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Original Research Article

Inducible Clindamycin resistance and MRSA amongst *Staphylococcus aureus* isolates: A phenotypic detection

Tanvi Panwala[1], Purvi Gandhi[1,*], Dipal Jethwa[1]

[1]*Dept. of Microbiology, Govt. Medical College, Surat, Gujarat, India*

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ABSTRACT

Introduction: Now a days clinicians switch over to drug Clindamycin to treat *Staphylococcus aureus* infections. Clindamycin is belonging to lincosamide group. As frequent use of this Clindamycin develops resistance among patients and ultimately treatment failure.
Aim: This present research is done to identify type of resistance like inducible or constitutive macrolide lincosamide – streptogramin B (iMLSB /cMLSB) resistance and MS (Macrolide lincosamide streptogramin) phenotypes among *Staphylococcus aureus* isolated from various samples received in Microbiology laboratory of tertiary care hospital of south Gujarat.
Materials and Methods: Among various samples total 232 *Staphylococcus aureus* were isolated. And all these isolates were subjected to routine antibiotic sensitivity testing by kirbey bauer disc diffusion method. Methicillin resistance *staphylococcus aureus* (MRSA) detected by using Cefoxitin disc. D test is performed as per Clinical and laboratory standards institute (CLSI) guidelines on all isolates.
Results: Total of 232 *Staphylococcus aureus* were isolated, among them 109 were Methicillin sensitive *Staphylococcus aureus* (MSSA) and 123 were Methicillin resistant *Staphylococcus aureus* (MRSA). Prevalence of iMLSB, cMLSB and MS phenotype were 59.34% ,15.44% and 13% in MRSA while 12.84%, 14.67% and 22.93% respectively in MSSA.
Conclusion: This research helps to detect Clindamycin resistance among *Staphylococcus aureus* and role of D test before starting the treatment with Clindamycin. By these knowledge clinician can choose correct treatment and we can prevent a treatment failure.

1. Introduction

Staphylococcus aureus (*S. aureus*) is a pluripotent pathogen. It is responsible for both nosocomial and community based infection. *S. aureus* is causing various infections that ranges from minor skin and tissue infection to life threatening consequences such as endocarditis, pneumonia and septicaemia.[1,2] There is an increased cases of Methicillin resistant *S. aureus* (MRSA) in recent years and it varies with geographical location and bacterial species.[3,4] For MRSA infection Vancomycin considered as drug of choice even though vancomycin usage is associated with considerable side effects and all of above more frequent use of this drug leads to emergence of Vancomycin resistance strain.[5] There is increasing frequency of MRSA infections and frequently changing antimicrobial resistance pattern make clinicians to jump on the macrolide lincosamide – streptogramin B (MLSB) antibiotics to treat infections.[6] One of the effective drug is Clindamycin which belongs to lincosamide group. The antibiotics belongs to MLSB family are chemically distinct but share a similar mode of action by binding to 23s rRNA –large ribosomal subunit and inhibit protein synthesis. Bacteria resist MLSB antibiotics in different ways like, 1.Target site modification by methylation or mutation that prevents the binding of the antibiotic to its ribosomal site. 2. Efflux of antibiotic 3. By inactivation of the drug.[7,8] Expression of MLSB resistance can be constitutive or inducible. For constitutive MLSB

* *Corresponding author*.
E-mail address: drpurvimodi@gmail.com (P. Gandhi).

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(cMLSB) phenotype resistance, erythromycin resistance methylase (erm) genes are consistently expressed and organisms show in vitro resistance to erythromycin(E) and clindamycin (CD), and also to other members of MLSB known as constitutive phenotype resistance while in case of inducible resistance, the erm genes require an inducing agent like erythromycin (E) ,act as a strong inducer of methylase synthesis to express resistance to clindamycin (CD). So isolates show in vitro resistance to E and susceptibile to CD. This type of resistance known as inducible phenotype. In this phenotype clindamycin therapy can lead to therapeutic failure.[2,9–12] Another mechanism of resistance is antibiotic efflux through *msrA* genes shows resistance to macrolides and streptogramin B only. In such cases Staphylococcal isolates appear erythromycin resistant and clindamycin sensitive both in vitro and in vivo.[13] This phenotype clindamycin can be given safely. Therefore, it is important to differentiate these mechanisms of resistance.

Double disk diffusion test is used for phenotypic detection of inducible resistance. It is also known as D test as D-Shaped zone of inhibition form around clindamycin if an erythromycin disc is placed adjacent to clindamycin disc. Double disk diffusion test is very simple and easy to perform test. It is inexpensive, sensitive and easy to interpret.[14] There are availability of molecular methods for detection of the erm genes, but they are costly and inconvenient for routine use. Thus, present study was done to detect the incidence of inducible clindamycin resistance in Staphylococci isolates by double disc diffusion test along with azithromycin and to study the relationship between clindamycin and methicillin resistance staphylococci in the tertiary care hospital of South Gujarat, India.

