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Research Article

PREVALENCE OF INDUCIBLE CLINDAMYCIN RESISTANCE IN CLINICAL ISOLATES OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* MEDIATED THROUGH GENE *ERMC* EXPRESSION

NIMISHA TANDON[1,2], MADHURI KULKARNI[1], SOWMYA GS[1], FAUZIA TABASSUM[3], MD SAYEED AKHTAR[2,4]*

[1]Department of Microbiology, JSS Medical college, Mysuru – 570 015, Karnataka, India. [2]Department of Microbiology, School of Allied Health Sciences, Sharda University, Knowledge Park-III, Greater Noida – 201 306, Uttar Pradesh, India.[3]Department of Pharmacology, All India Institute of Medical Sciences, Patna, Bihar, India. [4]Department of Clinical Pharmacy, College of Medicine and Health Sciences, Ambo University, Ambo-19, Ethiopia. Email: sayeedpp@gmail.com

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ABSTRACT

Objective: The objective of this study is to determine the phenotypic and genotypic expression of inducible clindamycin resistance due to the expression of *erm*A, *erm*B, and *erm*C genes in clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) by double disc diffusion and uniplex PCR.

Method: This cross-sectional study was conducted in microbiology department of an university teaching hospital. A total of 604 non-duplicate clinical isolates of *S. aureus* evaluated for MRSA and were subjected to uniplex PCR for *erm*A, *erm*B, and *erm*C genes, respectively.

Result: The analysis of 604 isolates showed that 220 (36.42%) were of MRSA. Out of which, 69 (11.42%) were demonstrated as inducible clindamycin resistance by double-disc diffusion method, and among inducible resistant isolates, 25 isolates of *erm*C (84%) were positive and 4 (16%) were negative, whereas, *erm*A and *erm*B genes could not be demonstrated by the genotypic method.

Conclusion: We observed that clindamycin may serve as a good alternative and advocated in severe MRSA infection based on susceptibility pattern. We observed D test as a mandatory method to detect inducible clindamycin *Staphylococcus*. Importantly, *erm*C gene is a major determinant of resistance to macrolides among MRSA.

Keywords: methicillin-resistant *Staphylococcus aureus*, Erm genes, D-test, Macrolide lincosamide streptograminB.

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INTRODUCTION

Staphylococci are Gram-positive cocci that occur in grape-like clusters [1]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a potent pathogenic organism that causes nosocomial as well as community-acquired infections with both endemic and epidemic spread [2]. Worldwide, there is a considerable increased prevalence of MRSA which was observed in last decade [3]. In India, MRSA prevalence rate ranges from 30% to 80% among all *S. aureus* infections [4]. MRSA-related death estimated to be 64% more likely in comparison to others. The increased cost of health care in low-income countries occurred due to increased hospitalization and this call for intensive care of the patient [5]. The cost of treating MRSA infections is four-fold more than its sensitive counterparts [6,7]. The strict surveillance and appropriate prevention measures during management of MRSA infections reduce the prevalence, rate of mortality, and treatment expenditure [8,9].

Clindamycin, a macrolide lincosamide streptogramin B (MLS_B) type of antibiotic is useful for penicillin-allergic cases for the treatment of skin and soft tissue infections of *S. aureus* [3,10]. Clindamycin is quite a good alternative and used as both parenteral and oral use and has good tissue distribution along with potent bacteriostatic action against *S. aureus* [11]. The therapeutic failure of clindamycin occurs due to lack of inducible clindamycin resistance [12,13]. Staphylococci labeling as clindamycin resistant could stop prescription of Clindamycin even it is only erythromycin resistant and susceptible to clindamycin [12,14].

MLS_B is presented as either constitutive ($MLSB_C$ phenotype) or inducible ($MLSB_I$ phenotype) due to enzyme encoded by a variety of erm genes. Alternatively, the resistance is mediated through active efflux pump encoded by msrA gene called MS phenotype [3]. ErmA, ermB, and ermC develop the resistance to MLS type B (MLS_B) by altering target site of the ribosome [15].

The use of *in vitro* susceptibility test for the identification of resistance to erythromycin in converse to clindamycin sensitivity by D test is a need of time [3].

