

Inducible Clindamycin Resistance, Glycopeptide Resistance and Mupirocin Resistance in Methicillin Resistant *Staphylococcus aureus* (MRSA) Isolated from Clinical Samples

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Staphylococcus aureus, a common pathogen is well known for its multidrug resistance. Existence of MRSA is further worsened by inducible clindamycin resistance and emerging glycopeptide resistance. Our aim of the study was to detect inducible clindamycin resistance, vancomycin resistance and mupirocin resistance among MRSA isolates. One hundred non-repetitive isolates were subjected to routine antibiotic susceptibility testing by Kirby Bauer's disc diffusion method including cefoxitin disc for MRSA. Inducible clindamycin resistance was detected by D-test, E-test for vancomycin MIC and mupirocin resistance by disc diffusion. Twenty three isolates showed inducible clindamycin resistance, one showed constitutive resistance and three showed MS phenotypes. Inducible clindamycin resistance, constitutive resistance and MS phenotype were found to be higher in MRSA as compared to MSSA. Only one isolate with vancomycin MIC 4 μ g/ml by E-test was considered as VISA. Forty one isolates were found resistant to mupirocin, which is a cause for concern. Study showed that D-test should be included as routine disc diffusion test to prevent therapeutic failure with clindamycin.

Key words: Clindamycin resistance, D-test; mupirocin resistance, constitutive MLS_B phenotype, inducible MLS_B phenotype.

Methicillin Resistant *Staphylococcus aureus* (MRSA) infection is common worldwide, which is further worsened by inducible clindamycin resistance.

Macrolide antibiotics are bacteriostatic agents which act by inhibiting protein synthesis by binding reversibly to 50 S ribosomal subunits of susceptible organism. Target site modification is the most common mechanism of acquired resistance to macrolide, lincosamide and streptogramin B (MLS_B) antibiotics, which are mediated by *erm* genes. These *erm* genes can be expressed constitutively (constitutive MLS_B phenotype) or inducibly (inducible MLS_B phenotype)¹. Strains with inducible resistance to clindamycin are difficult to detect in routine laboratory as they appear erythromycin resistant and clindamycin sensitive *in vitro* unless placed adjacent to each other. In such cases *in vivo* therapy with clindamycin may select constitutive

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erm mutants leading to treatment failure². Although vancomycin resistance in *S. aureus* is rare, decreased susceptibility and heteroresistance is being described more often than before^{3,4}.

MATERIAL AND METHODS

The study was conducted for a period of 6 months from May to October 2010. A total of 100 non-repetitive *S. aureus* isolates from various clinical specimens like pus (84), sputum (5), ear discharge (4), urine (3), blood (2), suction tip (1) and vaginal swab (1) were included in the study. The isolates were identified based on standard biochemical techniques and then subjected to susceptibility testing by Kirby Bauer's disc diffusion method on Muller Hinton agar plates using erythromycin (15µg), clindamycin (2µg), vancomycin (30µg), teicoplanin (30µg), cefoxitin (30µg), linezolid (30µg).

Methicillin resistance was detected by cefoxitin disc diffusion method as it is described to correlate well with detection of *mec A* gene. A zone size of less than 22mm indicated MRSA.

Inducible clindamycin resistance was detected by D-test in isolates resistant to erythromycin as per CLSI guidelines.⁽⁵⁾ Briefly, a 15µg erythromycin disc was placed 15mm (edge to edge) from a clindamycin (15µg) disc on a Muller Hinton agar, previously inoculated with 0.5 McFarland bacterial suspension. Following overnight incubation at 37°C, flattening of zone of inhibition around clindamycin adjacent to erythromycin disc was considered as D-test positive, indicating inducible clindamycin resistance. Three different phenotypes were interpreted as follows:

MS Phenotype

Staphylococcal isolates exhibiting resistance to erythromycin (zone size d" 13mm) while sensitive to clindamycin (zone size e" 21mm) giving circular zone of inhibition around clindamycin were labelled as having this phenotype.

Inducible MLS_B phenotype

Staphylococcal isolates exhibiting resistance to erythromycin (zone size d" 13mm) while being sensitive to clindamycin (zone size e" 21mm) and giving D shaped zone of inhibition

around clindamycin with flattening towards erythromycin disc were labelled as having this phenotype.

Constitutive MLS_B phenotype

Staphylococcal isolates exhibiting resistance to both erythromycin (zone size d" 13mm) and clindamycin (zone size d" 14mm) with circular shape zone of inhibition around clindamycin (whenever present) were labelled as this phenotype.

Vancomycin MIC was determined on Mueller Hinton agar using E-test strips (AB Bio-disk, Solna, Sweden) after incubation at 37°C for 24 hours. The test was performed as per manufacturers instructions. Performance of E-test strips were evaluated on known Vancomycin Resistant Strains (VRSA) Mu3 and vancomycin heteroresistant Strain (hVRSA) Mu50, both kindly provided by Hiramatsu, Japan.

