

Detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci - a study from South India

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Citation

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Abstract

Background: Inducible clindamycin resistance is a major concern for the use of clindamycin to treat staphylococcal infections. **Aims:** To determine the prevalence of inducible clindamycin resistance in clinical isolates of *Staphylococcus* spp. and the susceptibility pattern of the isolates.

Materials and Methods: A total of 300 isolates of *Staphylococci* spp. recovered from different clinical specimens were studied. All the *Staphylococcus* spp. were identified by conventional microbiological methods. Inducible clindamycin resistance was detected by double disk approximation test (D- test). **Results:** Of the 300 isolates, 176 were identified as *S. aureus*, while 124 were coagulase negative staphylococci (CoNS). The rates of inducible clindamycin resistance in methicillin resistant *S. aureus* (MRSA), methicillin sensitive *S. aureus* (MSSA), methicillin resistant CoNS (MR-CoNS) and methicillin sensitive CoNS (MS-CoNS) were 75.0%, 24%, 18.8% and 11.1%, respectively. The inducible clindamycin resistance was significantly more among MRSA compared to methicillin sensitive *S. aureus* (MSSA) (P value < 0.0001). Majority of the MRSA isolates were susceptible to clindamycin, vancomycin and linezolid, while most of them were resistant to erythromycin, gentamicin, ciprofloxacin, tetracycline and sulfamethoxazole-trimethoprim. **Conclusion:** In view of the significant in vitro inducible clindamycin resistance in *Staphylococcus* spp., we recommend that D test should be used as a mandatory method in microbiology laboratories to avoid misinterpretation of clindamycin result.

INTRODUCTION

Staphylococcus aureus and coagulase-negative staphylococci (CoNS) are important causes of nosocomial and community-acquired infections. Treatment of these infections is a growing problem because of the increasing methicillin resistance among staphylococci [1,2]. The macrolide-lincosamide-streptogramin B (MLS_B) family of antibiotics serve as an alternative, with clindamycin being the preferred agent due to its excellent pharmacokinetic properties [3]. However, widespread use of MLS⁺_B antibiotics has led to an increase in number of staphylococcal strains acquiring resistance to MLS_B antibiotics [4].

Although erythromycin and clindamycin are in separate antimicrobial agent classes, macrolides and lincosamides, respectively, their mechanisms of action (inhibition of protein synthesis) and mechanisms of resistance are similar

[5]. The cross-resistance for 3 antibiotic families (macrolides e.g., erythromycin, clarithromycin, azithromycin; lincosamides e.g., clindamycin; and group B streptogramins e.g., quinupristin) that share a common binding site is called as the MLS_B phenotype [6]. The two main mechanisms of resistance are production of methylase enzyme encoded by a multiallele plasmid-borne gene *erm* that alters the ribosomal binding site of the antimicrobial agents and efflux pumps. In staphylococci, the MLS_B resistance can be either constitutive (cMLS_B) or inducible (iMLS_B) [6]. If it is constitutive, in vitro susceptibility tests will show resistance to all 3 antibiotic classes, while if it is inducible, in vitro tests will show resistance to macrolides, but susceptibility to clindamycin will be retained, unless induced by a macrolide (i.e. erythromycin). Isolates that are erythromycin resistant but clindamycin susceptible may either possess inducible clindamycin resistance (iMLS_B) or

have efflux pumps that remove macrolides but not clindamycin from the microbe [6].

It is important to determine if resistance (whether inducible or constitutive) to clindamycin exists when it is being considered for therapy. Antimicrobial susceptibility data are important for the management of infections, but false susceptibility results may be obtained if staphylococci are not tested for inducible CL resistance by the disk diffusion induction test (D-test). We performed this study to determine the prevalence of inducible clindamycin resistance in clinical isolates of *Staphylococcus* spp. and the susceptibility pattern of the isolates.

MATERIAL AND METHODS

STUDY DESIGN AND SETTING

This cross-sectional study was conducted in the Department of Microbiology of Mahatma Gandhi Medical College and Research Institute (MGMC & RI), a 700-bedded tertiary care super-specialty hospital with teaching facility, located in Pondicherry, India. This study was approved by the Research and Ethical committees of our institute and informed consent was obtained from each patient.

