Inducible Clindamycin Resistance in Clinical Isolates of Staphylococci

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ABSTRACT

Background: The increasing frequency of methicillin-resistant Staphylococcus aureus (MRSA) infections and the changing patterns of antimicrobial resistance have led to renewed interest in the use of macrolide-lincosamide – streptogramin B (MLSB) antibiotics to treat such infections. However, resistance to MLSB antibiotics has been reported to be mediated by the msrA gene coding for efflux mechanism and erm gene which encoding the enzymes conferring resistance to MLSB antibiotics. Routine antibiotic sensitivity tests for clindamycin may fail to detect inducible clindamycin resistance due to the presence of the erm gene thereby resulting in failure of treatment. Aim: This study was undertaken to detect the presence of inducible clindamycin resistance among clinical isolates of staphylococci. Materials and Methods: A total of 247 strains of staphylococci (S. aureus - 158 and coagulase-negative staphylococci (CoNS) – 89), isolated from various clinical samples were included in this study. The isolates were identified using conventional methods. Inducible clindamycin resistance was tested by D test as per the CLSI guidelines. Results: Of total 247 isolates included in this study 52(21.05%) were MRSA, 67(27.13%) were Methicillin Resistant Coagulase Negative Staphylococci (MR-CoNS). Inducible clindamycin resistance was detected in 13.46 % of 52 MRSA and 2.99% of 67 MR-CoNS. Conclusion: Staphylococcal isolates, particularly MRSA, must be tested routinely by the D-test before treatment so that the clindamycin drug is used effectively and for maximum clinical utility. Keywords: Staphylococcus aureus, inducible MLSB, constitutive Clindamycin resistance, D-test, MRSA

1. INTRODUCTION

The increase in the occurrence of methicillin-resistant staphylococci, Vancomycin-intermediate resistant S. aureus (VISA)/Vancomycin-resistant S. aureus (VRSA) has left lower options for the treatment of infections. The macrolide-lincosamide- streptogramin B (MLSB) groups of antibiotics are commonly used in the treatment of Staphylococcal infections with clindamycin being the preferred agent. Good oral absorption makes it an important option in outpatient settings(1). However, resistance to MLSB antibiotics can occur due to drug interaction, target site modification, or efflux mechanisms(2). Macrolides such as erythromycin, roxithromycin, clarithromycin, and Lincosamides such as clindamycin and lincomycin belong to different classes of antimicrobials but act through the same mechanism that is by inhibition of protein synthesis(3). Macrolides resistance can be constitutive, where methylase is always produced, or inducible, where methylase is produced only in the presence of a macrolide inducer(4). Among MLSB drugs only macrolides are good inducers of the enzyme erythromycin ribosome methylase (erm). Once induced, the gene product confers cross-resistance to other members of the group including lincosamide and streptogramin B(5).
Detection of inducible clindamycin resistance in staphylococcal strains are difficult since these strains may appear sensitive to clindamycin and resistant to erythromycin. However, therapeutic failures are common with the emergence of selective constitutive erm mutants during clindamycin therapy. Expression of inducible resistance to clindamycin could limit the effectiveness of this drug. This resistance goes undetected by Kirby-Bauer method, however; it is detected by a simple D test. The result is observed as a flattening zone in the area between erythromycin and clindamycin disc, in shape of a ‘D’ which inducible clindamycin resistance.

Antimicrobial sensitivity testing is important for treating infections, but inducible clindamycin resistance test if not done, may lead to improper treatments.

The incidence of inducible resistance to Clindamycin may vary in different geographical regions. Since there is no substantial evidence of the Clindamycin resistance pattern in Sarat Abidah General Hospital, this study was done to detect the presence of inducible Clindamycin resistance (iMLSb) among clinical isolates of staphylococci by disc diffusion induction test (D-Test).

2. METHODS

A total 247 Staphylococcal isolates (158 S. aureus [SA] & 89 Coagulase-negative staphylococci [CoNS]) from pus/wound swab, blood, urine and others (tissue materials, body fluids & drain fluids) obtained from inpatients of tertiary care hospital over a period of one year (July 2012-June 2013) were included in this study. All the isolates were identified using conventional methods such as colony morphology, Gram stain, catalase and coagulase tests, etc. For the use of isolates, ethical approval was granted from Hospital Ethical Committee.

Antimicrobial Susceptibility Testing

Cefoxitin (FOX, 30µg, Mast Group Ltd, Merseyside, U.K.) disc was used to detect MRSA as per CLSI recommendations. Susceptibility to clindamycin (CD) and erythromycin (E) was determined by disc diffusion methods. For erythromycin, isolates exhibiting a zone of inhibition less than 13 mm were taken as resistant while those more than 21 mm were taken as sensitive. For clindamycin, the zone of inhibition less than 14 mm was taken as resistant while more than 21 mm was taken as sensitive. The isolates were studied for inducible clindamycin resistant as per CLSI guidelines, along with routine antibiotic susceptibility testing.

