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Original Research Article

Prevalence of Induced Clindamycin Resistance in Methicillin Resistant *Staphylococcus aureus* Strains Isolated in a Tertiary Care Hospital

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Abstract

Introduction: Clindamycin is an essential alternative antibiotic in the treatment of *Staphylococcus aureus* infections. The limited treatment choices for MRSA emphasise the necessity of selecting the correct antibiotic. The occurrence of inducible clindamycin resistance, which can lead to treatment failure, is the main issue with using clindamycin for such infections. The D-Test was used in this investigation to detect inducible clindamycin resistance in methicillin-resistant *S. aureus* isolates.

Methods: A D-test employing erythromycin and clindamycin was done according to CLSI standards to determine inducible clindamycin resistance. Four phenotypes were interpreted as methicillin-sensitive (MS) (D-test negative), inducible MLSB (iMLSB) (D-test positive), constitutive MLSB, and sensitive to both.

Results: Of the 470 isolates tested, 80 (17.02%) were MRSA. The prevalence of iMLSB, cMLSB phenotype, MS phenotype and sensitive phenotype in MRSA isolates was 22(28%), 24 (29%), 4 (5%) and 30 (38%), respectively. The majority of MRSA isolates originated from Skin & nasal swabs. All *S. aureus* isolates showed 100% sensitivity to vancomycin and Gentamycin.

Conclusion: This study highlights MRSA-induced clindamycin resistance. Thus, clinical microbiology laboratories should focus on D-zone assays to identify inducible MLSB resistance from constitutive. Increasing MRSA produced clindamycin resistance and antibiotic usage have increased sensitivity, prompting epidemiological investigation.

Keywords: Clindamycin, *Staphylococcus aureus*, D-Test.

Introduction

Staphylococcus aureus has been identified as a significant cause of community and hospital acquired infections [1]. The organism's potential to propagate and trigger outbreaks in hospitals varies. *Staphylococcus aureus* infections typically respond to lactam and related antibiotics. However, treatment of these infections has become difficult due to the emergence of methicillin resistance among *staphylococcus aureus* isolates (MRSA). A few key risk factors for MRSA acquisition are indiscriminate use of several antibiotics, prolonged hospital admissions, intravenous drug usage, and carriage of MRSA in the nose [2].

When lincomycin was first used in clinical trials in 1966, erythromycin was no longer thought to be a safe antianaerobic drug. Despite this, the clearly present "disappointed phenotype" was neglected in the research literature [3]. Clindamycin (Cd), a lincosamide, was commonly utilised to treat *Staphylococcus aureus* in cases of penicillin intolerance or methicillin resistance [4]. Recent results, however, suggest that failure may occur in the situation of inducible Clindamycin resistance despite invitro susceptibility to clindamycin. Clindamycin suppresses toxin and virulence factor generation in Gram-positive organisms by inhibiting protein synthesis [5,6]. The methylase expressed by the erythromycin resistant methylase (erm) and macrolides streptogramins resistance (msrA) genes mediates Clindamycin resistance in Staphylococcus.

Antibiotic efflux, drug inactivation, and target-site change by methylation or mutation cause bacterial resistance to macrolide and lincosamide antibiotics. The three processes affect pathogenic bacteria differently in incidence and clinical implications. Efflux and inactivation only impact some macrolides and lincosamides, whereas ribosomal target change confers broad resistance. Macrolides, lincosamides, and group B streptogramins (MLSB) all suppress bacterial protein synthesis and are used to treat Gram-positive infections.

Methylation of the 23s binding site is the resistance mechanism. If this happens, the bacteria become resistant to both macrolides and lincosamides. Erythromycin's binding to its target is weakened as a result of methylation. MLSB resistance expression might be constitutive or inducible. Many countries have reported the prevalence of induced clindamycin resistance in *S. aureus* [7-10]. Many papers from India [11-15] documented the establishment of induced clindamycin resistance in *S. aureus*.

Inducible clindamycin resistance may indicate mutational constitutive resistance. The D-test involves placing clindamycin and erythromycin discs 15–20 mm apart and looking for flattening of the zone nearest the erythromycin disc [16]. A positive D-test shows an erm gene, which may cause clindamycin resistance and clinical failure. Clindamycin resistance failures are rare [17-21].

