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PREVALENCE AND MOLECULAR CHARACTERIZATION OF INDUCIBLE CLINDAMYCIN RESISTANCE IN STAPHYLOCOCCUS AUREUS ISOLATES FROM A TERTIARY CARE CENTER IN SALEM, INDIA

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Abstract

Background: Staphylococcus species causes infections worldwide, including central nervous system, food poisoning, UTIs, eye, and metastatic diseases. It's the predominant bacterial pathogen in hospital and community settings. **Aim:** The objective is to detect the presence of Clindamycin-resistant Staphylococcus bacteria in different clinical samples and evaluate their resistance to inducible Clindamycin using the D-test as a regular practice to inform treatment decisions. **Materials & Methods:** Specimens were collected from several wards. The majority of them were from the pediatrics, orthopedics, general medicine, dermatology and sexually transmitted disease, and emergency departments. The samples were initially identified as Staphylococcus aureus using Gram Staining and Standard biochemical procedures. Additional tests, such as the Catalase test and Coagulase test, were done. **Result:** A total of 106 strains of Staphylococcus aureus were subjected to susceptibility testing for erythromycin and other antibiotics using the conventional disc diffusion method. Out of the 106 isolates that were resistant to Erythromycin, 54.7% (58/106) exhibited inducible resistance to clindamycin (confirmed by a positive D test), whereas 9.43% (10/106) showed constitutive resistance. Out of a total of 106 isolates, 46 of them, which is equivalent to 43.3%, exhibited the MS phenotype, as indicated by a negative D test result. Conclusion: The presence of Clindamycin resistance, specifically iMLSB and cMLSB, in the Staphylococcus aureus organism, particularly in Methicillin resistant Staphylococcus aureus, highlights the importance of routinely conducting the D-test. This test involves using Clindamycin against anti-staphylococcal antibiotics like Vancomycin and Linezolid for the treatment of Staphylococcal infections.

Keywords: Clindamycin Resistance in Staphylococcus Aureus, MRSA, Phenotype, D-test, Gram Staining.

INTRODUCTION

Staphylococcus aureus is the pathogen responsible for infecting the skin and mucous membranes. Clindamycin is an antibiotic that is commonly used to treat a variety of infections such as abscesses, pneumonia, osteomyelitis, endocarditis, arthritis, and sepsis. It is preferred due to its effective drug metabolism, availability in both intravenous and oral forms, high oral availability of 90%, lower cost, and strong ability to penetrate tissues. Additionally, it has the ability to inhibit toxin production in S. aureus, making it particularly effective in treating deep abscesses. (1)

The development of resistance occurs as a result of various infections caused by Staphylococcus aureus, particularly the Methicillin-resistant Staphylococcus aureus

(MRSA) strain acquired in hospitals. The MRSA staphylococcus strain exhibits resistance to β-lactam antibiotics, such as Macrolide, lincosamide, streptogramin (MLSB) medications, which are commonly used to treat staphylococcal infections. Excessive usage of the clindamycin medicine can result in the development of inducible clindamycin resistance, which in turn can lead to the failure of therapeutic treatment.

Clindamycin, an antibiotic belonging to the macrolide lincosamide streptogramin B (MLSB) class, is effective in treating skin and soft tissue infections caused by S. aureus in individuals with penicillin allergies (2). Clindamycin is a viable option for treating infections, since it may be administered through injections or taken orally. It is also effective in distributing throughout the body's tissues and has the ability to inhibit the growth of S. aureus bacteria. Treatment failure with clindamycin is caused by the absence of inducible clindamycin resistance (4, 5). Labeling Staphylococci as clindamycin resistant could result in the discontinuation of Clindamycin prescription, even if the bacteria are just resistant to erythromycin and susceptible to clindamycin. The user's text is simply "(6)".

The primary mechanisms of resistance to MLSB drugs include target site modification, efflux pump activation, and mutation. These three mechanisms are responsible for the MLSB phenotype, which can be either constitutive (cMLSB phenotype) where rRNA methylase is consistently produced, or inducible (iMLSB phenotype) where methylase is only produced in the presence of certain substances such as erythromycin.

