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DETECTION OF INDUCIBLE CLINDAMYCIN RESISTANCE IN STAPHYLOCOCCUS AUREUS IN CLINICAL ISOLATES

Microbiology

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ABSTRACT

Introduction:- Resistance of macrolides in Staphylococci spp. might be due to active efflux or ribosomal target modification .MLSB resistance can occur either constitutive or inducible manner. The induction tests are useby placing of closely disc of erythromycin and clindamycin; the plane side or flattening side of the clindamycin zone adjacent to the erythromycin disk whichshows inducible MLSB resistance.
The aim & objective of this study to detection of inducible Clindamycin resistance in Staphylococcus aureus in clinical isolates.
Methodology:-Population of this study was 206 in which we had isolated Staphylococcus spp infection.This study was conducted in Department of Microbiology in Heritage institute of Medical Sciences, Varanasi.
Result:- In this study we were included 206 cases. Among them 55.4% were male and rest were female. In this study, we found that 52.5% cases were coagulase positive Staphylococcus & 47.5% cases were Coagulase negative Staphylococcus. this study revealed that inducible resistant pattern found in 74 cases while consecutive pattern had seen in 70 cases.
Conclusion:-This study concludes that, D-test is a simple method detected by with routine antibiotic susceptibility testing, delineates inducible and constitutive clindamycin resistance. Early detection may help identification of truly Clindamycin susceptible Staphylococcus aureus and therefore it helps to avoid therapy failures.

KEYWORDS

Antibiotic susceptibility test, MRSA, D-test

INTRODUCTION

Staphylococci are Gram-positive cocci, which form clusters. Approximately 30% of the normal healthy population is exaggerated by Staphylococcus aureus as it asymptomatically colonizes on human hosts.[1]Staph. aureus is the common bacteria which cause of differentdisease whichincludes: skin & soft tissue infections such as impetigo, folliculitis, systemic infection such as infection of wound, osteomyelitis, bacteremia& septicemia with metastatic complications, etc.It also caused toxin mediated diseases such as food poisoning, toxic shock syndrome (TSS), scaled skin syndrome(SSS), etc.It seems common superficial infections which include carbuncles, impetigo, cellulitis, and folliculitis. hospital-acquired infections include bacteremia, endocarditis, osteomyelitis, and pneumonia.[2] Staphylococcus species are the major cause of nosocomial infections. Any Foreignbodies which are implanted such as sutures, indwelling catheters, and implanted joints are extremelyprone to Coagulase negative Staphylococci colonization because it is a normal flora of skin, and normally act as the point of entry for getting infection. By formation of biofilm, S. epidermidis is resistant to antibiotics, and can serve as a reservoir for antibiotic resistant genes that can be transferred tothe bacteria.[3]The first significantappearanceof antibiotic resistant Staphylococcus foundwith a specific spp.It refers to as Methicillin-Resistant Staphylococcus aureus (MRSA).[4]]This straincontainsa modified penicillinbinding protein encoded bymecAgeneand is appear in 4 forms of Staphylococcuscassette.[4]Clindamycin is considering as aalternate drugof choice in penicillin-allergic cases in the management of skin and soft tissue infections which is caused by S. aureus.It has an good tissue penetration, accumulates in abscesses, and no dosage adjustments are needed in the presence of renal disease. The excellent oral absorption of Clindamycin makes it an alternative option for use in patients or as follow-up treatment after intravenous therapy.[5]The most common mechanism is Target site modification of acquired resistance to macrolides, lincosamides, and streptogramin B (MLSB) antibiotics in Staphylococciand confers cross-resistance to the MLS antibiotics.[6]MLSB resistance are divided into constitutive or inducible;it may be either consecutive or inducible, if it is found inducible, then bacterial isolate often test give resistant to Erythromycin (E) butsensitive to Clindamycin.[7]