The prevalence of inducible clindamycin resistance varies in the different geographical region. Therefore, keeping these facts in mind, the present study was conducted to detect the prevalence of inducible clindamycin resistance by D-test and to investigate the presence of macrolide resistance along with the detection of ermA, ermB, and ermC genes in clinical isolates of *S. aureus*.

MATERIALS AND METHODS

This prospective study was conducted for 12 months from March 2014 to February 2015. A total of 604 non-duplicate clinical isolates were taken from various specimens such as pus, swab, and blood. All the samples were tested for its susceptibility by disc diffusion method (CLSI, 2007). All clinical isolates of MRSA during the study period were included. In contrast, all the specimens having *mec*A gene-negative isolates are excluded from the study.

Phenotypic method

For the detection of MRSA and inducible clindamycin resistance, samples were processed by standard microbiological methods and *S. aureus* was identified by standard laboratory procedures. The isolates were further characterised by oxacillin screen agar and cefoxitin disc diffusion to

identify it as MRSA. Lack of a D-shaped zone in erythromycin-resistant and clindamycin-susceptible isolates was interpreted as M/MS_B efflux phenotype.

Genotypic method

For the extraction of DNA, two-step lysostaphin and phenol-chloroform method was used as described by Anand *et al.* in 2009. The prepared pellets of MRSA isolates were dissolved in 50 µl of sterile water and DNA was stored at −20°C.

Detection of mecA and ermA, B, and C genes

Selected strains were subcultured on the top of blood agar and incubated overnight at 35 ºC. Bacterial DNA was extracted by phenol-chloroform protocol, and uniplex PCR was carried out using specific primers for the detection of *mec*A and *erm*A, B, and C genes.

Amplification of mecA, and ermA, B, and C genes using 1 primer/uniplex PCR method

Primers used for *erm*A, B, and C were procured from Sigma-Aldrich, Missouri, United States.

Preparation of PCR mix for mecA gene detection

Preparation of PCR master mix for 25 µl was done by taking 2.5 µl of ×10 Dream Taq Buffer (Thermo Fischer Scientific, California, USA) containing 25 mM $MgCl_2$ 2.5 µl of dNTPs containing all the 4 dNTPs at 2 mM concentration and 0.125 µl of Taq polymerase (5 units/µl). 2 µl of DNA and 0.1 µl each of forward and reverse primers of *mec*A gene (100 µM) and 18.875 µl of sterile water.

Preparation of PCR mix for ermA, B, and C by uniplex PCR

Preparation of PCR master mix for 25 µl was done by making 2.5 µl of ×10 Dream Taq Buffer containing 25 mM $MgCl_2$ (Thermo Fisher Scientific, California, USA).

Optimal cycling conditions for the PCR amplifications of ermA, B, and C genes

Biorad PCR equipment was used for PCR amplification. PCR master mix was dispensed in 200 µl PCR tubes. The optimal cycling conditions for *erm*A and C genes were as follows: 93°C for 3 min, 35 cycles of 93°C for 1 min, 37°C for 1 min, and 72°C for 1min, followed by a final extension of 72°C for 7 min, whereas, for *erm*B gene, the annealing temperature was at 42°C for 1 min.

Gel electrophoresis

Gel electrophoresis was done to separate amplicons, and detection of separated amplicons was done under UV light. Gene Ruler-1kb DNA ladder was used as the marker (Thermo Fischer Scientific, California, USA).

RESULT

The majority of the MRSA isolates were collected from the surgery department (21.68%), followed by ICU (11.75%) and orthopedics (7.45%) comprising 40.88% of the total isolates (Fig. 1).

The most common clinical conditions associated with MRSA were skin and soft tissue infections that account 29.77%. Among this, the majority were an abscess (13.9%) from different surgical site infection such as carbuncle, furuncle, and pyoderma. Bone infections and respiratory infections were comparatively rare (2%) (Table 1).

The antibiotic pattern for MRSA is shown in Fig. 2a and inducible clindamycin resistance can be depicted by the D-test which is shown in Fig. 2b.

As shown in Fig. 3, isolates 1, 5, 9, 15, 20, and 22 are not grown on oxacillin-resistant screen agar (ORSA).

The ORSA is used to differentiate between MRSA and methicillin-sensitive *Staphylococcus aureus* (MSSA). The presence of amplified *mec*A gene is significant for the detection of MRSA genotypically which is shown in Lane 2-15 (Fig. 4).