During the treatment of the patient, it is possible for the iMLSB phenotypes to undergo mutation and become cMLSB phenotypes. The Clinical and Laboratory Standards Institute (CLSI) suggests using the double-disk diffusion method, known as the D-test, to detect inducible clindamycin resistance in Staphylococcus aureus.

The primary mechanisms of resistance to MLSB medicines involve alterations in the target site, resulting in resistance mediated by an active efflux pump encoded by the msrA gene, known as the MS phenotype [7]. ErmA, ermB, and ermC provide resistance to MLS type B (MLSB) by modifying the target site of the ribosome [8]. The variation in ribosomal target is caused by the use of broad-spectrum resistance antibiotics like as macrolides and lincosamides, which is encoded by a cluster of erm (erythromycin ribosome methylase) genes. ErmA and ermC are commonly found in staphylococcal genomes. This process can exist in two forms: constitutive cMLSB or inducible iMLSB. The constitutive form produces methylase continuously, while the inducible form only produces methylase when an inductor is present (9).

Studies have shown that the use of clindamycin to treat patients with iMLSB can result in cMLSB and treatment failure (10). To prevent this failure, we can employ the use of an inducible clindamycin resistance (ICR) test, such as the D-test. Resistance mechanisms arise as a result of changes in phenotypes within hospital communities and various geographical regions, patient groups, bacterial strains, and bacterial susceptibility patterns (11).

The objective of this study was to ascertain the prevalence of Staphylococcus aureus isolates with inducible clindamycin resistance in our specific geographic region by the utilization of the D-test. The objective is to examine the occurrence of macrolide resistance and identify the ermA, ermB, and ermC genes in clinical isolates of S. aureus.

In order to treat patients infected with staphylococci that are typically resistant to clindamycin but merely resistant to erythromycin, alternative antibiotics are used instead of clindamycin. Additionally, a simple laboratory test called the D-zone test is recommended.

MATERIALS AND METHODS

This cross-sectional study collected a total of 211 S. aureus isolates from Vinayaka Mission KirupanandaVariyar Medical College and Hospitals, Salem during a period of 2 years, from November 2019 to February 2022.Isolates were acquired from various wards. The majority of them were from the pediatrics, orthopedics, general medicine, dermatology and sexually transmitted diseases, and emergency medicine departments.The samples underwent standard examinations such as Gram Staining and biochemical procedures. Additionally, specific tests such as the Catalase test and Coagulase test were conducted to confirm the presence of staphylococcus.

The antibiotic susceptibility testing was conducted using the modified Kirby Bauer's disc diffusion method on Mueller Hinton agar plates. The following antibiotic discs were used: erythromycin (15 µg), cefoxitin (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), and vancomycin (30 µg), in accordance with the CLSI guidelines. The user's text is "[12]". An inhibitory zone of 19 mm or smaller surrounding the cefoxitin disc confirms the presence of MRSA.

D Zone Test by disc diffusion method:

If the antibiotic exhibits inducible resistance to Clindamycin, it was evaluated. The Mueller-Hinton agar plate is prepared by inoculating bacterial suspensions. A disc of erythromycin (15 µg) is then placed 15 mm away from a disc of clindamycin (2 µg), with the edges of the discs touching.

After being incubated at a temperature of 37°C overnight, the presence of a flattened zone (formed like the letter D) around clindamycin, located between two discs, suggests the development of inducible clindamycin resistance (12). Following the testing, three distinct phenotypes were detected and subsequently evaluated. Only strains of S. aureus that were resistant to erythromycin were included. All strains that are susceptible to erythromycin are not included.

Amplification of erm genes:

The PCR amplification processes were conducted in a 25 µl volume, consisting of 10 µl of 2x (Promega) PCR mix, 0.2 pM (0.5µl) concentrations of each primer, 1 µl of DNA template, and the remaining volume was filled with molecular grade water. The process began with an initial denaturation at a temperature of 94°C for a duration of 10 minutes. This was followed by 30 cycles of amplification, with each cycle consisting of a denaturation phase at 94°C for 30 seconds, an annealing step at 53°C for 30 seconds, and an extension step at 72°C for 1 minute. The final cycle differed in that the extension stage lasted for 10 minutes. The PCR results were examined using a 1.5% agarose gel.

