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Detection of Methicillin Resistant Staphylococcus Aureus and Determination of Minimum Inhibitory Concentration of Vancomycin for Staphylococcus Aureus Isolated From Pus/Wound Swab Samples of Patients Attending Integral Insitute of Medical Sciences And Resarch Hospital, Lucknow"

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INTRODUCTION

Staphylococcus (staphyle , in Greek , meaning˙bunch of grapes' ; kokkos , meaning a berry) Staphylococci are Gram positive cocci that occur in grape – like clusters .Staphylococci were first observed in human pyogenic lesions by von Recklinghausen in 1871. Pasture (1880) obtained liquid cultures of the cocci from pus and produced abscesses by inoculating them into rabbits. Staphylococci were therefore classified into two groups ; Staph aureus (also called staph pyogenes) conataining strains giving a positive coagulase test , fermenting manitol and usually being pathogenic , and Staph.epidermidis (also called staph albus) containing coagulase – negative , mannitol nonfermenting and usually non pathogenic strain. S. aureus causes superficial , deep skin , soft – tissue infections , endocarditis , and bacteremia with metastatic absess formation and a variety of toxin – mediated. disease including gastroenteritis , staphylococcal scalded skin syndrome and toxic shock syndrome. Staphylococcus aureus resistance to antibiotics readily drugs should be prescribed according to the antimicrobial testingdone on the Muller hinton agar.Methicillin resistant in staphylococcus aureus is mediated by chromosomally coded gene called mecA gene , which alters penicillin – binding protein (PBP) present on S.aureus cell membrane to PBP 2a.PBP is essential protein needed for cell wall synthesis of bacteria . Beta lactam drug bind and inhibit this protein , there by inhibit the cell wall synthesis . The altered PBP 2a of MRSA strains has less affinity for beta lactam antibiotics ; hence , MRSA strain are resistance to all beta lactam antibiotics .MRSA are either community or hospital associated .Overuse of vancomycin has lead to the emergence of resistance to vancomycin . It may be of low grade resistance , known as VISA (vancomycin intermediate S. aureus)or high grade resistance , known as VRSA (vancomycin – resistant S. aureus).VRSA is very rare in India , it is reported from few places such as Hyderabad , Kolkata and Lucknow .VISA is more frequently reported than VRSA. VRsSA is mediated by Van A gene ;where as VISA is due to increase in cell wall thickness of S.aureus .The Van A gene is belived to be acquired from a vancomycin – resistant strain of Enterococcus faecalis by horizontal gene transfer.

High vancomycin MIC for MRSA which are susceptible to vancomycin indicate the drug resistance to many antibiotics MRSA is resistant to whole classes of beta lactam antibiotics including cephalosporins and carbapenems and higher danger of devlopement of protection from quniolones , aminoglycosides and macrolides .

AIM AND OBJECTIVES :

AIM :

To detect methicillin resistant staphylococcus aureus and determine minimum inhibitory concentration of vancomycin for staphylococcus aureus isolates from pus/ wound swab samples of the patients attendingIntegral Institute of Medical Sciences and Research, Hospital Lucknow.

OBJECTIVES :

1. To detect the methicillin resistance in staphylococcus aureus isolates .
2. To estimate the Minimum inhibitory concentration (MIC) for vancomycin in the Staphylococcus aureus isolates by broth micodilutionmethod .

MATERIAL AND METHODS

In clinically suspected patients , samples was collected such as pus / Wound swabs maintaining sterile conditions .

LABORATORY EXAMINATION

ISOLATION AND IDENTIFICATION OF STAPHYLOCOCCUS AUREUS-

The specimen was inoculated on blood agar and macconkey agar andincubates aerobically at 37 c for 48 hours . The strain of Staphylococcus aureus are identified on the basis of colony morphology , Gram stain and different biochemical test .

Confirmatory identification of the species was done by other biochemical tests like Indole (I) , Methyl red (MR),Voges –proskaure (VP) test , Citrate utilization test , Urease test , Triple Sugar Iron (TSI) test , Nitrate reduction test , Carbohydrate (ssugar) fermentation test , Huge and lefison oxidative fermentative test and Dnase test was done **.**

Culture Smear microscopy:

Gram stain:

Gram stainingfrom colonies shows gram positive cocci (1mm) , arranged in clusters.