D-Test

Suspension of organism equivalent to 0.5 McFarland was inoculated on Muller Hinton agar plates. Clindamycin (CD, 2µg, Mast Group Ltd, Merseyside, U.K.) and Erythromycin (E, 15µg, Mast Group Ltd, Merseyside, U.K.) discs were placed 15 mm apart edge to edge. S. aureus ATCC25923 strains were used as a standard reference. Plates were analyzed after 18 hours of incubation at 370С. Completely circular zone of inhibition was taken as negative D test while blunting of clindamycin zone adjacent to erythromycin was interpreted as positive D test. [Fig. 1]. Erythromycin-resistant and clindamycin resistant strains were interpreted as constitutive MLSB phenotypes (cMLSb), while erythromycin resistant, clindamycin sensitive and D-test positive strains were interpreted as inducible MLSB (iMLSb) phenotypes [Fig. 1]. Erythromycin-resistant, clindamycin sensitive and D test negative strains were interpreted as MS Phenotypes.

Fig. 1: Erythromycin resistant and Clindamycin sensitive staphylococcal isolate giving D-shaped zone of inhibition around clindamycin with flattening toward Erythromycin disc suggestive of iMLSb phenotype

3. RESULTS

Of total 247 isolates included in this study; 52 (21.05%) were MRSA, 106 (42.91%) were Methicillin Sensitive S. Aureus (MSSA), 67 (27.13%) were Methicillin Resistant Coagulase Negative Staphylococci (MR- CoNS), and 22 (8.91%) were
Methicillin Sensitive Coagulase Negative Staphylococci (MS-CoNS) [Table 1].

Inducible Clindamycin resistance was detected in 13.46% of the 52 MRSA, 2.83% of the 106 MSSA and 2.99% of the MR-CoNS isolates and none of the 22 MS-CoNS isolates. Constitutive resistance to MLSB was detected in 71 (28.75%) of the total isolates. It was more observed in MRSA 22 (42.31%) and MR-CoNS 32 (47.76%) [Table 1].

Table 1: Resistance phenotypes of isolates

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>MRSA n (%)</th>
<th>MSSA n (%)</th>
<th>MR-CoNS n (%)</th>
<th>MS-CoNS n (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-S, CD-S</td>
<td>19(36.45)</td>
<td>71(66.98)</td>
<td>16(23.88)</td>
<td>14(63.64)</td>
<td>120(48.58)</td>
</tr>
<tr>
<td>E-S, CD-R</td>
<td>1(1.92)</td>
<td>3(2.83)</td>
<td>1(1.49)</td>
<td>0(0)</td>
<td>5(2.02)</td>
</tr>
<tr>
<td>E-R, CD-R (cMLS\textsubscript{B})</td>
<td>22(42.31)</td>
<td>12(11.32)</td>
<td>32(47.76)</td>
<td>5(22.73)</td>
<td>71(28.75)</td>
</tr>
<tr>
<td>E-R, CD-S (D test negative MS phenotype)</td>
<td>3(5.77)</td>
<td>17(16.04)</td>
<td>16(23.88)</td>
<td>3(13.63)</td>
<td>39(15.79)</td>
</tr>
<tr>
<td>E-R, CD-S (D test positive - iMLS\textsubscript{B})</td>
<td>7(13.46)</td>
<td>3(2.83)</td>
<td>2(2.99)</td>
<td>0(0)</td>
<td>12(4.86)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>52(21.05)</td>
<td>106(42.91)</td>
<td>67(27.13)</td>
<td>22(8.91)</td>
<td>247(100)</td>
</tr>
</tbody>
</table>

\(E: \) Erythromycin, \(CD: \) Clindamycin, \(S: \) Sensitive, \(R: \) Resistant, \(cMLS\textsubscript{B}: \) Constitutive MLSB, \(iMLS\textsubscript{B}: \) Inducible MLSB

