Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 05, pp.15811-15817, May, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

PHENOTYPIC AND GENOTYPIC EXPRESSION OF CLINDAMYCIN RESISTANCE AMONG CLINICAL ISOLATES OF STAPHYLOCOCCUS AUREUS

***Geeta, S.H. and Rama, N.K.**

Department of Microbiology, MVJ Medical College and Research hospital, Hoskote, Bangalore

ARTICLE INFO

Article History:
Received 06th February, 2015
Received in revised form
06th March, 2015
Accepted 28th April, 2015
Published online 25th May, 2015

Key words:

Clindamycin,
D-test,
Inducible Macrolide-Lincosamide-Streptogramin B resistance,
Staph aureus,
ermA and ermC genes.

ABSTRACT

Resistance to antimicrobial agents among Staphylococcus aureus is an increasing problem. Emergence of Methicillin resistance in hospital acquired and also among community acquired Staphylococcus aureus has resulted with very few therapeutic options to treat staphylococcal infections. The Macrolide Lincosamide Streptogramin B [MLSB] family of antibiotics is one such alternative with clindamycin being the preferred drug due to its excellent pharmacokinetic properties. Hence clindamycin is commonly used to treat serious infections including skin and soft tissue infections produced by drug resistant Staphylococcus aureus including MRSA. As clindamycin is a safe drug to use in serious MRSA infections, it was continuously misused resulting in increased resistance to the drug. Clindamycin resistance may be constitutive or inducible. Two common genes responsible for resistance to macrolide, lincosamide and streptogramin B (MLSB) antibiotics are the ermA and ermC genes. In vitro routine tests for clindamycin susceptibility may fail to detect inducible clindamycin resistance due to erm genes resulting in treatment failure; thus necessitating the need to detect such resistance by a simple disc approximation test [D test] on a routine basis. The present study was undertaken to know the rate of inducible clindamycin resistance and erm genes among clinical isolates of Staphylococcus aureus in our hospital.

Materials and Methods: 355 Staphylococcal species were isolated from various clinical specimen in the department of microbiology over a period of 6 months. Of which, 81 S. aureus isolated and identified by standard protocol were included in the present study. MRSA & MSSA were detected using cefoxitin [30 µg] disc as per CLSI criteria. Antibiotic sensitivity to routine antimicrobial agents was done by Kirby Bauer's disk diffusion method. D test was performed on all erythromycin resistant MRSA and MSSA isolates to detect phenotypic expression of clindamycin resistance. ermA and ermC genome identified by PCR on D test positive isolates.

Results: Out of 81 isolates of S. aureus, 18 (22%) were found to be MRSA & 63(78%) MSSA. Erythromycin resistance was seen in 70(86%) & 11(14%) were erythromycin sensitive of S aureus. Out of the total 70 erythromycin resistant strains of Staph aureus, 22(31%) isolates showed inducible clindamycin resistance, 26(37%) showed constitutive clindamycin resistance and 22(31%) were MS phenotypes. Hence D test was positive among 16(73%) MSSA & 06(27%) MRSA isolates. D test positive strains (22) were tested for ermA and ermC genes. 4/22(18.2%) ermA genes and 15/22(68.2%) ermC in erythromycin resistant S aureus strains respectively.

Conclusion: Clindamycin is a very safe and effective drug which can be used against CA & HA MRSA infection. Development of in vivo therapeutic failure can be prevented by doing a D test routinely in microbiology lab.

INTRODUCTION

Staphylococcus aureus is one of the most common human pathogens which cause wide range of CA & HA infections. S aureus is a pathogen of greatest concern because of its intrinsic virulence, its ability to cause a diverse array of life threatening infections and its capacity to adapt to different environmental conditions (Lowy, 1998; Waldvogel, 2000).

****Corresponding author: Geeta, S.H.***
Department of Microbiology, MVJ Medical College and Research hospital, Hoskote, Bangalore.

In 1956, soon after the introduction of erythromycin into therapy, resistance emerged in staphylococci. However, resistance to erythromycin and clindamycin is increasing among clinical isolates of S aureus worldwide. (Lina *et al.,* 1999). The spread of methicillin-resistant clones in 1960s is reminiscent of the emergence of penicillin resistance in the 1940s. By the late 1960s, more than 80% of both community and hospital-acquired staphylococcal isolates were resistant to β lactams (Franklin, 2003; Schito, 2006; Rajaduraipandi, 2006). Also in the past decade, MRSA has emerged as pathogen for community acquired infections (CA-MRSA).

