

Original Research Article

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D-Test - A Useful Tool in Detection of Clindamycin Resistance among Staphylococcal Isolates in a Tertiary Care Hospital

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ABSTRACT

Keywords

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To demonstrate inducible clindamycin resistance among the staphylococcal isolates from various clinical samples by D- test. A total of 125 *Staphylococcus aureus* and *Coagulase Negative Staphylococcus* (CONS) isolates were collected from clinical samples. All the samples were tested for inducible clindamycin resistance by using the D -test as per CLSI guidelines. In this study, 11% inducible clindamycin resistant staphylococcal isolates (iMLSb), and 9% of Constitutive resistance (cMLSb) and 46% MS phenotype were detected and 34% were susceptible to both erythromycin and clindamycin (S phenotype) among the total isolates. The inducible clindamycin resistance in this study was 11%. The D-test is a very convenient test to perform. It can be done along with our routine antibiotic susceptibility testing in all the staphylococcal isolates. Accurate clindamycin resistance reporting is mandatory for a successful treatment outcome.

Introduction

Staphylococcus aureus and *Coagulase-Negative Staphylococci* (CONS) are recognized as important agents responsible for nosocomial and community acquired infections. In this era of super bugs, increasing antibiotic resistance is of more concern and selection of antibiotics to treat these infections has become more important. Rate of Methicillin Resistant *Staphylococcus aureus* (MRSA) has dramatically increased in recent years.^{1,2} This has led to renewed interest in the use of macrolide –lincosamide –streptogramin (MLSb) antibiotics.

Clindamycin is preferred for treating these infections because of its low cost, fewer severe side effects, availability of oral and parenteral forms, lack of need for renal adjustments, good tissue penetration and ability to directly inhibit toxin production. Clindamycin, furthermore, is a useful choice in cases of penicillin allergy.³

Macrolides, Lincosamides and Streptogramin (MLS) antibiotics are structurally unrelated; however, they are related microbiologically because of their similar mode of action. MLS antibiotics inhibit bacterial protein synthesis by binding to 23s rRNA (ribosomal RNA), which is a part of the large ribosomal subunit.

They have a spectrum of activity directed against Gram-positive cocci and intra-cellular bacteria such as chlamydiae and rickettsiae⁴

Resistance to MLS antibiotics occur either through target site modification, efflux of antibiotics, or drug modification.⁵ In target-site modification, methylation of the A2058 residue, located in the conserved domain V of 23s rRNA, takes place, which leads to cross resistance and results in the formation of the phenotype of resistance pattern known as MLS_B. This phenotype is encoded by erythromycin ribosome methylases (*erm*) genes that have been reported from a wide variety of microorganisms.⁶

The expression of the MLS_B phenotype can be constitutive (MLS_B c) or inducible (MLS_B i). Inducible resistance is observed when the inactive mRNA produced by the production of methylases becomes active in the presence of an inducer, while active methylase mRNA is produced in strains where constitutive expression is seen.⁵ The strains carrying the inducible *erm* gene are resistant to the inducer and remain susceptible to non inducer macrolides and lincosamides. Low levels of erythromycin is an inducer of the MLS_B i phenotype, which forms the basis of the D-test⁷. A second mechanism of resistance is due to active efflux pump encoded by *msrA* gene. They are the MS phenotype⁴ Strains with inducible clindamycin resistance are not detectable by the routine antimicrobial susceptibility tests. They appear to be erythromycin resistant and clindamycin susceptible *in vitro*. In these cases management with clindamycin will not be effective. To overcome this, the Clinical and Laboratory Standards Institute (CLSI) has recommended the erythromycin clindamycin disc approximation test (D – test)⁸. This study was done to find out the prevalence of erythromycin induced clindamycin resistance in *Staphylococcus aureus* and *coagulase*

negative Staphylococcus (CONS) isolates using this valuable, simple, reliable D-test.

Materials and Methods

This cross-sectional study was conducted in a tertiary care hospital in Tirunelveli after obtaining ethical committee approval. A total of 125 isolates of staphylococcus species from various clinical samples like pus, sputum, urine, blood, and body fluids were tested. The specimens were cultured on Nutrient agar, Blood agar and MacConkey agar plates and incubated aerobically at 37⁰C for 24 hours. The isolates were identified by using conventional laboratory methods- colony morphology, gram staining, catalase, coagulase test and other standard biochemical tests.¹⁰ Antibiotic sensitivity testing was performed by Kirby – Bauer disc diffusion method on Mueller - Hinton agar plates using the following antibiotics: Ciprofloxacin (5µgm), clindamycin (2 µgm), gentamicin (10µgm), erythromycin (15µgm), vancomycin (30µgm), cefoxitin (30µg), Doxycycline (30µgm), Co-Trimoxazole (1.25/23.75 µgm). Results were interpreted according to the CLSI guidelines.¹¹ Antibiotic discs were procured from HiMedia India, Private Limited. *Staphylococcus aureus* ATCC 25923 was used for quality control¹². Cefoxitin disc was used as a surrogate marker for detecting methicillin resistance.