Disk diffusion test is found to establish susceptibility, a distorted "D-shaped" zone called D zone in which inhibition is seen around Clindamycin, if an E disk is placed nearby the Clindamycin with the distance if 15mm.[7]Althoughbacteria isolatesshowedsensitive to Clindamycin in thenonexistence of an inducing agent. There is very much disinclinationto prescribedCDfortreatment of patients with infectioncaused by suchtype of organisms.8Staphylococcus aureus can cause of a wide range of infections ranging startingfromminor skin lesions to septicemia and endocarditis. In1944 and 1961 Penicillin and methicillin resistance was first recognized against Staphylococcus spp.MRSA are gradually more being reported at the present time with highly resistant to macrolides such as erythromycin, clarithromycin and lincosamides and after that leave the very few treatment option for a patientcare. Newer antibiotics such asvancomycin, linezolid, and quinupristindalfopristin have beenreportedin the management of therapy for suchtype of bacteria. Though thereports of resistance to thesebacteriaraise real concerns over how long these uniform susceptibilities will exist. Macrolides isuse as an alternative choice of drug for penicillin and cephalosporin in the treatment ofgram positiveisolates, but the development of resistance to macrolide has limited its use.In vitro, Staphylococcus aureus bacteria with the pattern of constitutive resistance are resistant to both erythromycin and clindamycin. however those resistancewhich areinduciblallyresistant to erythromycin andshowed as susceptibleto clindamycin (iMLSB). In such type of cases, patients harbouringiMLSB Staphylococci with in vivo treatment with clindamycin may select constitutive ermmutants and may leads to the development of constitutive resistance and therapeutic failure. Different studies have showed a wide difference in the rate of inducible clindamycin resistance in different places. Due to lack of data from this geographical area prompted us to carry out a study to establish the occurrence of inducible clindamycin resistance in clinical isolates of Staphylococcus aureus from a tertiary care hospital using D test, very simple method which may be used in routine microbiological practice and can help in the clinicians regarding

judicious use of clindamycin therapy. Staph. aureus may lead severe morbidity and fatal infections and the rapid evolution of antibiotic resistance in this pathogen is of considerable concern. Methicillin was usedagainst treatment of Staphylococcal infections due to penicillinase producing staphylococci. Methicillin resistant strains regularly evolved since last three decades which accounted for less than 0.1% of S.aureus in 1960s.

The Knowledge of susceptibility of a clinical isolate is vital for optimal antimicrobialtreatmentof infectedcases.This is particularly important while considering the appearanceof multidrug resistant organisms (MDR). There are so manyoptions existing for treatment of MRSA and MSSA infections, with clindamycin drug being one is the best alternatives option. Good oral absorption makes it an important option in outpatient therapy as a follow-up after intravenous therapy. Clindamycin is a good option of alternative antibiotic for the penicillin – allergic patients. [8]Though, tremendous use of clindamycin in infections can be develop therapeutic failure in inducible resistant phenotype (iMLSB) and from such isolates, spontaneous constitutively resistant mutants have arisen both in vivo and in vitro testing and during clindamycin therapy. [9]

MATERIAL &METHODS:-

StudyPopulation:-In this study, population was 206 in which we had isolated Staphylococcus aureus infection.

StudyArea:- This study was conducted in Department of Microbiology in Heritage institute of Medical Sciences, Varanasi.

Study Duration: - The duration of study was over a period of one and half years from January 2019 to 2020 July.

DATA COLLECTION:-

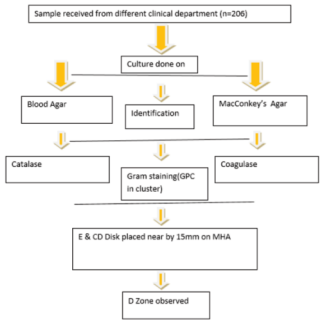
Sample was received in Microbiology Department. Total number of sample received was n=206 & culture was done on Blood Agar & MacConkey agar & incubated at 37C for 24-48hrs.Identification was done on the basis of Catalase & Coagulase test from isolated colonies. Antibiotic susceptibility test was done according to latest CLSI guidelines. Erythromycin& Clindamycin antibiotic disk were placed on MHA with the difference of 15mm between the disk. After that incubation of 24 hours zone of inhibition was measured & D-zone was also observed.

a) Inducible macrolide-lincosamidestreptogramin B (iMSLB) phenotype: D Test Positive: iMLSBStaphylococcus aureus bacteria which showingresistance to erythromycin (zone size ≤13 mm) while being susceptible to clindamycin (zone size ≥21 mm) and giving a Disorted shaped zone of inhibition around the clindamycin disk with flattening towards the erythromycin disc called D test positive.
b) Constitutive MSLB (cMSLB) phenotype: Staphylococcus aureus bacteria which showed resistant to both antibiotic erythromycin (zone size ≤13 mm) as well as clindamycin (zone size ≤14 mm) with circular shape zone of inhibition around clindamycin.
c) Methicillin – sensitivity (MS) phenotype: Staphylococcus aureus bacteria mayexhibitresistance to erythromycin (zone size ≤13 mm) but sensitive to clindamycin (zone size ≥21 mm) and giving a complete zone of inhibition around clindamycin disk that is D test negative.

DATAANALYSIS: -

Data was analysed by using Microsoft excel.