Data analysis:

The statistical analysis for the required sample size per group was carried out using Statistica version 9 (StatSoft, Inc, 1984-2009, USA). All other statistical analyses were performed using the Statistical Package for Social Sciences for Windows 8.0 software.

The results are presented as means with their respective standard deviations. The results were evaluated within a 95% confidence interval, and significance was determined with a probability level of less than 0.05.

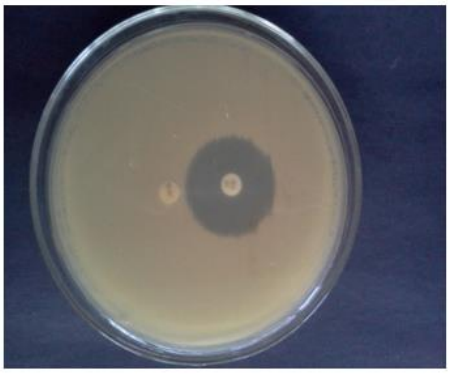
RESULTS

A total of 106 strains of Staphylococcus aureus were subjected to susceptibility testing for erythromycin and other antibiotics using the conventional disc diffusion method. Out of the 106 isolates that were resistant to Erythromycin, 54.7% (58/106) exhibited inducible resistance to clindamycin (confirmed by a positive D test), whereas 9.43% (10/106) showed constitutive resistance. Out of the 106 isolates, 46 of them, which is equivalent to 43.3%, exhibited the MS phenotype (D test negative).

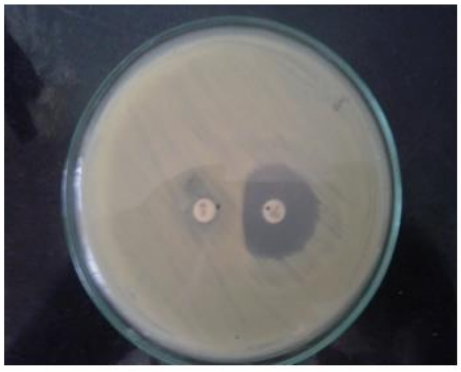
Among the 106 isolates that were resistant to Erythromycin, 39.6% (42/106) were Methicillin-Susceptible Staphylococcus aureus (MSSA) and 64.1% (68/106) were Methicillin-Resistant Staphylococcus aureus (MRSA). The prevalence of clindamycin resistance induced by MSSA (with positive D test) was 54%. Our analysis found that around 9.43% of the MSSA isolates exhibited constitutive resistance, as indicated in Table 1.

Table1: D test among Erythromycin Resistant Staphylococci isolates

	D test -ve (MS phenotype)	D test +ve (iMLSB)	cMLSB
MSSA (46)	18 (39.1%)	22 (47%)	4 (8.6%)
MRSA (60)	24 (41.17%)	32 (53.3%)	6 (9.8%)
TOTAL (106)	42 (39.6%)	54 (50.9%)	10 (9.4%)



D Test Negative



D Test Positive

Genotypic detection of Clindamycin Resistance by Polymerase Chain Reaction:

The PCR analysis revealed the presence of solely the erm A and erm B genes in the iMLSB isolates (16). Erm A genes are more frequent compared to erm B genes. Three isolates were discovered to contain both A and B genes. One isolate exhibited neither the erm A nor the erm B gene. Table 2 and Chart 2

Table 2: Prevalence of erm A gene & erm B gene (Isolate –iMLS B Staphylococcus aureus)

Genotype	PCR result	
ermA	Positive	50
	Negative	30.4
ermB	Positive	25
	Negative	50

Among ermA, ermB, and ermC genes by PCR, ermC is the predominant genetic determinant for the expression of resistance to macrolides among MRSA 21