2. Material and Methods

The present study was conducted during May– August 2018 at the Microbiology department at tertiary care center, South Gujarat, India. The study was approved by institutional ethical committee. A total of 232 non-duplicate *S. aureus* were isolated from different clinical samples like pus, vaginal swab, urine, throat swab, skin swab, body fluids/ aspirates, central line /umbilical catheter tips etc. were received at Microbiology department. *S. aureus* isolates were detected by standard manual methods. The isolates were screened for routine Antimicrobial susceptibility test by Kirby-Bauer's disc diffusion method using various antimicrobial agents like penicillin (5μg), amikacin (30μg), erythromycin (15μg), cotrimoxazole (1.25/23.75 μg), ciprofloxacin (5μg)/norfloxacin (10μg), Vancomycin (30μg), linezolid (30μg) as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Staphylococcal isolates were screened for MRSA (Methicillin resistant *Staphylococcus aureus*) with using 30 μg cefoxitin disc as per CLSI guidelines.[15] The plates were incubated at 33 to 35°C for 16 to 18h; strains showing a zone diameter of less than or equal to 21 mm were considered as having mec-A mediated oxacillin resistance. *S. aureus* ATCC 25923 was used as a quality control.[7] Chi-square test of significance is applied for correlating methicillin resistance in *S. aureus* and iCR. As per CLSI guidelines Erythromycin resistant *Staphylococcus aureus* were further studied for detection of inducible and constitutive clindamycin resistance by D test.[16] A 0.5 Macfarland suspension was prepared in normal saline for each isolate and inoculated on Muller Hinton agar plate. 2μg clindamycin and 15 μg erythromycin disc were placed 15 mm apart edge to edge manually followed by overnight incubation at 37°C, six different phenotypes were identified and interpreted as follows:

1. Constitutive Resistance (cMLSB Phenotype : Resistant to E and CD
2. D Positive E(iMLSB Phenotype): Inducible resistance to Clindamycin was manifested by flattening or blunting of the CD zone adjacent to E disc, giving a D shape.
3. D Negative (MSB Phenotype): No flattening of the CD zone; Resistant to E but susceptible to CD.
4. Sensitive (Phenotype : Sensitive to E and CL

3. Results

From various clinical samples total 232 *Staphylococcus aureus* were isolated (Table 1). Out of them 123 were Methicillin resistant *Staphylococcus aureus* (MRSA) and 109 were Methicillin sensitive *Staphylococcus aureus* (MSSA). From total 232 isolates, 163 isolates were resistant to Erythromycin drug. These 163 isolates were subjected to D test. Among them 41(17.67%) isolates showed MS phenotype, 87(37.5%) isolates were D test positive and 35(15.08%) isolates showed constitutive Clindamycin resistant (Table 2). Inducible Clindamycin resistance was significantaly ($p < 0.05$) higher in MRSA strains (59.34%) as compared to MSSA strains (12.84%). Constitutive Clindamycin resistance in MRSA and MSSA strains were 15.44% and 14.67% respectively (Table 2).

4. Discussion

For treating the skin and soft tissue infections caused by Staphylococci, Clindamycin is a good drug of choice as it is less costlier than other newer agents, having excellent tissue penetration and accumulates in abcesses.[1] It is not affected by higher bacterial load at the infection site and no renal dose adjustment required. Day by day treatment spectrum becoming narrow as increasing resistance to the Staphylococcal infection as this led to renewed interest in the use of Clindamycin.[2] It is useful drug in the treatment of Methicilin sensitive and Methicillin resistant *Staphylococcus aureus* infection.[14] tro susceptibility to Clindamycin in the case of inducible MLSB resistance.[9,11,17] Constitutive MLSB phenotypes can be

Table 1: *Staphylococcus aureus* isolates from various clinical samples

Sample	Quantity	Percentage of isolated *Staphylococcus aureus*
Swab	137	59.05%
Pus	62	26.74%
Urine	5	2.15%
Peritoneal fluid	1	0.43%
Pleural fluid	4	1.72%
Drain	8	3.44%
Blood	9	3.87%
Ascitic fluid	3	1.29%
ET secretions	2	0.86%
CSF	1	0.43%

Table 2: Findings of the disc diffusion test

Findings of the disc diffusion test	Erythromycin sensitive Clindamycin sensitive	Erythromycin resistant Clindamycin sensitive (D test negative)	Erythromycin resistant Clindamycin sensitive (D test positive)	Erythromycin resistant Clindamycin resistant
	No resistance n(%)	MS n(%)	iMLS$_B$ n(%)	cMLS n(%)
Staphylococcus aureus (232)	69 (29.74%)	41 (17.67%)	87 (37.5%)	35(15.08%)
MRSA (123)	15 (12.19%)	16 (13.00%)	73 (59.34%)	19(15.44%)
MSSA (109)	54(49.54%)	25(22.93%)	14(12.84%)	16(14.67%)

MS –Macrolide strptogramin B; iMLS$_B$- Inducible macrolide lincosamide streptogramin B phenotype; cMLS$_B$ – constitutive macrolide lincosamide streptogramin B phenotype; MRSA – Methicillin resistant *Staphylococcus aureus*; MSSA – Methicillin sensitive *Staphylococcus aureus*.