Study design:-



RESULT:-

In this study we were included 206 cases. Among them 55.4% were male and rest were female. In this study, prevalence were found in the <10 (29.1%) &>50 (29.1%) age group followed by other group. We were isolated staphylococcus mostly from Blood sample (44.7%) ,after that we got from pus (41.7%) and then from the rest of the sample. In this study, we found that 52.5% cases were coagulase positive Staphylococcus & 47.5% cases were Coagulase negative Staphylococcus. Among the all cases of Staphylococcus 56.3% were found MRSA & 43.7% MSSA. In this study it was observed that Erythromycin & Clindamycin is resistant in 70 cases and susceptible in 62 cases. While Erythromycin was resistant, Clindamycin susceptible with D zone found in 74 cases. So this study revealed that inducible resistant pattern found in 74 cases while consecutive pattern had seen in 70 cases.

Table:1 Distribution of cases according to gender

Gender	No. of cases	Percentage
Male	114	55.4%
Female	92	44.6%
Total	206	100%

Table:2 Distribution of cases according to samples

Sample	No. of cases	Percentage
Pus	86	41.7%
Swab	2	0.97%
Blood	92	44.7%
Pleural fluid	2	0.97%
Sputum	8	3.8%
Pleural fluid	2	0.97%
Urine	4	1.9%
Fluid	6	2.9%
Semen	4	1.9%
Total	206	100%

Table:3 Distribution of cases according to organisms

Organisms	No. of cases	Percentage
Coagulase positive Staphylococcus	108	52.5%
Coagulase negative Staphylococcus	98	47.5%
Total	206	100%

Table:4 This table showing interpretation according to Erythromycin & Clindamycin sensitivity

Phenotypic analysis	No. of cases
E-S,Cl-S	62
E-R,Cl-R	70
E-R,Cl-S,D+	74
Total	206

Table:7 Distribution of cases according to inducible & consecutive resistance pattern

Pattern of resistance	No. of cases	MSSA	MRSA
iMLSB	74	24	50
cMLSB	70	18	52

DISCUSSION:-

Clindamycin is a drug of choicewhich is most commonly usedin therapeutic treatment of skin and soft-tissue infections and also other infection caused by staphylococcal species, as well as for anaerobe infection. It has exceptional tissue penetration and accumulates in abscesses, and no need to adjustments in renal dosing [24].Best oral absorption makes it an important treatment option for OPD cases. Clindamycin has a specific importance as an alternative antibiotic in the penicillin-allergic patient.

For appropriate therapy decisions,accurate susceptibility data are important. Though,microdilution method and disc diffusion testing with erythromycin and clindamycin disc is nonadjacent positions may lead to false in vitro susceptibility result.Therefore, the routine testing of Staphylococcal isolates for inducible clindamycin resistance is highly recommended by the 2020 CLSI guidelines [10].

In this study, the prevalence of the MRSA strains was high (56.3%). These findings are supported by the results obtained by Gadepalli at al (52%), Mallick SR et al (51.6%) and S. Anuprabha et al (54.8%). [11-12]

Results of this study showed that total 206 isolates, 74 cases were of the

iMLSB phenotype. Similar findings were found by Angel et al from CMC Vellore (23.2%) and Fiebelkorn et al who have reported 28% iMLSB[13-14].

Several authors have highlighted the relationship of MRSA and MSSA with different phenotypes of S.aureus isolates. In the present study, 74% of the 50 MRSA isolates were found to be of the iMLSB phenotype. Several studies showed from India have been documented that 30% to 64% of the MRSA isolates to be of the iMLSBphenotype . Thefindings of this studyrevealed that 24 of the MSSA isolates showed iMLSB resistance. These results correlates with the findings by Delialiogu et al. they reported 10.7% iMLSB resistance in the MSSA isolates and Gupta at al who reported 17.3% iMLSB resistance in the MSSA isolates [15-16].

Our study found, 33.9% (70)S.aureus strains were of the cMLSB type, out of which 52were MRSA and 18 caseswere MSSA. Though Angel et al did not find any cMLSB resistance in the S.aureus strains. Gupta et al have reported 19% cMLB resistance, 46% of which were of the MRSA type and 10% were of the MSSA type [27,30].15.65% S.aureus strains showed the MSB phenotype in our study. In a study conducted Gadepalli et al, have been reported 12% strains of the MLSB phenotype among the S.aureus strains were found. Most of the studyshowedaelevatedincidenceof constitutive resistance than inducible resistance in Staphylococcus aureus. Theprevalencedepends on the population of patient studied, the geographical region, the hospital individualityand Methicillin susceptibility. This studyobservedahighincidence of iMLSB as compared to cMLSB in the S.aureusbacterial isolates. Mallick et al, reported 18.6% strains of iMLSB and 3.8% strains of cMLSB[25].

