Prevalence of Inducible Clindamycin Resistance in Isolates Staphylococcus Aureus from Various Clinical Specimen At Tertiary Care Centre

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Abstracts: Introduction: Staphylococcus aureus cases a variety of infections to skin and soft tissue infections to life threatening endocarditis. Therapeutic failure to Clindamycin has been reported due to mechanisms which confer resistance consitutively, or by the presence of low level inducers which can lead to therapeutic failure. Method: The present study January 2016 to 2016 November total 866 isolates of Staphylococcus aureus isolates from various specimens like pus, blood, fluids, wound swab and urine were tested. Antimicrobial susceptibility testing were done by Kirby Bauer's disc diffusion method .Inducible Clindamycin resistance was tested by Clindamycin disc induction test (D test) as per CLSI recommendations .Results: In present study was from January 2016 to November 2016 total sample 34,126 from their total 866 Staphylococcus aureus obtained from concecutive cilnical specimens were included, consisting of 97(11.2%) MRSA and 769(88.79%) MSSA. These isolates were subjected to D 121(13.97%) out of 866 Staphylococcus aureus Resistance showed Clindamycin 258(36%) and Erythromycin 453(63.44%).Sensitivity of Clindamycin 584(81.5%) and Erythromycin 423(59.2%). Conclusion: The D test is a simple effective and an important method for the phenotyping detection of inducible clindamycin resistance and it should be used routinely as it will help in guiding the empirical therapy. [Bhoya J NJIRM 2017; 8(2):39-43]

Key Words: D test, MRSA, Staphylococcus aureus

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Introduction: Staphylococcus aureus cause a variety of infections to skin and soft tissue infections to life threatening endocarditis. This study was undertaken to assess the frequency of the phenotyping expression of the inducible resistance to clindamycin which was due to the expression of erm gene in various clinical isolates of Staphylococcus aureus ,Methicillin Resistant Staphylococcus aureus infections and changing patterns in antimicrobial resistance have lead to renewed interest in use of macrolide lincosamide streptogramin B antibiotics to treat such infections. Three different mechanisms of resistance target site modification, enzymatic antibiotic inactivation and macrolide efflux pumps. Resistance occurs by different mechanisms to these microbiologically related antibiotics. Resistance due to active efflux encoded by msr(A) gene confers resistance to macrolides and streptogramin B (MS phenotype) but not to Clindamycin. Ribosomal target modification, another mechanism of resistance ,confers resistance to macrolide, type B streptogramin and also to Clindamycin MLSB phenotype. MLSB resistance in staphylococci is either constitutive cMLSB where rRNA methylase is always produced or inducible ,where methylase is only produced in the presence of inducer, and encoded by erm(A) and erm(C)gene. Inducible MLSB phenotype in isolates that susceptible to Clindamycin and resistance to Erythromycin is possible by using D test .As MRSA infections have become increasingly common in the community setting the development of empirical antimicrobial therapeutic strategies for staphylococcus infections has become more problematic. clindamycin has long been an option for treating both MSSA AND MRSA infections.

Isolates with inducible clindamycin resistance are found to be resistant to erythromycin but susceptible to Clindamycin when these discs are not placed adjacent to each other during antimicrobial sensitivity testing. These isolates can be detected by the D TEST ,a disc diffusion test in which induction of clindamycin resistance by erythromycin is tested. D TEST is simple ,reliable, inexpensive and easy to interpret with high sensitivity and specificity. Erythromycin is an effective inducer whereas an clindamycin is weak inducer.

Thus the aim of the present study was to detect the inducible Clindamycin resistance in clinical isolates of Staplylococcus aureus by the disc diffusion induction test.

Methods: The present study was conducted in the Department of Mcrobiology, B.J Medical College Ahmedabad, Gujarat, India. The study is conducted from January 2016 to November 2016 .Isolates of Staphylococcus aureus total 866 from samples of pus, swab, blood, respiratory tract infections, urine, body,

fluids, etc. The Staphylococcus aureus strains were identified by using standard microbiological procedures. Antibiotic susceptibility tests were performed by modified Kirby-Bauer disc diffusion method.

All the samples were inoculated on Nutrient agar and Blood agar media, incubated at 37° C for 48 hours and growth recorded after 24 and 48 hours.

Identification of Staphyloccocus aureus based on :

Culture characteristics: Colonies are large ,circular, convex, opaque, smooth, shiny.

Morphology: Determined by performing the Grams stain. Staphylococcus aureus appers as gram positive cocci arranged in pairs, tetrads or irregular clusters,giving it characteristic grap like clusters.

