Prevalence of Clindamycin resistance among *Staphylococcus aureus* in a tertiary care hospital in South India

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Abstract

Background: *Staphylococcus aureus,* a pyogenic bacterium, causes a wide spectrum of diseases, ranging from minor skin infections to fatal necrotizing pneumonia. The emergence and spread of *MRSA* (Methicillin Resistant *Staphylococcus aureus*), *VISA* (Vancomycin Intermediate *Staphylococcus aureus*) and *VRSA* (Vancomycin Resistant *Staphylococcus aureus*) has left us with very few antibiotics to treat *Staphylococcal* infections. Clindamycin is the most important antibiotic to treat infections with Methicillin Resistant *Staphylococcus aureus* (*MRSA*). *In vitro* routine diagnostic tests for Clindamycin susceptibility fail to detect inducible Clindamycin resistance due to *erm* genes resulting in treatment failures and Clindamycin sensitive strains possess *msr* gene. Such resistance is detected by phenotypic methods like D – test and Agar dilution method and genotypic methods like PCR detect the *ermA, ermC* and *msrA* gene.
Methods: A total of 200 *Staphylococcus aureus* isolates from various clinical samples were subjected to study by D - Test and Agar Dilution as per CLSI guidelines and gene detection was done by PCR.
Result: Among the 200 *Staphylococcus aureus* isolates, 103 (51.5%) were erythromycin resistant. Out of which, 72 (69.9%) were *MRSA* and 31 (30.1%) were *MSSA*. Among the 72 *MRSA* isolates 30 (41.7%) were iMLS$_B$ phenotype, 17 (23.6%) were cMLS$_B$ phenotype and 25 (34.7%) were MS phenotype. Among the 31 (30.1%) *MSSA*, 14 (45.3%) were iMLS$_B$ phenotype, 15 (48.3%) were MS phenotype and 2 (6.4%) were cMLS$_B$ phenotype. Among the 63 (31.5%) Clindamycin resistant isolates 43 (68.2%) showed the presence of *ermA* gene and 20 (31.2%) had *ermC* gene. Among the 40 MS phenotype, 36 (90%) showed *msrA* gene.
Conclusion: Keeping the mode of action, adverse reactions and pharmacokinetics of certain antibiotics like Vancomycin in mind, Clindamycin should be preferred as the drug of choice for the treatment of severe and resistant *Staphyloccous aureus* infections.

Key Words: Inducible Clindamycin Resistance, *MRSA*, D – Test, Agar Dilution, iMLS$_B$ phenotype, PCR

Introduction

Staphylococcus aureus is a recognized pathogen responsible for nosocomial and community-acquired infections in every region of the world[1]. The emergence and spread of *MRSA* (Methicillin Resistant *Staphylococcus aureus*), *VISA* (Vancomycin Intermediate *Staphylococcus aureus*) and *VRSA* (Vancomycin Resistant *Staphylococcus aureus*) has left us with very few antibiotics to treat *Staphylococcal* infections. The Macrolide-Lincosamide-Streptogramin B (MLS$_B$) family of antibiotics serves as one such alternative[2]. Erythromycin was discovered in 1952 by McGuire and co-workers in the metabolic products of a strain of *Streptomyces erythreus*. Clarithromycin and Azithromycin are semisynthetic derivatives of Erythromycin. *Staphylococci* were reliably sensitive to Erythromycin until the discovery of Erythromycin

resistance in 1969, Erythromycin sensitivity no longer can be relied upon unless *in vitro* susceptibility has been documented.[3] Clindamycin, available for use since 1966, is classified as a lincosamide antimicrobial agent as it is chemically similar to Lincomycin.[4]

Clindamycin is an important antibiotic to treat infections with Community Acquired *Methicillin Resistant Staphylococcus aureus* (*CA-MRSA*). It is also used as an alternate drug in patients allergic to Penicillin to treat skin and soft tissue infections. Clindamycin has been used to treat serious infections caused by susceptible *Staphylococcus aureus* strains in children for more than 30 years. Absorption after oral administration is nearly complete, yielding serum concentrations that approximate those of intravenous (IV) administration. This permits early transition to outpatient management of susceptible infections without the complications of continued IV access[10]. Clinical isolates resistant to Clindamycin were first recognized in 1968[5]. Clinical and bacteriologic relapse in a patient with *Staphylococcus aureus* endocarditis during the fourth week of Clindamycin therapy after initial improvement was reported in 1976. The initial isolate was susceptible to Erythromycin and Clindamycin while that from the relapse was resistant to both. This led to abandonment of Clindamycin for

treatment of endocarditis[6]. The MLS_B group antibiotics act by binding to 23S rRNA of 50S ribosome, thus inhibiting protein synthesis. Resistance to macrolide, lincosamide and streptogramin B (MLS_B) antibiotics most commonly results from acquisition of erythromycin resistance methylase (*erm*) genes which encode enzymes that methylate the 23S rRNA[7].