Chart 2: Distribution of *erm A* and *erm B* genes among iMLSB phenotypes

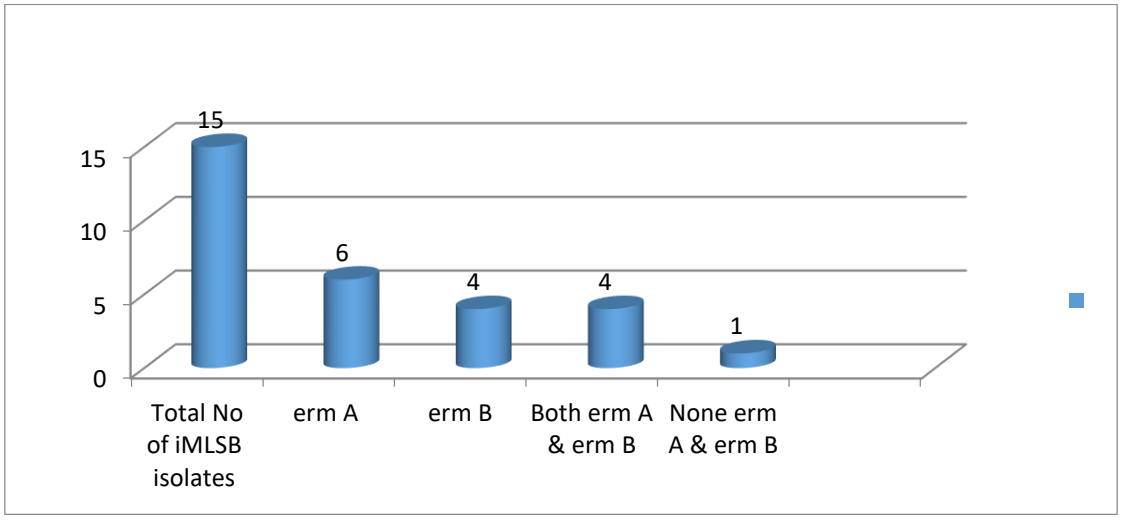
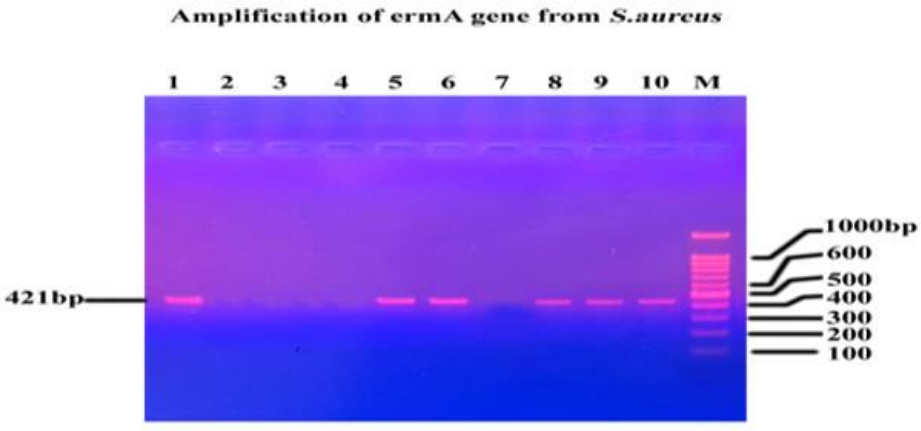
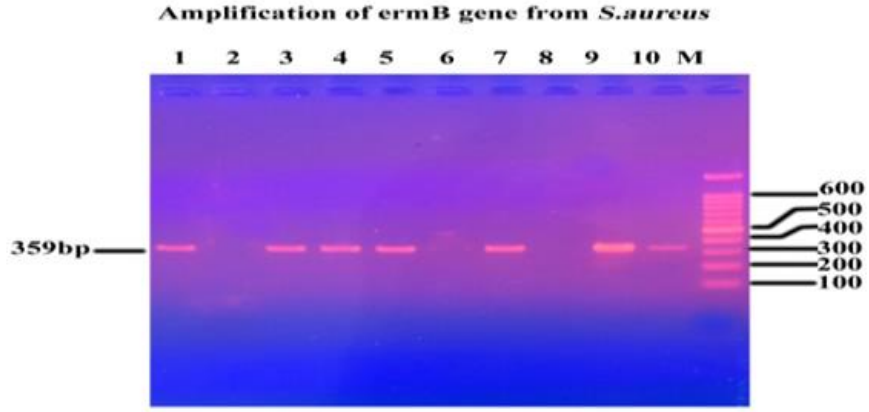


Table 3: Primers and PCR Cycle conditions

Gene	Target Sequence Product	Product size (bp)
erm A	GTTCAAGAACAATCAATACAGAG GGATCAGGAA AAGGACATTTTAC	**421**
erm B	CCGTTTACGA AATTGGAACA GGTAAAGGGC GAATCGAGAC TTGAGTGTGC	**359**





DISCUSSION

During our research, we found that 60% of S. aureus infections were caused by MRSA, which was identified using cefoxitin. Anupurba et al. (2003) found a prevalence of 54.82%, while Joshi et al. (2013) observed 54% MRSA among S. aureus infections [14, 15]. The variation in incidence of inducible clindamycin could be attributed to geographic locations and different hospital setups [16].

