

Original Research Article

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Inducible Clindamycin Resistance among Clinical Isolates of *Staphylococcus aureus* in a Tertiary Care Hospital, Nanded, Maharashtra, India

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ABSTRACT

Keywords

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Staphylococcus aureus is one of the most common organisms causing nosocomial and community acquired infections worldwide. Antibiotic resistance in this organism has become an ever-increasing problem. Present study was undertaken to determine the percentage of *Staphylococcus aureus* isolates having inducible clindamycin resistance. A total of 270 *Staphylococcus aureus* isolated from different specimens were subjected to routine antibiotic sensitivity testing by Kirby Bauer disc diffusion method. All isolates were tested for Methicillin resistance by using Cefoxitin 30 µg disc. Inducible clindamycin resistance was detected by 'D' test as per CLSI guidelines. Among the 270 *S. aureus* isolates, 124 (45.92%) were detected as MRSA, 116 *S. aureus* isolates (42.96 %) were resistant to Erythromycin and 41 (15.16 %) isolates were D-test positive. Both inducible and constitutive clindamycin resistance was higher among MRSA compared to MSSA. High prevalence of strains with inducible clindamycin resistance particularly among MRSA indicates that inducible clindamycin resistance testing (D-test) should be included as a part of routine antibiotic susceptibility. These isolates may be missed in routine antibiotic testing by disk diffusion method.

Introduction

Staphylococcus aureus is one of the most common organisms causing nosocomial and community acquired infections worldwide (Prabhu *et al.*, 2011). These bacteria can cause a wide range of infections from mild folliculitis to potentially fatal systemic illnesses such as bacteremia or endocarditis (Seifi *et al.*, 2012). Antibiotic resistance in this organism has become an ever-increasing problem. In *Staphylococcus aureus*, penicillin resistance was recognized first in 1944 and methicillin resistance was recognized first in 1961 (Gade *et al.*, 2013). This has led to the usage of macrolide – lincosamide – streptogramin B (MLS_B) antibiotics to treat

S. aureus infections, with Clindamycin being the preferable agent due to its excellent pharmacokinetic properties (Kaur *et al.*, 2013). However, widespread use of MLS_B antibiotics has led to an increase in the number of staphylococcal strains acquiring resistance to MLS_B antibiotics (Dhanalakshmi *et al.*, 2012).

Macrolide and lincosamide resistance is mainly due to one of these three mechanisms (Leclercq, 2002).

a) Target site modification: Ribosomal methylation or mutation which prevents

binding of antibiotic to its ribosomal target. This is the most prevalent mechanism of resistance to macrolides and lincosamides encoded by *erm* genes.

b) Efflux of antibiotic: encoded by *msrA* gene

c) Drug inactivation: encoded by *lnu* genes

Modification of ribosomal target which confers broad-spectrum resistance to macrolides and lincosamides is encoded by a variety of *erm* (erythromycin ribosome methylase) genes. *ErmA* and *ermC* are typically *Staphylococcal* genes. This mechanism can be constitutive (cMLS); always producing the rRNA methylase, or inducible (iMLS), that is producing methylase only in the presence of an inducer (Saderi *et al.*, 2009). *In vitro*, *S. aureus* isolates with constitutive resistance are resistant to both Erythromycin and Clindamycin whereas those with inducible resistance are resistant to Erythromycin and appear sensitive to Clindamycin (iMLS_B) (Lyll *et al.*, 2013). The treatment of patients harboringi MLS_B *Staphylococci* with Clindamycin leads to the development of constitutive resistance, subsequently leading to therapeutic failure.

Frequencies of different resistance phenotypes vary by hospital and geographical regions, patient group, bacterial strains and bacterial susceptibility pattern (Zorgani *et al.*, 2009). Thus, the present study was undertaken to determine the percentage of *Staphylococcus aureus* isolates having inducible clindamycin resistance in our geographical area.