Hanging drop: Hangining drop revealed non- motile cocci**.**

BIOCHEMICAL TESTS FOR IDEENTIFICATION

CATALASE TEST : The enzyme catalase mediates the breakdown of hydrogen peroxide (H2O2) into oxygen and water . The presence of the enzyme in a bacterial isolates was evident when a small inoculums is introduced into hydrogen peroxidase ((30% of the slide test) and the rapid elaboration of oxygen bubbles occuars. The lack of catalase was evident by a lack of or weak bubble production .

COAGULASE TEST :

The enzyme coagulase produced by S. aureus binds plasma fibrinogen and activates a cascades of reaction that cause plasma to clot . An organism can produced two types of coagulase .

Slide coagulase test :-It detects clumping factor (i.e.bound coagulase)

Tube coagulase test :-It detects free coagulase .

DNase test agar with methyl green:DNase producing organism exhibit clear zone around growth against green background.

Detection of Strains of MRSA by Cefoxitin Disc Diffusion Method

Susceptibility of Staphylococcus auerus isolates to Cefoxitin (30) will be determined by modified Kirby-Bauer disc diffusion method following CLSI guidelines .Cefoxitin is a surrogate marker for oxacillin resistance.The strains of S. aureus found to be resistant to Cefoxitin will be reported as MRSA.

ANTIMICROBIAL SUSPETIBILITY TESTING –

The antimicrobial susceptibility testing will performed by modified Kirby Bauer disk diffusion technique using Mueller's Hinton Agar (Hi Media Laboratories Private limited, India). Following Clinical and Laboratory Standards Institute (CLSI guidelines).

Antibiotics discs used were ciprofloxacin (5), Clindamycin (2), Chloramphenicol(3), Erythromycin (15), Gentamycin (1), Tetracycline (3), Cotimoxozole(25), Rifampin (5), and penicillin G.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS OF VANCOMYCIN.[15]

MIC of Vancomycin for all isolates of Staphylococcus aureus was determined by broth micro dilution method.

The result was interpreted according to CLSI Guidelines.[14]

The concentration of vancomyic use 0.5µg/ml to >32 µg/ml.

ANTIBIOTICS STOCK SOLUTION

Stock solutions will be prepared following the manufacture recommendations.

Freeze and thaw stock solutions only once and then discard them.

Stock solutions will be prepared using the formula:

1OOO ÷P×V×C=W

Where

P = potency given by the the manufacture.

V = Volume required (ml)

C = Final concentration of solution (multiple of 1000)
W= Weight of antibiotics (mg) to be dissolved in volume V (ml)

PREPARATION OF ANTIBIOTICS DILUTION RANGE.
Dilution range : 0.25 – 128 mg/L
11 universal containers was belabelled as follows ; 128, 64 , 32, 16, 8,4, 2, 1,0.5, 0.25 and 0 mg/L.

Preparation of inoculum
This method was used for non fastidious organisms eg. Staphylococcus.
Touch at least four morphologically similar colonies with a sterile loop.
Transfer the growth into ISB OR equivalent that has been shown not to affect the performance of the test, and incubate broth with shaking at 35 – 37℃. Visible turbidity should be equal to or greater then the 0.5 McFarland standards. Alternatively, an overnight broth culture will be used.

QUALITY CONTROL
Appropriate controls, depending on genera, must be included with every batch of MIC determination.

ORGANISM	QUALITY CONTROL
STAPHYLOCOCCUS AUREUS	ATCC 29213

INCUBATION CONDITION

ORGANISM	INCUBATION CONDITION
STAPHYLOCOCCUS AUREUS	35 - 37℃ IN INCUBATER FOR 18 – 20 hrs

Labelled 11 test tubes with the appropriate antibiotics solution.
Add 500µl of each antibiotic dilution to two rows of wells.
Dispense 50µl of organism suspension into the second row of wells.
Include inoculated and uninoculated tubes of antibiotics free broth (the first controls the adequacy of the organisms, the second for sterility cheacking.)
Incubate at 35-37℃ for 18-20 hours in incubater.

