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RESEARCH ARTICLE

MecA and *ermA* Gene Discrepancy from Their Phenotypic Profile in *Staphylococcus aureus* Isolates

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ABSTRACT

Objectives: *Staphylococcus aureus* is one of the most important bacterial pathogens in clinical practice and a primary diagnostic focus for the routine microbiology laboratory. The aim of this study was to find out the phenotypic and genotypic variations in *Staphylococcus aureus* isolates at a tertiary care center in Lucknow.

Methods: 140 clinical isolates of *S. aureus* were taken in the study. Kirby–Bauer disc diffusion method was performed to identify antibiotic susceptibility testing, phenotypically methicillin-resistant *Staphylococcus aureus* (MRSA) were identified by using cefoxitin disc (30 µg), and inducible clindamycin resistance was identified by the presence of D-shaped zone around clindamycin and by using conventional PCR method mecA and ermA genes were identified.

Results: Out of 140 clinical isolates *S. aureus*, 93 (66.4%) were MRSA, and 47 (33.6%) were methicillin-sensitive *Staphylococcus aureus* (MSSA). Phenotype iMLSb was 41 (29.3%), cMLSb phenotype was 37 (26.4%), mecA gene was present in 84 (60%), and none of the samples showed ermA gene positivity.

Conclusion: As we know, the presence of the mecA gene is the major evidence for the detection of MRSA isolates. Their presence in low numbers opens the door to search for other mechanisms that may compete with mecA gene in producing resistance phenomenon. The absence of ermA gene in strains *S. aureus* with iMLSb and cMLSb phenotypes concluded that some other erm gene is responsible for this MLS type of resistance. Due to the frequency of MRSA strains showing the iMLSb phenotype, the use of clindamycin in erythromycin-resistant strains cannot be recommended due to the high possibility of failure in treatment with this antibiotic. *J Microbiol Infect Dis 2021; 11(4):6-11.*

Keywords: *Staphylococcus aureus, mecA gen, ermA gene, MRSA, MLSb phenotype*

INTRODUCTION

Resistance in *Staphylococcus aureus* has become a primary global health concern. In the mid-1970s and late 1990s, methicillin-resistant *S. aureus* (MRSA) emerged as a severe threat [1]. Since then, *Staphylococcus aureus* has shown an increasing trend of resistance towards β-lactam antibiotics along with other classes of drugs. Management became further complex with the emergence of community-associated MRSA (CA-MRSA). These strains show varied susceptibility to trimethoprim-sulfamethoxazole, clindamycin, fluoroquinolones, doxycycline, or minocycline [2,3]. Since most of the CA-MRSA infections cause skin and soft-tissue infections, in that case, clindamycin seems to be a practical option, can be given both orally and intravenously, distributes well into the skin, and has inhibitory action towards certain toxins and virulence factors in Staphylococci [4]. However, one of the significant drawbacks of the use of clindamycin is that it shows inducible resistance with erythromycin.

Moreover, this resistance cannot be detected by the standard broth dilution, disc diffusion, and E-strip methods. Furthermore, this has created a dilemma in clinicians regarding

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whether to use clindamycin when erythromycin resistance is reported. Thereby, introducing a simple D test on a routine basis helps in the detection of inducible clindamycin resistance in that isolates [5].

An active efflux pump encoded by the msrA gene causes macrolides and type B streptogramins resistance, not clindamycin [6]. erm gene encodes enzymes that confer inducible or constitutive resistance to the MLS group of antibiotics via methylation of the 23S rRNA, reducing the binding of these antibiotics to the ribosome. ermA, ermB, and ermC are the three main rRNA methylase genes that have been detected in Staphylococci.

In this study, we have tried to find out the burden of methicillin-resistant *S. aureus* (MRSA) and inducible clindamycin resistance in our hospital and their correlation with each other.

The hypothesis was that the ribosomal target modification was the primary resistance mechanism in clindamycin and ermA, the most predominant gene responsible for inducible resistance. Therefore, this study will show the prevalence of clindamycin resistance pattern in *S. aureus* at our setup, and the clinician may choose other options for treatment of patients.