According to the investigations conducted by Kavitha Prabhu and others, the percentages of inducible resistance and constitutive clindamycin resistance were higher in MRSA compared to MSSA, with rates of 20% and 16.66% for inducible resistance, and 6.15% for constitutive resistance in MRSA and MSSA, respectively. My study demonstrates that the prevalence of inducible clindamycin resistance is greater among methicillin-resistant Staphylococcus aureus (MRSA) compared to methicillin-sensitive Staphylococcus aureus (MSSA). This represents a rare occurrence where just a small number of previous investigations have revealed similar findings. Several investigations have demonstrated a remarkably high prevalence of MRSA with inducible resistance. In contrast, several investigations have demonstrated a higher prevalence of inducible resistance in methicillin-susceptible Staphylococcus aureus (MSSA) compared to methicillin-resistant Staphylococcus aureus (MRSA).

The study found that the department of surgery had the highest number of MRSA isolates (25.2%), followed by the emergency department (11.75%). This could be attributed to the fact that surgical interventions were required in a majority of the cases. Adam et al. (2009) and Valsesia et al. (2010) found that 35% of cases originated from the surgical ward, and 86% of MRSA infections were frequently found in skin and soft tissue infections [17, 18]. Another study found that skin and soft tissue infections were the most often reported cases in emergency wards. Among these infections, MRSA was the most prevalent strain isolated from abscesses (61%), wounds (53%), and cellulitis with purulent discharge (47%) [19]. Concurrently with the previous study, most of the cases originated from surgical wards, and MRSA was frequently found in skin and soft tissue infections.

Out of the 106 S. aureus isolates, 32 (53.33%) showed inducible resistance [20,21]. Another study, with 288 Staphylococcal isolates, revealed that 116 (40.27%) of them exhibited resistance to cefoxitin and were classified as MRSA. A D-test was conducted, which revealed that 21 individuals (18.1%) had erythromycin resistance and clindamycin sensitivity, confirming the presence of inducible clindamycin resistance [22-25]. Simultaneously, we have reported that out of 106 S. aureus isolates, 60 (56.6%) were identified as MRSA. Out of the total, 32 (30.2%) showed evidence of inducible clindamycin resistance.

Kavitha Prabhu, along with others, conducted research that also performed the same test to corroborate the inclusion of the D-test as a standard procedure for antimicrobial testing. Administering clindamycin without screening for inducible resistance in S. aureus may lead to the use of incorrect clindamycin treatment. Conversely, a negative result for inducible clindamycin resistance suggests that clindamycin is effective and offers a highly favorable treatment choice. The user's text is "[26]".

Due to the fact that the iMLSB resistance mechanism is not detected using normal susceptibility test methods and its frequency differs depending on the geographic region, the D-test is essential for routine antimicrobial susceptibility testing.

Devi Thapa and colleagues conducted a study that emphasizes the same point. The significant prevalence of MRSA and iMLSB phenotypes in S. aureus highlights the necessity of including methicillin resistance testing and D-test in routine susceptibility testing to ensure efficient management of S. aureus.

The resistance shown in Subsini Majhi, Muktikesh Dash, and others is mostly attributed to factors such as age groups, antibiotic prescription patterns, and methicillin susceptibility. Identifying the phenotypic pattern of inducible Clindamycin resistance and establishing a clear antibiotic policy might be beneficial in decreasing the prevalence of MRSA infections and improving the success rate of Clindamycin therapy in the hospital.

