Research Article

Inducible clindamycin resistance in Staphylococcus aureus isolatedfrom clinical samples

K. Vidyasagar[1*], R. Ravikumar[2]

[1]Assistant Professor, Department of Microbiology, Adichunchanagiri Institute of Medical Sciences BG Nagar, Taluka Nagamangala, Dist Mandya-PIN:571448, Karnataka, INDIA.
[2]Professor and HOD, Department of Neuromicrobiology, NIMHANS, Hosur Road, Bangalore-560029 Karnataka, INDIA.
Email: drsagar81@gmail.com

Abstract

Purpose: Clindamycin is commonly used in the treatment of erythromycin resistant *Staphylococcus aureus* causing skin and soft tissue infections. *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 D test on routine basis. **Materials and Method:** 100 *Staphylococcus aureus* isolates from various clinical samples subjected to routine antibiotic susceptibility testing by Kirby Bauer disc diffusion method. Inducible clindamycin resistance was detected by D test, as per CLSI guidelines on erythromycin resistant isolates. **Results:** 42 (42%) isolates showed inducible clindamycin resistance, 13 (13%) showed constitutive resistance while 10(10%) showed MS phenotype. Inducible resistance was found to be higher in Methicillin resistance Staphylococcus aureus (MRSA) as compared to Methicillin sensitive Staphylococcus aureus (MSSA) (56.6%, 13.2% and 25.53%, 12.7% respectively) **Conclusion:** Study showed that D test should be used as a mandatory method in routine disc diffusion testing to detect inducible clindamycin resistance.

Keywords: D-test, macrolide résistance, clindamycin resistance

[*]**Address for Correspondence**
Dr. K. Vidyasagar, Assistant Professor, Department of Microbiology, Adichunchanagiri Institute of Medical Sciences BG Nagar, Taluka Nagamangala, Dist Mandya-PIN:571448, Karnataka, INDIA.
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INTRODUCTION

Clindamycin has been used to treat serious infections caused by susceptible *Staphylococcus aureus* strains [1]. It remains effective for many infections caused by community-acquired Methicillin-resistant *S. aureus* (CAMRSA) [2]. Clindamycin resistance is common among health care-associated MRSA strains[1, 2]. Macrolide-inducible resistance to clindamycin was first recognized in the laboratory in the early 1960s. Clinical isolates resistant to clindamycin were first recognized in 1968[3]. Clindamycin is considered an useful alternate drug in penicillin-allergic patients in the treatment of skin and soft tissue infections caused by *Staphylococcus aureus*. It has excellent tissue penetration (except for the central nervous system), accumulates in abscesses, and no dosage adjustments are required in the presence of renal disease[1]. The good oral absorption of clindamycin makes it an attractive option for use in outpatients or as follow-up treatment after intravenous, therapy. Emergence of methicillin resistance in *Staphylococcus aureus* has left us with very few therapeutic alternatives 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 due its excellent pharmacokinetic properties.[4] However, widespread use of MLS_B antibiotics has led to an increase in number of staphylococcal strains acquiring resistance to MLS-

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$_B$ antibiotics[5]. The most common mechanism for such resistance is target site modification mediated by *erm* genes which can be expressed either constitutively (constitutive MLS $_B$ phenotype) or inducibely (inducible MLS $_B$ phenotype). Strains with inducible resistance to clindamycin are difficult to detect in the routine laboratory as they appear erythromycin resistant and clindamycin sensitive *in vitro* when not placed adjacent to each other. In such cases, *in vivo* therapy with clindamycin may select constitutive *erm* mutants leading to clinical therapeutic failure. In case of another mechanism of resistance mediated through *msrA* genes i.e. efflux of antibiotic, Staphylococcal isolates appear erythromycin resistant and clindamycin sensitive both *in vivo* and *in vitro* and the strain does not typically become clindamycin resistant during therapy.[6] This study demonstrates a very simple method of detecting inducible resistance to clindamycin in erythromycin resistant staphylococcal isolates. I.e. D test as described by Fiebelkorn *et al*.[4]

MATERIAL AND METHODS

The study was conducted from Jan 2013 to Jun 2013. A total of 100 isolates of *Staphylococcus aureus* isolated from various clinical specimens like pus, wound swab, tracheal secretions ,bone flaps, blood, External ventricular drainage shunt tips, Extradural pus, pus from brain abscess, cystic fluid, sub galeal collection, sub cutaneous collection, epidural pus, shunt CSF and Ventricular peritoneal shunt tip were tested [table 1]. The isolates were first identified as *S. aureus* by standard biochemical techniques[7] and then subjected to susceptibility testing by Kirby Bauer's disc diffusion method on Mueller Hinton agar plates using erythromycin (15µg), asperCLSI, guidelines.[8] Those isolates which were erythromycin resistant were further subjected to 'D test' as per CLSI guidelines. Briefly, erythromycin (15 µg) disc was placed at a distance of 15mm (edge to edge) from clindamycin (2 µg) disc on a Mueller Hinton agar plate previously inoculated with 0.5 McFarland bacterial suspension. Following overnight incubation at 37 ^{0}C, flattening of zone (D shaped) around clindamycin in the area between the two discs, indicated inducible clindamycin resistance

Three different phenotypes were observed after testing and interpreted as follows:

