Original Research Article

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

Hira Padekar¹, Badhuli Samal¹*, Lona Dash¹, Jayanthi Shastri¹

¹Dept. of Microbiology, Topiwala National Medical College, Nair Hospital, Mumbai, Maharashtra, India

ARTICLE INFO
Article history:
Received 24-07-2020
Accepted 07-08-2020
Available online 13-10-2020

Keywords:
Prevalence
Inducible clindamycin resistance
MRSA

ABSTRACT

Introduction: Clindamycin is an excellent drug for skin and soft tissue Staphylococcus aureus infections, but resistance mediated by inducible macrolide-lincosamide-streptogramin B (iMLS₉) 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 iMLS₉ 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 iMLS₉ or cMLS₉ was determined through double disk diffusion method or D-test by using erythromycin (2 μg) and clindamycin (15 μg) 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, iMLS₉, cMLS₉ 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 test. Therefore, clinical microbiology laboratory should routinely perform D-test in all clinically isolated Staphylococcus aureus to guide clinicians for the appropriate use of clindamycin.

© 2020 Published by Innovative Publication. This is an open access article under the CC BY-NC license (https://creativecommons.org/licenses/by-nc/4.0/)

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 MLS₉ antibiotics has led to an increase in number of staphylococcal strains acquiring resistance to MLS₉ 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
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 up to 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. 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 ceftoxitin 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), netilmicin (30µg) vancomycin (30µg) were tested.

Inducible resistance to clindamycin was tested by ‘D test’ as per CLSI guidelines.[CLSI] 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 ≤13 mm), while sensitive to clindamycin (zone size ≥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 ≤13 mm) while being sensitive to clindamycin (zone size ≥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 ≤13 mm) and clindamycin (zone size ≤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 (cMLS) 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. However, clindamycin resistance can develop in staphyloccocal isolates with inducible phenotype, and from such isolates, spontaneous constitutively resistant mutants have arisen both in vitro testing and in vivo during clindamycin therapy. 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 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. 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
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
al9 reported 43.3% in MRSA and 2.3% in MSSA, Gadepellier et
al2 reported 30% in MRSA and 10% in MSSA, Yilmaz
et al7 found inducible resistance of 24.4% in MRSA and
14.8% in MSSA. Whereas Ajantha et al10 showed very high
frequency of inducible resistance 74% in MRSA and 45% in
MSSA. On the contrary, in another study Schreckenberger et
al11 and Levin et al12 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 al1 reported (3.6%) in
MRSA isolate. While Yilmaz et al7 and Ciraj et al13 here
reported in (14.8%) and (15.3%) respectively. On the
contrary, one study by Angel et al14 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.

References
Clindamycin Resistance in Staphylococcus aureus and Coagulase-
2. Gadeppalli R, Dhawan B, Mohanty S, Kapil A, Das BK, Chaudhry
R, et al. Inducible clindamycin resistance in clinical isolates of
3. Leclercq R. Mechanisms of resistance to macrolides and

Table 1:

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

(ERY- Erythromycin, CL- Clindamycin, S- sensitive, R- resistant, CMLS B- Constitutive MLS B phenotype, iMLS B - Inducible MLS B phenotype, MS-MS phenotype, MRSA- Methicillin resistant staphylococcus aureus, MSSA- Methicillin sensitive staphylococcus aureus.)


**Author biography**

**Hira Padekar** Resident

**Badhuli Samal** Assistant Professor

**Lona Dash** Associate Professor

**Jayanthi Shastri** Professor and HOD