CLINICAL SAMPLES AND BACTERIAL ISOLATES

Three hundred isolates of *Staphylococcus* spp. recovered from pus, sputum, tracheal aspirate, body fluids and high vaginal swab, over a period of 9 months from March 2010 to November 2010, were included in the study.

LABORATORY PROCEDURES

Identification of staphylococcal isolates was done based on colony morphology on 5% sheep blood agar, Gram stain and catalase test. Coagulase test by the plasma tube method and sugar fermentation tests were done to distinguish between *S. aureus* and coagulase negative staphylococci. The isolates were subjected to susceptibility testing by Kirby Bauer disc diffusion method on Mueller Hinton agar plates using erythromycin, (15 µg), clindamycin (2 µg), penicillin (10 IU), ciprofloxacin (5 µg), gentamicin (10 µg), ceftiofur (30 µg), vancomycin (30 µg) and linezolid (30 µg) as per Clinical Laboratory Standards Institute (CLSI) guidelines [7]. Methicillin resistance was detected by oxacillin disc diffusion method and oxacillin screen agar (5% NaCl, 6 µg/ml oxacillin) [7].

D-TEST

Those isolates which were erythromycin resistant were

subjected to 'D test' as per CLSI guidelines [7]. A 0.5 McFarland suspension of staphylococci was inoculated on Mueller Hinton agar plate. The test was performed with erythromycin (15 µg) disc placed at a distance of 15mm (edge to edge) from clindamycin (2 µg) disc, followed by overnight incubation at 37°C. Three different phenotypes were interpreted as follows [8]:

1. cMLS_B phenotype – isolates showing resistance to both erythromycin (zone size ≤13mm) and clindamycin (zone size ≤14mm) with circular shape of zone of inhibition if any around clindamycin.
2. iMLS_B phenotype – isolates showing resistance to erythromycin (zone size ≤13mm), while being sensitive to clindamycin (zone size ≥21mm) with a D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc.
3. MS phenotype – isolates showing resistance to erythromycin (zone size ≤13mm) while being sensitive to clindamycin (zone size ≥21mm) with a circular zone of inhibition around clindamycin.

RESULTS

The demographic details of the patients included in the study are summarized in Table 1. A total of 300 staphylococci were isolated from various types of clinical samples obtained from these patients. Of these 300 isolates, 176 were identified as *S. aureus*, while 124 were CoNS. Of the 176 *S. aureus*, 35 (19.89%) were methicillin resistant *S. aureus* (MRSA), while 25 of the 124 (20.2%) CoNS were methicillin resistant. The erythromycin and clindamycin resistance patterns of the isolates based on disc diffusion method are shown in Table 2 & Fig1. Majority of the MRSA (80.0%) were erythromycin resistant and clindamycin sensitive, while most (63.8%) of the MSSA were sensitive to both erythromycin and clindamycin.

Of the 300 staphylococcal isolates, 121 (40.33%) were erythromycin resistant and clindamycin sensitive (Table 2). These were subjected to D- test for detecting inducible clindamycin resistance. The rates of inducible clindamycin resistance of the different staphylococcal isolates are shown in Table 3. The inducible clindamycin resistance was significantly more among MRSA compared to methicillin sensitive *S. aureus* (MSSA) (P value < 0.0001).

The antibiotic susceptibility patterns of the different staphylococcal isolates are summarized in Table 4. Majority

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of the MRSA isolates were susceptible to clindamycin, vancomycin and linezolid, while most of them were resistant to erythromycin, gentamicin, ciprofloxacin, tetracycline and sulfamethoxazole-trimethoprim.

