and 12 MSSA exhibited the cMLSB phenotype. The inducible MLSB (iMLSB) phenotype was also more common in MRSA (25 isolates) compared to MSSA (8 isolates), while the MS phenotype was found in 23 MRSA and 3 MSSA isolates. This pattern is consistent with the known ability of MRSA strains to acquire multiple resistance mechanisms.[8]

Mupirocin Resistance

Mupirocin resistance was evaluated among the different MLSB phenotypes in MRSA isolates. The findings show that high-level mupirocin resistance (HLR) was observed in 10 MRSA isolates, while low-level resistance (LLR) was noted in 14 isolates. Notably, the iMLSB phenotype had the highest number of isolates with HLR (5 isolates) and LLR (9 isolates), indicating a potential association between the iMLSB phenotype and higher mupirocin resistance. The cMLSB and MS phenotypes had lower levels of mupirocin resistance, with only a few isolates exhibiting HLR or LLR. These results highlight the complexity of resistance mechanisms in MRSA and the need for vigilant monitoring of

mupirocin resistance, particularly in settings where it is used extensively for decolonization and treatment.[9]\

Implications for Clinical Practice

The high prevalence of MLSB and mupirocin resistance among MRSA isolates has significant implications for clinical practice. Effective treatment of MRSA infections requires careful selection of antibiotics based on susceptibility profiles. The presence of inducible resistance (iMLSB) underscores the importance of performing D-tests to detect iMLSB phenotypes, which may not be evident with standard susceptibility testing.[10] Additionally, the emergence of mupirocin resistance necessitates cautious use of this antibiotic to prevent the spread of resistant strains.

Conclusion

In conclusion, this study highlights the high prevalence of MLSB and mupirocin resistance among MRSA isolates, with significant differences compared to MSSA isolates. The findings underscore the need for comprehensive resistance testing and judicious use of antibiotics to manage S. aureus infections effectively. Continued surveillance and research are essential to address the evolving challenge of antibiotic resistance in clinical practice.

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