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Original Research Article

Prevalence of inducible clindamycin resistance among Staphylococcus aureus isolates from a tertiary care hospital

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ABSTRACT

Introduction: Clindamycin is an excellent drug for skin and soft tissue Staphylococcus aureus infections, but resistance mediated by inducible macrolide-lincosamide-streptogramin B (iMLS$_B$)phenotype leads to in vivo therapeutic failure even though they may be in vitro susceptible in Kirby–Bauer disk diffusion method (KBDDM). Hence the study was undertaken to detect the prevalence of iMLS$_B$ phenotype among Staphylococcus aureus isolates by double disk approximation test (D-test) in a tertiary care hospital.
Materials and Methods: A total of 100 consecutive Staphylococcus species isolates were identified by standard microbiological methods and subjected to antimicrobial susceptibility testing by KBDDM. Clindamycin-resistance either in the form of iMLSB or cMLSB was determined through double disk diffusion method or D-test by using erythromycin (2 μg) and clindamycin (15μg) as per the CLSI guidelines.
Results: Out of 100 Staphylococcus species studied, 50(50%) were methicillin sensitive Staphylococcus aureus, 30(30%) were Methicillin resistant Staphylococcus aureus and 20 (20%) were Coagulase negative Staphylococci. Out of 80 Staphylococcus aureus studied, iMLSB, cMLSB and MS phenotype were 32.5%, 1.25%, 5% respectively. Inducible resistance and MS phenotype were found to be higher in MRSA as compared to MSSA (60%, 6.66% and 16%, 4% respectively).
Conclusion: The study revealed 32.5% of Staphylococcus aureus isolates were inducible clindamycin resistant, which could be easily misidentified as clindamycin susceptible in Kirby–Bauer disk diffusion method. Therefore, clinical microbiology laboratory should routinely perform D-test in all clinically isolated Staphylococcus aureus to guide clinicians for the appropriate use of clindamycin.

1. Introduction

Macrolides, lincosamides and streptogramin (MLS) antibiotics are structurally unrelated although related microbiologically because of their similar mode of action. These antibiotics serves as one such alternatives for treatment of staphylococcal infections especially of MRSA. Clindamycin being the preferred agent due its excellent pharmacokinetic properties.[1]

However, widespread use of MLS $_B$ antibiotics has led to an increase in number of staphylococcal strains acquiring resistance to MLS $_B$ antibiotics.[2] Staphylococcus spp. can be resistant to erythromycin through either erm or msr A genes. Strains with erm -mediated erythromycin resistance may possess inducible clindamycin resistance but may appear susceptible to clindamycin by the in vitro disc diffusion test, while Staphylococcus aureus isolates with constitutive resistance appear resistant to erythromycin and clindamycin.[3,4]

This study demonstrates a very simple method of detecting inducible resistance to clindamycin in erythromycin resistant staphylococcal isolates. i.e. D test which is mentioned in CLSI.[5]

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2. Material and Methods

This prospective study was carried out in the Department of Microbiology at a Tertiary Care Hospital, over one year. From among inpatients and outpatients those who attended the services a total of 1300 clinical specimens were tested. Those included pus, wound swabs, ear swab, conjunctival swab, blood culture, plural fluid and urine from patients. Staphylococcus isolates recovered from these samples were identified upto species level by conventional methods such as Gram stain, cultural characters, growth on mannitol salt agar, slide and tube coagulase test, DNAse test and other biochemical tests.[66] All Staphylococcus aureus isolates were subjected to antimicrobial susceptibility testing using Kirby–Bauer disk diffusion method on Mueller-Hinton agar (MHA) plates as per CLSI guidelines.

Methicillin resistance was determined by disk diffusion method using 30μg cefoxitin disks. The results were interpreted according to CLSI guidelines. Antimicrobial susceptibility to penicillin (10U), ampicillin (10μg), erythromycin (15μg), gentamicin (10μg), tetracycline (30μg), amoxicillin-clavulanic acid (30μg), clindamycin (2μg), cefazolin (30μg), linezolid (30μg), netilmycin (30μg) vancomycin (30μg) were tested.

Inducible resistance to clindamycin was tested by 'D test' as per CLSI guidelines.[CLSI][5] Briefly, erythromycin (15μg) disc was placed at a distance of 15 mm (edge to edge) from clindamycin (2μg) disc on a Mueller-Hinton agar plate, previously inoculated with 0.5 McFarland standard bacterial suspensions. Following overnight incubation at 37°C, flattening of zone (D-shaped) around clindamycin in the area between the two discs, indicated inducible clindamycin resistance. Three different phenotypes were appreciated after testing and then interpreted. This interpretation was done only for erythromycin-resistant Staphylococcus aureus strains.

2.1. MS phenotype

Staphylococcus aureus isolates exhibiting resistance to erythromycin (zone size $\leq$13 mm), while sensitive to clindamycin (zone size $\geq$21 mm) and giving circular zone of inhibition around clindamycin (D test negative).