METHODS

This study was done in the postgraduate department of microbiology, King George's Medical University, Lucknow, a tertiary care hospital in northern India. The study was done over a period of one year, from July 2018 to June 2019. Total 140 non-repeated isolates of *S. aureus* from various clinical specimens (pus, blood, urine, sputum, and body fluids endotracheal aspirate, CSF) of the patients attending OPD irrespective of their gender and age groups. All specimens were inoculated on blood agar and MacConkey agar plates. Plates were prepared by reconstituting the commercially available powder from Hi-media Mumbai India as per the manufacturer's instructions. Inoculated plates were incubated at 37 ^{0}C aerobically for 24 hours. *S. aureus* was identified morphologically using colony characteristics, Gram stain, catalase test, coagulase test, and DNAase test by standard microbiological techniques [7]. Antibiotic susceptibility testing was performed on MHA plates by the Kirby–Bauer disc diffusion method using different antibiotics; ampicillin (10 µg), cotrimoxazole (1.25/23.75 µg), ciprofloxacin (5 µg), vancomycin (30 µg), cephalexin (30 µg), and gentamycin (10 µg) discs. In addition, Cefoxitin (30 µg) for the detection of methicillin resistance and erythromycin (15 µg) clindamycin (2 µg) discs at 15 mm apart were also used on the same plate for the detection of inducible clindamycin resistance as per CLSI guidelines 2019 [5].

Detection of methicillin resistance

Isolates with cefoxitin zone size ≥22 mm were considered methicillin-susceptible, and those with ≤21 mm were considered methicillin-resistant according to CLSI guidelines 2019 [5]. Classification of clindamycin resistance as shown in figure-1 clinically isolated *S. aureus* strains that demonstrated clindamycin resistance and cefoxitin resistance phenotypes were used as quality control.

Detection of mecA gene & ermA gene: Detection of mecA and ermA gene was done from phenotypically confirmed MRSA and iMLSB isolates. Oligonucleotide primers for mecA and ermA were self-designed and were synthesized at Eurofins Genomics India Pvt Ltd, Bangalore (Karnataka):

•Mec A forward primers:
AAAATCGATGGTAAAGGTTGGC (530bp)

•Mec A Reverse primers:
AGTTCTGCAGTACCGGATTTGC

•Erm A Forward primers:
AAGCGGTAAACCCCTCTGA (190bp)

•Erm A Reverse primers:
TTCGCAAATCCCTTCTCAAC

Positive controls for mecA and ermA were taken internally from a clinical isolate.

DNA Extraction

First, 200 µl nuclease-free water in a 1.5 ml Micro Centrifuge Tube (MCT) was taken, and the isolated colonies of bacterial culture were dissolved in that MCT after touching ten single isolated colonies. These tubes were placed in a water bath with a 95 ^{0}C temperature for 15 minutes and then centrifuged at 2000 rpm for 5 min [8]. The supernatant was transferred to another MCT and used as a DNA template for PCR amplification.

PCR: Amplification reaction was carried out in total volume of 25 µl, which constitutes universal PCR master mix (12 µl) (Thermo

Fischer Scientific Baltics UAB, Vilnius, Lithuania), forward and reverse primer (1.0 µl), nuclease-free water (5.5 µl) and 5 µl of template DNA of the isolates. It was taken in 0.2 ml thin-walled PCR tubes and one positive control and one negative control. After sealing the tubes with caps, it was placed into a thermal cycler. Amplification process was started with an initial denaturation step (95 ^{0}C for 10 min) each PCR reaction consisted of 35 cycles (denaturation at 95 ^{0}C for 30 sec, annealing at 55 ^{0}C (mecA) & 56.5 ^{0}C (ermA) for 40 sec, final extension was done at 72 ^{0}C for 1 min. After amplification for 35 cycles, the PCR products were recovered by gel electrophoresis using 1.5% agarose gel containing 0.5 µl/ml of ethidium bromide (0.5 mg/ml, Medox Biotech Pvt. Ltd.) with molecular weight marker (100 bp DNA ladder; Bangalore Ganei, India) and PCR products of negative and positive control electrophoretically. A constant current of 100 V was maintained for 1 hour, and amplified DNA was analyzed by 264 nm wavelength UV transillumination.

