Original Article

Inducible Resistance to Clindamycin in *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* Isolated from Clinical Samples in Tertiary Care Hospitals in Guwahati City

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Abstract

Background: Methicillin-resistant *staphylococcus aureus* (MRSA) are responsible for many hospital-acquired infections. Clindamycin (CL) is used to treat methicillin-sensitive *S. aureus* (MSSA) and MRSA. Antibiotic sensitivity testing (AST) can miss out the inducible CL resistance (CL-R) and result in failure of treatment. *D*-test detects inducible CL-R. **Subjects and Methods:** One hundred and ten *S. aureus* strains were tested. *D*-test was performed using erythromycin (ER) (15 mcg) and CL (2 mcg). The absence of inhibition around ER and a zone of inhibition around CL with flattening of the zone facing ER side is taken as positive *D*-test. **Results:** Of the total *S. aureus* strains, 36% were MRSA and 74% were MSSA. A total of 20 (18.18%) strains out of 110 were found to have inducible CL-R. In this study, MRSA (19.4%) were found to have higher percentage of *D*-test positivity as compared to MSSA (17.56%). **Conclusion:** *D*-test must be performed routinely as a part of AST for the presence of inducible phenotype.

Keywords: *Clindamycin, D-test, erythromycin, inducible, macrolide–lincosamide, streptogramin B*

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Introduction

Staphylococcus aureus is known to be a major cause of wide range of infections including skin and soft tissue infection. Healthy young people have lesser risk of getting this infection, but they can be potential careers of this infection.[1] *S. aureus* is also a common pathogen that causes serious nosocomial or hospital-acquired and community-acquired infections, which leads to a high morbidity rate worldwide.[2] *S. aureus* infection can involve multiple organs and very commonly found as a nasal carriage in hospital staffs, which is the reason of most of the hospital-acquired infections.[3] The first case of methicillin-resistant *S. aureus* (MRSA) was reported in 1961 in England from a case of hospital-acquired infection.[4] The first case of community-acquired MRSA was reported in 1980 in the United States.[5] Clindamycin (CL) is used for treating both *S. aureus* infections and MRSA infections; the advantage of using CL is that it can be administered both orally and intravenously and has a good tissue penetration ability.[6] It is also used to treat diseases such as pneumonia due to MRSA; however, the one drawback of CL treatment failure is macrolide–lincosamide–streptogramin B (MLSB)-inducible resistance.[7] Mainly, there are two types of macrolide–lincosamide–streptogramin (MLS) resistance, one is constitutive and the other one is inducible. Constitutive resistance can be seen by standard antibiotic susceptibility testing that is done routinely; however, the inducible resistance to CL can be missed out by routine antibacterial sensitivity testing as it requires an inducer of methylase synthesis such as erythromycin (ER).[8] The detection of inducible MLSB (iMLSB) requires a double-disk diffusion method using CL (2 mcg) and ER disk (15 mcg); in this method, the inducible MLS-resistant strains of *S. aureus* will show a zone of inhibition with a flattened end toward

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the ER side, giving it an appearance of the letter D. This test is also known as the *D*-test. The determination of a positive *D*-test or inducible CL resistance (CL-R) is very important, because even though CL is effectively working against the inducible resistant strains of *S. aureus,* there is a high chance of development of resistance and failure in the therapy. Hence, a *D*-test must be routinely done to rule out the iMLSB types and CL must not be used to treat such cases.[9]

In this study, we have performed *D*-test on the strains of *S. aureus* and MRSA obtained from tertiary care hospitals of Guwahati, Assam. All the strains are obtained from various clinical samples.

Subjects and Methods

This study was done on samples collected from 10 months (April 2019 to January 2020); all the samples that were collected were from hospitals located in Guwahati city of Assam. In this study, we have tried to establish that *D*-test must be used routinely in laboratories along with the antibiotic sensitivity testing (AST) to rule out inducible resistance to CL to avoid therapy failure.

Sample

S. aureus isolates from various clinical samples were included in this study.

Inclusive criteria

S. aureus grown from various clinical samples.

Exclusion criteria

Anything that is not coagulase-positive *staphylococcus*.

Identification of *S. aureus*

Gram staining

Gram staining was performed on all the strains using ATCC S. aureus (25923) as the positive control and ATCC Escherichia coli (25922) as the negative control. All the Gram-positive cocci was included in the study for further testing.

Catalase test

Catalase test was performed using ATCC *S. aureus* (25,923) and ATCC *Enterococcus faecalis* (29,212) as positive and negative controls, respectively. Catalase-positive strains were only selected for further identification.

