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Original Research Article

Inducible Clindamycin resistance among clinical isolates of *Staphylococcus aureus* at a Rural tertiary care teaching hospital of western Uttarpradesh

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ABSTRACT

Background: *Staphylococcus aureus* a notorious pathogen is rapidly acquiring resistance against most of the major group of antibiotics including Clindamycin. Inducible Clindamycin resistance is difficult to detect and is often missed in routine antibiotic susceptibility testing. Prompt and timely detection of such resistance in the clinical isolates of *S.aureus* is imperative to formulate appropriate strategy for the effective treatment of such infections.

Aims & Objectives: To determine the percentage of inducible Clindamycin resistance among clinical isolates of S.aureus in our geographical area using D-test and to distinguish different susceptibility patterns/resistance phenotypes in Erythromycin resistant clinical isolates of S.aureus.

Materials and Methods: A total of 244 consecutive non-duplicate isolates of *S.aureus* recovered from variousclinical specimens(Urine,pus,wound swab,blood,body fluids,aspirates etc.) were subjected to Antibiotic Susceptibility Testing by Standard disc diffusion method and Methicillin resistance testing using Cefoxitin disc(30¼gm). Erythromycin resistant *S.aureus* isolates were subjected to D-test as per CLSI 2014 guidelines.

Result: Out of 244 clinical isolates of *S.aureus*, 49(20%) were found to be MRSA and 195(80%) MSSA strains.74(30.33%) were Erythromycin resistant and were subjected to D-test.16 isolates(6.56%) exhibited resistance against both Erythromycin and Clindamycin indicative of Constitutive $cMLS_B$ phenotype, 21(8.6%) were found to be D-test positive for inducible resistance($iMLS_B$ phenotype) while 39(15.98%) were D-test negative and hence truly susceptible to Clindamycin(MS phenotype).

Conclusion: The observations of this study had clearly revealed that if D-test would've not been performed then a significant proportion of Erythromycin resistant isolates would have been reported wrongly as Clindamycin sensitive and this could have resulted in the treatment failure.So,D-test must be incorporated in routine Antibiotic susceptibility testing for *S.aureus* isolates so as to ensure the judicious and rational use of this valuable drug.

KEY WORDS: D-Test, Methicillin Resistant *Staphylococcus aureus* (MRSA), Methicillin Sensitive *Staphylococcus aureus* (MSSA), Constitutive Macrolide Lincosamide Streptogramin B Phenotype ($cMLS_B$), Inducible Macrolide Lincosamide Streptogramin B Phenotype ($iMLS_B$), MS phenotype.

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BACKGROUND

Staphylococcus aureus has been recognised throughout the world as one of the most common pathogen causing a wide range of nosocomial as well as community acquired infections [1]. But such infections are now rapidly emerging as a grave threat to the public health as the

pathogen is rapidly acquiring resistance against most of the major group of antibiotics being rendered refractory to therapy leading to treatment failure. Clinicians are struggling hard to treat such infections with very few alternatives left in their arsenal.

One such alternative left which is quiet effective and preferred by clinicians in treating such infections is Macrolide-Lincosamide-Streptogramin (MLSB) group of antibiotics. In this group a lincosamide Clindamycin has emerged as the most popular and most frequently used option due to its excellent pharmacokinetic properties, 100% bioavailability on oral administration, good tissue penetration, tendency to accumulate in abscesses, tolerability and low cost [1-4].

However it has been observed that resistance towards Clindamycin can develop in S.aureus strains. This resistance is mainly attributed to the erm gene mediated target site modifications which can be either Constitutive or Inducible [3-6].

Inducible Clindamycin resistance in iMLSB phenotypes is not easy to detect and is often missed as the isolates appear Clindamycin sensitive but erythromycin resistant in vitro if their discs are not placed adjacent to each other in Standard disc diffusion test[1].

Treatment of such cases with Clindamycin leads to selection of Constitutive erm mutants (cMLSB phenotypes) further worsening the situation [1-6].

But there is another mechanism of resistance towards this group mediated via msrA genes through efflux of antibiotics which presents apparently a similar picture where isolates appear Clindamycin sensitive but erythromycin resistance not only in vitro but also in vivo(MS phenotypes) and respond well to Clindamycin therapy [1,2].

Prompt and timely detection of inducible Clindamycin resistance in the clinical isolates of S.aureus is becoming increasingly important to formulate appropriate strategy for the effective treatment of such infections.