CONCLUSION

Staphylococcus aureus, specifically the strain that is resistant to Methicillin Staphylococcus aureus, namely Methicillin-resistant Staphylococcus aureus (MRSA), has emerged as a significant worldwide health issue, affecting both community settings and hospitals. Therefore, resistance to antibiotics arises as a result of their widespread usage. Consequently, it is imperative to implement antibiotic stewardship control strategies within our healthcare settings. The sensitivity pattern of MRSA to vancomycin and adherence to an antibiotic policy are effective in lowering hospital-acquired infections. If both inducible macrolide-lincosamide-streptogramin B (iMLSB) and constitutive macrolide-lincosamide-streptogramin B (cMLSB) resistance mechanisms are present, the recommended treatment choices for methicillin-resistant Staphylococcus aureus (MRSA) include Linezolid and Vancomycin medicines. In order to identify these resistance mechanisms through observable characteristics, we must regularly do the D-test. This will assist clinicians in determining the appropriate use of Clindamycin.

Limitations

As this was a single center study with a comparatively short sample size, results of this study cannot be generalized. Generalization requires the support of results from similar large studies.

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Conflicts of interest: There are no conflicts of interest**.**

Ethical statement

Institutional ethical committee accepted this study. The study was approved by the institutional human ethics committee, Vinayaka Mission's Kirupananda Variyar Medical College & Hospital, Salem. Informed written consent was obtained from all the study participants and only those participants willing to sign the informed consent were included in the study. The risks and benefits involved in the study and the voluntary nature of participation were explained to the participants before obtaining consent. The confidentiality of the study participants was maintained.

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Authors’ contributions:

Mrs.M.Nithya - conceptualization, data curation, investigation, methodology, project administration, visualization, writing—original draft, writing—review and editing; ***Dr.A. Sowmya*** -conceptualization, methodology, writing—original draft, writing—review and editing; ***Dr.S.Mathavi*** - conceptualization,

visualization, supervision, writing—original draft; ***Mrs.P.Hemapriyadharshini***- methodology, writing—original draft, writing, review and editing. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. All authors have read and agreed to the published version of the manuscript***.***

Data Availability

All datasets generated or analyzed during this study are included in the manuscript.

Informed Consent

Written informed consent was obtained from the participants before enrolling in the study

References

1) S. Majhi, M. Dash, D. Mohapatra, A. Mohapatra, and N. Chayani, "Detection of inducible and constitutive clindamycin resistance among Staphylococcus aureus isolates in a tertiary care hospital, Eastern India," Avicenna Journal of Medicine, vol. 6, no. 3, pp. 75–80, 2016

2) Forrest GN, Oldach DW. Macrolides and clindamycin. In: Gorbach SL, Barlett JG, Blacklow NR, editors. Infectious Diseases. 3rd ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2004. p. 223.

3) Zelazny MA, Ferraro JM, Glennen A, Hindler FJ, Mann ML, Munro S, et al. Selection of strains for quality assessment of the disk induction method for detection of inducible clindamycin resistance in staphylococci: A CLSI collaborative study. J Clin Microbiol 2005;43:2613-5.

4) Chelae S, Laohaprertthisarn V, Phengmak M, Kongmuang U, Kalnauwakul S. Detection of inducible clindamycin resistance in staphylococci by disk diffusion induction test. J Med Assoc Thai 2009;92:947-51.

5) Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical disk diffusion method for detection of inducible clindamycin resistance in Staphylococcus aureus and coagulase-negative staphylococci. J Clin Microbiol 2003;41:4740-4.

6) Gupta KY, Gupta G, Bishnoi RB, Binnani A, Garg PS. Phenotypic detection of inducible clindamycin resistance in Staphylococcus aureus. IJPRBS 2013;2:267-72

7) Jindal N, Singh S, Grover P, Malhotra R. Prevalence of inducible clindamycin resistance among clinical isolates of MRSA in Malwa region of Punjab (North India), PARIPEX. Indian J Res 2013;10:133-4.

8) Lina G, Quaglia A, Reverdy EM, Leclercq R, Vandenesch F, Etienne J. Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. Antimicrob Agents Chemother 1999; 43:1062-6.

9) Saderi H, Owlia P, Eslami M. Prevalence of Macrolide-Lincosamide-Streptogamin B (MLSB) resistance in S.aureus isolated from patients in Tehran, Iran. Iran J Pathol 2009; 4: 161-166.