Materials and Methods

This study was conducted for the period of 1 year and 6 months, from July 2015 to December 2016 in the Department of Microbiology, Dr. Shankarrao Chavan Government Medical College, Nanded. A

total of 270 isolates of *S. aureus* isolated from clinical specimens such as pus, wound swab, blood, urine, cerebrospinal fluid, sputum and other body fluids were considered for the study.

Isolates were identified on the basis of colony characteristics, Gram staining, catalase test, slide coagulase test, tube coagulase test, growth on mannitol salt agar and DNase test (Collee, 2012). Antibiotic susceptibility tests were performed using Kirby Bauer's disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines 2014.

Drugs used were Erythromycin (15µg), Clindamycin (2µg), Penicillin (10 Units), Linezolid (30µg), Cefoxitin (30µg), Cotrimoxazole (1.25/23.75µg), Ciprofloxacin (5µg), Vancomycin (30µg). MRSA were screened using Cefoxitin (30µg) disc by disc-diffusion technique (M 100-S 25.CLSI, 2014). The results were interpreted according to CLSI guidelines. *Staphylococcus aureus* ATCC 25923 was taken as the positive control strain.

Isolates that were Clindamycin susceptible and Erythromycin resistant were tested for inducible resistance by the use of D-zone test.

D-zone test: A 0.5 McFarland equivalent suspension of organisms was incubated on Muller-Hinton agar (MHA) plate as described in the CLSI recommendations. Clindamycin and Erythromycin disks were placed 15-26mm apart from each other on the MHA plates. After 18hours incubation at 37° C, plates were checked. Flattening of inhibition zone (D-shaped) around clindamycin was considered as inducible clindamycin resistance.

The test allows for identification of three different phenotypes:

- a) Inducible MLS_B phenotype: i MLS_B *S. aureus* isolates which showed 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 (D test positive)
- b) Constitutive MLS_B phenotype: c MLS_B *S. aureus* isolates which showed resistance to both Erythromycin (zone size ≤ 13 mm) and Clindamycin (zone size ≤ 14 mm) with circular shape zone of inhibition around Clindamycin.
- c) Methicillin-sensitive (MS) phenotype: *S. aureus* isolates exhibiting resistance to Erythromycin (zone size ≤ 13 mm), while sensitive to Clindamycin (zone size ≥ 21 mm) and giving circular zone of inhibition around Clindamycin (D test negative).

Results and Discussion

A total of 270 *S. aureus* isolates were obtained from specimens such as pus and wound swabs, blood, urine, sputum, aspirates, and body fluids. Majority of these were detected from pus and wound swabs (54.07%), followed by urine (19.6%), blood (9.63%), body fluids / aspirates (9.26%) and sputum (7.41%). Most of these specimens were from inpatients (62.22%) (Table 1).

Out of 270 *S. aureus* isolates, 146 (54.07%) were Methicillin-sensitive *Staphylococcus aureus* (MSSA), and 124 (45.92%) were Methicillin-resistant *Staphylococcus aureus* (MRSA) (Figure 1).

All 270 isolates were subjected to antimicrobial susceptibility testing. These showed 100% sensitivity to vancomycin and linezolid, 116 *S. aureus* isolates (42.96 %) were resistant to Erythromycin, out of which

57.26% (71) were MRSA and 30.82% (45) were MSSA (Table 2 and Figure 2).

The result of the D- test revealed that 42(11 MSSA, 31 MRSA) isolates were resistant to both Erythromycin and Clindamycin indicating c MLS_B phenotype. 33 (21 MSSA, 12 MRSA) isolates were D- test negative, indicating MS phenotype. These isolates were truly susceptible to Clindamycin and 41 (13 MSSA, 28 MRSA) isolates were D- test positive, indicating i MLS_B phenotype. These isolates were actually resistant to Clindamycin which would have been easily missed and reported as Clindamycin susceptible in regular Kirby–Bauer disk diffusion susceptibility testing. The study also showed that higher percentage MRSA isolates were both constitutive and inducible clindamycin resistant in comparison to MSSA (Table 3 and Figure 3).