4. DISCUSSION

Accurate detection of antimicrobial resistance in a microbe is an essential factor in determining appropriate therapeutic regimens\(^2\). The increasing frequency of staphylococcal infections among patients and changing patterns of antimicrobial resistance have led to renewed interest in the use of clindamycin therapy to treat such infections\(^{13,14}\). Clindamycin is frequently used to treat skin and bone infections because of its tolerability, cost, oral form and excellent tissue penetration, and the fact that it accumulates in abscesses and no renal dosing adjustments are needed\(^{14}\). Good oral absorption makes it an important option in outpatient therapy or as a follow-up after intravenous therapy. Clindamycin is a good alternative for the treatment of both methicillin-resistant and susceptible staphylococcal infection\(^{15,16}\). It is of particular importance as an alternative antibiotic in penicillin-allergic patients\(^{17}\). However, one important issue in clindamycin treatment is the risk of clinical failure during therapy. The therapeutic failure caused by MLSB inducible resistance is being more commonly reported\(^{13}\). MLSB resistance can be either constitutive (cMLS\textsubscript{B}) or inducible (iMLS\textsubscript{B}). The erythromycin resistance methylase (erm) genes encode enzymes that confer inducible or constitutive resistance to MLSB agents. Constitutively resistant isolates are resistant to all MLSB antibiotics and are detected readily by standard susceptibility testing methods. Inducible resistance is expressed in the presence of strong inducers of methylase synthesis, such as 14 membered (e.g. erythromycin), and 15 membered (e.g. azithromycin) macrolides. The 16 membered macrolides (e.g. spiramycin), lincomamide (e.g. clindamycin) and streptogramin B antibiotics may appear active when susceptibility is tested by the standard method as they are only weak inducers of methylase synthesis, but inducible resistance can be detected by disc diffusion induction test (D test)\(^{18}\).

Reporting staphylococci as susceptible to clindamycin without checking for inducible clindamycin resistance may thus result in inappropriate clindamycin therapy. On the other hand, negative results for inducible clindamycin resistance confirm clindamycin susceptibility and provide a very good therapeutic option.

In the present study; out of 247 staphylococcal isolates tested, 12 (4.86%) isolates showed inducible clindamycin resistance. The inducible clindamycin resistance was more observed in MRSA, 7 (13.46%). Reports from the different region have shown a different pattern of resistance, some reports have...
indicated a higher prevalence of inducible phenotype\(^8\). While others have indicated the frequency of incidence shifting from inducible to constitutive type.

In this study; out of 247 isolates, 71(28.75\%) showed constitutive resistance, which was more frequent in MRSA 22(42.31\%) and MR-CoNS 32(47.76\%). These findings were in accordance with Date et al., (2012) who found 26.43\% of total isolates showed constitutive resistance, which was more frequent in MRSA 52.63\% and MR-CoNS 78.95\% and Shantala et al., (2011) who found a higher incidence of cMLS B in S. aureus 18.26\%(19,20). Gupta et al., (2009); have reported cMLS B resistance in 19\% of total isolates of which 46\% were MRSA type and ten\% were MSSA type\(^21\).

In this study, higher incidence of cMLS B resistance was found in CoNS 41.57\%. It was more in MR-CoNS 47.76\% as compared to MS-CoNS 22.73\%. These finding in accordance with Date et al., (2012); who recorded higher incidence of cMLS B in CoNS 32.86\% which was more in MR-CoNS 78.95\% as compared to MS-CoNS 15.69\%(19). Delialioglu et al., (2005), also reported higher constitutive resistance 40.2\% among CoNS\(^44\).

There is a high variation for constitutive clindamycin resistance between various studies, as it depends on the overuse of the drug and conversion of inducible phenotype to constitutive phenotype during treatment\(^22\).

Inducible clindamycin resistance was detected in13.46\% of MRSA and 2.99\% of MR-CoNS in this study. Date et al., (2012) in their study have also reported 10.52\% MLS B resistance among MRSA isolates\(^19\). Whereas Deotale et al., (2010) reported 45\% of isolate of S. aureus to be MLS B\(^23\).

It was observed that percentages of inducible resistance and constitutive clindamycin resistance were higher among MRSA as compared to MSSA (13.46\%, 42.31\% and 2.83\%, 11.32\%, respectively). This was in concordance with few of the studies reported before\(^1,13\).

Some studies have shown a very high frequency of inducible resistance MRSA\(^24\). On the contrary, few studies have shown a higher percentage of inducible resistance in MSSA as compared to MRSA\(^96,25\).

The true incidence of cMLS B and MLS B depends on patient population studied, the geographical region, the methicillin susceptibility and hospital characteristics\(^19\).

Furthermore, the D-test was performed as a routine test on all Staphylococcal strains which were isolated in the laboratory, whereas most of the published D-test studies select only isolates that are E-resistant and CD susceptible for testing. The author was concerned that if the D-test was delayed until E resistance and CD susceptibility was noted in the isolate, the results may not be available for maximum clinical utility.

5. CONCLUSION

D-test must be done before reporting clindamycin susceptibility as clindamycin is not a suitable drug for D-test positive isolates while it can be definitely proving to be a drug of choice in case of D-test negative isolates. Therefore, regular surveillance of antimicrobial susceptibility pattern of MRSA, determination of the phenotypic pattern of inducible clindamycin resistance and formulation of a definite antibiotic policy may be helpful in reducing the burden of MRSA infection and failures in clindamycin treatment in the hospital.

REFERENCES