10) Siberry GK, Tekle T, Carrol K, Dick J. Failure of clindamycin treatment of methicillin resistant Staphylococcus aureus expressing inducible clindamycin resistance in vitro. Clin Infect Dis 2003; 37: 1257-1260.

11) Clinical and Laboratory standards institute. Performance standard for antimicrobial susceptibility testing; nineteen informational supplement M100-S19.Wayne, PA: CLSI 2009.

12) Clinical and Laboratory Standards Institute. 2012. Performance standards for antimicrobial susceptibility testing; twenty-second informational supplement Clinical and Laboratory Standards Institute Wayne, PA.

13) Reddy SP, Suresh R. Phenotypic detection of inducible clindamycin resistance among the clinical isolates of staphylococcus aureus by using the lower limit of inter disk space. JMicrobiol Biotechnol Res Jul-Sep 2008; 51(3):376-8.

14) Joshi S, Ray P, Manchanda V, Bajaj J, Chitnis SD, Vikas G, et al. Methicillin resistant Staphylococcus aureus (MRSA) in India: Prevalence and susceptibility pattern. Indian J Med Res 2013;137:363 9.

15) Anupurba S, Sen MR, Nath G, Sharma BM, Gulati AK, Mohapatra TM. Prevalence of methicillin resistant Staphylococcus aureus in a tertiary referral hospital in eastern Uttar Pradesh. Indian J Med Microbiol 2003;21:49-51.

16) Jindal N, Singh S, Grover P, Malhotra R. Prevalence of inducible clindamycin resistance among clinical isolates of MRSA in Malwa region of Punjab (North India), PARIPEX. Indian J Res 2013;10:133-4.

17) Adam JH, Allen GV, Currie A, McGeer JA, Simor EA, Richardson ES, et al. Community-associated methicillin resistant Staphylococcus aureus: Prevalence in skin and soft tissue infections at emergency departments in the Greater Toronto Area and associated risk factors. CJEM 2009;11:439-46.

18) Valsesia G, Rossi M, Bertschy S, Pfyffer EG. Emergence of sccmec type IV and sccmec Type V Methicillin resistant Staphylococcus aureus containing the panton-valentine leukocidin genesina large academic teaching hospital in Central Switzerland: External invaders or persisting circulators? J Clin Microbiol 2010;48:720-7.

19) Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, et al. Methicillin resistant Staphylococcus aureus infections among patients in the emergency department. N Engl J Med 2006;355:666-74.

20) Valsesia G, Rossi M, Bertschy S, Pfyffer EG. Emergence of sccmec type IV and sccmec Type V Methicillin resistant Staphylococcus aureus containing the panton-valentine leukocidin genesina large academic teaching hospital in Central Switzerland: External invaders or persisting circulators? J Clin Microbiol 2010;48:720-7.

21) Spiliopoulou I, Petinaki E, Papandreou P, Dimitracopoulos G. erm(C) is the predominant genetic determinant for the expression of resistance to macrolide among methicillin resistance Staphylococcus aureus clinical isolates in Greece. J Antimicrob Chemother 2004;53:814-7.

22) Vidhya R, Parimala S, Beena PM. Inducible clindamycin resistance in Staphylococcus aureus isolates from a rural tertiary care hospital, Kolar. J Clin Biomed Sci 2013;3:125-8.

23) Vidhya R, Parimala S, Beena PM. Inducible clindamycin resistance in Staphylococcus aureus isolates from a rural tertiary care hospital, Kolar. J Clin Biomed Sci 2013;3:125-8.

24) Upadhya A, Biradar S. Prevalence of inducible clindamycin resistance in Staphylococcus aureus in a tertiary care hospital in north-east Karnataka, India. Health Sci Int J 2011;1:21-4.

25) Adwan G, Adwan K, Jarrar N, Amleh A. Molecular detection of nine antibiotic resistance genes in methicillin resistant Staphylococcus aureus isolates. Roum Arch Microbiol Immunol 2014;73:9-17.

26) Kavitha Prabhu, Sunil Rao, Venkatakrishna Rao.Inducible Clindamycin Resistance in Staphylococcus aureus Isolated from Clinical Samples. Journal of Laboratory Physicians Jan-Jun 2011-3-1.