D- Test

The susceptibility testing was performed among the isolates by Kirby –Bauer disc diffusion method on Mueller- Hinton agar plates inoculated with 0.5 Mac Farlands standard bacterial suspension. Erythromycin (15µg) disc was placed at a distance of 15mm (edge to edge separation) from Clindamycin (2µg) disc on the same plate and were incubated at 37⁰C overnight⁵ (Figure 1).

Interpretation of D-test

Four different phenotypes were interpreted as follows.

Inducible iMLS_B phenotype

Isolates showing resistance to erythromycin (zone size ≤ 13 mm) and sensitive to clindamycin (zone size ≥ 21 mm) with a D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc.-D-test positive. Same inhibitory diameter as above with no D shaped zone-D-test negative.

Four different phenotypes were interpreted as follows.

- 1. Inducible iMLS_B phenotype** – Isolates showing resistance to erythromycin (zone size ≤ 13 mm) and sensitive to clindamycin (zone size ≥ 21 mm) with a D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc.-D-test positive. Same inhibitory diameter as above with no D shaped zone-D-test negative.
- 2. Constitutive cMLS_B phenotype** – Isolates showing resistance to both erythromycin (zone size ≤ 13 mm) and clindamycin (zone size ≤ 14 mm) with circular shape of zone of inhibition around clindamycin.
- 3. MS phenotype** – Isolates showing resistance to erythromycin (zone size ≤ 13 mm) while being sensitive to clindamycin (zone size ≥ 21 mm) with a circular zone of inhibition around clindamycin.
- 4. S phenotype** – Isolates showing sensitivity to both erythromycin zone size ≥ 23 mm and clindamycin ≥ 21 mm.¹³

Results and Discussion

Among the 125 isolates studied, 74 (59%) were *Staphylococcus aureus* and 51 (41%) were COagulase Negative Staphylococci (CONS). Of the 74 *Staphylococcus aureus* strains, 51 (69%) were Methicillin Resistant *Staphylococcus aureus* (MRSA) and 23 (31%) were Methicillin Sensitive *Staphylococcus aureus* (MSSA). Out of 51 CONS, 46 (90%) were Methicillin Resistant Coagulase Negative Staphylococci (MRCONS) and 5 (10%) were Methicillin Sensitive COagulase Negative Staphylococci (MSCONS) (Table 1).

In the present study, out of the total 125 staphylococcal isolates 14 (11%) inducible clindamycin resistant strains was detected. *Staphylococcus aureus* isolates were 74 among them MRSA isolates were 51 and MSSA isolates were 23. Among the 51 MRSA isolates 8 (16%) were iMLSB, 6 (12%) were cMLSB, 22 (43%) MS phenotype and 15 (29%) S phenotype. Among the 23 (18%) MSSA isolates 1 (5%) was iMLSB, there was no cMLSB isolate detected, 10 (44%) MS phenotype and 12 (51%) S phenotype.

Out of the total 51 CONS isolates 46 (37%) were MRCONS and 5 (4%) MSCONS. Among the 46 (37%) MRCONS isolates 5 (11%) were iMLSB, 5 (11%) were cMLSB, 23 (50%) MS phenotype and 13 (28%) S phenotype. Among the 5 (4%) MSCONS isolates there was no cMLSB and iMLSB, isolate detected, 2 (40%) was MS phenotype and 3(60%) S phenotype. In this study out of the total 125 staphylococcal were 14 (11%) iMLSB, 11 (9%) were cMLSB, 57 (46%) MS phenotype and 43 (34%) S Phenotype (Table 2).

