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Original Article

D-Test – Its role in detection of inducible resistance to Clindamycin in Staphylococcus aureus with special reference to MRSA.

Veena Manjunath[a*], Eshwar Singh[a], Ramya.T.G[b], Mridula Raj Prakash[b], Archana Sharma[b]

[a*]*Asst Professor,* [b]*Postgraduate,Dept.of Microbiology, Dept of Microbiology, J.J.M.Medical College, Davangere-577004,Karnataka*

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ABSTRACT

Context: Staphylococcus aureus is a very widely occurring pathogen causing both nosocomial and community acquired infections globally. It is developing resistance to a wide variety of drugs tested routinely. Clindamycin which was very effective for most of the skin and soft tissue infections is also receding in its effect due to evolution of resistant strains. Aim: This study aims to know the details of different phenotypes involved in inducible resistance to clindamycin in erythromycin resistant isolates and also the association of MRSA and the inducible resistance. Materials and Methods: 96 S.aureus isolates were subjected to routine antibiotic susceptibility testing including Erythromycin, Clindamycin and Cefoxitin by Kirby Bauer disc diffusion method. Inducible clindamycin resistance was detected by disc approximation test (D test) as per CLSI guidelines. Results: Among the 96 isolates tested, 59 (61.45%)were found to be MRSA and 37(38.54%) were MSSA. Of the 59 isolates,34(57.63%)showed inducible clindamycin resistance,14(23.73%) constitutive resistance and 11(18.64%). Conclusion: High incidence of inducible and constitutive resistance was observed in MRSA as compared to MSSA. We suggest use of D test routinely to detect true resistance to clindamycin and to avoid treatment failure.

1. Introduction

Staphylococcus aureus is one of the most common bacteria infecting man [1] . It is also known to acquire antimicrobial resistance promptly after the introduction of new antibiotics [2]. Emergence of increasing resistance in Gram positive bacteria in the recent years has led to the use of the macrolide ,lincosamide ,and streptogramin (MLS) antibiotics in the treatment of Gram positive infections[3]. Increasing frequency of methicillin resistant staphylococcus aureus (MRSA) infections and changing patterns in antimicrobial resistance have led to renewed interests in the use of MLS antibiotics to treat such infections with Clindamycin being the preferred drug because of its excellent pharmacokinetic properties [4]. However, recently there has been increasing resistance pattern to MLS antibiotics because of their indiscriminate use. The determination of antimicrobial susceptibility pattern is very crucial for the optimal therapy of infected patients [5].

Erythromycin is an effective inducer of inducible MLSB resistance. It will induce production of the methylase, which allows Cd resistance to be expressed. To detect inducible Cd resistance strains,the disk approximation test (D-test) has been used by several authors [6-9].From past two to three decades, Clindamycin is being used to treat serious infections caused by Staphylococcus aureus. It is also found to be effective for many infections caused by community acquired methicillin resistant Staphylococcus aureus.Clindamycin resistance is common among health care-associated MRSA strains but the resistance pattern vary by region. Staphylococcal resistance to MLS antibiotics may be due to an active efflux mechanism encoded by msrA (conferring resistance to macrolides and inducible resistance to type B streptogramins only and susceptibility to clindamycin) or may be due to ribosomal target modification mediated by erm genes affecting macrolides,lincosamides and type B streptogramins (MLSB resistance).erm genes encode enzymes that confer inducible or constitutive resistance to MLS agents via methylation if 23S rRNA thereby reducing binding by MLS agents to the ribosome [10,11,12].