Ethical approval was obtained from the Institute's Ethics committee, King George's Medical University, Lucknow, India. Ref no: 90th ECM II B- Thesis/P23.

Data analysis

The statistical analysis was done using SPSS version 21.0 statistical analysis software. The values were represented in number (%) and mean ± SD. The statistical tools used were mean, standard deviation, Chi-Square test, analysis of variance (ANOVA, F ratio), level of significance, p <0.05 was considered significant.

RESULTS

A total of 140 *S. aureus* isolates were collected from different clinical specimens, which included pus/wound swab (n=83), blood (n =50), other body fluids (n=7). Out of the strains isolated, 93 (66.4%) were MRSA, and 47 (33.6%) were MSSA. In the present study mean age of patients was 27.8 ± 19.4, with more male preponderance (52.1%), and greater samples input were from IPD patients (70.7%). Resistance pattern was observed maximum for penicillin (98.6%), followed by erythromycin (80.7%), levofloxacin (38.5%), gentamycin (34.3%), clindamycin (26.4%), tetracycline (21.4%), amikacin (25%) cotrimoxazole (20.0%) and no resistance was seen in vancomycin and linezolid (0.0%).

Out of 140 *S. aureus* isolates 41 (29,3%) were inducible MLSb phenotype (iMLSb), 37 (26.4%) cases were constitutive MLSb phenotype (cMLSb), 35 (25,0%) had MS phenotype while remaining 27 (19.3%) were both erythromycin and clindamycin sensitive (Figure 2). It was found that inducible MLSb phenotype was most common among MRSA, 35 (35.4%) and only 8 (17%) iMLSBb found in MSSA strains (Figure-3, Table 2). On evaluating the data statistically, the difference between MRSA and MSSA was significant (p=0.001). The mean age of patients was maximum for constitutive MLSb Phenotype (33.3 ± 20.0 years) followed by erythromycin sensitive, clindamycin sensitive (30.95 ± 17.3 years), inducible MLSb phenotype (27.3 ± 18.3 years), and MS Phenotype (19.9 ± 20.7 years) respectively. Statistically, there was a significant difference in the mean age of patients in different groups (p=0.042) (Table 2). MecA gene was present in 84 (60%) (Figure 4). None of the samples showed ermA gene positivity (Figure 5).

Among isolates that were positive for mecA genotype, maximum showed constitutive MLSb (39.7%) phenotype, whereas those negative isolates for mecA genotype showed a maximum of inducible MLSb phenotype (32.9%). Statistically, the difference between the two groups was significant X2=13.6 (df=3); p=0.004.

DISCUSSION

Our study showed higher male preponderance (52.1%), which was similar to the study conducted by Diwakar et al. [9]. The prevalence was higher among males (56.3%) than females (43.8%). There was more samples input from the inpatient department (70.7%) than the outpatient department (29.3%), and a higher proportion of MRSA was isolated from hospitalized patients (65.6%). Inducible clindamycin resistance was detected more in inpatients (30.3%) than in outpatients (26.8%). This was in support of the study done by Mokta K et al. [10], where the proportion of inpatients (54.9%) was more than the outpatients (45.2%) and reported a higher proportion of MRSA (76.8%).

In the present study, the proportion of MRSA was found to be 66.4%, similar to the study

conducted by Sah P et al. [11], as they reported that out of 140 isolates, MRSA was found in 61.4%, and MSSA was 38.6%. A similar study by Kumar A et al. [12] detected MRSA in 81 (60.9%) out of 133 isolates. Variation in the prevalence of MRSA in different areas and countries might be due to the different study populations, use of antibiotics, different samples types, sample size, and infection control practices.