In the light of above facts we had undertaken this study to determine the percentage of inducible Clindamycin resistance among clinical isolates of *S.aureus* in our geographical area using D-test.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Dept.of Microbiology of a rural tertiary care teaching hospital of western Uttarpradesh from Jan.2016 to Dec.2016 for a duration of one year.

A total of 244 consecutive non-duplicate isolates of S.aureus recovered from various clinical specimens(Urine, pus,wound swab,blood,body fluids, aspirates etc.) sent to Bacteriology Lab. for routine culture sensitivity as a part of standard diagnostic protocol. All the samples were processed using Standard microbiological techniques [7].

Antibiotic Susceptibility Test [8]: In vitro antibiotic sensitivity test for all S.aureus isolates was conducted by Kirby Bauer Standard Disc diffusion method on MHA plates as per CLSI-2014 guidelines using following antibiotic discs:

1)Amoxycillin, 2)Erythromycin, 3)Clindamycin

4)Cefoxitin, 5)Doxycyclin, 6)Gentamycin, 7)Ciprofloxacin/Norfloxacin, 8)Cotrimoxazole

Methicillin Resistance Test: Test for mecA mediated Oxacillin resistance [8]: Those S.aureus isolates which exhibited resistance to Cefoxitin disc(30µg) with Zone of inhibition diameter $\leq$ 21mm by Standard disc diffusion method as per CLSI guidelines were reported as MRSA strains.

D-Zone Test for screening Inducible Clindamycin resistance in *S.aureus* isolates[1,2,3,6,8,9,10]: All the clinical isolates of S.aureus which were found to be resistant to Erythromycin(15 µg) by Standard disc diffusion method were further tested for inducible Clindamycin resistance by D-Zone test as per CLSI-2014 guidelines.

0.5 McFarland standard suspension of test strains inoculated on prewarmed MHA plates. Erythromycin(15 µg) and Clindamycin(2 µg) discs were placed over the seeded plate spaced 24mm.apart edge to edge. After overnight incubation at 37^0C for 18 hrs.

Interpretation of D-Test was done as follows:

1) Inducible iMLS$_B$ phenotype (D-test positive): Test strains of *S.aureus* exhibiting resistance against Erythromycin(15 µg) with zone size ≤ 13mm. but being apparently sensitive to Clindamycin with zone size ≥ 21mm. and demonstrating D shaped inhibition zone around Clindamycin disc with the flattening towards Erythromycin disc were considered to be D-test positive for inducible resistance(iMLS$_B$ phenotype).

2) MS phenotype(D-test Negative): Test strains of *S.aureus* showing resistance towards Erythromycin with zone size ≤ 13mm but sensitive to Clindamycin with zone size ≥ 21mm, zones of inhibition being circular were considered to be D-test negative for inducible resistance(MS phenotype).

3) Constitutive cMLS$_B$ phenotype: Test strains of *S.aureus* exhibiting resistance towards both Erythromycin with zone size ≤ 13mm and Clindamycin with zone size ≤ 14mm with a circular zone of inhibition if any around Clindamycin.

Quality control of antibiotic discs was performed with *S.aureus* ATCC 25923 as per CLSI guidelines.

RESULTS AND DISCUSSION

Out of 244 clinical isolates of *S.aureus*, 49(20%) were found to be MRSA and 195(80%) were MSSA strains.

Out of all 244 isolates 74(30.33%) were Erythromycin resistant and were subjected to D-test.16 isolates(6.56%) exhibited resistance against both Erythromycin and Clindamycin indicative of Constitutive cMLS$_B$ phenotype.

Out of the 57 isolates which appeared to be sensitive to Clindamycin, 21(8.6%) were found to be D-test positive for inducible resistance (iMLS$_B$ phenotype) while 39(15.98%) were D-test negative and hence truly susceptible to Clindamycin(MS phenotype).

Amongst MRSA strains Constitutive cMLS$_B$ phenotypes were 8.16% and Inducible iMLS$_B$ phenotypes came out to be 12.2% while amongst MSSA strains cMLS$_B$ phenotypes were 6.15% and iMLS$_B$ phenotypes came out to be 7.69% .