Additionally, Clindamycin Disc induction test wasexecuteas a routine diagnostic testforallStaphylococcal strains whichwere isolatedfromthe clinical specimen in the laboratory, while most of the studies which have been published on D-test, select only isolate that are Erythromycin-resistant and Clindamycinsensitivefor testing. Scientist worriesthat if theClindamycin disk induction test was delayed until ER resistance and CL susceptibility were noted in thebacterial isolate. Resultsmightnot be available for maximum clinical therapeutic utility.

CONCLUSION:

It can be conclude that, execution of the Clindamycin disk induction test, aneasy, supplementarydiagnostic method with routine antibiotic susceptibility test, describe inducible and constitutive clindamycin resistance. The highincident of inducible resistance in bothmethicillin sensitive S. aureus and methicillin resistant. S. aureusstrains raise concerns that clindamycin treatment failures may occur with MSSA and with MRSA infection.Therefore, timelyidentification may helps in the use of CL only in infections caused by truly CL susceptible S.aureus and assistto avoidfailure ofclinical therapy.

REFERENCES

1. Gill SR, Fouts DE, Archer GL, Mongodin EF, Deboy RT, Ravel J, et al. Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistantStaphylococcus aureus strain and a biofilm-producing methicillin-resistant Staphylococcus epidermidis strain. J Bacteriol 2005; 187:2426-38.
2. Frank AL, Marcinak JF, Mangat PD, Schreckenberger PC. Increase in community-acquired methicillin-resistant Staphylococcus aureusin children. Clin Infect Dis 1999; 29:935-6.
3. Leid JG, Shirtliff ME, Costerton JW, Stoodley P. Human leukocytes adhere to, penetrate, and respond to Staphylococcus aureusbiofilms. Infect Immun 2002; 70:6339-45.
4. Frank AL, Marcinak JF, Mangat PD, Schreckenberger PC. Community-acquired and clindamycin-susceptible methicillinresistant Staphylococcus aureusin children. Pediatr Infect Dis J 1999; 18:993-1000.
5. Descheemaeker P, Chapelle S, Lammens C, Hauchecome M, Wijdooghe M, Vandamme P, et al. Macrolide resistance and erythromycin resistance determinants among Belgian Streptococcus pyogenes and Streptococcus pneumoniae isolates. J Antimicrob Chemother 2000; 45:167-3.
6. Leclercq R, Courvalin P. Bacterial resistance to macrolide, lincosamide, and streptogramin antibiotics by target modification. Antimicrob Agents Chemother 1991; 35:1267-72.
7. Schreckenberger PC, Ilendo E, Ristow KL. Incidence of constitutive and inducible clindamycin resistance in Staphylococcus aureusand coagulase-negative Staphylococci in a community and a tertiary care hospital. J Clin Microbiol 2004; 42: 2777-9.
8. Vidhya R, Parimala S, Beena PM. Inducible clindamycin resistance in Staphylococcus aureus isolates from a rural tertiary care hospital, Kolar. J Clin Biomed Sci2013;3: 125-8.
9. Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I. Detection and prevalence ofinducible clindamycin resistance in Staphylococci. J Med Microbiol 2007;56: 342-5.
10. Gadepalli R, Dhawan B ,Mohanty S, Kapil A, Das BK, Chaudhary R. Inducible clindamycin resistance in clinical isolates of Staphylococcus aureus. Indian J Med Res. 2006;123:571-3.
11. Mallick SK, Basak S, Bose S. Inducible clindamycin resistance in Staphylococcus aureus-A therapeutic challenge. Journal of clinical and diagnostic research 2009; 3:1513-18
12. Anuprabha S., Sen MR, Nath G, Sharma BM, Gulati Ak, Mohapatra TM. Prevalence of Methicillin Resistant Staphylococcus aureus in a tertiary referral hospital in Eastern Utter Pradesh. Indian J.Med. Microbiol. 2003;90.
13. Angel MR, Balaji V, Prakash JAJ, Brahmadathan KN, Mathews MS. Prevalence of inducible clindamycin resistance in gram positive organisms in a tertiary care centre. Indian J Med Microbiol. 2008; 26:262-4.
14. Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical disk diffusion method for detection of inducible clindamycin resistance in Staphylococcus aureus and Coagulase negative Staphylococci. J Clin Microbiol. 2003; 41:4740-4.
15. Delialioglu N, Aslan G, Özturk C ,Baki V, Sen S, Emekdas G. Inducible clindamycin resistance in Staphylococci isolated from clinical samples. Jpn J Infect Dis. 2005; 58:104-6
16. Gupta V, Datta P , Rani H, Chander J. Inducible clindamycin resistance in Staphylococcus aureus: A study from North India. J Postgrad Med. 2009; 55:176-9.