In our study, MRSA was predominantly isolated from pus/wound samples (51.6%) followed by blood (46.2%). This was in concordance with the study by Shetty J et al. [13]. In the present study, inducible MLSb phenotype (iMLSb) was found in 41 isolates (29.3%) and was most common among MRSA 33 (35.4%), and it was statistically significant ($p< 0.001$). The result was in concordance with a study by Reddy et al. [14], where iMLSb was found in 28.5% isolates, and 26.2% iMLSb strains were found to be MRSA. Similarly, a study done by Saxena S et al. [15] reported 25.8% of inducible clindamycin resistance, out of which 30% were methicillin-resistant.

As far as the antibiotic susceptibility pattern of different antibiotics is concerned, All the isolates in our study showed susceptibility to vancomycin and linezolid, which has been reported in several other studies like Diwakar et al. [9]. Resistance of other antibiotics ranges from 20% to 80%, similar to a study done by Shetty J et al. [13].

In our study, mecA gene was expressed in 60% of MRSA isolates. In a similar study conducted by Davoodi et al. [16], where they took 100 isolates of *S. aureus*, and 56% carried mecA gene. Another study by Alli OA et al. [17] reported the prevalence of mecA gene as 42.3% in their study. However, in their study, Kareem MS et al. [18] reported a higher prevalence (82.43%) of mecA gene out of total *S. aureus* isolates, which was higher than our study. Lower detection in MRSA isolates may call for a search of other resistance gene mechanisms (mecB, mecC) prevailing in *S. aureus*.

Resistance to MLS antibiotics in Staphylococci is mainly mediated by erm genes. In our study, ermA gene was not found in any of the D test positive isolates. A similar study conducted by Tandon N et al. [19] reported ermC as the predominant gene for the resistance in inducible clindamycin resistant, and they could not demonstrate ermA and ermB genes in *S. aureus* isolates by the genotypic method. Another study by Rajkumar S et al. [20], where they had also reported that in inducible MLSb resistant isolates, ermC was the predominant resistance determinant followed by ermA while ermB genes were not detected. On the contrary, a study by Kareem MS et al. [18] reported the prevalence of ermA gene (7.4%) and ermC (5.9%) in their 84 Staphylococci isolates.

This study has also the following limitations. The samples under investigation were less and other genes responsible for resistance like ermB, ermC, msrA were not included in the study.

CONCLUSION

This study studied the discrepancy between phenotypic and genotypic variations in *Staphylococcus aureus* isolates in our hospital. As we know, the presence of mecA gene is the major evidence for detecting MRSA isolates, and in our study, we detected a lesser proportion of mecA gene (60%). This observation opens the door to search for other intrinsic factors that may compete with mecA gene in producing resistance. We hypothesized that ermA gene is a common gene that produces inducible clindamycin resistance in *Staphylococcus aureus* isolates. Nevertheless, surprisingly, it was not found in any of the isolates. It concluded that another erm gene is responsible for the expression of inducible clindamycin resistance. Therefore, clinicians should not prescribe clindamycin if clinical samples show resistance to erythromycin.

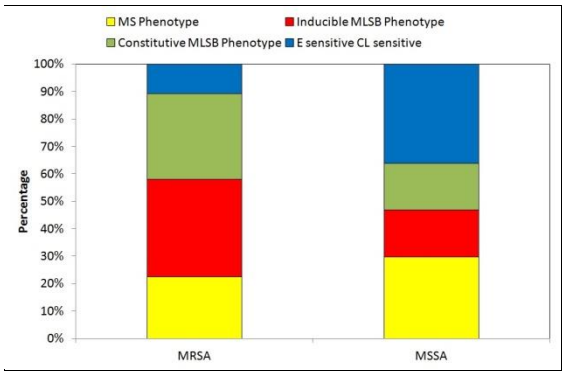

Figure 3. Phenotype distribution in MRSA and MSSA.