Unlike hospital acquired MRSA, the CA MRSA are known to be sensitive to drugs other than vancomycin, such as, ciprofloxacin, doxycycline, minocycline, trimethoprim sulphamethoxazole and clindamycin although susceptibility to these agents may vary by geographic area (Naimi *et al*., 2003).
The traditional recommendations of various b-lactam antibiotics for first line therapy, including use of oral b-lactams for treatment of outpatients with staphylococcal infection, requires reassessment in the era of CA-MRSA. Because CA-MRSA strains often do not demonstrate the same degree of multidrug resistance, there has been a resurgence of interest in other antibiotics, often older antimicrobial agent classes for the management of these infections (Charlebois *et al.,* 2004). MLSB antibiotics have different structure, but similar mode of action. These antibiotics inhibit bacterial protein synthesis by binding to 23s rRNA in 50S ribosomal subunits (Horieh Saderi, 2011; Leclercq, 2002). They have a spectrum of activity directed against gram positive bacteria & intracellular bacteria such as Chlamydiae and Rickettsiae, gram negative bacteria i.e., Bordetella, Campylobacter, Chlamydia, Helicobacter and Legionella species (Lina *et al.,* 1999). Clindamycin represents an attractive option for several reasons. Firstly, good oral absorption of clindamycin makes it suitable for outpatient therapy or as follow-up after intravenous therapy. Secondly, it has high tissue penetration (except for the central nervous system) and accumulation in abscesses, and no need for renal dosing adjustments. Thirdly, clindamycin can be used as an alternative antibiotic in patients allergic to penicillin. Fourthly, community-acquired methicillin-resistant S. aureus, which has rapidly emerged in recent years as a cause of skin and soft-tissue infections, has shown susceptibility to clindamycin. Finally, it has been shown that clindamycin inhibits the production of toxins and virulence factors in gram-positive organisms through inhibition of protein synthesis (Fiebelkorn *et al.,* 2003; Kasten, 1999). Three mechanisms have been reported for resistance to MLSB antibiotics: target site modification, efflux of antibiotics, and drug modification. Methylation of the A2058 residue, located in the conserved domain V of 23s rRNA, takes place in target-site modification and prevents the binding of MLSB antibiotics to their ribosomal target. This phenomenon leads to cross-resistance to these antibiotics, and produces the MLSB phenotype that was encoded by erythromycin ribosome methylase (erm) genes (Leclercq, 2002; Weisblum, 1995; Roberts *et al.,* 1999). While strains with cMLSB resistance can be detected by routine disk diffusion testing, a special disk diffusion method, the D-test, was developed for the detection of iMLSB (Fiebelkorn *et al*., 2003; Clinical and laboratory standard institute, 2011). On the other hand, labelling all erythromycin-resistant S. aureus as clindamycin-resistant may prevent the use of clindamycin in cases where it would be effective therapy (Fiebelkorn *et al*., 2003). Thus, accurate detection of iMLSB-resistant strains is very important. Inducible clindamycin resistance is not detected by standard broth micro-dilution testing, automated susceptibility testing devices, the standard disk diffusion test or Etest (Jorgensen *et al*., 2004). So far, nearly 40 erm genes have been reported. Among the 4 major classes of erm genes (ermA, ermB, ermC and ermF) in different bacteria, ermA and ermC are the primary genes responsible for MLSB resistance in S. aureus (Leclercq, 2002; Fiebelkorn *et al*., 2003; Weisblum, 1995; Roberts *et al*., 1999). An erm gene, usually erm(C) or erm(A), encodes methylation of the 23S rRNA–binding site that is shared by these 3 drug classes. Phenotypically, resistance can be expressed constitutively (the cMLSB phenotype) or only when induced into production (the iMLSB phenotype) (Weisblum, 1995). These determinants are mostly borne by plasmids and transposons that are self-transferable. These modifications can include deletions, duplications, or other mutations and they result in constitutive expression of the methylase gene with obvious resistance to MLSB drugs. (Weisblum, 1995; Werckenthin *et al.,* 1999). Molecular markers for the erm genes are available only at few places, are expensive also and inconvenient for everyday use. In developing countries with high burden of MRSA, where health associated finances is borne by the patient, alternatives to vancomycin are need of the hour. Clindamycin is a good option but prevalence of inducible resistance should be known, as it varies by geographical location, hospital conditions and bacterial species (Diekema *et al.,* 2001). In patients with non – iMLSB S. aureus infection, clindamycin can be used safely and effectively. If clindamycin is used for treatment of infections with iMLSB producing isolates, close follow-up and monitoring for failure or relapse is needed. In more-severe infections, the presence of the iMLSB phenotype should preclude the use of clindamycin **(**Weisblum, 1995**,** Leclercq, 2002).