These strains are Ery-resistant because erythromycin (Ery) induces this methylase, but modifications in the promoter region of erm allow methylase synthesis without an inducer [22]. Clindamycin cannot bind to this residue after methylation. MRSA is becoming resistant to macrolides and lincosamides, limiting treatment options. Low-dose erythromycin induces MLSB resistance best. Ery induction of Cd resistance can detect MLSB strains in disc approximation experiments [22]. These strains use erythromycin and Cd or lincomycin discs. As Erythromycin diffuses through the agar, lincosamide resistance is developed, flattening or blunting the zone of inhibition near to the disc, giving it a D shape (D zone effect). In January 2004, NCCLS devised a Cd induction testing approach [23] that places Cd discs 15 to 26 mm from an Ery disc in a normal disc diffusion process or on an inoculum check agar plate.

Different phenotypic appearances of D-zone are demonstrated in hospital acquired-MRSA isolates from medical wards, surgical wards, and intensive care units at Viswabharathi Hospital, Kurnool in the current study.

Materials and Methods

This study was carried out over the course of a year, from November 2021 to October 2022 in the Department of Microbiology, Viswabharathi Medical College & Hospital, Kurnool, In this study a total of 470 samples were obtained from patients of medical wards (n=280), surgical wards (n=140) and intensive care units (ICU) (n=50), at Viswabharathi Hospital, Kurnool. Samples comprised of blood

(=28), urine (n=49), pus (n=44), respiratory tract swabs (n=39), ear swabs (n-88), eye swabs (n=49), skin infection swabs (n=79), and anterior nasal swabs (n=94)

Culture

Patients' swabs and bodily fluids were put into blood agar plates, with each plate holding a single patient's sample. These inoculation plates were incubated at 37o C for 18 to 24 hours. The swabs were placed in 7.5% sodium chloride brain heart infusion broth (BHI) after being inoculated on blood agar and incubated at 37°C for 18-24 hours. BHI broth that had been subcultured was inoculated into blood agar plates. *S. aureus* colonies on blood agar plates that were opaque, spherical, and stained with hemolysis were identified by Grams staining, Catalase, and Coagulase (Slide and Tube) assays [24]. Appropriate controls were installed at each level. 153 coagulase positive *S. aureus* strains were recovered and identified from 478 clinical samples.

Testing for antibiotic susceptibility

The medicines Oxacillin (1g), Gentamycin (10g), Erythromycin (15g), Cotrimoxazole (25g), and Vancomycin (30g) were tested for susceptibility using the Kirby- Bauer disc diffusion technique with the quality control strain *S. aureus* ATCC25923 (Hi-media). On Muller-Hinton agar with 4% NaCl and 6g/ml oxacillin, bacterial suspensions with turbidity standards of 0.5 McFarland were inoculated. MRSA was found in isolates that demonstrated visible growth after 24 hours of incubation at 33-35oC. Oxford strains of *S. aureus* NCTC 6571 sensitive to methicillin and *S. aureus* NCTC 12493 resistant to methicillin were used as control organisms. MRSA has finally identified thanks to the finding of the mecA gene by PCR.

D-zone evaluation

When doing the Erythromycin and Clindamycin double disc susceptibility test, the NCCLS guideline2004 [26] was followed (D-zone test). Muller-Hinton agar plates were used to culture all of the isolates (Hi media, India). The clindamycin disc (2 g) was manually placed around 12 mm (edge to edge) from the erythromycin disc (15 g) (Hi media, India) [27]. The induction test results (D-shaped zone) were read using transmitted and reflected light at 16 to 18 hours.

Ethical Approval: Obtained from the Institutional Ethics Committee (IEC).

Results

150 clinical samples out of 470 were found to be *S. aureus* positive. Testing the sensitivity to oxacillin allowed researchers to distinguish 80 of 150 samples that tested positive for *S. aureus* as MRSA strains. Tables 1 and 2 indicate the overall number of MRSA isolates as well as their pattern of antibiotic susceptibility.

Table 1: Showing total no of isolates of *Staphylococcus aureus* and MRSA isolated from various samples of patients from 3 groups (Medical wards, surgical wards and Intensive care units (ICU).