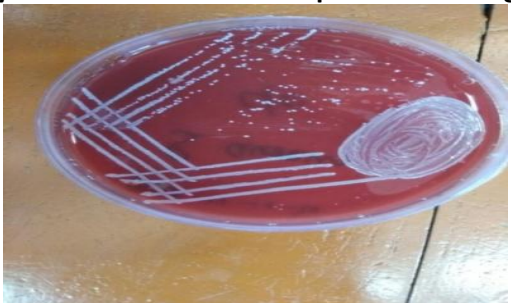
Catalase test: was done for all gram positive cocci which were catalase positive.

Coagulase test: Clot formation of any size is a positive result and indicative of the isolate being Staphylococcus aureus.

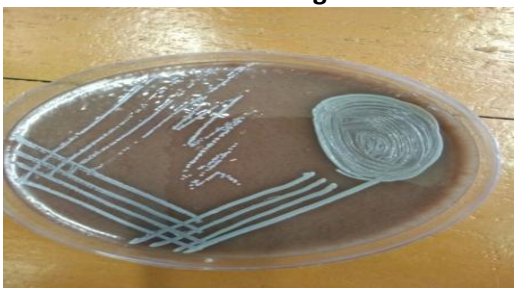
Mannitol: fermentation test : Organism such as Staphylococcus aureus that can grow in presence of salt and ferment mannitol change the colour of media to yellow.

Staphylococcus aureus colonies positive on nutrient agar

Staphylococcus aureus colonies positiveblood agar



Staphylococcus aureus colonies positive on chochalate agar



Antimicrobial Susceptibitlity test: IN the D test positive patient in our laboratory use BIO-DISC -12 Circle 112 and 512.It has 12 rings .Made in india by PATHOTEQ BIOLOGICAL LABORATORIES And Clindamycin 100/vial and Erythromycin 100/vial. For MRSA Cefoxitine 100/vial and Mupirocin 100/vial. Manufraction by Himedia.
To identify MLSBi phenotype, the D-test was performed.A lawn culture of the isolate which was adjusted to 0.5 Macfarland's concertration was made on a Mueller Hinton agar plate and discs of Clindamycin(2ug) and Erythromycin(15ug) were placed at distance of 15mm(edge to edge) as per the CLSI recommendations, along with routine antibiotic susceptibility testing. The disc diffusion test, based on the D test, showed four phenotypes. D Positive (iMLSB Phenotype) Inducible resistance to Clindamycin was manifested by flattening or blunting of Clindamycin zone adjacent to the erythromycin disc, giving a D SHAPE.

D test positive isolate, inducible resistance to Clindamycin and Erythromycin Methicillin: resistance was detected by using a 30ug cefoxitin disc. Staphylococcus ATCC 25923 was used as the control strain for the disc diffusion method. MRSA Cefoxitine 100/vial and Mupirocin 100/vial. Manufraction by Himedia.

Resistant to CX and Sensitive to MU

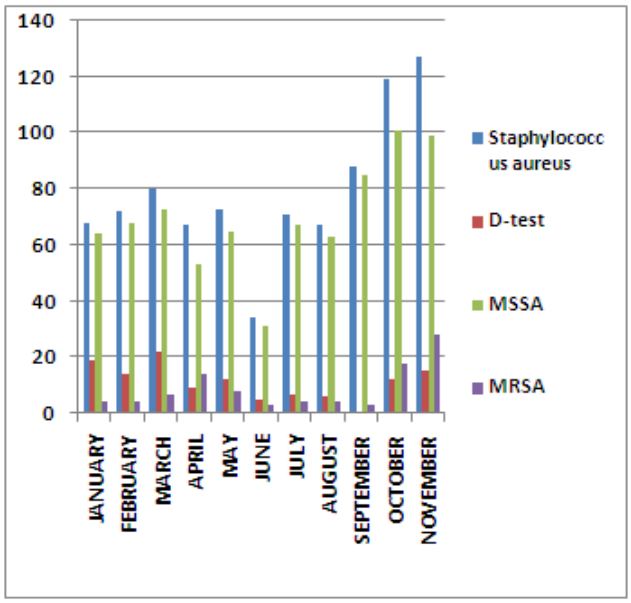
Quality control for erythromycin and clindamycin discs were done by using Staphylococcus aureus ATCC 25923 according to standard disc diffusion QC procedure. Negative and positive control strains-ATCC BAA-976(MS phenotype, msrA gene positive) and BAA-977(iMLSB phenotype, ermA gene positive)were inoculated on each plate.

Result: In present study was from January 2016 to October 2016 total sample 34,126 from their total 866 Staphylococcus aures obtained from consecutive cilnical specimens were included, consisting of 97(11.2%) MRSA and 769(88.79%) MSSA. These isolates were subjected to D 121(13.97%) out of 866 Staphylococcus aureus. Resistance showed Clindamycin 258(36%) and Erythromycin 453(63.44%).Sensitivity of Clindamycin 584(81.5%) and Erythromycin 423(59.2%).