Aim of the study

The present study was undertaken to:

- To determine the prevalence of MLSB phenotypes among erythromycin resistant strains of S. aureus.
- To identify the genotypes of D test positive strains of S. aureus by PCR.

MATERIALS AND METHODS

The present study was a prospective study conducted for a period of 6 months from Jan 2014 – June 2014. Out of a total of 355 Staphylococcal species, 81 strains of S. aureus isolated from various clinical samples as pus, swabs, aspirates, blood, urine and sputum at our institution were included in the study. Repeated isolates of S. aureus from same patients were excluded from the study. Study protocol was duly approved by Institutional Ethics Committee. All the isolates were identified as S. aureus using standard conventional microbiological methods. (Clinical and laboratory standard institute, 2011**,** Winn *et al.,* 2006**).** Detection of MRSA was done by Kirby Bauer disc diffusion test using cefoxitin (30 µg) as per CLSI guidelines. **(**Clinical and laboratory standard institute, 2011**).** Routine antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion method as per CLSI criteria. The antibiotics used were Penicillin (10units), Amoxyclav (30mcg), Gentamicin (10mcg), Tetracycline (30mcg), Doxycycline (30mcg), Linezolid (30mcg), Cotrimoxazole (25mcg), Cefoxitin (30mcg), Erythromycin (15mcg), Clindamycin (2mcg), Ciprofloxacin (5mcg), Chloramphenicol (30mcg). (Clinical and laboratory standard institute, 2011**).** Phenotypic expression of erm gene activation was detected by performing D-test. Erythromycin resistant S. aureus strains [zone of inhibition < 13mm] were subjected to erythromycin and

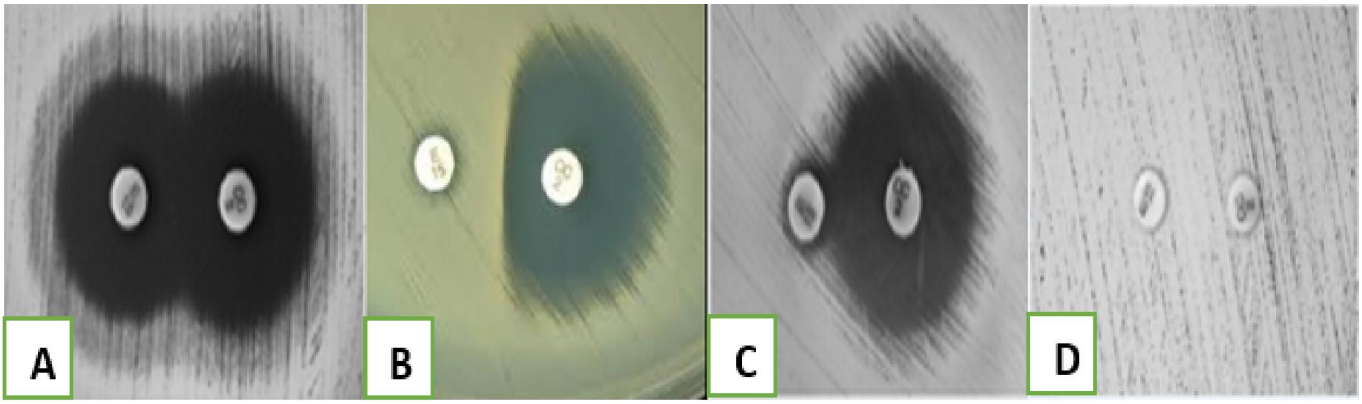
clindamycin disc approximation test [D test] for detection of inducible clindamycin resistance as per CLSI **(**Clinical and laboratory standard institute, 2011**).** A 0.5 McFarland equivalent suspension of organisms was inoculated onto a Mueller - Hinton agar (MHA) plate, the ER (15µg) disk was placed 15-22 mm (edge to edge) apart from CD (2 µg) disk on MHA. Plates were analysed after 18 hours of incubation at 35°C.When tested in close proximity, ER (inducing agent) diffuses into the media and induces the erm gene expression. This effect extends up to the sensitivity zone on one side of the CD disc leading to a D-shaped zone of inhibition (Clinical and laboratory standard institute, 2011). Three different phenotypes were identified among the Staphylococcus aureus strains:

- Isolates with resistance to both erythromycin and clindamycin are cMLSB (constitutive clindamycin resistance).
- iMLSB (inducible clindamycin resistance) phenotype strains show sensitive zone around clindamycin disc [zone size > 21 mm]with a flattening towards erythromycin disc.
- MS phenotype strains are sensitive to clindamycin [zone >21 mm] with a circular zone w/o flattening towards erythromycin disc.