Source of sample	Total No. of samples	Coagulase Positive *S. aureus*	MRSA
Blood	28	03	01
Urine	49	10	02
Pus	44	20	13
Nasal swabs	94	44	25
Respiratory tract swabs	39	02	00
Eye swabs	49	01	00
Ear swabs	88	30	13
Skin swabs	79	40	26
Total	470	150	80

Table 2: Susceptibility pattern of the coagulase positive *Staphylococcus aureus*

Antibiotic	Resistance %	Intermediate%	Sensitivity%	Total
Oxacillin	53	-	47	150
Gentamycin	-	-	100	150
Erythromycin	21	21	58	150
Co-trimoxazole	40	-	60	150
Vancomycin	-	-	100%	150

Inducible clindamycin resistant-Phenotypes

Two induction and four non-induction phenotypes were found in 80 MRSA isolates by disc diffusion (Table 2 and Fig.1). 19 isolates showed the D-zone phenotype after induction, with a blunt edge but a clear zone of inhibition surrounding the Cd disc (at different distances Fig. 2). Three isolates were D+. These isolates had zone of inhibition blunting and small colonies around the Cd disc. Cd induction supports D and D+ results (inducible MLSB resistance). Four isolates showed Ery-resistant and Cd-susceptible zone widths without blunting. Negative phenotype. Hazy D (HD phenotype) was seen in 17 isolates, with some blunting. Because growth reached the disk's edge, the HD trait was not considered inducible (indicating Cd resistance). Seven isolates showed Ery and Cd resistance and confluent growth around both discs with no inner zone of inhibition, suggesting the R phenotype. HD and R phenotypes are cMLSB resistant. 30 showed significant inhibition around Ery and Cd discs, confirming the S phenotype.

D and D+ isolates had similar Ery and Cd zone diameters. The inducible D zone was visible at 16–18 hrs, but the small colonies extending up to the Cd were more visible at 24 hrs, especially when using transmitted light. Neg and D+ phenotypic isolates had equal Cd and Ery zone sizes. For most HD isolates, the hazy zone surrounding the Cd disc separated it from the solid growth in R phenotype. (Table 3)

Table 3: Characteristics of clindamycin induction test phenotypes as tested by disk diffusion

Induction test	Resistance phenotype	Cd result	Ery result	No. of isolates
D	Inducible MLSB	S	R	19
D+	Inducible MLSB	S	R	03
Neg	MLSB	S	R	04
HD	Constitutive MLSB	R	R	17
R	Constitutive MLSB	R	R	07
S	No resistance	S	S	30

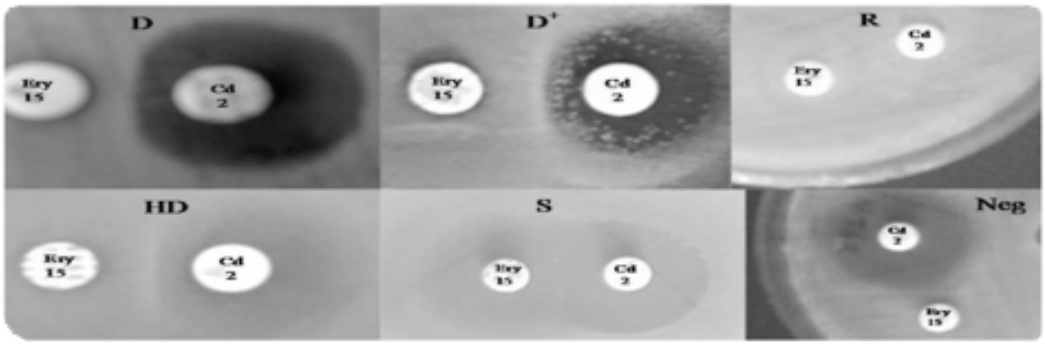

Figure 1: Showing the six phenotypes observed during Cd induction testing of *S.aureus* by disk diffusion. Ery (15 µg) and Cd (2 µg) disks.