1. Constitutive MLSB Phenotype - Staphylococcal isolates which showed resistance to both erythromycin (zone size ≤13mm) and clindamycin (zone size ≤14mm) with circular shape of zone of inhibition if any around clindamycin [Figure1]
2. Inducible MLS $_B$ Phenotype - Staphylococcal isolates showing resistance to erythromycin (zone size ≤13mm) while being sensitive to clindamycin (zone size ≥21mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc were labeled as having this phenotype (Figure 2)

MS Phenotype - Staphylococcal isolates which showed resistance to erythromycin (zone size ≤13mm) while sensitive to clindamycin (zone size ≥21mm) and giving circular zone of inhibition around clindamycin was labelled as having this phenotype (figure 3)

Table 1: Source and categorization of clinical isolates

Specimen	MSSA	MRSA	Total
Pus	28	27	55
Tracheal secretions	5	12	17
Bone flaps	3	6	9
Blood	3	2	5
External ventricular drainage shunt tips	3	1	4
Extradural pus	1	2	3
Pus from brain abscess	1	0	1
Cystic fluid	0	1	1
Sub galeal collection,	1	0	1
Sub cutaneous collection	0	1	1
Epidural pus	1	0	1
Shunt CSF	1	0	1
Ventricular peritoneal shunt tip	0	1	1
Total	**47**	**53**	**100**

Table 2: Distribution of isolates

	Susceptibility pattern(phenotype)	MSSA	MRSA	Total
E- S,	CD – S	23	12	35(35%)
E- R	CD-R (Constitute MLS$_B$)	6	7	13(13%)
E-R	CD-S D test positive(Inducible MLS$_B$)	12	30	42(42%)
E-R	CD-S D test negative(MS phenotype)	6	4	10(10%)
	Total	**47**	**53**	**100**

E- Erythromycin, D-Clindamycin, S-sensitive, R-resistance

Figure 1: Constitutive MLSB Phenotype

Figure 2: Inducible MLS B Phenotype

Figure 3: MS Phenotype

RESULTS

Out of 100 *Staphylococcus aureus* isolates, 53 isolates (53%) were MRSA and 47 (47%) were MSSA. All isolates tested for susceptibility to erythromycin by routine disc diffusion testing; 65 (65%) of them were erythromycin resistant. These isolates when subjected to D test showed 13 (13%) isolates resistant to both erythromycin and clindamycin indicating constitutive MLS_B Phenotype; 52 (52%) isolates showed clindamycin sensitivity. Out of these, 42 (42%) isolates showed positive D test indicating inducible MLS_B phenotype while 10(10%) isolates showed negative D test indicating MS phenotype [Table 2].

The overall percentage resistance for all three phenotypes was as follows.

1. constitutive clindamycin resistance - 13%
2. Inducible clindamycin resistance - 42%
3. MS Phenotype - 10%

Percentage of both inducible and constitutive resistance was higher amongst MRSA isolates as compared to MSSA (56.6%, 13.2% and 25.53%, 12.7% respectively) [Table 2].

DISCUSSION

The determination of antimicrobial susceptibility of a clinical isolate is often crucial for optimal antimicrobial therapy of infected patients. This is particularly important considering the increase of resistance and the emergence of multidrug resistant organisms. There are many options available for treatment of MSSA and MRSA infections, with clindamycin being one of the good alternatives.[4] However, clindamycin resistance can develop in staphylococcal isolates with inducible phenotype, and from such isolates, spontaneous constitutively resistant mutants have arisen both *in vitro* testing and *in vivo* during clindamycin therapy.[9]

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 option[9]. In the present study we found high percentage of erythromycin resistant isolates [65(65%)]. Amongst them 42 (42%) isolates tested positive for inducible clindamycin resistance by D test while rest of the isolates were negative for D test, out of which 13(13%) were shown to have constitutive clindamycin resistance and 10 (10%) showed true sensitivity to clindamycin (MS phenotype). These observations suggest that had D test not been performed, nearly half of the erythromycin resistant isolates would have been misidentified as clindamycin sensitive resulting in therapeutic failure. It was also observed that percentages of inducible resistance and constitute resistance were higher amongst MRSA (56.6% and 13.2% respectively) as compared to MSSA (25.53% and 12.7% respectively).

This was in comparison with a few of the studies reported before - Yilmaz *et al*[9] found inducible resistance of 24.4% in MRSA and 14.8% in MSSA; Gadepalli *et al.*[5] showed it to be 30% in MRSA and 10% in MSSA, while Mohamed Rahabar *et al*[10] reported 22.6% in MRSA and 4% in MSSA. Another study from India[11] showed very high frequency of inducible resistance (63%) in erythromycin -R, clindamycin sensitive isolates being 74% in MRSA and 45% in MSSA. In the light of the restricted range of antibiotics available for the treatment of methicillin-resistant staphylococcal infections and the known limitations of vancomycin, clindamycin should be considered for the management of serious soft tissue infections with methicillin-resistant staphylococci that are sensitive to clindamycin.[12] The true sensitivity to clindamycin can only be judged after performing D test on the erythromycin resistant isolates. The prevalence of inducible clindamycin resistance may vary from hospital to hospital. In the present study, there is a fairly high percentage of inducible clindamycin resistance amongst the staphylococcal isolates which shows erythromycin resistance.

CONCLUSION

Study showed that D test should be used as a mandatory method in routine disc diffusion testing to detect inducible clindamycin resistance. Use of D test in a routine laboratory will help 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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