Detection of erm genes

Applied Biosystems by Life Technologies (Gradient PCR); Primers (Bioserve), Ladder/Dntps (Gbiosciences) Inducible clindamycin resistance in the S. aureus isolates was identified genotypically through multiplex PCR by detection of the ermA and ermC genes (Kasten, 1999; Sutcliffe *et al.,* 1996). **(**Kasten, 1999; Sutcliffe *et al.,* 1996**,** Schmitz *et al.,* 2000**)**. A direct colony suspension of the culture equivalent to a 1.0 McFarland standard was prepared in 500 µL of 10 mM Tris-1 mM EDTA (pH 8.0), vortexed, and boiled for 10 min an aliquot of 5 µL of the suspension was used for each 25 µlL reaction mixture. PCR assays and primers specific from the ermA and ermC resistance genes used in this study have been previously described. (CLSI 2011; Schreckenberger *et al.,* 2004; Francis Martineau *et al.,* 2000; Schmitz, 2000) PCR products were visualised following electrophoresis in agarose 1.5% w⁄v gels under UV light and the sizes of the amplification products were estimated by comparison with 139/190bp molecular size standard ladder. (Sutcliffe *et al.,* 1996; Nawaz *et al.,* 1997). An internal control was integrated into the PCR assay to verify the efficiency of the amplification and to ensure there was no significant PCR inhibition.

Phenotypes of MLSB shown by S. aureus



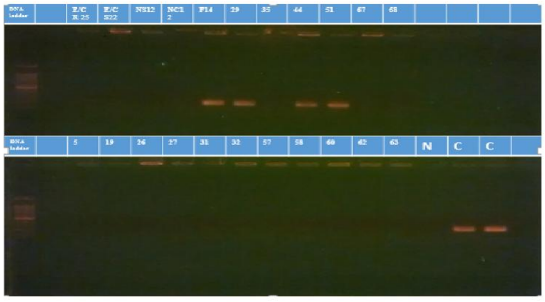
A – ER/CD sensitive; B – Inducible clindamycin resistant; C – MS Phenotype; D – ER/CD resistant

DNA Isolation

The DNA isolation kit used was [AMNION, AMPURE Bacterial DNA Mini Spin Isolation Kit. # AMRK017]. For nucleic acid isolation from staphylococcal isolates, the frozen samples were thawed rapidly and were cultivated in BHI broth at 37°C with shaking overnight. Total DNA was isolated from 5 ml of a broth culture grown overnight. After incubation, bacterial cells were harvested by centrifugation at 3000 × **g** for 10 min, the cell pellet was re-suspended in phosphate-buffered saline with 100 µg of lysostaphin per ml and incubated at 37°C for 30 min. The phenol/chloroform extraction method was used for nucleic acid extraction and DNA was precipitated in 1 ml 70 per cent ethanol. The DNA precipitate was dissolved in 50 µl of TE buffer [10 mM Tris chloride-1 mM EDTA (pH 8.0)], and stored at -20°C until processing (Strommenger *et al.,* 2003).

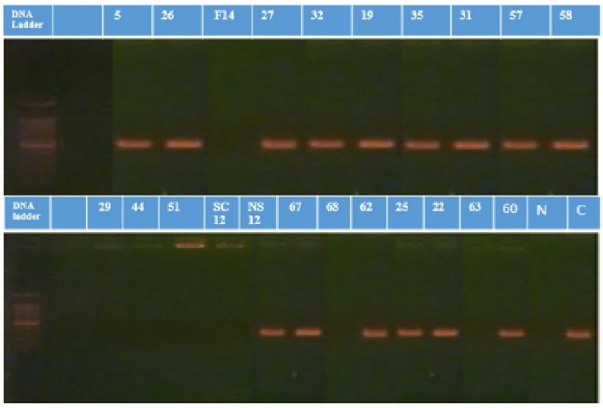
Genotypes [ermA and ermC] of Inducible clindamycin resistant S. aureus strain

Figure 1



Lane 1: DNA ladder of 139bp [ermA]
Lane 2 – 12: S. aureus strains
Lane 13, 14, 15: Negative & positive controls.

Figure 2



Lane 1: DNA ladder of 190bp [ermC]
Lane 2 – 11 & 2 – 13: S. aureus strains
Lane 14 – 15: Negative & positive controls.