According to sample distribution for MRSA, pus is the most common specimen followed by an abscess, blood, urine, ear swab, pleural fluid, sputum, endotracheal tube, ENT, catheter tip, and others (Table 2). Of which, 69 were demonstrated as inducible clindamycin resistance by D-test (11.42%) (Table 2).

Among ermA, *erm*B, and *erm*C genes by PCR, *erm*C is the predominant genetic determinant for the expression of resistance to macrolides among MRSA 21 (84%) (Figs. 5 and 6).

Table 1: Clinical conditions associated with MRSA

S. No.	Clinical Conditions	Total Isolates (%)
1.	Abscess	84 (13.9)
2.	Skin infection	63 (10.43)
3.	Others	37 (6.12)
4.	Ear infection	25 (4.13)
5.	Bone and joint infections	13 (2.15)
6.	Wound infection	12 (1.98)
7.	Respiratory infection	10 (1.65)
8.	Ulcer	10 (1.65)
9.	Diabetic foot	6 (0.99)
10.	Surgical site infection	3 (0.49)
11.	Gangrene	3 (0.49)
12.	Necrotizing fasciitis	2 (0.33)
13.	Urinary tract infection	0
14.	Bacteremia	0
15.	Diagnosis not known	336 (55.62)

MRSA: Methicillin-resistant *Staphylococcus aureus*

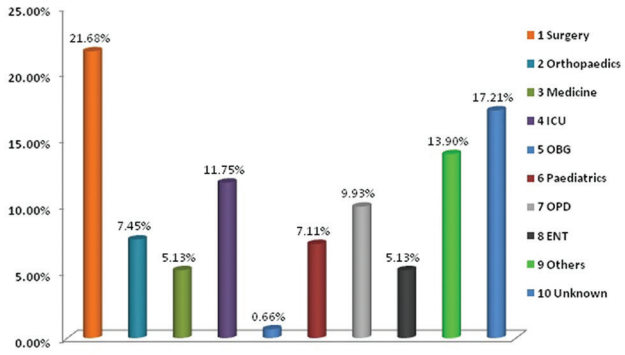

Fig. 1: Methicillin-resistant *Staphylococcus aureus* isolated pattern from different department of the hospital

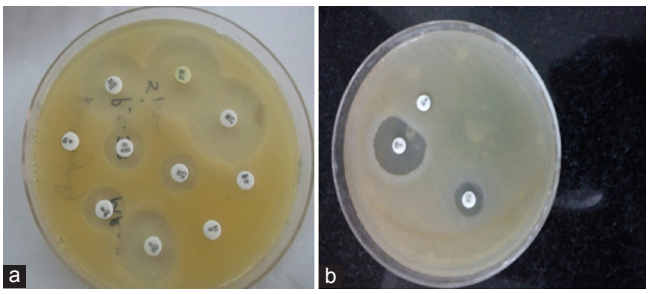

Fig. 2: (a) Antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (b) cefoxitin resistance and D- test positive for clindamycin

Table 2: Sample-wise distribution of the *S. aureus*, MRSA isolates having inducible clindamycin resistance

Samples	*S. aureus* (%)	MRSA (%)	MRSA+IR Genotype (%)	MRSA+IR Phenotype (%)
Pus	442 (73.17)	163 (26.98)	17 (68)	58 (9.6)
Abscess	44 (7.28)	17 (2.81)	01 (4)	02 (0.33)
Blood	24 (3.97)	10 (1.65)	00	01 (0.16)
Urine	09 (1.47)	06 (0.99)	00	0
Ear swab	26 (4.3)	07 (1.15)	01 (4)	01 (0.16)
Pleural fluid	09 (1.49)	01 (0.16)	00	0
Sputum	16 (2.64)	08 (1.32)	01 (4)	02 (0.33)
ET	28 (4.63)	01 (0.16)	01 (4)	03 (0.49)
ENT	01 (0.16)	03 (0.49)	00	01 (0.16)
CT	04 (0.66)	03 (0.49)	00	0
Others	01 (0.16)	01 (0.16)	00	01 (0.16)

CT: Catheter tip, ET: Endotracheal tube, MRSA: Methicillin-resistant *Staphylococcus aureus*