Clindamycin, a lincosamide, is one of the most efficient antibiotics in treating staphylococcal skin and soft tissue infections. It is available in oral and parenteral formulations, 90% oral bioavailability, less costly in comparison to newer drugs, good tissue penetration and is able to inhibit production of toxins and virulence factors in *Staphylococci* (Majhi *et al.*, 2016).

However, resistance to Clindamycin is highly variable, and incidence of its resistant phenotypes varies by geographic regions and even between hospitals (Fiebelkorn *et al.*, 2003). These isolates have a high rate of spontaneous mutation during the therapeutic process which would enable them to develop resistance to Clindamycin (Prabhu *et al.*, 2011). Thus, the empirical treatment options against *S. aureus* infections have become more limited.

Therefore, this study was undertaken to detect and report the presence of Clindamycin-

resistant phenotypes in a tertiary care hospital.

In this study, majority (54.07 %) of the *S. aureus* isolates was detected from pus and wound swabs. It is in accordance to other studies done by Lyall *et al.*, (2013) and Majhi *et al.*, (2016). Most of the isolates (62.22%) were obtained from hospitalized patients as also observed by Majhi *et al.*, (2016).

Prevalence of MRSA in our study is 45.92 % which resembles with the reports of Gade *et al.*, (2013). The prevalence of MRSA isolates among *S. aureus* was high in studies done by Lyall *et al.*, (2013), Majhi *et al.*, (2016) and Sah *et al.*, (2015). Lack of awareness, indiscriminate and improper use of antibiotics before coming to the hospital might be the contributory factors for a high prevalence of MRSA. In our study, none of the isolates

were resistant to Vancomycin or Linezolid and 42.96% of *S. aureus* isolates were resistant to Erythromycin. Similar high prevalence of resistance to Erythromycin has reported by Mittal *et al.*, (2013) and Sasirekha *et al.*, (2014).

Our study revealed 41 (15.16 %) *S. aureus* isolates were D- test positive. It was observed that percentage of inducible clindamycin resistance was higher among MRSA (22.58%) compared to MSSA (8.9%). This finding conforms to many published studies such as Gade *et al.*, (2013), Majhi *et al.*, (2016) and Lall *et al.*, (2014). On the contrary, Sasirekha *et al.*, (2014) and Bottega *et al.*, (2014) had shown a higher percentage of inducible resistance in MSSA compared to MRSA.

Table.1 Distribution of specimens

Specimen	OPD No. (%)	IPD No. (%)	Total No. (%)
Wound swab/pus	55 (20.37 %)	91 (33.70 %)	146 (54.07 %)
Urine	23 (8.52 %)	30 (11.11 %)	53 (19.63 %)
Blood	05 (1.85 %)	21 (7.77 %)	26 (9.63 %)
Sputum	09 (3.33 %)	11 (4.07 %)	20 (7.41 %)
Body fluids/aspirates	10 (3.70 %)	15 (5.55 %)	25 (9.26 %)
Total	102 (37.78 %)	168 (62.22 %)	270 (100%)

Table.2 Antibiotic resistance pattern among MSSA and MRSA isolates

Antibiotics	MSSA (146)	MRSA (124)
Vancomycin (30µg)	00	00
Linezolid (30µg)	00	00
Cefoxitin (30µg)	00	100 % (124)
Clindamycin (2µg)	16.44 % (24)	47.58 % (59)
Erythromycin (15µg)	30.82 % (45)	57.26 % (71)
Cotrimoxazole(1.25/23.75µg)	40.41 % (59)	58.06 % (72)
Ciprofloxacin (5µg)	32.19 % (47)	59.68 % (74)
Penicillin (10 Units)	81.51 % (119)	88.71 % (110)