Quality control

- ATCC 25923 S. aureus strain.
- In house strains of S. aureus showing D – test positive.
- The viability of the isolates was maintained by periodic subculture in semisolid nutrient agar.
- Results were statistically analysed using p value and Chi-square test.

RESULTS

Eighty one S. aureus (23%) were obtained from a total of 355 Staphylococcal species from various clinical samples over a period of 6 months from Jan 2014 – June 2014.

Table 1. Specimen – wise distribution of S. aureus isolates

Clinical samples	S. aureus	MRSA	MSSA
Pus / swab	65 (80%)	13(20%)	52(80%)
Blood	12 (15%)	05(42%)	07(58%)
Sputum	02 (03%)	--	02(100%)
Urine	01 (01%)	--	01(100%)
Body fluid	01 (01%)	--	01(100%)
TOTAL	81 (100%)	18(22%)	63(78%)

Table 2. Shows the age and sex distribution of the patients

Age / Sex	< 1 Yrs	1–20 Yrs	20-40 Yrs	>40 Yrs	Total
Male	09	05	18	14	46
Female	06	06	15	08	35
Total	15	11	33	22	81

Table 4. Genotypes of iMLSB phenotypes in S. aureus

MLSB Genotypes	Total (22)	iMLSB Resistance	
		MRSA (06)	MSSA(16)
ermA	04	03 (50%)	01(17%)
ermC	15	02(12.5%)	13 (81.3%)

Eighty one S. aureus strains were tested for susceptibility to erythromycin and other antibiotics by routine disc diffusion testing; 70/81 (86%) were erythromycin resistant and 11/81 (14%) were erythromycin sensitive. Out of the total (70) erythromycin resistant strains of S. aureus, MRSA were 16/70 (23%) and MSSA were 54/70 (77%) respectively. Among the erythromycin sensitive strains of S aureus, MRSA were 2/11 (18%) & MSSA were 9/11 (81%) respectively. Out of 81 isolates, 18/81 (22%) were MRSA & the remaining 63/81 (78%) isolates were MSSA. Table I shows the specimen-wise distribution of the S. aureus. Pus and wound swabs 65(80%) accounted for majority of the isolates followed by blood 12(15%), respiratory specimen 02(03%), urine 01(01%) and body fluids 01(01%). Table II shows the demographic characteristics of 81 isolates where S. aureus infections were predominant in males 46(57%) and 20 – 40 age group showed higher incidence of these infections in both men and women. Table III shows distribution of isolates according to clindamycin resistance phenotypes. D test was performed on erythromycin resistant S. aureus isolates; 22(31%) isolates showed D test positive (iMLSB phenotype), 26(37%) were cMLSB phenotypes and 22(31%) showed D test negative; suggesting MS phenotypes.

DISCUSSION

The global prevalence rate varies worldwide, with as low as <10% in the developing countries to as high 60 – 70% in the developed countries (Yilmaz *et al.,* 2007). The distribution of 2 erm genes (ermA and ermC) among erythromycin-resistant S. aureus isolates have been studied. These genes were reported as being the most prevalent genes responsible for resistance to MLSB antibiotics within S. aureus strains (Lina *et al.,* 1999). A relationship between use of antibiotics and acquisition of resistance is generally accepted and studies suggest that stronger antibiotic pressure is exerted on MRSA than on MSSA (Otsuka *et al.,* 2007; Seifi *et al*., 2012). In the present study, among the total S aureus (81), the prevalence of MRSA (22%) was lower compared to MSSA (78%) which is in concordance with few studies (Ciraj *et al*., 2009; Ravikumar Gupta *et al*., 2014; Nizami Duran *et al*., 2012). Few studies have shown higher prevalence of MRSA compared to MSSA (Lyall *et al.,* 2013; Dejan *et al.,* 2014). A higher percentage of resistance of S aureus to erythromycin 70 (86%) was observed in our study.

Table 3. Clindamycin resistance phenotypes among Erythromycin resistant S. aureus isolates

ER – R Phenotypes of S. aureus	MRSA [16]		MSSA [54]		Total [70]	
	No	%	No	%	No	%
Inducible clindamycin resistance ER-R, CD-S, D test +ve	06	09	16	23	22	31.5
MS Phenotypes ER-R, CD-S, D test –ve	02	03	20	28	22	31.5
Constitutive resistance ER-R, CD-R	08	11	18	26	26	37
Total No (%)	16	23	54	77	70	100

Table 5. Phenotypic and genotypic parameters of inducible clindamycin resistance in the studies by various authors