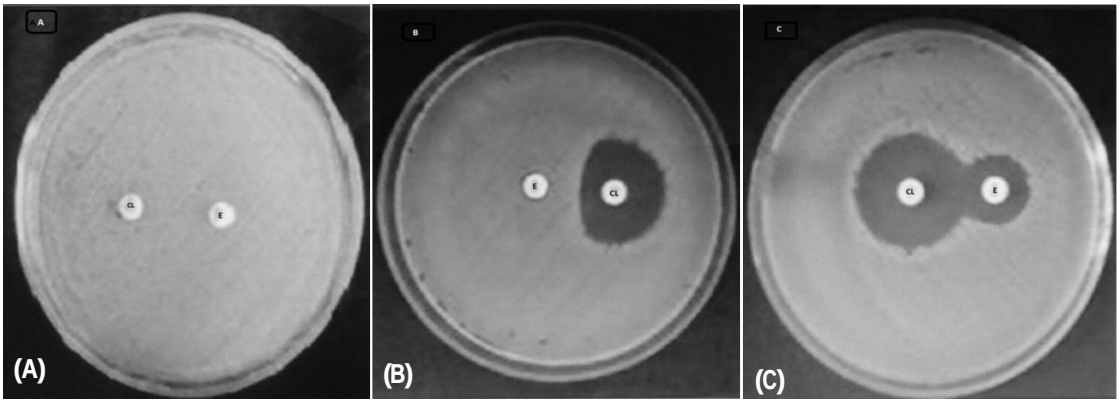
Erythromycin and Clindamycin Susceptibility patterns among S.aureus isolates and interpretation of D-test is depicted in Table-1 and Fig-1 respectively.

In this study we had found a significantly high percentage of Erythromycin resistant isolates(30.33%) and amongst them 21(28.38%) were D-test positive for inducible Clindamycin resistance.

The observations of this study had clearly revealed that if D-test would've not been performed then a significant proportion of Erythromycin resistant isolates would have been reported wrongly as Clindamycin sensitive and this could have resulted in the treatment failure.

It has also been found in this study that the percentages of inducible and constitutive Clindamycin resistance were higher among MRSA strains(12.2% & 8.16%) as compared to MSSA strains(7.69% & 6.15%). This finding is in accordance with a number of other studies [1,4,12-14] (Yilmaz et al, Gadepalli et al, Mohammad Rahabar et al, Ajantha et al, Prabhu K et al.).

Fig. 1: Interpretation of D-zone test for inducible Clindamycin resistance.



(A) Constitutive cMLSB phenotype, (B) Inducible iMLSB phenotype, (C) MS phenotype

Table 1: Erythromycin and Clindamycin Susceptibility patterns among S.aureus isolates.

Susceptibility pattern	D-Test	Phenotype	No. of isolates (%)		
			MRSA (n=49)	MSSA (n=195)	Total (n=244)
Ery-S Clind-S		Susceptible	32(65.3%)	136(69.74%)	168(68.85%)
Ery-R Clind-R	Negative	$cMLS_B$	4(8.16%)	12(6.15%)	16(6.56%)
Ery-R Clind-S	Positive	$iMLS_B$	6(12.2%)	15(7.69%)	21(8.6%)
Ery-R Clind-S	Negative	MS	7(14.3%)	32(16.41%)	39(15.98%)

Ery-Erythromycin, **Clind**- Clindamycin, **S**- Susceptible, **R**-Resistant, **D-Test**: Disc Approximation Test, **MRSA**- Methicillin Resistant Staphylococcus aureus, **MSSA**- Methicillin Sensitive Staphylococcus aureus, **$cMLS_B$**- Constitutive Macrolide Lincosamide Streptogramin B Phenotype, **$iMLS_B$**- Inducible Macrolide Lincosamide Streptogramin B Phenotype.

CONCLUSION

As a routine diagnostic practice,we go on reporting *S.aureus* isolates as susceptible to Clindamycin on the basis of Standard disc diffusion method without incorporating any test to check for the inducible Clindamycin resistance and such inappropriate reporting may miss a significant proportion of resistant cases resulting in irrational Clindamycin therapy of such infections leading to treatment failure.We can avoid this simply by incorporating a test like D-test which is very simple, easy and cost effective for detecting inducible Clindamycin resistance amongst Staphylococcal isolates.

We therefore strongly recommend that each and every Microbiology Laboratory should include D-test in routine antibiotic susceptibility testing for S.aureus which will enable the judicious and rational use of Clindamycin.

ABBREVIATIONS

D-Test: Disc Approximation Test
MRSA- Methicillin Resistant Staphylococcus aureus
MSSA-Methicillin Sensitive Staphylococcus aureus
$cMLS_B$ - Constitutive Macrolide Lincosamide Streptogramin B Phenotype
$iMLS_B$- Inducible Macrolide Lincosamide Streptogramin B Phenotype
ATCC- American Type Culture Collection
CLSI- Clinical and Laboratory Standards Institute

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