For mupirocin resistance 5µg and 200µg disc were used. A zone diameter e" 14 mm for 5 µg and 200 µg disc were considered susceptible. Isolate that showed zone diameter less than 14 mm in the 5 µg disc but more than or equal to 14mm in 200 µg disc were considered to be MuL (Low level) strains. All isolates with zone diameters less than 14 mm for both 5 µg and 200 µg were considered to be MuH (High level) strains⁶.

RESULTS

Among the 100 *S. aureus* isolates tested, 42 were identified as MRSA and 27 were resistant to erythromycin. Among the erythromycin resistant isolates, 23 (85.2%) belonged to iMLS_B phenotype, 3 (11.1%) belonged to MS phenotype and 1 (3.7%) belonged to cMLS_B phenotype. Percentage of both inducible and constitutive phenotype was higher among MRSA isolates than MSSA (Table 1).

All the isolates were sensitive to linezolid and teicoplanin.

All the MRSA isolates had their vancomycin MIC < 3µg/ml. The only exception, which had MIC 4µg / ml has been considered as VISA. Our study detected mupirocin resistance in 11 (26.1%) MRSA and 30 (51.72%) MSSA isolates. Among the mupirocin resistant strains, the percentage of low level resistance and high level resistance (Table 2).

Table 1. Comparison of inducible, Constitutive and MS phenotype among MRSA and MSSA isolates

Resistance phenotype	MRSA (42)	MSSA (68)
iMLS _B	15(35.7%)	8(11.7%)
cMLS _B	1(2.3%)	0(0%)
MS	3 (7.1%)	0(0%)

iMLS_B – inducible resistance to clindamycin.
 cMLS_B – Constitutive resistance to clindamycin.
 MS – MS phenotype.

Table 2. Comparison of low level and high level mupirocin resistance among MRSA and MSSA isolates

Total	MuL	MuH
MRSA (11)	4 (36.3%)	7 (63.6%)
MSSA (30)	7 (23.3%)	23 (76.6%)

MuL - Low level resistance to mupirocin
 MuH- High level resistance to mupirocin

DISCUSSION

The determination of antimicrobial susceptibility of clinical isolate is often crucial for optimal antimicrobial therapy of infected patients. This is particularly important when there is increase in resistance and emerging multidrug resistance. There are many alternatives available for treatment of MRSA infections with clindamycin being one of the good alternatives⁷. However, clindamycin resistance can develop in *staphylococcal* isolate with inducible phenotypes and from such isolates, spontaneous constitutively resistance mutants have evolved both *in vitro* and *in vivo* during clindamycin therapy⁸. Reporting *staphylococcus aureus* as susceptible to clindamycin without checking for inducible resistance may result in institution of inappropriate clindamycin therapy. On the other hand negative result for inducible clindamycin resistance confirms clindamycin susceptibility and provides a very good therapeutic alternative⁹.

In our study it was observed that percentage of inducible and MS phenotype were higher among MRSA (35.7% and 7.14% respectively) as compared to MSSA (11.7% and 0%) respectively. This was in agreement with few studies reported before, KE Vandana *et al.* reported inducible resistance of 48.7% in MRSA and 9.5% in MSSA¹⁰. Mohammed Rahabar *et al.* reported 22.6% in MRSA and 4% in MSSA Gadepalli et al. showed it to be 30% in MRSA and 10% in MSSA.⁽¹¹⁾ Another study in Thailand showed 35.9% in MRSA and 4.7% in MSSA.⁽¹²⁾ On the contrary Schreckenberger *et al.*¹³ and Levin *et al.*¹⁴, showed a higher percentage of inducible resistance in MSSA as compared to MRSA, (19-20% in MSSA and 7-12% in MRSA; 68% in MSSA and 12.5% in MRSA respectively).

True sensitivity to clindamycin can only be judged after performing D-test on erythromycin resistant isolates. From our study we can conclude that since there is fairly high percentage of inducible clindamycin resistance in *staphylococcal* isolate, D-test showed be included in routine disc diffusion. VRSA isolates are rare and infrequently reported. The exact mechanism of resistance in vancomycin intermediately susceptible *S. aureus* (VISA) is still not clear; it has been suggested that co-operative effect of the clogging and cell wall thickening enables VISA to prevent vancomycin from reaching its true target in the cytoplasmic membrane¹⁵. Detection of VISA is challenging as its detection fails in disc diffusion test. There have been instances of treatment failure associated with VISA infections¹⁶. In our study only one strain, which had MIC 4µg/ml has been considered as VISA. VISA may demonstrate heteroresistance or there may be subpopulation that are resistant. Screening for hVISA requires additional testing to reveal its heterovariant phenotype and these methods are more labor intensive and costly than routine susceptibility testing.

Mupirocin is a topical antibiotic that interferes with protein synthesis by competitive inhibition of bacterial isoleucyl tRNA synthetase. It is used as topical antibiotic for elimination of MRSA in carriers¹⁷. Development of resistance to mupirocin should be a cause of concern as it is used to eradicate nasal carriage.

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