Figure 1

Table 1. Demographic data of the patients included in the study

Demographic data	Number (%)	Mean ± SD
Age		
Newborn	6 (2.0)	12.0 ± 7.2 (days)
Children (1 – 12)	19 (6.3)	5.9 ± 3.5 (years)
Adolescent (13 – 18)	11 (3.7)	16.5 ± 1.6 (years)
Adult (19 and above)	264 (88.0)	41.8 ± 15.4 (years)
Sex		
Male	177 (59.0)	
Female	123 (41.0)	
Clinical diagnosis		
Abscess	133 (44.3)	
Genital infection	15 (5.0)	
Cellulitis	19 (6.3)	
Diabetic foot	26 (8.7)	
Respiratory infection	8 (2.7)	
Fournier's gangrene	5 (1.7)	
Fracture	30 (10.0)	
Bone infections	28 (9.3)	
Trophic ulcer	18 (6.0)	
Others	18 (6.0)	

Figure 2

Table 2. Macrolide resistance of the isolates based on disc diffusion method

Organism	Total no. of isolates	Both erythromycin and clindamycin sensitive	Erythromycin resistant and clindamycin sensitive	Both erythromycin and clindamycin resistant
MRSA	35	6 (17.1%)	28 (80.0%)	1 (2.9%)
MSSA	141	90 (63.8%)	50 (35.5%)	14 (4.7%)
MR-CoNS	25	7 (28.0%)	16 (64.0%)	2 (8.0%)
MS-CoNS	99	62 (62.6%)	27 (27.3%)	10 (10.1%)

MRSA – Methicillin resistant *Staphylococcus aureus*

MSSA – Methicillin sensitive *Staphylococcus aureus*

MR-CoNS – Methicillin resistant coagulase negative staphylococci

MS-CoNS – Methicillin sensitive coagulase negative staphylococci

Figure 3

Table 3. Inducible clindamycin resistance among the isolates based on D test

Organism	Total no. of isolates tested	Inducible clindamycin resistance (D test)		P value
		Positive	Negative	
MRSA	28	21 (75.0%)	7 (25.0%)	< 0.0001
MSSA	50	12 (24.0%)	38 (76.0%)	
MR-CoNS	16	3 (18.8%)	13 (81.3%)	0.6546
MS-CoNS	27	3 (11.1%)	24 (88.9%)	

MRSA – Methicillin resistant *Staphylococcus aureus*

MSSA – Methicillin sensitive *Staphylococcus aureus*

MR-CoNS – Methicillin resistant coagulase negative staphylococci

MS-CoNS – Methicillin sensitive coagulase negative staphylococci

Figure 4

Table 4. Antibiotic susceptibility pattern of the isolates

Isolate	No. of isolates	No. of susceptible isolates (%)						
		ERY	CLI	GEN	CIP	TET	SXT	VAN/ LNZ
MRSA	35	6 (17)	34 (97)	7 (20)	1 (3)	12 (34)	3 (9)	35 (100)
MSSA	141	90 (64)	140 (99)	120 (85)	62 (44)	128 (91)	70 (50)	141 (100)
MR-CoNS	25	7 (28)	23 (92)	11 (44)	5 (20)	17 (68)	3 (12)	25 (100)
MS-CoNS	99	62 (63)	89 (90)	82 (83)	76 (77)	74 (75)	47 (48)	99 (100)

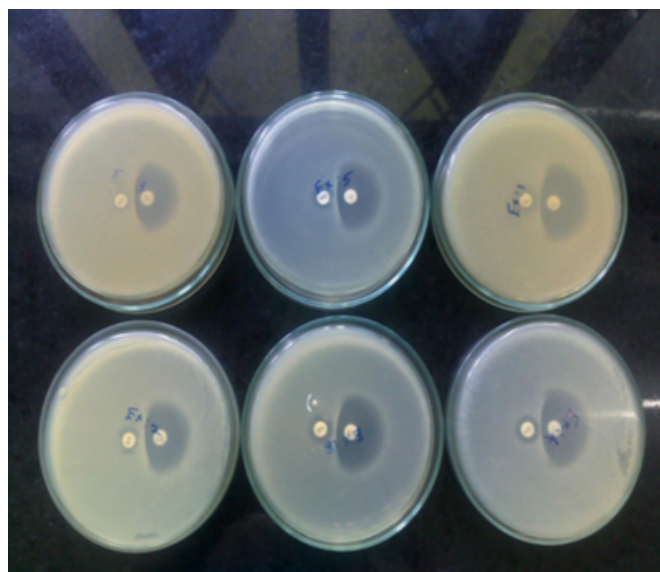
ERY – erythromycin, CLI – clindamycin, GEN – gentamicin, CIP – ciprofloxacin, TET –

tetracycline, SXT – trimethoprim/ sulfamethoxazole, VAN – vancomycin, LNZ –

linezolid

Figure 5

Figure 1: Positive D Test



DISCUSSION

The performance of antimicrobial susceptibility testing remains a crucial component of the microbiology laboratory.