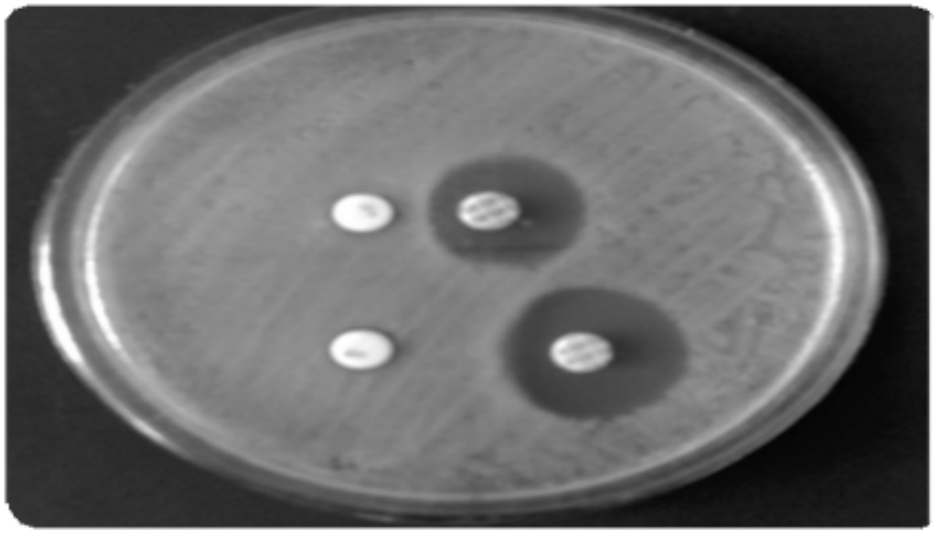

Figure 2: Inducible clindamycine resistance expressed by *S.aureus* at different distances.

Discussion

Methicillin-resistant *S. aureus* causes high morbidity and mortality. MRSA strains account for 53.6% of hospital-acquired infections in Indian hospitals, and antibiotic sensitivity testing has indicated 30% to 80% methicillin resistance [28]. Antibiotic resistance is usually triggered by inhibitor deactivation or target change (mutations of ribosomal proteins or rRNA genes). Active efflux (msrA gene) and ribosomal target modification (erm gene) can resist MLSB (MLSB resistance). The MLS group's binding to the 23s region of the 50S subunit of bacterial ribosomes prematurely dissociates the peptidyl-tRNA, giving an antibiotic action. Peptide bond formation is promoted by the 23s region of the 50S (peptidyl trans- ferase centre), and an aminoacyl-tRNA is linked to a developing peptide chain via the P site of the donor tRNA. A site. MLS antibiotics stop this. Resistance is 23s binding site methylation. This makes bacteria resistant to macrolides and lincosamides. Methylation weakens erythromycin's target binding. Inducible or constitutive MLSB resistance. Bacteria generate dormant mRNA without methylase to induce resistance. Only macrolide inducers activate mRNA. The constitutive expression generates active methylase mRNA without erythromycin. Non-inducible macrolides and lincosamides affect inducible erm gene-carrying organisms but not inducers.

Mutations in the erm promoter region allow methylase synthesis without erythromycin [29,30]. These mutants resist erythromycin and clindamycin forever. Gram-positive, spirochete, and anaerobic bacteria express erm methylases, which macrolides and lincosamides target.

Erythromycin-inducible clindamycin resistance was found in 28% of MRSA strains. As a result of the fact that the binding sites for macrolides, lincosamides, and streptogramins B in 23S rRNA are highly conserved and often overlap, induced clindamycin-resistant *S. aureus* is multidrug resistant to many antimicrobial agents, limiting treatment options and increasing the risk of inadequate treatment and morbidity and mortality. Inducible clindamycin resistance has complicated multidrug-resistant organism treatment in recent years. Clindamycin was an effective treatment for skin and soft tissue infections caused by these strains, but using it alongside erythromycin produced clindamycin resistance [31].

This investigation validated phenotypic presentations of the Cd zone next to a conventional 15 g Ery disc in a classic disc diffusion test. Induced Cd resistance strains with the ermA or ermC gene constitutively expressed have a flattening Cd disc diffusion zone (D-zone effect). Cd-resistant strains differ in Cd zone shape [32]. After 16–18 hours, positive disc diffusion induction findings (D and D+) were read using reflected light, while transmitted light helped distinguish non-inducible phenotypes like HD and R. Disk tests incubated for up to 24 hours helped distinguish D from D+ phenotypes, but they were not needed to distinguish Cd inducible from non-inducible isolates.

Most induction test studies find inducible Cd resistance in Ery-resistant but Cd-susceptible isolates [33]. Even before results for Ery and Cd resistance are known, some labs do the D-zone test on susceptibility testing purity plates. Many isolates resistant or susceptible to both erythromycin and clindamycin were examined to determine phenotypes. The HD zone is actually a Cd-induced phenotype. If the Cd test was not first interpreted phenotypically, our isolates had several macrolide resistance genes, making predictions unreliable. For therapeutic reasons, it is important to differentiate between erm-mediated inducible MLSB (D and D+ phenotypes) and msrA-mediated cMLSB resistance in a clinical laboratory. However, differentiating D from D+ phenotypes may help epidemiologists characterise isolates in health care and community settings.