READING AND INTERPRETATION
Read the MIC end point as the lowest concentration of antibiotics at which there is no visible growth.
RANGE - <2 µg/ml to>16 µg/ml.
Susceptible - <2µg /ml,
Intermediate - 4-8µg/ml,
Resistance - >16µg /ml[8]

OBSERVATION AND RESULTS

Among 87 S. aureus isolates processed during the study,49 (56.32%) isolates were detected MRSA by Cefoxitin disc diffusion method. Out of 87 patients, 53 (61%) were males and 34 (39%) were females From IIMS&R, Lucknow.87 patients include in the study , maximum number of patient age group 20-29 year 22 %, followed by 11% patients who belonged to 0-9 years , 20%patients belong to 10-19 years, 20% patients belong to age group 30-39 , 10% patients belong to 40-49 years age group , 50-59 year age group include10%patients,6%patients belong to the 60-69 year. 0 % patient belong to age 70-79 years age group while the least number of patients 1%belong to age group 80-89.87 patients include in the study , maximum number of patient age group 20-29 year 22 %, followed by 11% patients who belonged to 0-9 years , 20%patients belong to 10-19 years, 20% patients belong to age group 30-39 , 10% patients belong to 40-49 years age group , 50-59 year age group include 10%patients,6%patients belong to the 60-69 year. 0 % patient belong to age 70-79 years age group while the least number of patients 1%belong to age group 80-89.Out of 87 S. aureus sample, most frequent MIC was 0.25 mg/L(36.78%) followed by 0.5 mg/L(34.48%), 1 mg/L(26.44%) and 2 mg/L.(2.30%).Out of 49 MRSA sample , most frequent MIC was 0.5 mg/L(36.73%) followed by 0.25 mg/L(32.65%), 1 mg/L(28.57%) and 2 mg/L (2.04) .All S. aureus had MIC of vancomycin below 4 mg/L (0.25 mg/L to 2 mg/L) that is susceptible to vancomycin irrespective to Methicilin resistance.

DISCUSSIONS

S. aureus is a flexible pathogen , which may be responsible for causing community acquired as well as nosocomial infections. Despite the years of efforts to devlope the new antibiotics for the eradication of MRSA , it has established itself as the commonest cause of skin and soft tissue infections. The difference in the prevalence of MRSA among different studies may be due to difference in the location and time period of the study . The prevalence of MRSA may differ from one hospital to another hospital , depending upon the types of the patients it receives , hygienic condition of the hospital and the health care workers.In our study 56.32 % of the isolates were found to be MRSA by cefoxitin disc diffusion method, which was comparable with the finding by khanal and Jha (68%) and Tiwari et al .(69.1%).The main objective of our study is to determine the prevalence of MRSA in Pus / wound swab Samples and determination ofMinimum inhibitory Concentration (MIC) of Vancomycin in S. aureus to IIMS&R, Lucknow.The current study explores the prevalence of S.aureus in pus / wound swab sample in our hospital. Our results demonstrated a prevalence rate of MRSA in pus / wound swab (56.32%). Also high prevalence of MRSA (56.32%) was found .Similar observations found by other researchers.

CONCLUSION

The results of **87** isolates of S. aureus of this study clearly demonstrated a high prevalence rate of MRSA (**56%).**Out of **87** S. aureus Minimum inhibitory concentration of vancomycin was **(0.25 mg/L to 2 mg /L).** Maximum number of Minimum inhibitory concentration (**MIC**) in S. aureus **32 (36.8%)** was obtained **0.25** mg/L .Minimum number of **(MIC)** of vancomycin in S. aureus **2 (2.36%)** was obtained **2 mg/L** . which are susceptible to vancomycin **. 49 MRSA** were demonstrated by cefoxtin disc diffusion method . Out of 49 MRSA Minimum inhibitory concentration of vancomycin was (0.25 mg/L to 2 mg/L). Maximum number of Minimum inhibitory concentration (MIC) of vancomycin in MRSA strains **18 (36.5%)** was obtained 0.5 mg/L . Minimum number of (MIC) of vancomycin in MRSA strains **1 (2.36%)** was obtained **2 mg/L .**

In our study the rate of isolation of MRSA among all the strains of S.aureus was very high . But none of the S . aureus strains were found to be vancomycin intermediate or vancomycin resistant.