The prevalence of Inducible Clindamycin resistance has been increasing at an alarming rate and so the present study was conducted to study the prevalence of Inducible Clindamycin resistance in clinical isolates of *Staphylococcus aureus* by phenotypic and genotypic methods, as the prevalence of such resistance in this region was unknown.

Materials & Methods

This prospective cross sectional study was done in the Department of Microbiology, V.M.K.V.Medical College, Salem on 200 consecutive *Staphylococcus aureus* isolates from various clinical samples like pus, urine, blood, sputum, vaginal swab and endotracheal aspiration fluid received in the lab over a period of 18 months (Nov' 2013 to April 2015). The clinical samples were cultured on routine culture media like Blood agar and Mac Conkey agar and the isolates were identified as *Staphylococcus aureus* by Gram Staining and biochemical tests like Catalase test, Tube coagulase test and Mannitol fermentation test[8].

Antibiotic Susceptibility testing: After confirmation, these isolates were subjected to antimicrobial susceptibility testing by Kirby Bauer disc diffusion method according to CLSI guidelines[8]. The following antibiotics Penicillin, Erythromycin, Cefoxitin, Linezolid, Ciprofloxacin, Co-Trimoxazole, Vancomycin and Clindamycin were tested for susceptibility after making a lawn culture of the organism on Mueller Hinton agar plate at a distance of 6mm from each disk and using not more than 7 disks per plate after standardizing the broth to 1 Mc Farlands[9,10].

Fig. 1: Antibiotic Sensitivity Testing

Phenotypic Detection of Methicillin Resistant *Staphylococcus aureus*: Methicillin susceptibility was detected by using Cefoxitin 30µg disc and was classified as Methicillin Susceptible *Staphylococcus aureus* (*MSSA*) if the zone of inhibition was $> 22\ mm$ and Methicillin Resistant *Staphylococcus aureus* (*MRSA*) if the zone of inhibition was $\leq 21\ mm$ as per CLSI guidelines[8].

Fig. 2: Phenotypic detection of *MRSA* using Cefoxitin disc (30 ug)

Phenotypic detection of Clindamycin resistance by D-test: Inducible resistance to Clindamycin was tested by D-test. Erythromycin (15 µg) disc was placed at a distance of 15 mm (edge to edge) from Clindamycin (2 µg) disc on a Mueller-Hinton agar (MHA) plate, previously inoculated with 1 McFarland standard bacterial suspensions. Following overnight incubation at 37°C, flattening of zone (D-shaped) around Clindamycin in the area between the two discs, indicates inducible Clindamycin resistance[9]. Three different phenotypes are appreciated after testing and then interpreted. This interpretation was done only for Erythromycin-resistant *Staphylococcus aureus* strains. All the Erythromycin-sensitive strains were excluded.

1. **MS Phenotype** - *Staphylococcal* isolate exhibiting resistance to Erythromycin (zone size ≤13 mm) while sensitive to Clindamycin (zone size ≥21 mm) and giving circular zone of inhibition around Clindamycin is labeled as having this phenotype.

2. **Inducible MLS_B Phenotype** - *Staphylococcal* isolate showing resistance to Erythromycin (zone size ≤13 mm) while being sensitive to Clindamycin (zone size ≥21 mm) and giving D-shaped zone of inhibition around Clindamycin with flattening towards Erythromycin disc was labeled as having this phenotype.

3. **Constitutive MLS_B Phenotype** - This phenotype was labeled for those *Staphylococcal* isolates, which showed resistance to both Erythromycin (zone size ≤13 mm) and Clindamycin (zone size

≤14 mm) with circular shape of zone of inhibition if any around Clindamycin[10].