Therapy suggestions require proper susceptibility data. If created resistance can be consistently recognised in clinically significant isolates on a regular basis, Cd can be safely and effectively delivered to patients with true Cd-susceptible strains. Knowing the incidence of inducible resistance in clindamycin-erythromycin *S. aureus* might reduce adverse effects while maintaining Cd effective. Inducible MLSB resistance occurs when bacteria produce latent mRNA that cannot encode methylase, hence clinical microbiology laboratories should perform the D-zone test to distinguish it from constitutive resistance.

Only macrolide inducer erythromycin activates the mRNA. Constitutive expression generates active methylase mRNA without Erythromycin. Inducible erm gene strains withstand inducers but not noninducer macrolides and lincosamides. Mutations in the erm promoter region allow methylase synthesis without erythromycin [30]. Lincosamides should not be taken with erythromycin, chloramphenicol, or most bactericidal medicines since they have a shared site of action and may interact.

Conclusion

This study shows MRSA-induced clindamycin resistance. Since MLSB medicines share a site of action, clindamycin and macrolide erythromycin

should not be given simultaneously. Clinical microbiology laboratories should use D-zone tests to distinguish inducible MLSB resistance from constitutive. MRSA-induced clindamycin resistance and antibiotic use have increased sensitivity, necessitating epidemiological inquiry.

References

1. Sheagren, J.N. Staphylococcus aureus. The persistent pathogen (first of two parts). N Engl J Med. 1984; 310: 1368-1373.
2. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev. 1997; 10: 505-520.
3. Reig M, Fernandez M.C, Ballesta J.P, Baquero F. Inducible expression of ribosomal clindamycin resistance in Bacteroides vulgatus. Antimicrob Agents Chemother. 1992; 36: 639-642.
4. Daurel C, Huet C, Dhalluin A, Bes M, Etienne J, Leclercq R. Differences in potential for selection of clindamycin-resistant mutants between inducible erm(A) and erm(C) *Staphylococcus aureus* genes. J Clin Microbiol. 2008; 46: 546-550.
5. Herbert S, Barry P, Novick R.P. Subinhibitory clindamycin differentially inhibits transcription of exoprotein genes in Staphylococcus aureus. Infect Immun. 2001; 69: 2996-3003.
6. Marcinak J.F, Frank A.L. Epidemiology and treatment of community- associated methicillin-resistant *Staphylococcus aureus* in children. Expert Rev Anti Infect Ther. 2006; 4: 91-100.
7. Jenssen W.D, Thakker-Varia S, Dubin D.T, Weinstein M.P. Prevalence of macrolides-lincosamides-streptogramin B resistance and erm gene classes among clinical strains of staphylococci and streptococci. Antimicrob Agents Chemother. 1987; 31: 883-888.
8. Braun L, Craft D, Williams R, Tuamokumo F, Ottolini M. Increasing clindamycin resistance among methicillin-resistant *Staphylococcus aureus* in 57 northeast United States military treatment facilities. Pediatr Infect Dis J. 2005; 24: 622-626.
9. Delialioglu N, Aslan G, Ozturk C, Baki V, Sen S, Emekdas G. Inducible clindamycin resistance in staphylococci isolated from clinical samples. Jpn J Infect Dis. 2005; 58: 104-106.
10. Fokas S, Tsironi M, Kalkani M, Dionysopouloy M. Prevalence of inducible clindamycin resistance in macrolide- resistant Staphylococcus spp. Clin Microbiol Infect. 2005; 11: 337-340.
11. Goyal R, Singh N.P, Manchanda V, Mathur M. Detection of clindamycin susceptibility in macrolide resistant phenotypes of Staphylococcus aureus. Indian J Med Microbiol. 2004; 22: 251-254.
12. Gadepalli R, Dhawan B, Mohanty S, Kapil A, Das B.K, Chaudhry R. Inducible clindamycin resistance in clinical isolates of Staphylococcus aureus. Indian J Med Res. 2006; 123: 571-573.
13. Navaneeth B.V. A preliminary in vitro study on inducible and constitutive clindamycin resistance in *Staphylococcus aureus* from a South Indian tertiary care hospital. Int J Infect Dis. 2006; 10: 184- 185.
14. Angel M.R, Balaji V, Prakash J, Brahmadathan K.N, Mathews M.S. Prevalence of inducible clindamycin resistance in gram positive organisms in a tertiary care centre. Indian J Med Microbiol. 2008; 26: 262-264.
15. Ciraj A.M, Vinod P, Sreejith G, Rajani K. Inducible clindamycin resistance among clinical isolates of Staphylococci. Indian J Pathol Microbiol. 2009; 52: 49-51.
16. Schmitz F.J, Petridou J, Fluit A.C, Hadding U, Peters G, von Eiff C. Distribution of macrolide-resistance