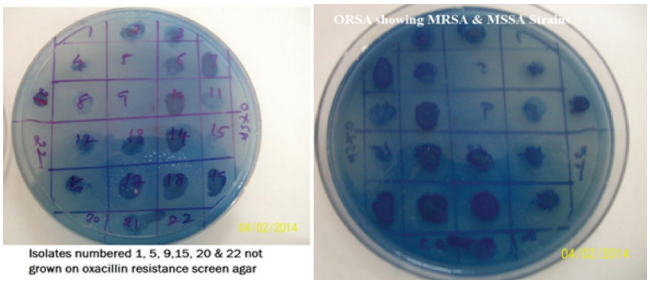

Fig. 3: (a and b) Oxacillin resistance screening of *Staphylococcus aureus* isolates. Methicillin-resistant *S. aureus* strains appeared blue colored on oxacillin-resistant screen agar. Blocks without growth represent methicillin-sensitive *S. aureus* isolates

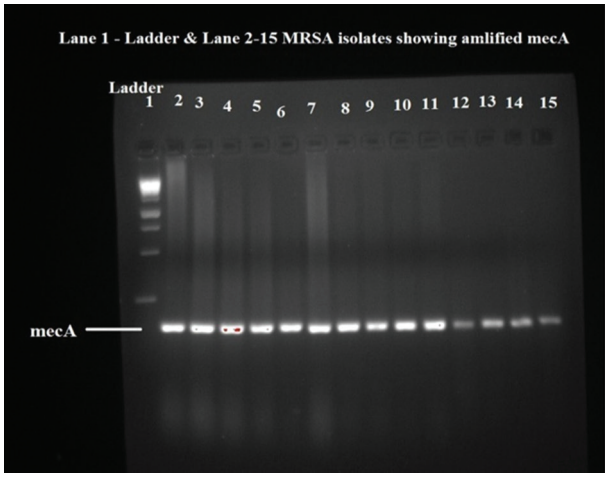

Fig. 4: Amplification of *mec*A locus. lane 1 is ladder and lanes 2–15 are methicillin-resistant *Staphylococcus aureus* isolates

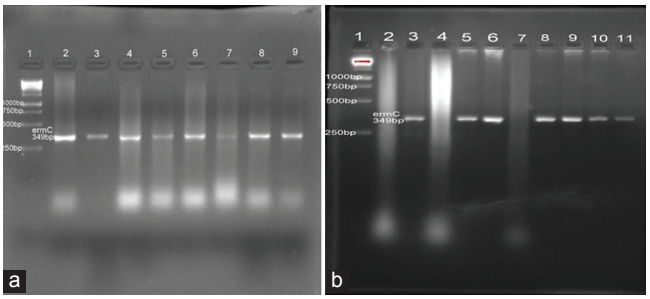

Fig. 5: (a) Amplification of *erm*C, Lane 1 - Marker, Lanes 2–7 test strains, Lane 8 - positive control. Gel doc showing separation, Lane 2–7 isolates are *erm*C positive and Lane - 8 positive strain as a control for PCR. (b) Amplification of *erm*C, Lane 1 - Marker, Lane 2–10 test strains, Lane 11 - positive control. Gel doc showing separation, Lane - 3, 5, 6, 8, 9, 10, and 11 isolates are *erm*C positive and Lane 11 standard strain as control for PCR