Fig. 3: Phenotypic detection of iMLS$_B$ Phenotype

Fig. 4: Phenotypic detection of MS Phenotype

Fig. 5: Phenotypic detection of cMLS$_B$ Phenotype

Phenotypic detection of Clindamycin resistance by Agar dilution method: Mueller-Hinton agar containing both 1 mg/liter Erythromycin and 0.5 mg/liter Clindamycin was prepared. In addition, agar plates with 0.5 mg/liter Clindamycin alone or with 1 mg/liter Erythromycin alone and agar plates without antibiotics were prepared, the latter two serving as growth controls. MHA is poured into 90 mm plates to a depth of 3 mm.[11] These plates were spot inoculated with a bacterial concentration of 1 x 10^4 bacteria/mL. Interpretation of Agar Dilution method was as follows:

1. **iMLS$_B$ phenotype**, if there was any visible growth on the Erythromycin-only and combined plates but not on the Clindamycin-only plate.
2. **MS resistance phenotype**, if growth was found on the Erythromycin-only plate but not on the combined or Clindamycin-only plate.
3. **cMLS$_B$ phenotype**, if there was growth on all the three plates[11].

Fig. 6a: Combined Erythromycin and Clindamycin plate showing iMLS$_B$ phenotype

Fig. 6b: Erythromycin only plate showing MS phenotypes

Fig. 6c: Clindamycin only plate showing cMLS$_B$ phenotype

Genotypic detection of Clindamycin resistance by PCR: The MLS$_B$ phenotype (both iMLS$_B$ and cMLS$_B$) shows the presence of *erm* genes, whereas, MS phenotype has *msr* gene. Conventional PCR for *ermA, ermC and msrA* genes by using oligonucleotide primers specific for the *ermA, ermC and msrA* genes. Amplifying 640 bp (*ermA*), 520 bp (*ermC*) and 940 bp (*msrA*)[12]. (M denotes the Marker)

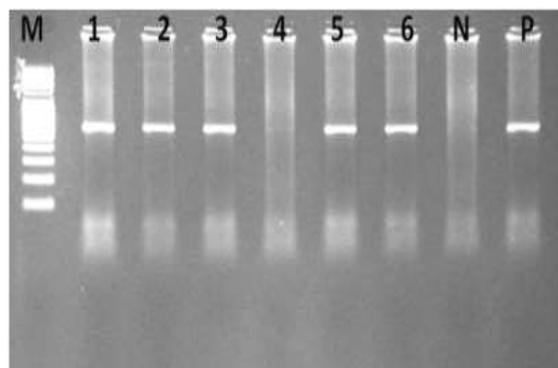**Fig. 7: Genotypic detection of *ermA gene* by PCR**

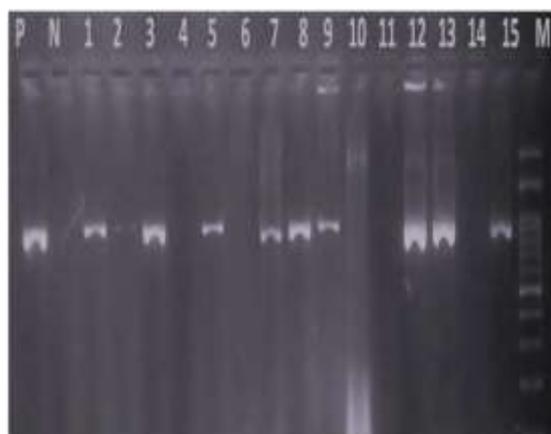**Fig. 8: Genotypic detection of *ermC gene* by PCR**

Fig. 9: Genotypic detection of *msrA gene* by PCR

Results

Out of the 200 *Staphylococcus aureus* isolated, majority were from pus, followed by urine, blood, sputum, vaginal swab and endotreacheal tube aspiration. (Table 1)

Table 1: Sample wise isolation of *Staphylococcus aureus*

Sl. No.	Sample	Total no. of samples	Percentage
1.	Pus	138	69
2	Urine	28	14
3.	Blood	18	09
4.	Sputum	8	04
5.	Vaginal swab	6	03
6.	Endotracheal tube	2	01
Total		**200**	**100**

Staphylococcus aureus isolates were highly sensitive to Linezolid followed by Vancomycin and Clindamycin. High resistant was observed to Penicillin, Ciprofloxacin and almost 50% of Staphylococcus aureus isolates were resistant to Cefoxitin and Erythromycin. (Table 2)

