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EVALUATION OF CONVENTIONAL AND MOLECULAR ASSAYS IN THE DETECTION OF MRSA: A DIAGNOSTIC CHALLENGE

Microbiology

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ABSTRACT

MRSA has been causing increased morbidity and mortality both in the hospital and community .The aim of the present study was to know the prevalence of MRSA and its antimicrobial susceptibility pattern and to analyse the conventional and molecular detection methods . All the clinical samples submitted for Culture and sensitivity were processed following standard laboratory protocols. All the Staphylococcus aureus isolates were tested for Cefoxitin screen (30µg) by Kirby Bauer disc diffusion method and simultaneously processed in Vitek2 Compact and then were subjected to PCR for mecA gene detection. Of the 639 Culture positive isolates 43 were S. aureus . Of these 13% were identified as MRSA by Cefoxitin disc diffusion method, 27% in Vitek2 and all these 27% isolates were found positive for mecA gene by PCR. Automation is highly effective in detection of MRSA and is a best alternative for the gold standard PCR.

KEYWORDS

MRSA, Vitek 2, PCR, Prevalence

INTRODUCTION:

Staphylococcus aureus is responsible for causing variety of human infections varying from minor skin disease to life threatening infections(HareKrishna Tiwari et al). Staphylococci are uniformly sensitive to Penicillin in the pre-antibiotic era, with very few strains producing penicillinase. Soon after the penicillin came to be used clinically resistant strains emerged (Sangeetha Joshi et al).

Betalactamase (penicillinase) producing strains emergence became the reason for introduction of betalactamase resistant penicillins like Methicillin, Cloxacillin. Methicillin was though the first drug used, but it had disadvantage of being acid-labile . Hence it was superceded by the acid stable isoxazoyl penicillin Oxacillin.

Soon after the introduction of methicillin in 1960 (Jevons MP), Methicillin resistant Staphylococcus aureus (MRSA) strains emerged. MRSA were first reported in 1961 in United kingdom (Ajmal AN et al). Resistance to Methicillin is chromosomally mediated by a gene called “mecA”, which alters the Penicillin binding protein present on the cell wall of S.aureus to PBP2a (Apurba Sastry).

MRSA is becoming endemic in India . The incidence of MRSA varies from 25% in western part of India to 50% in South India (Sangeetha Joshi et al, Patel Ak et al). From the time of its first reporting, over years MRSA has been causing increased morbidity and mortality both in hospital and community.

With this back ground the present study has taken up to know the prevalence of MRSA and its antimicrobial susceptibility pattern , to study the role of Automated ID AST system over Conventional Cefoxitin disc diffusion in the detection of MRSA and to evaluate and analyse the efficacy of Automated ID AST system and Molecular assay in the confirmation of MRSA that helps in the accurate diagnosis and early intervention of these infections.

Materials and methods:

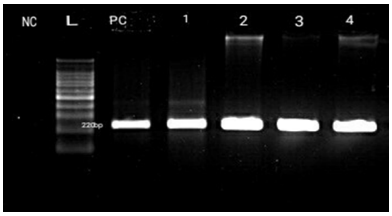
This is a Prospective Observational study done in the Clinical Microbiology lab over a period 6 months (from May 2018 to Oct 2018). A total of 4548 samples which include Pus swabs ,body fluids, blood ,sputum etc., submitted for Culture and Sensitivity were processed following standard laboratory protocol. Urine and stool samples were not included in the study. Ethical committee approval was obtained from the institute .

Of all the culture positive isolates, Staphylococcus aureus was identified based on Gram staining, β-hemolysis and golden yellow pigmentation on sheep blood agar, catalase positive, coagulase positive and mannitol fermentation. All the Staphylococcus aureus isolates were tested for Cefoxitin screen (30µgHimedia Lot: 0000315985 ,Exp :2/2019)by Kirby Bauer disc diffusion method. Zone of inhibition of >22mm is considered sensitive and <21mm is considered resistant as per CLSI 2017 guidelines . S.aureus ATCC25923 ,ATCC 33592 were used as reference control strains.

All the Staphylococcus aureus isolates were also simultaneously processed in Vitek2 Compact (version08.01) a fully automated microbial ID and AST system using GP (LOT NO:2420595103, EXP : 2020-02-06) and AST P628 cards (5380802203,EXP : 2020-02-06). Results of Cefoxitin screen (6µg/mL) in the AST panel were interpreted by Advanced Expert System of Vitek as modification of PBP (mecA) , which is positive for MRSA.

All these Staphylococcus aureus isolates were subjected to PCR for mecA gene detection. Colonies of Staphylococcus aureus grown on mannitol salt agar were used for DNA extraction by High salt method. Constituents of various components used in PCR were TKM-1 (PH - 7.6 Tris HCl, KCl, MgCl2, EDTA) , TKM-2 (PH -7.6 , lysis buffer Tris HCl, KCl, MgCl2, EDTA, NaCl), Phosphate buffer saline(PBS - NaCl, KCl, Potassium dihydrogen orthophosphate, Disodium hydrogen orthophosphate, distilled water) and 2X Master mix (Buffer , MgCl2, distilled water, dNTPs mix, Taq Polymerase). Emerald Amp GT PCR Master Mix (Lot:AH2002ON) include 12.5µl of 2X master mix, 1.0 µl each of Forward primer and Reverse primer , 4.0 µl of DNA template and 6.5 µl of Distilled water. A final volume of 25 µl of master mix was used for PCR run .The following forward and reverse primers were used for amplification of 220bp fragments (Bioserve ,Hyderabad). F: 5'-TCCAGATTACAACTTCACCAGG-3' ; R: 5'-CCACTTCATATCTTGTAACG-3' (M Stegger et al). The reaction is set as denaturation , annealing ,extension in ThermoFisher scientific veriti Thermal cycler. The amplified products were visualised by Electrophoresis in 2% agarose gel stained with Ethidium bromide. Amplicon at 220bp was identified as mecA gene(Figure1).