2.2. Inducible MLS $_B$ phenotype

iMLS $_B$ Staphylococcus aureus isolates which showed resistance to erythromycin (zone size $\leq$13 mm) while being sensitive to clindamycin (zone size $\geq$21 mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc (D test positive).

2.3. Constitutive MLS $_B$ phenotype

cMLS $_B$ Staphylococcus aureus isolates which showed resistance to both erythromycin (zone size $\leq$13 mm) and clindamycin (zone size $\leq$14 mm) with circular shape zone of inhibition around clindamycin.

3. Results

In the present prospective study, a total of 100 isolates of Staphylococcus were studied. Out of 100 staphylococcus isolates, 30(30%) were methicillin resistant Staphylococcus aureus (MRSA) while 50(50%) were methicillin sensitive Staphylococcus aureus (MSSA). The remaining 20 (20%) were coagulase negative staphylococcus species (C0NS). In the present study, inducible clindamycin resistance i.e. positive D test was detected in 18(60%) MRSA isolates and 8(16%) isolates strains showing resistance to both clindamycin and erythromycin i.e. MSLB (cMLSB) resistance was detected to be 1(3.33%) in MRSA isolates and not in MSSA isolates.MS phenotypes was detected among two (6.66%) MRSA and two (4%) in MSSA isolates.(Table 1). The overall percentage resistance for all three phenotypes was as follows**:**

Inducible clindamycin resistance - 32.5%

Constitutive clindamycin resistance - 1.25%

MS Phenotype – 5%

Percentage of inducible resistance was higher amongst MRSA isolates (60%) as compared to MSSA.Table 1

4. 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.[1]

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.[7] 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. Since the iMLS$_B$ resistance mechanism is not recognized by using standard susceptibility test methods and its prevalence varies according to geographic location, D-test becomes an imperative part of routine antimicrobial susceptibility test for all clinical isolates of Staphylococcus aureus.[8] In this study 80 isolates of Staphylococcus aureus were studied over a period of one year. Erythromycin resistance was seen in 31(38.75%) isolates. Amongst them 26(83.87%) isolates tested positive for inducible clindamycin resistance by D test while rest of the isolates were negative for D

Table 1:

Susceptibility pattern (Phenotype)	MRSA (%) Total (30)	MSSA (%) Total (50)	Total
ERY-S,CL-S	9 (30%)	40(80%)	49 (61.25%)
ERY-R,CL-R (Constitutive MLSB)	1 (3.33%)	Nil	1 (1.25%)
ERY-R,CL-S, D test positive (Inducible MLSB	18(60%)	8 (16%)	26 (32.5%)
ERY-R,CL-S,Dtest negative (MS)	2(6.66%)	2 (4%)	4 (5%)
Total	30 (37.5%)	5 (62.5%)	80

(ERY-Erythromycin, CL-clindamycin, S-sensitive, R-resistant ,C MLS_B : Constitutive MLS_B phenotype, $iMLS_B$- Inducible MLS_B phenotype, MS-MS phenotype, MRSA-Methicillin resistant staphylococcus aureus, MSSA- Methicillin sensitive staphylococcus aureus.)

test. Out of these 1(3.22%) was shown to have constitutive clindamycin resistance in MRSA isolates only not in MSSA and 4 (12.90%) 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 MS phenotype were higher amongst MRSA (60% and 6.66% respectively) as compared to MSSA (16% and 4%).

In the present study, inducible clindamycin resistance was found to be 26 (32.5%) of this 18 (60%) were from MRSA and 8(16%) from MSSA. The study by Deotale et al[9] reported 43.3% in MRSA and 2.3% in MSSA, Gadepalli et al[2]reported 30% in MRSA and 10% in MSSA, Yilmaz et al[7]found inducible resistance of 24.4%in MRSA and 14.8% in MSSA. Whereas Ajantha et al[10] showed very high frequency of inducible resistance 74% in MRSA and 45% in MSSA. On the contrary, in another study Schreckenberger et al[11] and Levin et al[12] showed higher percentage of inducible resistance in MSSA as compared to MRSA,7-12% in MRSA and 19-20% in MSSA;12.5% MRSA and 68% MSSA respectively.

In our study constitutive resistance was observed in 1(3.33%) MRSA isolate. This was in concordance with one study reported before Deotale et al[9]reported (3.6%) in MRSA isolate. While Yilmaz et al[7]and Ciraj et al[13]here reported in (14.8%) and (15.3%) respectively. On the contrary, one study by Angel et al[14]which did not find it in any of the strains.

5. Conclusion

High prevalence of clindamycin resistance among both MRSA & MSSA isolates, especially inducible resistance, in our community shows that antimicrobial susceptibility test is essential when clindamycin is an option for therapy of Staphylococcus aureus infection. So, clinical microbiology laboratories should report inducible clindamycin resistance in Staphylococcus aureus. D-test can be used as a simple, auxiliary, and reliable method to delineate inducible and constitutive clindamycin resistance in routine testing.

6. Source of Funding

None.

7. Conflict of Interest

None.

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