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Compare phenotypic and genotypic methods to detect MERSA in tertiary care center in central India

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Abstract

Staphylococcus aureus is significant human pathogen which cause various kinds of infection ranging from minor skin diseases to life- threating endocarditis. It has acquired resistance to previously effective antimicrobials including the methicillin. Methicillin resistant Staphylococcus aureus (MRSA) is prototype of bacteria which is resistant and associated with long hospital stay, more mortality, raised costs & trouble-some to patient when compared with methicillin -sensitive Staphylococcus aureus (MSSA). The present study was prospective study conducted for a period 1 year 8 month (from Nov 2011-June 2013) all staphylococcal isolate in Microbiology Laboratory, Shri-Aurobindo Medical-college & P.G. institute Indore India. Gram staining of each specimen (except blood) was performed & findings noted. Each specimen was cultured on Blood agar & MacConkey's agar aerobically incubated over-night at 37 ˚C. Staphylococcal isolate were identified by phenotyping methods like Gram stain, catalase test, slide &tube coagulase test growth on manitol-salt agar, VP test Phosphates test and bacitracin susceptibility test. The antimicrobial susceptibility testing was conducted by Kirby-Bauer disc diffusion method as per guidelines of CLSI. Sensitivity, specificity & Positive predictive valve of chromogenic-agar for identification of MRSA detection was 83.7%, 80.2% and 71.9% respectively. In our study out of 100 cefoxitin resistant strain 89 were mec a positive and 11 were mec a negative

Keywords: Phenotypic and genotypic methods, MERSA, Staphylococcus aureus

Introduction

Staphylococcus Aureus is significant human pathogen which cause various kinds of infections ranging from minor skin infections to life- threating endocarditis. It has acquired resistance to previously effective antimicrobials including the methicillin [1]. Methicillin resistant Staphylococcus aureus (MRSA) is a prototype of resistant bacteria associated with prolonged hospital stay, raised mortality, higher costs &troublesome to patient when compared with methicillin-sensitive Staphylococcus-aureus (MSSA) [2-6].
B-Lactams are 1st choice antibiotics for treatment of Staphylococcal infections. Recently, the raised resistance against anti-bacterial drugs is primary public health concern & one of the

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biggest threats faced by physicians. In case of resistance to methicillin, due to of alteration of constitutive penicillin-binding-protein (PBPs)/expression of mecA [7, 8]. This has triggered alarm to this community as S. aureus causing life-threatening infections in hospitals & community. MRSA is one of commonest causes of nosocomial infections causing 40% to 70% S. aureus infections in intensive-care-units (ICU) [9].
The Clinical and Laboratory Standards Institute (CLSI) [11] has recommended the cefoxitin disk test for prediction of mecA–mediated resistance. Detection of mec A gene by Polymerase chain reaction (PCR) is considered to be gold standards but it is not yet available in all clinical laboratories. Therefore phenotypic methods still remain a method of choice in resource limited settings [10]. The present study was done to characterize and to determine the prevalence of MRSA isolates obtained from clinical specimens in a health care setup.

Material and Methods

The present study was a prospective study conducted for a period 1 year 8 month (from Nov 2011- June 2013) all staphylococcal isolate in Microbiology Laboratory, Shri Aurobindo Medical College & P.G. institute Indore India. The specimens like pus and wound swab, blood, bronchoalveolar lavage, sputum, tracheal aspirate, suction tip, urine, vaginal swab, tissue, body fluids/ CSF submitted to microbiology laboratory were processed as per standard procedures [12]. Gram staining of each specimen (except blood) was performed and findings noted. Each specimen was cultured on Blood agar and MacConkey's agar aerobically incubated overnight at 37 ˚c. Staphylococcal isolate were identified by phenotyping methods like Gram stain, catalase test, slide and tube coagulase test growth on manitol salt agar, VP test Phosphates test and bacitracin susceptibility test.
All Staphylococcus aureus isolates were tested for methicillin resistance by Cefoxitin (30 µg) disc diffusion test (CDD), Chromogenic agar method. E test to know minimum inhibitory concentration (MIC) of Oxacillin and Vancomycin, Latex agglutination test based on detection of PBP2a in 100 strains of S.aureus. Polymerase chain reaction (PCR) for detection of mec A gene in 100 cefoxitin resistant strains of MRSA. The antimicrobial susceptibility testing was performed by Kirby -Bauer disc diffusion method as per CLSI guidelines [13]. lawn culture of the test strain was prepared on Mueller- Hinton agar (MHA), plate. With all aseptic precautions the antibiotics discs were placed on Mueller Hinton agar plate and incubated at 37 ˚C overnight. Following antibiotics discs (Hi Media Pvt. Ltd, Mumbai) were used according to CLSI guidelines 2010 and ATCC 25923 (MSSA), 43300(MRSA) strain was used as a control strain [13].