Table 1. Association of Clindamycin resistance with Methicillin resistance.

SN	Susceptibility pattern (Phenotype)	Total	MRSA (n=93)		MSSA (n=47)	
			No.	%	No.	%
1.	MS Phenotype (E resistant and CL sensitive with D-test negative)	35	21	22.5	14	29.7
2.	Inducible MLSB Phenotype (E resistant and CL sensitive with D-test positive)	41	33	35.4	8	17
3.	Constitutive MLSB Phenotype (E resistant and CL resistant with D-test negative)	37	29	31.2	8	17
4.	E sensitive CL sensitive	27	10	10.8	17	36.2

Table 2. Association of Clindamycin resistance with age.

SN	Susceptibility pattern (Phenotype)	Total	Age (Yrs)	
			Mean	SD
1.	MS Phenotype (E resistant and CL sensitive with D-test negative)	35	19.85	20.74
2.	Inducible MLSB Phenotype (E resistant and CL sensitive with D-test positive)	41	27.30	18.33
3.	Constitutive MLSB Phenotype (E resistant and CL resistant with D-test negative)	37	33.25	19.95
4.	E sensitive CL sensitive	27	30.95	17.30

F=3.242; p=0.042 (S)

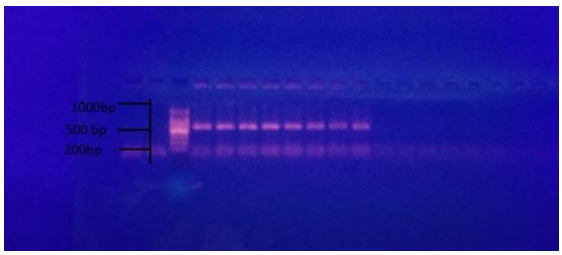

Figure 4. Gel electrophoresis of mecA gene positive isolates.

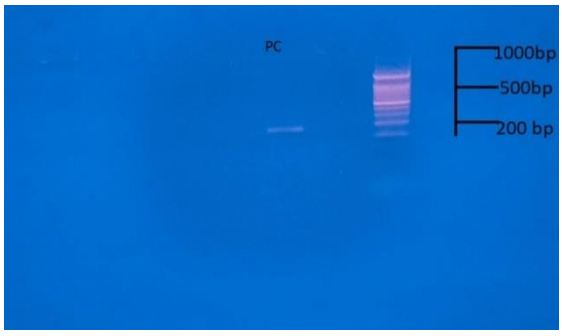

Figure 5. Gel electrophoresis of ermA gene positive control.

Abbreviations

CD: clindamycin; E: erythromycin; cMLSB: constitutive clindamycin resistance phenotypes; iMLSB: inducible clindamycin resistance phenotypes; MLSB: macrolide-lincosamide streptogramin B; MRSA: methicillin resistant *Staphylococcus aureus*; MSSA: Methicillin sensitive *Staphylococcus aureus*; µg: microgram;CLSI :Clinical and Laboratory Standard Institute; PCR: Polymerase Chain Reaction; SPSS: Statistical software Package for Social Sciences; OPD-out patient department; IPD: in patient department.

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REFERENCES

1. Verma S, Joshi S, Chitnis V, Hemwani N, Chitnis D. Growing problem of methicillin resistant Staphylococci, Indian scenario. Indian J Med Sci 2000; 54(12):535-40.

2. Naimi TS, LeDell KH, Como-Sabetti K, et al. Comparison of community-and health-care–associated methicillin-resistant *Staphylococcus aureus* infection. JAMA 2003; 290:2976–84.

3. Charlebois, ED, Perdreau-Remington F, Kreisworth B, et al. Origins of community strains of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 2004; 39:47–54.

4. Coyle EA, Lewis RL, Prince RA. Influence of clindamycin on the release of *Staphylococcus aureus* a-hemolysin from methicillin resistant *S. aureus*: could MIC make a difference [abstract 182]? Crit Care Med 2003; 31 (Suppl):A48.