In vitro,S.aureus isolates with constitutive resistance are resistant to erythromycin and clindamycin while isolates with inducible resistance are resistant to erythromycin but appear susceptible to clindamycin [8].Failure to identify inducible MLSB

* Corresponding Author : Dr.Veena Manjunath
Asst Professor, Dept of Microbiology,
J.J.M.Medical College,
Davangere-577004
Karnataka
Cell: 9480599771
E.mail: veenaarush@gmail.com

resistance may lead to clinical failure of clindamycin therapy,a frequent choice for Staphylococcal skin and soft tissue infection[13].Inducible MLSB resistance can be detected by disc approximation test–(D test) by placing erythromycin and clindamycin discs in adjacent positions [8,10].Hence this study was undertaken to detect inducible clindamycin resistance in Staphylococcus aureus isolates from skin and soft tissue infections.

2. Materials and Method

This study was undertaken from November 2011 to January 2012.A total of 164 non repetitive, clinical samples from patients attending dermatology OPD with various infections like boils, folliculitis, acne, wound infections, abscesses etc were taken .Two swabs were used to collect the sample, of which one was used for culture and the other for microscopy. The samples were collected under aseptic precautions.Informed consent of the patient was obtained before collecting the samples. The samples were processed as per standard CLSI guidelines.96 Staphylococcus aureus were isolated .The remaining 68 comprised CONS (46),no growth (10) and samples contaminated (12).Hence they were all excluded from the study. The Staphylococcus aureus isolates were identified by microscopy-Gram's stain, their growth on blood agar, catalase test, slide and tube coagulase tests and finally on mannitol fermentation. The confirmed isolates were routinely tested for antibiotic susceptibility by Kirby–Bauer's disc diffusion method .The drugs tested are as follows-

Tetracycline (30µg), cotrimoxazole (25µg), Cephalexin(30µg),Cefoxitin(30µg),Clindamycin (2µg) and Erythromycin(15µg).All the discs were procured from Hi-Media, Mumbai.

Clindamycin and erythromycin discs were placed adjacent to each other, the distance from edge to edge being 21±1 mm (mean of the recommended range).Staphylococcus aureus ATCC25923 was used as quality control .Following incubation at 370c for 18-24 hours a flattening of the zone in the area between the discs where both drugs have diffused indicates that the organism has inducible clindamycin resistance(iMLSB Phenotype) [10,14].Three different phenotypic patterns were seen .The interpretation was done only for erythromycin resistant Staphylococcus aureus strains and all the sensitive strains were excluded

1. D-test Positive (iMLSB Phenotype): Isolates shoeing resistance to Erythromycin(≤ 13mm) and sensitive to clindamycin(≥ 21mm) and showing D shaped zone of inhibition around Clindamycin with the flattening towards Erythromycin.

2. D-test Negative (MS Phenotype): Isolates showing resistance to Erythromycin (≤ 13mm) but susceptible to Clindamycin(≥ 21mm) and showing circular zone of inhibition around clindamycin.

3. Constitutive Resistance (cMLSB Phenotype): Isolates showing resistance to both Erythromycin (≤ 13mm)and Clindamycin(≤ 14mm)with circular zone of inhibition if any around Clindamycin.

It was observed that many of the isolates were showing resistance to cefoxitin (≤19mm) on routine testing. Suspecting them as MRSA producers, these isolates were further tested for oxacillin (1µg) resistance or susceptibility pattern. The results were tabulated and analysed.

3.Results

Of the 164 samples processed, 96 (58.53%) were Staphylococcus aureus,32(33.33%) isolates showed D test positive indicating inducible MLSB(iMLSB) phenotype,18(18.75%)of the isolates showed constitutive MLSB(cMLSB) phenotype and 46(47.9%)were D test negative indicating MS phenotype.

Out of 96, 59(61.45%) were oxacillin resistant (zone size≤ 10mm) and considered to be MRSA,37 (38.54%) were sensitive and considered to be MSSA. The correlation between MRSA and MSSA with different phenotypes were analysed and interpreted as shown in the table.

Fig I D- test showing blunting of zone of inhibition around Clindamycin towards Erythromycin disc indicating iMLS$_B$ phenotype.