Due to the emergence of methicillin resistance in *Staphylococcus* spp., only a few therapeutic alternatives are available to treat staphylococcal infections. The macrolide-lincosamide-streptogramin B (MLS_B) family of antibiotics serves as one such alternative, with clindamycin being the preferred agent. Clindamycin has excellent tissue penetration, accumulates in abscesses, and no renal dosing adjustments are needed [9]. Also, it has good oral bioavailability making it a good option for outpatient therapy and changeover after intravenous antibiotics [10]. However, one of the major concerns regarding the use of clindamycin to treat staphylococcal infections is the possible presence of inducible resistance to clindamycin [11,12].

In *S. aureus* and CoNS, resistance to macrolides (e.g. erythromycin), lincosamides (e.g. clindamycin) and type B streptogramins (MLS_B) can be the result of ribosomal target modification in which enzymes encoded by *erm* genes confer constitutive or inducible resistance to MLS drugs through methylation of the 23S rRNA [2]. Also, staphylococci can have an active efflux mechanism (encoded by *msrS* genes) that confers resistance to MLS_B only, but not to lincosamides [7,13]. Isolates with constitutive resistance can be detected readily by standard susceptibility testing methods [14]. When tested by standard methods, clindamycin may appear active against staphylococci with inducible clindamycin resistance, and so this mode of resistance is identified by the disk-diffusion induction test (D-test) [2,13,15].

Among the 176 *Staphylococcus aureus* strains, we found 35 (19.9%) to be MRSA, which is lower than that reported by Gupta et al in North India [16]. In our study, 33 (42.3%) *S. aureus* were of the iMLS_B phenotype, whereas in other studies only about 20 – 30% of the *S. aureus* showed iMLS_B phenotype [3,17,18]. In our study, 24.0% MSSA isolates were of the iMLS_B phenotype, which is higher than that reported by other workers who have found that 4 – 15% of their MSSA isolates were of the iMLS_B phenotype [19-21]. But in study from Thailand, 22% of MSSA were noted to be of the iMLS_B phenotype similar to our study [22]. Several studies from different parts of India have reported that 30-64% of their MRSA isolates were of iMLS_B phenotype [4,17,19,21,23]. Our study showed a higher value with 75% MRSA isolates found to be of iMLS_B phenotype.

In the present study the constitutive clindamycin resistance was present in 2.9% of MRSA and 4.7% of MSSA isolates. This trend is in contrast with other studies from Korea where

the majority of MRSA had constitutive resistance (cMLS_B) [24]. This indicates that the incidence of constitutive and inducible resistance in staphylococcal isolates varies widely by hospital and geographic region. The low constitutive clindamycin resistance in our study may also be attributed to the fact that drug is not commonly used and hence there is less selection of resistant strains.

In our study, majority of the MRSA isolates were susceptible to clindamycin, vancomycin and linezolid, while most of them were resistant to erythromycin, gentamicin, ciprofloxacin, tetracycline and sulfamethoxazole-trimethoprim, similar to the study by Mallick et al [18]. In our study, *S. aureus* was commonly isolated from hospitalized patients with surgical site infections and orthopedic patients with fracture and bone infection. Similar observations have been reported elsewhere that *S. aureus* is the cause of most wound infections among hospitalized patients [25].

In conclusion, resistance to antimicrobials such as macrolides might not be readily apparent by routine testing. The D-test is easy to perform and inexpensive for practical work. We feel that this test should be made mandatory as a routine work in clinical microbiology laboratories. Therapeutic failures can be prevented if clindamycin is not used for treatment of patients with infections caused by staphylococci with inducible clindamycin resistance.

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