Coagulase test

Slide and tube coagulase test was performed using the standard protocols and only coagulase-positive strains were included and identified as *S. aureus*.

Growth on mannitol salt agar

The organism was grown on mannitol salt agar (MSA) and only *S. aureus* showed yellow colonies on MSA.

Methicillin-resistant Staphylococcus aureus detection

Once the *S. aureus* were identified, the next step was to identify the MRSA among the total 110 strains of *S. aureus*. MRSA detection was done by disk diffusion method using cefoxitin disk. Interpretation was made using the CLSI guidelines.

Zone of inhibition ≥22 mm was considered as methicillin-sensitive *S. aureus* (MSSA) and ≤21mm was considered as MRSA.

Of the total 110 strains of *S. aureus,* 74 were identified as MSSA and 36 were MRSA.

Performing D-test

For the *D*-test, all the isolates of *S. aureus* that are erythromycin resistance (ER-R) and CL-S were only included and isolates that are ER-S were excluded.

D-test was performed using the protocol given by Fiebelkorn *et al*. (2003); standard protocol of the National Committee for Clinical Laboratory Standards was followed for the disk diffusion test. Mueller–Hinton agar without any supplements was used (HiMedia). For the antibiotics, antibiotic disk of 15 mcg for ER and 2 mcg for CL was used (HiMedia). The Mueller–Hinton agar plate was streaked with an inoculum of the test organisms that is standardized using 0.5 McFarland standard. Antibiotic disks of ER and CL were kept 15–20 mm apart. The plates were incubated for 16 to 18 h at 37°C. After the incubation, the test was interpreted, and appearance of growth of organisms near the edges of the disk was considered as resistant [Figure 1].

- Resistance to both ER and CL was considered as constitutive resistance to MLS
- ER-R and CL showing a zone of inhibition with slightly blunt or flattened zone at one side was an indication of positive *D*-test [Figure 1].

Results

In this study, we have taken a total of 110 strains of *S. aureus*; these strains were isolated from various clinical samples such as pus, blood, bronchoalveolar lavage, wound swab, tracheal aspirate, catheter tip, discharge fluid, urine, and sputum [Figure 2].

Of these 110 samples of *S. aureus,* 36 (32.7%) strains were found to be MRSA and 74 (67.2%) strains were found to be MSSA by cefoxitin disk diffusion method. *D*-test was performed on all the samples, and a total of 19 (17.27%) of the total samples were constitutive MLSB (CMLSB) phenotypes; of these, 8 (22.22%) were MRSA and 11 (14.86%) were MSSA. A total of 20 (18.18%) strains were iMLSB, of which 7 (19.4%) were MRSA and 13 (17.56%) were MSSA. As given in Table 1, the last group is of strains that are resistant to

ER and sensitive to CL but *D*-test negative which means no flattening or blunting of the zone of inhibition around CL is seen. These strains were 71 (64.54%) in number, and of these, 21 (58.33%) were MRSA and 50 (67.56%) were MSSA. These strains are sensitive to CL.

Discussion

In the treatment of an infectious agent, the antibiotic sensitivity results play a very important role. It rules out the drugs that the organism is resistant to and also helps in finding multidrug resistance in the organism. With the emergence of MRSA, the options for treatment are very limited and a proper AST is very useful to avoid treatment failure.[10,11] Because of high resistance to most of the antibiotics in case of MRSA, vancomycin is the preferred drug of choice; however, due to its side effects, other families of antibiotics are considered for the treatment like MLSB.[12] Clindamycin, which belongs to the Lincosamide family is an excellent antibiotic for treating MSSA as well as MRSA infections. Its versatility lies in the fact that it is well absorbed even if administered orally or intravenously and it is an alternative to cases that are allergic to penicillin.[13-15] Development of resistance to CL can lead to failure of treatment; this resistance can be seen *in vitro* or *in vivo* as a result of development of an inducible phenotype and leads to rise of constitutive phenotype with resistance to CL.[7] Considering CL as a sensitive drug without finding out the inducible resistance can lead to failed or inappropriate treatment. The good thing about *D*-test is that even if it is negative, it assures that CL therapy is going to be successful.[16]

In this study, we included a total of 110 isolates of *S. aureus*. Thirty-six (32.7%) out of the total strains were MRSA; out of these, 36 were MRSA strains, 8 (22.22%) CMLSB, 7 (19.4%) iMLSB, and 21 (58.33%) sensitive to CL and resistant to ER or *D*-test negative. The rate of iMLSB is higher in MRSA as compared to the CMLSB, which is similar to the study conducted by Prabhu *et al*., Mallikarjun K *et al*., and V Deotale *et al.* The rest 74 (67.2%) of the total isolates were MSSA, out of which 11 (14.86%) were CMLSB, 13 (17.56%) iMLSB, and 50 (67.56%) sensitive to CL and resistant to ER or *D*-test negative. As a whole, a total of 19 (17.27%) of the isolates were CMLSB, 20 (18.18%) were iMLSB, and 71 (64.54%) were both CL and ER sensitive.