Table 2: Antibiotic Susceptibility Profile of *Staphylococcus aureus*

Sl. No.	Antibiotics	Sensitive	Resistant
1.	Penicillin	40 (20%)	160 (80%)
2.	Ciprofloxacin	40 (20%)	160 (80%)
3.	Linezolid	182 (91%)	18 (09%)
4.	Co-trimoxazole	92 (46%)	108 (54%)
5.	Vancomycin	160 (80%)	40 (20%)
6.	Cefoxitin	97 (48.5%)	103 (51.5%)
7.	Erythromycin	103 (51.5%)	97 (48.5%)
8.	Clindamycin	137 (68.5%)	63 (31.5%)

Among the 200 *Staphylococcus aureus* isolates, 103 (51.5%) were Erythromycin resistant. Inducible Clindamycin resistance (iMLS$_B$ phenotype) among Erythromycin resistant *Staphylococcus aureus* was 44 (42.7%), Constitutive Clindamycin resistance (cMLS$_B$ phenotype) was 19 (18.5%) and MS phenotype was 40 (38.8%), by both D- test and agar dilution method.

Out of the total 200 *Staphylococcus aureus* isolates, 97 (48.5%) were *MRSA* and 103 (51.5%) were *MSSA*. Among the 103 (51.5%) Erythromycin resistant isolates, 72 (69.9%) were *MRSA,* out of which 30 (41.7) isolates were iMLS$_B$ phenotype, 17 (23.6%) isolates were cMLS$_B$ phenotype and 25 (34.7%) isolates were MS phenotype. Among the 103 (51.5%) Erythromycin resistant isolates, 31 (30.1%) were *MSSA*, out of which 14 (45.3%) were iMLS$_B$ phenotype, 2 (6.4%) were cMLS$_B$ phenotype and 15 (48.3%) were MS phenotype. (Table 3)

Table 3: Distribution of Clindamycin Resistant Phenotypes among *MRSA* and *MSSA*

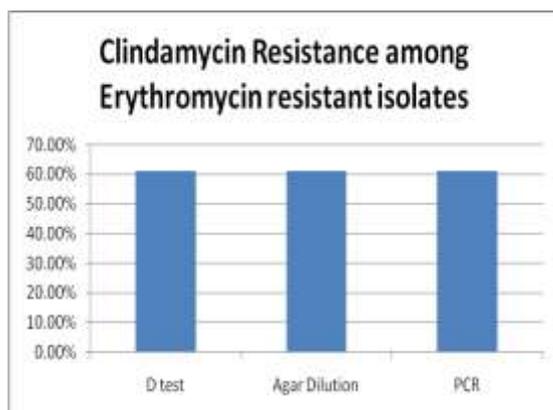| Sl. No	Erythromycin Resistant *Staphylococcus aureus* (103)	iMLS$_B$ Phenotype (44)	cMLS$_B$ Phenotype (19)	MS Phenotype (40)
1	MRSA 72 (69.9%)	30 (41.7%)	17 (23.6%)	25 (34.7%)
2	MSSA 31 (30.1%)	14 (45.3%)	2 (6.4%)	15 (48.3%)
3	Total 103 (100%)	44 (42.7%)	19 (18.5%)	40 (38.8%)

All the 103 erythromycin resistant strains were subjected to genotypic detection of Clindamycin resistance by conventional PCR. Among the 103 Erythromycin resistant isolates, 63 (61.2%) were Clindamycin resistant isolates (both iMLS$_B$ and cMLS$_B$), 43 (68.2%) showed the presence of *ermA* gene (Fig. 7) and 20 (31.2%) had *ermC* gene (Fig. 8). Among the 40 (38.8%) MS phenotype isolates, 36 (90%) showed *msrA* gene (Fig. 9).

Fig. 10: Comparison of Phenotypic and Genotypic Results

Sixty one point two percent Clindamycin resistance among the erythromycin resistant *Staphylococcus aureus* isolates was detected by both phenotypic and genotypic methods (Fig. 10).