BIBILIOGRAPHY

[1]. A.O.Kshetry, N.D. Pant ,R.Bhandri et al.,"Minimum inhibitory concentration of vancomycin to methicillin resistant Staphylococcus aureus isolated from different clinical sample at a tertiary care hospital in Nepal, "Antimicrobial Resistance &infection Control, vol.5.no.1, article 27,2016.
[2]. Ananthnarayan&Paniker ; Text book of Microbiology 9 th edition .2013
[3]. Apporva Shankar shastri 8 thed 2018
[4]. Bailey & Scott s Diagnostic Microbiology , 12 edition .2007.
[5]. Barber, M . Methicillin – resistant Staphylococci . J Clipatho 1961; 14 385.
[6]. Barrett , F . F ., M cghee , R.F and Finland , M. Methicillin – resistant Staphylococcus aureus at boston city hospital . N Eng j Med 1968 ; 279 ,448.s
[7]. Chambers , H.F. The changing epidemiology of Staphylococcus aureus .Emerg Infect Dis 2001;(2)178-182
[8]. Deresinski S . Methicillin – resistant Staphylococcus aureus : an evolutionary , epidemiologic and therapeutic odyssey . Clin Infect Dis 2008 ;(6) : 747 -63.
[9]. F.Rossi ,L.Diaz, A.Wallom et al." Transferable of vancomycin resistance in a community – associated MRSA lineage,"The New England Journal of Medicine , vol. 370 , no .16,pp.1524-1531 , 2014.
[10]. Gordon , R., & Lowy , F . Pathogenesis of Methicillin – resistant Staphylococcus aureus infection .Cli Infect Dis 2008;46(5):350-359.
[11]. GoyalR,Das , Mathur M. colonization of Methicillin – resistant Staphylococcus aureus among health care workers in a tertiary care hospital of Delhi . Ind J Med Sci 2002 ; 56(7): 321-324.
[12]. Grundmann , H., M. Aires – de –Sousa , J Boyce , and E. Timersma. Emergence and resurgence of Methicillin – resistant Staphylococcus aureus as a public health threat . Lancet 2006 ;368: 874-885.
[13]. J.M. Andrews , "Determination of minimum inhibitory concentration" journal of Antimicrobial Chemotherapy,vol 48, no . 1,pp.5-16,2001.
[14]. Mackie & McCartney Partical Medical Microbiology.14 e .2006.
[15]. Mahon C.R , Lehman D. C, Manuselis . G; Text book of Diagnostic Microbiology , London : W.B. Saundar Company . 1995 , 1134p.
[16]. PalavecinoE .Clinical , epidemiological , and laboratory aspects of methicillin – resistant Staphylococus aureus (MRSA) infections . MethoMol Bio 2007 ; 391 : 1-19.

[17]. P.C.Pahadi,U.T. Shrestha ,N .Adhikari , P.K.Shah, and R.Amatya,"Growing resistance to vancomycin among methicillin resistant Staphylococcus aureus isolates from different clinical samples," Journal of Nepal Medical Association, vol.52,no.196,pp.977-981,2014.
[18]. RaghabendraAdhikari et all 2017.
[19]. R.Amatya, P. Devkota , and A.Gautam ,"Reduced susceptibility tovancomycin in methicillin resistant Staphylococcus aureus a time for action,"Nepal Medical College Journal, vol.16,no.1,pp.42-44,2014.
[20]. Stryjewski ME, Corey GR. Methicillin – resistant Staphylococcus aureus : an evolving pathogen . Clin Infect Dis 2014; 58(1): 10-19.
[21]. S. Gardete and A.Tomsaz,"Mechanism of vancomycin resistant in Staphylococcus,"Journal of Clinical Investigation , vol.124,no 7,pp.2836-2840,2014.
[22]. TortoraG.J ,Funke B . R, Case Ch.L: Microbiology : An introduction , Redwood city , California : Benjamin Cummings Publishing Company , 1992 Inc , 810p.
[23]. TreakleAM .Thom , KA. Furuno, JP .Strauss , SM . Harris ,AD.Perencevich, EN .(2009)Mar. Bacterial contamination of health care workers white ccoats . Am J Infeccont 2009;37: 101-5.
[24]. TisingerCK , Empowering your patients in the fight against methicillin- resistant Staphylococcus .J Am Acad Nurse pract .2008:20(4): 204-11.

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