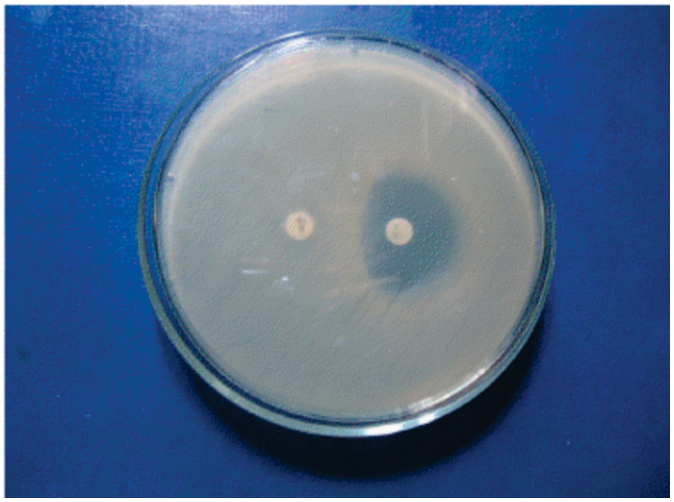

Table depicting different phenotypes of MRSA and MSSA

Phenotype	MRSA	MSSA
E-R,Cd-S(D-test positive, iMLS$_B$)	34 (57.63%)	6 (16.22%)
E-R,Cd-S(D-test negative, MS)	11 (18.64%)	23(62.16%)
E-R,Cd-R (cMLS$_B$)	4 (23.73%)	8(21.62%)
Total (96)	59 (61.45%)	37(38.54%)

a] E-Erythromycin, b] Cd-Clindamycin, c] cMLSB-constitutive, d] iMLSB-inducible.

4. Discussion

Macrolide inducible resistance to clindamycin was first recognized in the laboratory in early 1960s [15].Clinical isolates resistant to clindamycin were first recognized in 1968[16].Clindamycin has been the drug of choice to treat serious infections caused by susceptible staphylococcus aureus and also for many infections caused by CA –MRSA [17,18]. Widespread use of MLSB antibiotics has led to an increase in number of Staphylococcal strains acquiring resistance to MLSB antibiotics [4].

Inducible MLSB resistance is not recognized by using standard susceptibility test methods including standard broth based or agar dilution susceptibility tests [10]. The combination of resistance to erythromycin with susceptibility to clindamycin in

S.aureus (and other Gram negative microbes) can be due to iMLSB genotypes or efflux pumps. The D-test, based on disc diffusion susceptibility testing, is recommended to determine if the iMLSB genotype is present [8].The D-test positive isolates in our study were 32(33.33%) out of 96 Erythromycin resistant Staphylococcus aureus . A study done by Kavitha Prabhu et al,showed 37.52% of D-test positive isolates which is similar to our findings.[19] V Deotale et al found 45% of isolates to be D-test positive in their study [20].Another study done by Gadepalli Ravishekhar et al noticed 21% of iMLSB phenotypes in their study [4].cMLSB and MS phenotypes were 18(18.75%) and 46(47.9%) respectively in our study.

Various studies have shown the prevalence of cMLSB phenotype to be ranging from 11-27% and MS phenotype to be from 12-44% [4,19,20].It was noted that the percentage of inducible clindamycin resistance was highest among MRSA as compared to MSSA.57.63% and 16.22% respectively. This correlates with studies done by various researchers elsewhere [3,9,21,22]. However the true incidence depends on the patient population studied, The geographical region, the hospital characteristics and Methicillin susceptibility [23].

5.Conclusion

In conclusion, the D-test or Disc induction test, with routine antibiotic susceptibility testing can be used as a reliable and cost effective with an ease of performance to detect inducible and constitutive clindamycin resistance routinely. Hence the early detection of clindamycin resistance helps the clinicians to use clindamycin judiciously for infections caused by truly susceptible strains of S.aureus thus avoiding treatment failure.

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