Discussion

In this study, the majority of the isolates were from pus samples (69%), which is in correlation with Mojtaba Moosavin[13] in 2014 (64.6%). Our study shows 51.5% prevalence of Erythromycin resistant *Staphylococcus aureus,* which is in concordance with the study by P. Sreenivasulu Reddy[14] in 2012 (54%). The prevalence of Methicillin resistant *Staphylococcus aureus* (*MRSA)* was 48.5%, which is in this study and is in concordance with the studies by Moojtaba Moosavin[13] in 2014 and Yilmaz[15] in 2007 (48.4% and 52.5%) respectively. Infections with Methicillin resistant strains have poorer outcomes, longer hospitalization and increased costs compared to infections with Methicillin susceptible strains. Hence, screening of *MRSA* is today's necessity[16].

The antibiogram of *Staphylococcus aureus* showed high sensistivity to Linezolid (91%), followed by Vancomycin (80%) and Clindamycin (68.5%), whereas

high resistance was seen to Penicillin (80%) and Ciprofloxacin (80%). A similar result was also observed by Moojtaba Moosavin[13] and P. Srinivasulu Reddy[14]. Since Vancomycin is known to produce side effects and Linezolid is a "reserve antibiotic", Clindamycin is advised as an antibiotic for sensitive Staphylococcus aureus isolates[17].

Our study showed 42.7% inducible Clindamycin resistant (iMLS$_B$ phenotype) *Staphylococcus aureus*, 38.9% Clindamycin sensitive (MS phenotype) *Staphylococcus aureus* and 18.4% constitutive Clindamycin resistant (cMLS$_B$ phenotype) *Staphylococcus aureus*. Similar studies conducted by P. Sreenivasulu Reddy[14] in 2012 and Mallikarjuna Reddy[17] in 2014 showed iMLS$_B$ Phenotype to be 38.27% and 46.34%, MS Phenotype to be 40.7% and 40.8% and cMLS$_B$ Phenotype to be 21% and 12.86% respectively and the results correlates with our study.

In our study, 42.7% of the isolates belonged to iMLS$_B$ phenotype, 18.5% belonged to cMLS$_B$ phenotype and 38.8% to the MS phenotype by both D – test and Agar dilution methods. A similar result was also observed by Clarence. J. Fernandes[11] in 2007 and Christian Lavalle[18] in 2010. This shows that D - test and Agar dilution have the same efficacy in detecting inducible Clindamycin resistance. This shows that D - test can be used in daily routine diagnostic to detect inducible Clindamycin resistance.

There is an almost equal distribution of inducible Clindamycin resistance among both *MRSA* and *MSSA* (41.7% and 45.3% respectively), which is in correlation with the study by Mallikarjuna Reddy[17] in 2014 which showed inducible Clindamycin resistance to be 46.3%.

Out of the total MLS$_B$ isolates, 68.2% showed the presence of *ermA* gene and 31.8% showed the presence of *ermC* gene. Mojtaba Moosavin[13] in 2014 showed 57.2% of the isolates contained *ermA* and 42.8% contained *ermC*. The *ermA* gene has a higher prevalence when compared to *ermC* gene. Among the 40 MS phenotype, 90% carried *msrA* gene in them. Gerard Lina[19] in 1998 showed a prevalence of 86% of *msrA* gene. There is a maximum prevalence of *msrA* gene among *Staphylococcus aureus*, as seen in our study and other studies[13,19].

All the three methods (D-test, Agar Dilution and PCR) show the same efficacy (61.2%) in detecting Clindamycin resistance in our study. This correlates with studies done by Clarence J Fernandes[11] and Christian Lavalle[18].

Conclusion

Staphylococcus aureus is the single most frequently isolated bacterial pathogen in hospitals and the most common etiological agent of wound infections. Keeping in mind the limitations of certain antibiotics like Vancomycin, Clindamycin should be preferred for the treatment of severe and resistant Staphyloccal skin infections due to its excellent pharmacokinetic properties. There is a need for *in-vitro* detection of macrolide resistance and correct interpretation of susceptibility tests to guide the therapy. Various methods like D–test, agar dilution and genotypic methods have equal efficacy in detection of Inducible Clindamycin resistance. Agar Dilution method requires skilled labor and is time consuming process and genotypic method is very expensive, but D-test is a cost-effective and a simple method to detect inducible Clindamycin resistance.

We conclude that D-Test should be used as a mandatory method in routine Disk Diffusion testing for detection of Inducible Clindamycin resistance. All D-test positive isolates should not be treated with Clindamycin but it is the drug of choice for all D-test negative isolates (MS phenotypes).

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