Conclusion

S. aureus is a potential pathogen that causes infection, which is hospital acquired as well as community acquired. With the emergence of MRSA, the options for treating the patient with antibiotics are limited. Vancomycin and CL are the drugs used for most of the resistant strains of *S.* or MRSA. CL is known to work well for both MSSA and MRSA. For a precise antimicrobial therapy to start a reliable report of

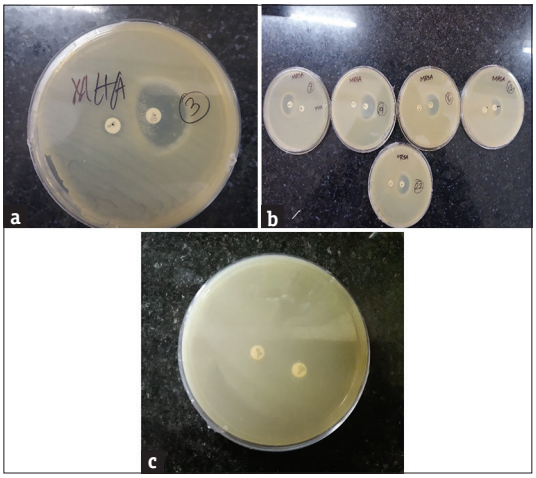

Figure 1: (a) D-test positive. (b) D-test positive. (c) Constitutive MLS resistant

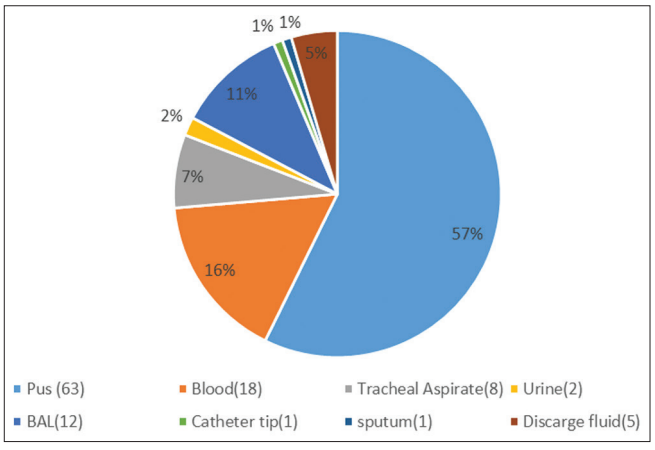

Figure 2: Distribution of samples

Table 1: Findings of *D*-test

Samples (110)	$_{c}$MLS$_{B}$ (ER-resistant, CL-resistant)	$_{i}$MLS$_{B}$ (ER-resistant, CL-sensitive) *D*-test positive	(ER-resistant, CL-sensitive) *D*-test negative
MRSA (*n*=36; 32.7%)	8 (22.22)	7 (19.4)	21 (58.33)
MSSA (*n*=74; 67.2%)	11 (14.86)	13 (17.56)	50 (67.56)
Total (*n*=110)	19 (17.27)	20 (18.18)	71 (64.54)

$_{i}$MLS$_{B}$: Inducible macrolide-lincosamide-streptogramin B, $_{c}$MLS$_{B}$: Constitutive macrolide-lincosamide-streptogramin B, MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin-sensitive *Staphylococcus aureus*, ER: Estrogen receptor, CL: Clindamycin

antibacterial sensitivity, testing is required. CL being one of the few antibiotics available to treat MRSA must be used carefully, i.e., when it is found truly sensitive to the strain. In this study, we observed that many strains of MRSA and MSSA were showing the pattern of CL-S and ER-R on the antibacterial sensitivity test report, but CL also showed inducible resistance or *D*-test positive for the same strain, which indicates that *D*-test is very important and must be included as a part of routine antibacterial sensitivity test in diagnostic laboratories.

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Conflicts of interest

There are no conflicts of interest.

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