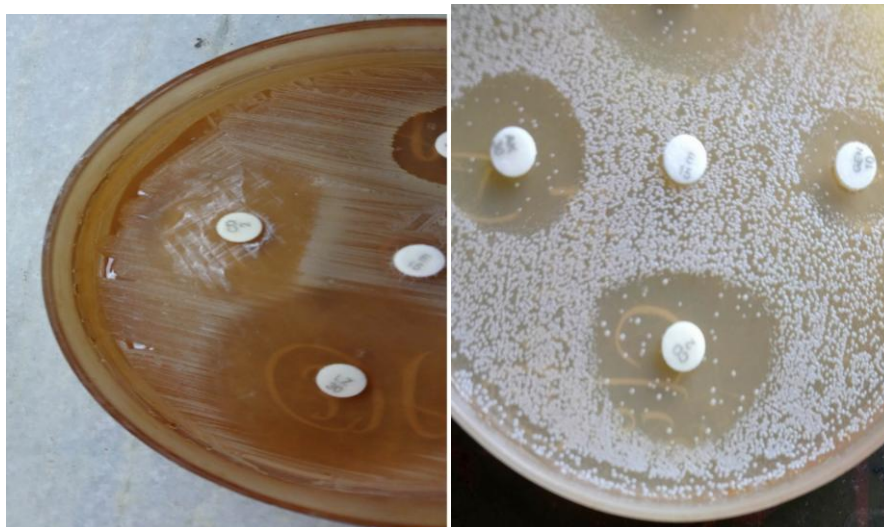
Table.1 Distribution of methicillin sensitive and resistant *Staphylococcus aureus* and cons

<i>Staphylococcus aureus</i> (n=74)		CONS (n=51)	
MRSA	51(69%)	MRCONS	5 (10%)
MSSA	23(31%)	MSCONS	46(90%)
Total n=125	74 (100%)		51(100%)

Table.2 Distribution of phenotypes among methicillin sensitive and resistant *Staphylococcus aureus* and cons

Organism	Total Isolates	iMLSb	cMLSb	MS Phenotype	S Phenotype
MRSA	51(41%)	8(16%)	6(12%)	22(43%)	15(29%)
MSSA	23(18%)	1(5%)	-----	10(44%)	12(51%)
MRCONS	46(37%)	5(11%)	5(11%)	23(50%)	13(28%)
MSCONS	5(4%)	-----	-----	2(40%)	3(60%)
TOTAL	125	14(11%)	11(9%)	57(46%)	43(34%)

Fig.1



The highlight among staphylococcal infections is their emerging multidrug resistant patterns. Most of them are Methicillin Resistant *Staphylococcus aureus* isolates. Treatment of these infections is a difficult task for the clinicians, unless detected appropriately. Clindamycin can be the drug of choice in these situations. Cross

resistance among the MLSb group is another challenging factor. A precise antibiotic susceptibility testing is of utmost importance in treating patients successfully with clindamycin. The D-test is a convenient test to detect inducible clindamycin resistance along with our routine antibiotic susceptibility testing and helps to overcome

misinterpretation and treatment failures. It serves as a useful tool for MLSb phenotyping also. The prevalence of inducible clindamycin (iMLSb) resistance varies from area to area, and also from hospital to hospital. In the present study 11 % (of the total isolates) were iMLSb isolates. Inducible resistance pattern in this study is in concordance with that reported by Sureerat Chelae *et al.*,¹³ with 9.9%. and Fiebelkorn *et al.*,¹⁴ had 10% of iMLSb phenotype and Ciraj AM *et al.*,¹⁵ from Karnataka report 13.1% of iMLSb. A north Indian study showed higher incidence of inducible resistance (69%) compared to the present study.¹⁶ The low constitutive clindamycin resistance in this study is in range with the study of Deotale *et al.*,¹⁷ It may be due to the minimal usage of clindamycin in our hospital.

In conclusion, this study will help in documentation and understanding of inducible clindamycin resistance in a tertiary care center. This is not only important in the clinicians point of view but also for epidemiological purposes. Hence this simple, easy to perform D-test is recommended routinely for the detection of iMLSb isolates. Blind prescription of clindamycin without antibiotic susceptibility testing can lead on to treatment failures. Therefore in any hospital set up, regular surveillance of resistance pattern will be useful in formulating institutional antibiotic policies and for a better antibiotic approach.

Abbreviation

MRSA: Methicillin Resistant *Staphylococcus aureus*; MSSA: Methicillin Sensitive *Staphylococcus aureus*; MRCONS: Methicillin Resistant COagulase Negative Staphylococci; MSCONS: Methicillin Sensitive Coagulase Negative Staphylococci; iMLSb: Inducible phenotype; cMLSb: Constitutive phenotype; MSphenotype:

Erythromycin resistant Clindamycin sensitive; S phenotype: Erythromycin and Clindamycin sensitive

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