Figure 1 : Gel electrophoresis showing 220bp amplicons of test strains with positive and negative controls



Results:

Table:1 - Overview of the clinical samples processed

Type of sample	No. of samples processed	No. of Culture positives
Blood	2359	165 (6.9%)
Exudates (Pus, Pus swab, body fluids, Sputum)	2189	474 (21%)
Total	4548	639 (14%)

Table:2 - Distribution of isolates

Organisms isolated	Blood (n=165)	Exudates(n=474)	Total (n=639)
Klebsiella species	80 (48%)	209 (44%)	289 (45.2%)
Acinetobacter species	12 (7.2%)	8 (1.6%)	20 (3.1%)
Pseudomonas aeruginosa	15(9 %)	93 (19.6%)	108 (16.9%)
Escherichia coli	17 (10 %)	51(10 %)	68 (10.6%)
Staphylococcus aureus	8(4.8 %)	35(7.3%)	43 (6.7%)
CONS	8(4.8%)	4(0.8%)	12 (1.8%)
Others	25(15 %)	73(15 %)	98 (15 %)

Table:3 - Analysis of conventional and molecular assays in the detection of MRSA (The study is statistically significant with X2 value is 3.127 and P= 0.209)

Method (n=43)	MRSA	MSSA
Cefoxitin disc diffusion	6 (13.9%)	37 (86.1%)
Vitek 2 compact	12 (27.9%)	31 (72.1%)
PCR	12 (27.9 %)	31 (72.1 %)

DISCUSSION :

Staphylococcus aureus is one of the most common nosocomial pathogen, frequently changing its antibiotic resistance pattern because of indiscriminate use of antibiotics and poor hand hygiene practices in the hospital. MRSA is emerging as a major clinical and epidemiological threat in the hospitals as it cannot be killed by β-lactam group of antibiotics.

Basic mechanism of action of β-lactam antibiotics in S. aureus is , acting on the bifunctional transglycolase-transpeptidase PBP2 (Giesbrecht P et al). The active site of the transpeptidase enzyme is blocked ceasing the peptidoglycan biosynthesis . resistance to Methicillin and Oxacillin is through acquisition of mecA gene that encodes a homologue of the PBP2 called PBP2a (Hartman BJ et al), which is not susceptible to drug action. This is because the active site serine of the transpeptidase is located in a deep pocket which is not accessible to β-lactams(Lim D et al) .PBP2a is encoded by the mecA gene which is located within the family of distinct but related Staphylococcal chromosome cassette(scc) elements. SCC is a large genetic mobile element which varies in size and genetic composition among the strains of MRSA (Katayama Y et al).

In the present study all the S. aureus isolates has shown highest resistance to Penicillins (60%) and Fluoroquinolones(74%) and 100% sensitive to Vancomycin Similar pattern was reported by Dr. Bandaru Narasinga Rao et al from Andhra Pradesh and in 2011 by INSAR group from various centers in India. In the present study the prevalence of MRSA was 13.9% by conventional method , whereas 41% was reported by INSAR group in 2009 , 34% by Anila A Mathews et al in 2010 and 5% by Arun Kumar V et al in 2015 at Vellore by using similar disc diffusion method. While evaluating the Vitek2 results prevalence of MRSA was 27.9% in our study, whereas Harleen kaur et al from Punjab in 2012 reported 38% and Gandhiraj D et al from Tamilnadu in 2016 reported 13% using Vitek2 automation method.

When comparing the Vitek2 results with the PCR for detecting mecA gene , all the MRSA positive (27.9%) in automation were found to have mecA gene in PCR with a similar prevalence percentage of 27.9%. while comparing other studies a prevalence of 64% was reported by KB Anand et al from Pune in 2009 , 34% by Anila A Mathews et al from Coimbatore in 2010 and 25.45% by Susmita Battacharya et al from Kolkatta in 2010.

Conclusion : Methicillin resistant Staphylococcus aureus (MRSA) has come into limelight as it is extending to obtain endemicity in India. Automated assay is highly effective in detection of MRSA. Molecular confirmation though gold standard , but in India due to resource constraints most of the laboratories are not fully furnished or poorly accessed to molecular facility. Hence automation can be best alternative to the technically demanding PCR. Moreover conventional cefoxitin screen for detection of MRSA can be done to some extent , which can be opted for low budget setups. The present situation of MRSA with the prevalence of 27.9% alerts the Clinicians, Microbiologists, Epidemiologists to work together in combating the spread of MRSA in hospital and community by early detection and intervention.

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