The present study was conducted for period of from January2016 November 2016

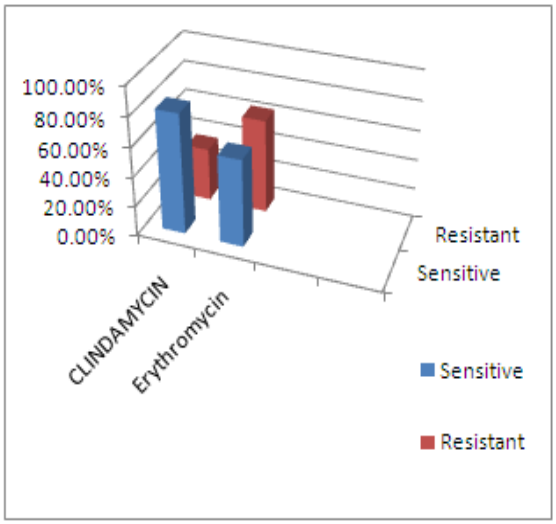
	Staphlococcus Aures	D Test	MSSA	MRSA
January	68	19	64	4
February	72	14	68	4
March	80	22	73	7
April	67	9	53	14
May	73	12	65	8
June	34	5	31	3
July	71	7	67	4
August	67	6	63	4
September	88	0	85	3
October	119	12	101	18
November	127	15	99	28
Total	866	121	769	97

Resistance showed Clindamycin 258(36%) and Erythromycin 453(63.44%).



Sensitivity of Clindamycin 584(81.5%) and Erythromycin 423(59.2%)

Drug name	Sensitive	Resistant
Clindamycin	(81.5%)	(36%)
Erythromycin	(59.2%)	(63.44%)



Discussion: Clindamycin is drug which is useful for treating skin and soft tissue infections which are caused by Staphylococcus aureus. It has excellent tissue penetration, it accumulates in abscesses, it is not impeded by high bacterial burden at the infection site and no renal dose adjustments are needed. Good oral absorption makes it an important option in outpatient therapy as follow up after intravenous therapy. Clindamycin is also a good alternative

antibiotic for the penicillin allergic patients and in infections due to MRSA. Clindamycin is less costly than some of the newer agents that might be considered for the infection.

Agar dilution testing to determine the MIC of induction phenotypes

Resistance phenotype	MIC for ERY (ug/ml)	MICfor CLI (ug/ml)	MIC for CLI & ERY combination
D	≥8	≤0.5	CLI(0.5ug/ml)+ ERY(1-2ug/ml)
D+	≥8	≤0.5	CLI(0.5ug/ml)+ ERY(1-4ug/ml)
Negative	≥8	≤0.5	CLI(0.5ug/ml)+ ERY(≥8ug/ml)
R	≥8	≥4	CLI(0.5ug/ml)+ ERY(≥8ug/ml)
S	≤0.5	≤0.5	CLI(0.5ug/ml)+ ERY(≤0.5ug/ml)

CLI=Clidamycin ERY=Erythromycin

In present study was from January 2016 to November 2016 total sample 34,126 from their total 866 Staphylococcus aures obtained from consecutive cilnical specimens were included, consisting of 97(11.2%) MRSA and 769(88.79%) MSSA. These isolates were subjected to D 121(13.97%) out of 866 Staphylococcus aureus.

In incidence of iMLSB and cMLSB is higher among 97(11.2%) MRSA compared 769(88.79%) MSSA respectively, in our setting. On the contrary showed a higher percentage of inducible resistance in MSSA.

Conclusion: The D test is a simple, effective and an important method for the phenotypic detection of inducible clindamycin resistance and it should be used routinely , as it will help in guiding the empirical therapy. The possible clinical failures can thus avoided. The high rates of occurrence of inducible resistance in both the MSSA as well as with MRSA infection. Consequently early detection helps in the

use of Clindamycin only in infections caused by truly Clindamycin susceptible Staphylococcus aureus and thus helps to avoid treatment failures.

Treatment of staphylococcal infections has always been a challenge for the treating physician, particularly in backdrop of changing resistance patter. Keeping the mode of action, side effects and pharmacokinetics in mind of certain drugs like vancomycin and linezoid, clindamycin should be considered for the treatment of sever and resistant staphylococcal infections. Different studies that prevalence of inducible cilndamycin resistance varies from place to place .Therefore, we recommend that

whenever clindamycin is intended for treatment of staphylococcal infection the clinical microbiology laboratory should test the isolated organism by D test, before clindamycin susceptibility is reported. Present study giving magnitude f clindamycin resistance among clinical isolates of Staphylococcus aureus from this region of the country will help clinicians choose an appropriate therapy.

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