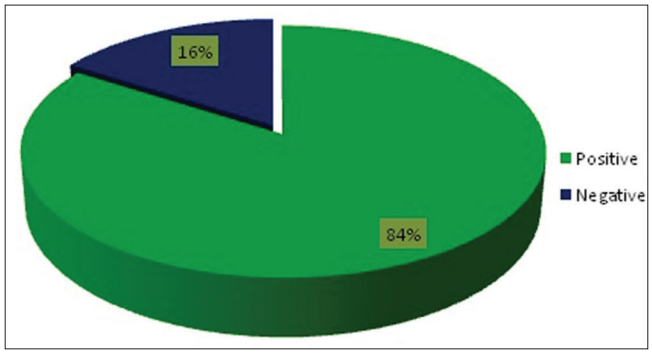

Fig. 6: Result of *ermC* gene detection by PCR

DISCUSSION

Antimicrobial resistance is a worldwide problem, particularly among pathogens related to hospital-acquired infections. *Staphylococci* have become one of the most common causes of both hospital- and community-acquired infections [16-18]. The increased frequency of *Staphylococcal* infections along with the augmented problem of antimicrobial resistance has led to renewed interest in clindamycin usage [19]. Clindamycin is a good alternative drug for the treatment of skin, soft tissue, and bone infections because of its tolerability, oral bioavailability, accumulation in abscesses, and cost-effectiveness [3]. There has been a number of reported clindamycin therapy failure in *Staphylococcal* infections due to inducible resistance phenotype [19].

In accordance with the previous studies, we also observed 36.42% prevalence of MRSA among *S. aureus* infections as this was detected by cefoxitin (D-test). In 2003, Anupurba *et al.* reported 54.82% prevalence, and in another study conducted by Joshi *et al.* in 2013 [4], 42% MRSA was detected among *S. aureus* infections [4,20]. This difference could be due to the prevalence of inducible clindamycin which may vary by geographic regions and even in different hospital setup [3].

In the current study, the majority of the MRSA isolates were observed from the department of surgery (21.68%) followed by ICU (11.75%) which may be because surgical interventions were needed in most of the cases. In a study conducted by Adam *et al.* in 2009 and Valsesia *et al.* in 2010, 35% of cases were from the surgical ward and 86% of MRSA were commonly isolated from skin and soft tissue infections [21,22]. In another study, it was reported that skin and soft tissue infections as most commonly reported cases in emergency wards and MRSA were the most common strains isolated from abscesses (61%), wounds (53%), and cellulitis with purulent exudate (47%) [23]. Concurrent with the previous study, the majority of the cases were from surgical wards and also MRSA was commonly isolated from skin and soft tissue infections.

Among *erm*A, *erm*B, and *erm*C genes by PCR, *erm*C is the predominant genetic determinant for the expression of resistance to macrolides among MRSA. In a previous study, of 1477 *S. aureus* isolates, 173 expressed resistance to erythromycin, constitutive phenotypes in 106 isolates, the inducible phenotype in 53, and MS phenotype in 13 cases. Most of the MRSA isolates carried the *erm*C gene followed by *erm*A gene and the *msr* [24].

Similarly, among 285 *S. aureus* isolates, inducible resistance was found in 38 (13.33%) [25]. In another similar study, of 288 Staphylococcal isolates, 116 (40.27%) were found to be MRSA resistant to cefoxitin. D-test was performed and showed erythromycin resistant and clindamycin sensitive in 21 (18.1%) indicating inducible clindamycin resistances [3]. Concurrently, we reported that, among 604 *S. aureus* isolates, 220 (36.42%) were of MRSA. Among them, 69 (11.42%) were demonstrated as inducible clindamycin resistance.

In accordance to the previous report, we also observed 69 (11.42%) isolates for MRSA + IR demonstrated by double-disk diffusion method phenotypically, whereas, 25 isolates were analyzed for the presence of *erm*A, B, and C genes. Of these, 21 (84%) were observed to be as *erm*C positive by PCR. In a previous study, among 414 *S. aureus* isolates, 150 were MRSA of which 35.33% were demonstrated as inducible clindamycin resistant, and of 264 MSSA, 7.95% were inducible clindamycin resistant [26]. In another study, 55 MRSA isolates showed that there was no discordance between conventional susceptibility testing by Kirby-Bauer disk diffusion method and gene detection by multiplex PCR assay. The study showed the prevalence of *erm*(A) and *erm*(C) genes at the rate of 30.9% and 74.5%, respectively [27].

CONCLUSION

We concluded that MRSA can be demonstrated by double disc diffusion method which is an appropriate treatment for the patients whenever clindamycin is to be used for *S. aureus* infections. Among *erm*A, *erm*B, and *erm*C genes, *erm*C is the predominant genetic determinant for the expression of resistance to macrolides among MRSA. We recommend that the isolates which are erythromycin resistant and clindamycin sensitive should be checked phenotypically for inducible resistance by D-zone test and genotypically for the presence of *erm* gene.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

AUTHOR'S CONTRIBUTION

All the authors have substantially contributed in the research and publication of this study.

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