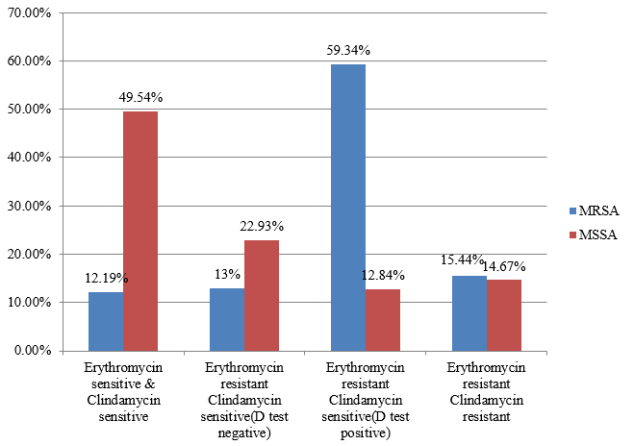

Fig. 1: D test result among MRSA (resistant *Staphylococcus aureus*) and MSSA (Methicillin sensitive Staphylococcus aureus)

detected easily in a routine Antimicrobial susceptibility testing but inducible MLSB resistance may be missed if Erythromycin and Clindamycin discs are kept apart. Hence present study follow the routine testing of inducible MLSB, Constitutive MLSB and MS phenotypes as recommended in CLSI guidelines for Staphylococcus isolates.[18] Methicillin resistance was seen in 53.01% isolates of *Staphylococcus aureus* as compared with other studies in India.[19,20] Of the 232 isolates of Staphylococci, 70.25% were Erythromycin resistance which is comparable to Dalela et al (66.34%).[1] Erythromycin resistance reported by Lyall et al[21] was 51.7% and Pal et al[22] was 50.52% where as lower percentage was reported by Prabhu et al[23] 28.4%. In present study Inducible Clindamycin resistance rate was 37.5% which is similar to Dalela et al[1] 36.63% and Lyall et al[21] 33.3% whereas higher percentage was observed by Ajantha et al[24] 49%, Goyal et al[25] 50.6% and lower percentage of iMLSB was reported by Prabhu et al[23] 10.5% and Ciraj et al[26] 13.1%. In present study constitutive Clindamycin reistance (cMLSB) rate was 15% which is accordance with Mokta et al[1] 17.14% and Lall et al[27] 16.6%. Higher percentage was reported by Pal et al[22] 46.9% where as lower percentage was observed in Patil et al[28] 3.55% and Mittal et al[29] 6.15%. In the present study 17.6% isolates showed true Clindamycin susceptibility (MS phenotype) which is similar to Patil et al[28] 15.33% and Mittal et al[29] 15%. Lower rate of MS phenotype was reported in Mokta et al[1] 8% and Dalela et al[2] 5.94%. These all studies shows that there is a wide variation in incidence of Clindamycin resistance among clinical isolates of *Staphylococcus aureus* in different geographical areas. The rate of inducible Clindamycin resistance in MRSA and MSSA in present study is 59.34% and 12.84% respectively which is comparable to Pal et al[22] and Mittal et al.[29] Higher incidence of ICR positive cases in MRSA was reported by Angel at al[30] (64% in MRSA). However higher percentage of ICR in MSSA as compared to MRSA have been reported by other studies like Schreckenberger et al[31] and Levin et al[10] 9.25% and 68% respectively. In this

present study percentage of cMLSB in MSSA and MRSA is observed 14.67% and 15.44% respectively. Gadepalli et al[7] reported 38% in MRSA and 15% in MSSA while Lall et al[27] had reported 16.6% in MRSA and 4.8% in MSSA. Lowe percentage of cMLSB was found in Prabhu et al[23] (16.7% in MRSA and 6.2% in MSSA) and Patil et al[28] (9.6% in MRSA & 0% in MSSA).MRSA is now growing public health problem. The relationship between MRSA and ICR appears to be clinically insignificant eventhough a highly positive correlation coefficient is in present study observed. This is an alarming sign that Clindamycin therapy failure may occur without prior testing for inducible resistant phenotypes. It should be necessary to prepare local sensitivity data which help in guiding empiric therapy and for preparing antibiotic policy.

Production of erm gene and its subtypes detected by molecular methods like DNA probing, Polymerase chain reaction, RFLP etc. These tests have not done in present study. These tests are available at research institute only. This is a limitation of present study.

5. Conclusion

Now a days therapeutic treatment for *Staphylococcal* infection become challenging job for physicians as changing of antibiotic susceptibility pattern, so they start the treatment of severe staphylococcal infection with use of either Vancomycin or Linezolid or tegicycline or Clindamycin. But before to start the treatment with Clindamycin ICR test become necessity as prevalence of ICR varies in different studies at different places. D test is simple and cost effective test with high sensitivity. Hence each laboratory should implement the D test for detection of ICR on a routine basis. Clindamycin can't be a choice of drug in D test positive isolates. So, result of D test is important for clinicians to choose a correct drug.

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7. Conflict of Interest

The authors declare they have no conflict of interest.

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Author biography

Tanvi Panwala, Assistant Professor

Purvi Gandhi, Tutor

Dipal Jethwa, Assistant Professor

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