Table.3 Results of D-Zone test

	MS phenotype (%)	Inducible MLS _B phenotype (%)	Constitutive MLS _B phenotype (%)
MRSA (n= 124)	12 (9.68%)	28 (22.58%)	31 (25%)
MSSA (n=146)	21 (14.38%)	13 (8.9%)	11 (7.5%)
Total (n=270)	33 (12.22%)	41 (15.16%)	42 (15.56%)

Fig.1 Prevalence of MRSA

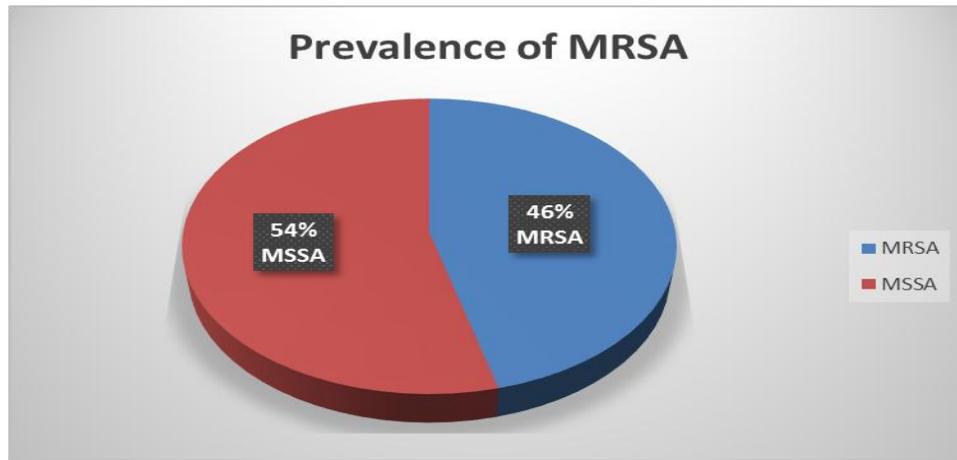


Fig.2 Antibiotic resistance pattern among MSSA and MRSA isolates

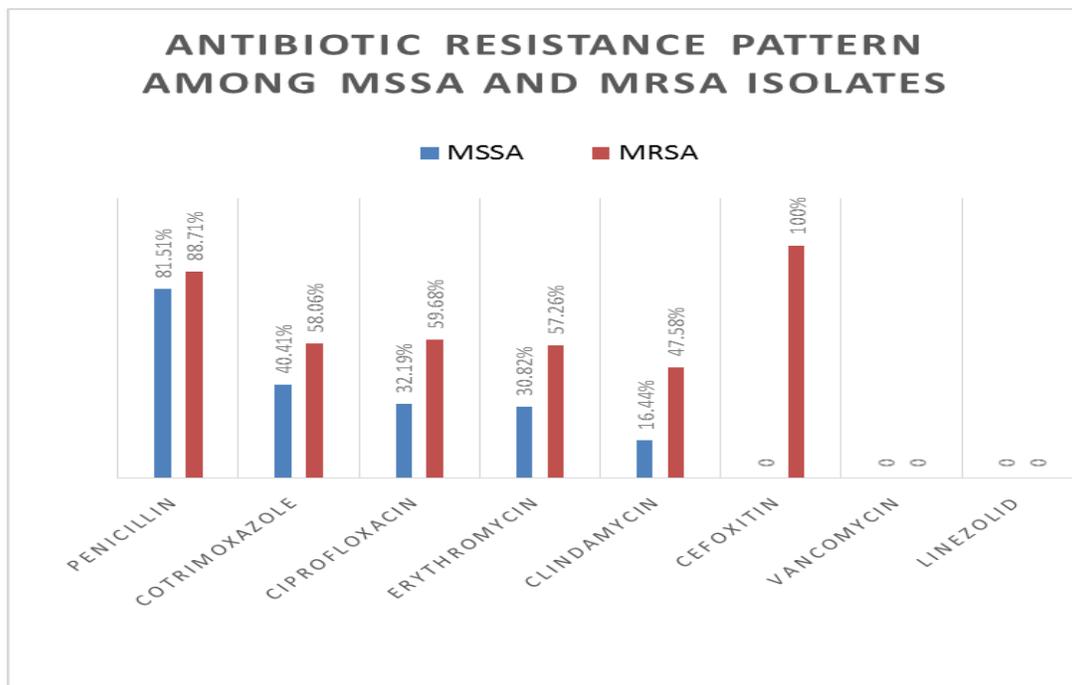
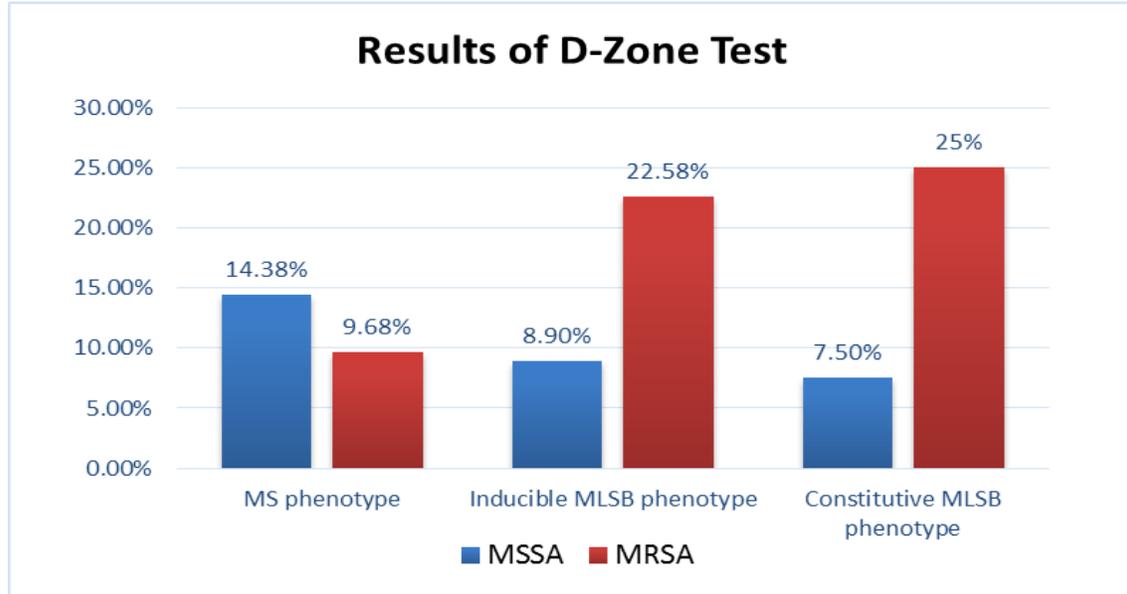


Fig.3 Results of D-Zone test



The different patterns of resistance observed in various studies are due to the fact that resistance varies by geographical regions, age groups, antibiotic prescription patterns, methicillin susceptibility and even from hospital to hospital.

Constitutive clindamycin resistance in our study was observed in 7.5% of MSSA and 25% of MRSA isolates, which is similar to Majhi *et al.*, (2016) and Chudasama *et al.*, (2014). Truly clindamycin-sensitive isolates, which exhibit MS phenotype, were present in 14.38% of MSSA and 9.68% of MRSA isolates in our study. This result is similar to Banik *et al.*, (2015) and Phukan *et al.*, (2015).

In conclusion, from the current study, we can conclude that there is a high percentage of inducible clindamycin resistance amongst the staphylococcal isolates. If D- test would not have been performed, many inducible clindamycin resistant *S. aureus* could have been easily misidentified as clindamycin susceptible leading to therapeutic failure.

Thus, simple and reliable D- test can be incorporated into routine Kirby–Bauer disk diffusion method in clinical microbiology laboratory. This will enable us in guiding the clinicians regarding judicious use of Clindamycin in skin and soft tissue infections as Clindamycin is not a suitable drug for D test positive isolates; while it can definitely prove to be a drug of choice in case of D test negative isolates.

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