5. Clinical Laboratory Standards Institute (CLSI) guidelines. Performance standards for antimicrobial susceptibility testing: twenty second informational supplement. CLSI document M100-S22. Clinical and Laboratory Standards Institute. Pennsylvania; Wayne; 2019.

6. Roberts, MC, Sutcliffe J, Courvalin P, Jensen LB, Rood J, Seppala H. Nomenclature for macrolide-lincosamide-streptogramin B resistance determinants. Antimicrob. Agent Chemother 1999; 43:2823–2830.

7. Isenberg HD. Clinical microbiology procedures handbook. 2nd ed. Washington D.C.: ASM Press; 2004.

8. Farrow KA, Lyras D, Rood JI. The macrolide-lincosamide-streptogramin B resistance determinant from Clostridium difficile 630 contains two erm(B) genes. Antimicrob Agents Chemother 2000; 44(2):411-413.

9. Diwakar MK, Goyal A, Verma S, Srivastava N. Prevalence of Inducible Clindamycin Resistance among Nasal Carriage *Staphylococcus aureus* among Healthy Population. Int J Curr Microbiol App Sci 2018; 7(5): 2509-2517.

10. Mokta KK, Verma S, Chauhan D, et al Inducible Clindamycin Resistance among Clinical Isolates of *Staphylococcus aureus* from Sub Himalayan Region of India. J Clin Diagn Res 2015; 9(8):DC20-23.

11. Sah P, Khanal R, Lamichhane P, Upadhaya S, Lamsal A, Pahwa VK. Inducible and constitutive clindamycin resistance in *Staphylococcus aureus*: An experience from Western Nepal. Int J Biomed Res 2015; 6(3); 16-19.

12. Kumar A, Kumar A. Prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) In A Secondary Care Hospital In North Eastern Part of India. Archiv Infect Dis & Therap 2018; 2(1):1-2.

13. Shetty J, Afroz Z. Prevalence of constitutive and inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus* in a tertiary care institute in North India. Int J Res Med Sci 2017; 5(7):3120-3125.

14. BS. Reddy,S. Basak, S. Das, Kaushik P. inducible clindamycin resistance-*Staphylococcus aureus* and its therapeutic-implications; Int J Curr Researc 2017; 9 (10): 59930-59933.

15. Sexena S, Singh T, Rakshit P, Dutta R, Gupta RK. Prevalence of inducible clindamycin resistance in *Staphylococcus aureus* at a tertiary care hospital: implications for clinical therapy. Int J Curr Microbiol App Sci 2014; 3(3):720-725.

16. Davoodi NR, Jalil V, Harz N, Hajrafi A, Rajaei B, Gerayesh-Nejad S. Molecular detection of methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin resistant coagulase negative *Staphylococcus* (CoNS) in Iran. Afr J Microbiol Res 2012; 6: 3716.

17. Alli OA, Ogbolu DO, Shittu AO, Okorie AN, Akinola JO, Daniel JB. Association of virulence genes with mecA gene in *Staphylococcus aureus* isolates from Tertiary Hospitals in Nigeria. Indian J Pathol Microbiol 2015; 58:464-471.

18. Kareem SM, Al-Jubori SS, Ali M. Prevalence of erm Genes among Methicillin Resistant *Staphylococcus aureus* MRSA Iraqi Isolates. Int J Curr Microbiol App Sci 2015; 4(5):575-585.

19. Tandon N, Kulkarni M, Sowmyags, tabassum F, Akhtar M. Prevalence of inducible clindamycin resistance in clinical isolates of methicillin-resistant *Staphylococcus aureus* mediated through gene *ermC* expression. Asain J Pharmaceutic Clin Res 2018; 11(9):106-109,.

20. Rajkumar S, Sistla S, Manoharan M, et al. Prevalence and genetic mechanisms of antimicrobial resistance in Staphylococcus species: A multicentre report of the Indian council of medical research antimicrobial resistance surveillance network. Indian J Med Microbiol 2017; 35(1):53-60.