Author	ER (%)	S.aureus (%)		iMLSB(%)		cMLSB (%)		MS types(%)		ermA(%)		ermC(%)	
		MRSA	MSSA	MRSA	MSSA	MRSA	MSSA	MRSA	MSSA	MRSA	MSSA	MRSA	MSSA
Present study	86	22	78	8.6	23	11.4	25.7	2.9	28.6	50	17	12.5	81.3
Prabhu K	28.42	31.57	68.42	20	6.15	16.6	6.15	13.33	6.15	--	--	--	--
Deotale	32.4	--	--	14.5		3.6		14.2		--	--	--	--
Lyall KS	51.7	91.5	8.51	33.2	34.6	--	--	--	--	--	--	--	--
Gadepalli	40.5	52	48	30	10	38	15	12	12	--	--	--	--
Ciraj AM	32	17.3	82.6	38.4	12.9	5.1	--	--	--	--	--	--	--
Ravikumar Gupta	100	25.4	74.6	28.9	12.6	47.4	33.9	_	--	--	--	--	--
Naima Fasih	36	36	64	64.2	62	10.2	72	30		6		--	--
Schreckenberger	5-13	29	51	3-12	19-20	--	--	--	--	--	--	--	--
Levin TP	54	72	28	12.3	68	--	--	--	--	--	--	--	--
BD Dejan	40	82		50		26		24		40		55	
Martineau		55								12		1.2	
Parviz	68	68.3	31.7	6.4	--	93	--	08	--	81.4	15	74.4	12.5
Schmitz	69	93	44	--	--	--	--	--	--	88	38	5	47
Otsuka T	58.5	38	62	39	94	61.3	1.3	--	--	97	51	11.5	42

It is similar to some of the reported studies. (Ravikumar Gupta *et al*., 2014; Horieh Saderi *et al.,* 2011; Dhanalakshmi *et al.,* 2012) Lower rates were reported by few authors. (Ciraj *et al*., 2009, Prabhu *et al.,* 2011; Deotale *et al*., 2010; Gadepalli *et al*., 2006). Our study showed higher incidence of MRSA infections in male patients with the age group of 20–40 years. Male predominance was most likely due to the fact that exposure to the environment is greater (Sasirekha *et al*., 2014).

We found that iMLSB resistance is higher in MSSA (23%) compared to MRSA (8.5%).This is in concordance with some reported studies (Levin *et al*., 2005; Paul *et al*., 2004; Delialioglu *et al*., 2005; Lyall *et al*., 2013) and the contrary findings were reported by (Seifi *et al.,* 2012; Ciraj *et al*., 2009; Prabhu *et al.,* 2011; Ravikumar Gupta *et al*., 2014; Gadepalli *et al.,* 2006). In the present study, isolation rates of MS phenotypes showing true clindamycin sensitivity were higher in MSSA (37%) compared to MRSA (13%) which was contrary to other authors' findings (Seifi *et al.,* 2012; Prabhu *et al.,* 2011; Levin *et al.,* 2005) Gadepalli *et al* showed the prevalence of MS phenotype of 12% in both MRSA and MSSA. (Gadepalli *et al.,* 2006). The usefulness of polymerase chain reaction (PCR) based assays for the rapid detection of methicillin resistant Staphylococci is well established. (Strommenger, 2003; Sutcliffe *et al.,* 1996, Otsuka *et al.,* 2007, Francis Martineau *et al.,* 2000; Schmitz *et al.,* 2000; Spiliopoulou *et al.,* 2004). Erythromycin resistance in Staphylococci is predominantly mediated by erythromycin resistance methylase encoded by erm genes. (Weisblum, 1995). In human infections caused by Staphylococci, ermA & ermC are the most common methylase genes. (Lina, 1999; Horieh Saderi *et al.,* 2011; Leclercq, 2002; Nawaz *et al.,* 1997). In the present study, results regarding predominance of the ermA among MRSA (50%) isolates are consistent with previous reports but predominance of the ermC among MRSA isolates has not been reported, except from Greece. In our study, ermC was predominant among MSSA (81.3%) which correlated with some previous studies. [Otsuka *et al.,* 2007; Horieh Saderi *et al.,* 2011; Francis Martineau *et al.,* 2000; Schmitz *et al.,* 2000). There was a very good correlation between the genotypic analysis by PCR and the phenotypes determined by standard methods of susceptibility testing and identification of Staphylococcal species. High prevalence of erm genes in S. aureus emphasizes the need for performing antimicrobial susceptibility testing when clindamycin is considered for use in treatment of infections caused by them (Otsuka *et al.,* 2007).