genes in *Staphylococcus aureus* blood-culture isolates from fifteen German university hospitals. M.A.R.S. Study Group. Multicentre Study on Antibiotic Resistance in Staphylococci. Eur J Clin Microbiol Infect Dis. 2000; 19: 385-387.
17. Gopal Rao G. Should clindamycin be used in treatment of patients with infections caused by erythromycin-resistant staphylococci? Journal of Antimicrobial Chemotherapy. 2000; 45: 715.
18. Drinkovic D, Fuller E.R, Shore K.P, Holland D.J, Ellis-Pegler R. Clindamycin treatment of *Staphylococcus aureus* expressing inducible clindamycin resistance. J Antimicrob Chemother. 2001; 48: 315-316.
19. Frank A.L et al. Clindamycin treatment of methicillin- resistant *Staphylococcus aureus* infections in children. Pediatr Infect Dis J. 2002; 21: 530-534.
20. Fiebelkorn K.R, Crawford S.A, McElmeel M.L, Jorgensen J.H. Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase- negative staphylococci. J Clin Microbiol. 2003; 41: 4740-4744.
21. Siberry G.K, Tekle T, Carroll 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.
22. Weisblum B, Siddhikol C, Lai CJ, Demohn V. Erythromycin- inducible resistance in Staphylococcus aureus: requirements for induction. J Bacteriol. 1971; 106: 835-847.
23. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial Susceptibility testing, 17th informational supplement (M100-517). 2007, Wayne Pa: Clinical and Laboratory Standards Institute. 2007.
24. Cadness-Graves B W.R, Harper G.H, Miles A.A. Slide-test for coagulase-positive staphylococci. Lancet i: 1943; 736-738.
25. NCCLS. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard M7- A6. NCCLS. Wayen PA. USA. 2003.
26. NCCLS. Performance standards for antimicrobial susceptibility testing; 14th informational supplement. M100-S14. NCCLS, Wayne PA. 2004.
27. Sutcliffe J, Tait-Kamradt A, Wondrack L. Streptococcus pneumoniae and Streptococcus pyogenes resistant to macrolides but sensitive to clindamycin: a common resistance pattern mediated by an efflux system. Antimicrob Agents Chemother. 1996; 40: 1817-1824.
28. Anupurba S, Sen M.R, Nath G, Sharma B.M, Gulati A.K, Mohapatra T.M. Prevalence of methicillin resistant *Staphylococcus aureus* in a tertiary referral hospital in eastern Uttar Pradesh. Indian J Med Microbiol. 2003; 21: 49-51.
29. Weisblum B. Insights into erythromycin action from studies of its activity as inducer of resistance. Antimicrobial Agents and Chemotherapy. 1995; 39: 797–805.
30. Leclercq R. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clinical Infectious Diseases. 2002; 34: 482-492.
31. Sanchez M.L F.K, Jones R.N. Occurrence of macrolide-lincosamide-streptogramin resistances among staphylococcal clinical isolates at a university medical center. Is false susceptibility to new macrolides and clindamycin a contemporary clinical and in vitro testing problem. Diagnostic Microbiology and Infectious Disease. 1993; 16: 205-213.
32. Westh H H.D, Vuust J, Rosdahl V.T. Prevalence of erm gene classes in erythromycin-resistant *Staphylococcus aureus* strains isolated between 1959

and 1988. Antimicrobial Agents and Chemotherapy. 1995; 39: 369-373.
33. Wondrack L, Massa M, Yang B.V, Sutcliffe J. Clinical strain of *Staphylococcus aureus* inactivates and causes efflux of macrolides. Antimicrob Agents Chemother. 1996; 40: 992-998.