Conclusion

Without the double-disc test, all the S. aureus isolates with inducible clindamycin resistance would have been misinterpreted as clindamycin susceptible, resulting in an underestimated clindamycin resistance rate. In view of therapeutic implication, D-test was found to be a simple effective test that should be performed on all S. aureus isolates showing clindamycin-erythromycin discordance on disc diffusion in order to use clindamycin as the drug of choice. Detection of erm genes is the gold standard for confirmation of iMLSB strains of S. aureus.

REFERENCES

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

Ciraj, A.M., Vinod, P., Sreejith, G., Rajani, K. Inducible clindamycin resistance among clinical isolates of staphylococci. *Ind. J. Pathol. Microbiol.,* 2009; 52: 49-51

Clinical and laboratory standard institute. Performance standards for antimicrobial susceptibility testing; Twenty-first informational Supplement. CLSI document. Wayne, PA. 2011; CLSI: M100-S21.

Dejan, BD *et al*. 2014. Prevalence of inducible clindamycin resistance among CA Staph isolates in central Serbia. *Indian J of Medical Microbiology.,* Vol32, issue 1: 49-52

Delialioglu, N., Aslan, G., Ozturk, C., Baki, V., Sen, S. and Emekdas, G. 2005. Inducible Clindamycin Resistance in Staphylococci Isolated from Clinical Samples. Jpn. *J. Infect. Dis*., 58: 104- 106

Deotale, V, Mendiratta, DK, Raut, U. and Narang, P. 2010. Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. *Indian J Med Microbiol.*, 28: 124-6.

Dhanalakshmi *et al*. 2012. Prevalence of inducible clindamycin resistance in S aureus. *J of Academy of Med Sciences*., Vol 2, Issue 2: 73-75.

Diekema, D.J. *et al*. 2001. Survey of infections due to Staphylococcus species: frequency of occurrence and

antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997–1999. *Clin. Infect. Dis*. 32 (Suppl. 2):S114–S132.

Fiebelkorn, KR, Crawford, SA, McElmeel ML. and Jorgensen, JH. 2003. Practical disk diffusion method for detection of inducible clindamycin resistance in Staphylococcus aureus and coagulase-negative staphylococci. *J Clin Microbiol*., 41: 4740–44

Francis Martineau *et al*. 2000. Multiplex PCR assay for the detection of clinically relevant antimicrobial resistance genes in Staph isolated from patients infected after cardiac surgery. *J of Antimicrobial Chemotherapy*., 46: 527-533.

Franklin D. Lowy. 2003. Antimicrobial resistance: the example of Staphylococcus aureus. *J. Clin. Invest*., 111:1265–1273.

Gadepalli, R., Dhawan, B., Mohanty, S., Kapil, A., Das, B.K. and Chaudhry, R. 2006. Inducible clindamycin resistance in clinical isolates of S. aureus. *Ind. J. Med. Res*., 123: 571-3

Horieh Saderi, Behzad Emadi, Parviz Owlia. Phenotypic and genotypic study of macrolide, lincosamide and streptogramin B (MLSB) resistance in clinical isolates of Staphylococcus aureus in Tehran, Iran. *Med Sci Monit*. 2011; 17(2): BR48-53

Jorgensen JH, Crawford SA, McElmeel ML, Fiebelkorn KR. Detection of inducible clindamycin resistance of staphylococci in conjunction with performance of automated broth susceptibility testing. *J Clin Microbiol*. 2004; 42:1800–2.

Kasten MJ. Clindamycin, metronidazole, and chloramphenicol. Mayo Clin Proc. 1999; 74: 825–33

Leclercq R. 2002. Mechanisms of resistance to macrolides and lincosamides: Nature of the resistance elements and their clinical implications. *Clin Infect Dis*, 34: 482–92.

Levin TP, Suh B, Axelrod P *et al*. 2005. Potential clindamycin resistance in clindamycin-susceptible, erythromycin-resistant Staphylococcus aureus: report of a clinical failure. Antimicrob Agents Chemother., 49(3): 1222–24

Lina G, Quaglia A, Reverdy ME *et al*. 1999. Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. Antimicrob Agents Chemother., 43(5): 1062–66

Lowy, F.D. 1998. Staphylococcus aureus infections. N. Engl. *J. Med*., 339:520–532.

Lyall KS *et al*. 2013. Inducible clindamycin resistance among clinical isolates of S aureus. *J of Mahatma Gandhi Inst. of Med Sciences.*, Vol 18; issue 2: 112-115.

Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J, *et al*. 2003. Comparison of community- and health-care-associated methicillin resistant Staphylococcus aureus infection. JAMA., 290: 2976-84.

Nawaz MS, Khan AA. and Cerniglia CE. 1997. Detection of erythromycin resistant methylase gene by the polymerase chain reaction. *Mol Cell Probes*.,11: 317–322.

Nizami Duran *et al*. 2012. Antibiotic resistance genes & susceptibility pattern in Staphylococci. *Indian J of Medical Research*., 135: 389-396.

Otsuka T., H. Zaraket, T. Takano, K. Saito, S. Dohmae, W. Higuchi and T. Yamamoto Research notes Macrolide–lincosamide–streptogramin B resistance phenotypes and genotypes among Staphylococcus aureus clinical isolates in Japan, Clin Microbiol Infect. 2007; 13: 325–327.

Paul C. Schreckenberger *et al*. 2004. Incidence of Constitutive and Inducible Clindamycin Resistance in Staphylococcus aureus and Coagulase-Negative Staphylococci in a Community and a Tertiary Care Hospital. *Journal of clinical microbiology.*, 2777-2779.

Prabhu, K., Rao, S. and Rao, V. 2011. Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. *J. Lab. Physicians.*, 3(1): 25 27

Rajaduraipandi K, Mani KR, Panneerselvam K, Mani M, Bhaskar M. and Manikandan P. 2006. Prevalence and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus: a multicentre study. *Indian J Med Microbiol*., 24: 34-8.

Ravikumar Gupta *et al*. Prevalence of Inducible clindamycin resistance in S aureus at a tertiary care hospital: Implications for clinical therapy. *International J of current microbiology and applied sciences.* 2014; Vol 3, No 3: 720-725

Roberts MC, Sutcliffe J, Courvalin P *et al*. 1999. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. Antimicrob Agents Chemother., 43: 2823–30

Sasirekha *et al*. Incidence of constitutive and inducible clindamycin resistance among hospital-associated Staphylococcus aureus. 3 *Biotech.* 2014; 4:85–89

Schito GC. The importance of the development of antibiotic resistance in Staphylococcus aureus. *Clin Microbiol Infect*. 2006; 12 Suppl 1: 3-8.

Schmitz, F.-J., J. Petridou, A. C. Fluit, U. Hadding, G. Peters, and C. von Eiff. Distribution of macrolide-resistant genes in Staphylococcus aureus blood-culture isolates from fifteen German university hospitals. *Eur. J. Clin. Microbiol*. Infect. Dis. 2000; 19:385–387.

Seifi N *et al* Inducible clindamycin resistance in Staphylococcus aureus isolates recovered from Mashhad, Iran. *Iranian J of Microbiology*. 2012; Volume 4, No 2: 82-86.

Spiliopoulou I, Petinaki E, Papandreou P. and Dimitracopoulos G. 2004. erm(C) is the predominant genetic determinant for the expression of resistance to macrolides among methicillin-resistant Staphylococcus aureus clinical isolates in Greece. *J Antimicrob Chemother*., 53(5): 814–17

Strommenger B, Kettlitz C, Werner G, Witte W. Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in Staphylococcus aureus. J Clin Microbiol. 2003; 41:4089–94.

Sutcliffe, J., T. Grebe, A. Tait-Kamradt, and L. Wondrack. 1996. Detection of erythromycin-resistant determinants by PCR. Antimicrob. *Agents Chemother*., 40:2562–2566.

Waldvogel, F.A. 2000. Staphylococcus aureus (including staphylococcal toxic shock). In Principles and practice of infectious diseases. G.L. Mandell, J.E. Bennett, and R. Dolin, editors. Churchill Livingstone. Philadelphia, Pennsylvania, USA. 2069–2092.

Weisblum B: Erythromycin resistance by ribosome modification. Antimicrob Agents Chemother. 1995; 39: 577–85

Werckenthin C, Schwarz S. and Westh H. 1999. Structural alterations in the translational attenuator of constitutively

expressed ermC genes. Antimicrob Agents Chemother. 1999; 43:1681–5.

Winn Jr. W, Allen S, Janda W, Koneman E, Procop G, Schrekenbeger P *et al*. 2006. Chapter 12. Gram positive cocci: Part I: Staphylococci and Related Gram Positive Cocci. In: Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th edition. Lippincott Williams and Wilkins, 623-671.

Yilmaz G, Aydin K, Iskender S, Caylan R. and Koksal I. 2007. Detection and prevalence of inducible clindamycin resistance in staphylococci. *J Med Microbiol*., 2007; 56:342-345.

****