Cefoxitin disk-diffusion test

Procedure: All strains were subjected to phenotypic antimicrobial susceptibility tests by Kirby Bauer disc diffusion method using cefoxitin 30 µg disc (Hi-Media, India) following CLSI guidelines 2010.

Chromogenic agar

Hi-Chrome Me Re Sagar (Hi-Media) was used as chromomeric medium for the identification of MRSA.

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Latex agglutination test
Principal

It is a qualitative slide latex agglutination test for the detection of PBP2a, determinant of mecA gene encoding methicillin resistance. This test was performed by using commercially available MRSA-screen kit manufactured by Denka- Seiken, Tokyo, Japan.

E-Test103

Oxacillin-vancomycin Ezy MIC strips (Hi media Laboratories, Mumbai) were used. The strip was having concentration gradient of oxacillin from 0.064µg-8µg on one half and vancomycin concentration from 0.019 µg-16 µg on other half. Simultaneously oxacillin and vancomycin MIC was detected.

Detection of the *mecA* Gene by PCR

Considering cefoxitin as a surrogate marker for mecA gene, 100 strains which were methicillin resistant by cefoxitin disc diffusion, were subjected to genotypic confirmation using PCR for mecA gene detection.
Conventional PCR was done to detect mecA gene in methicillin resistant staphylococcal strains. Three steps for doing PCR and identification of target DNA.

Result

Table 1: Comparison of various methods for detection of resistant staphylococcal aureus isolate

Method	Specificity	Sensitivity	PPV	NPV
Chromogenic agar	80.2	83.7	71.9	89.01
E test	96.8	91.4	94.5	94.9
Latex test	97.6	96.5	98.2	95.35
PCR	96.78	89	87.25	97.26
CCD	100	100	100	100

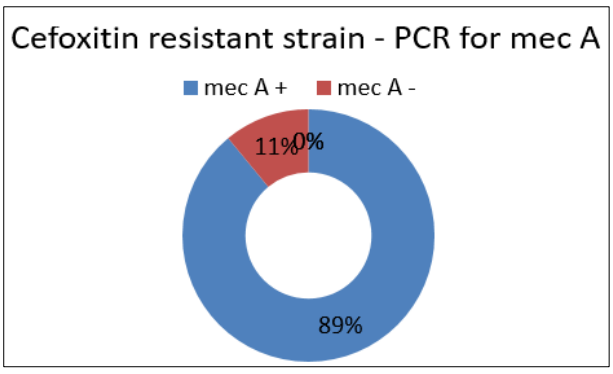

Fig 1: Showing mecA gene existence in cefoxitin resistant strain (n=100)

Discussion

Testing of oxacillin (methicillin) resistance in *S.aureus* has been a challenge for clinical laboratories in recent years. Detection of *mecA* gene is a gold standard method for diagnosis of MRSA in clinical microbiology laboratories.[14] However, most laboratories in developing countries are not in position to perform molecular methods. Cefoxitin is considered as a surrogate marker for mec a gene various studies recommend cefoxitin disc diffusion as a

reliable marker for detection of MRSA [14].

Sensitivity, specificity & Positive predictive valve of chromogenic – agar for identification of MRSA detection was 83.7%, 80.2% and 71.9% respectively. Our results was in concordance with other studies. (Oberoy L *et al*. 2012 [15]. MIC by E-test is useful for quantitative determination of susceptibility of bacteria to antibacterial agents. Of the penicillinase-stable penicillins, oxacillin is preferred in-vitro testing as it is more likely to detect heteroresistant strains of staphylococci. In the present study MIC by E-test for oxacillin showed a sensitivity 91.4% and specificity 96.8%, positive predictive value 94.5% similar to 90.9% sensitivity and 98.1% specificity by Oberoy L *et al*. 2012 [15].

The MRSA screening latex-agglutination test designed to detect PBP2a is rapid, easy to perform and has similar accuracy to PCR for *mecA* gene detection with respect to sensitivity (100%) and specificity (99.1%) (Louie *et al*. 2000 [16].

Two isolates that are cefoxitin resistant and mec a positive are negative by latex agglutination test for PBP2a. This may indicate that only small amounts of PBP 2a present and that the amounts are too small to be detected by the latex agglutination test.

Iraz M *et al*. 2011 [17] reported 96.5% sensitivity and 98.4% specificity of Latex agglutination test in comparison to PCR. Most of the studies showed 100% correlation of mecA gene with cefoxitin (CDD)(Rao V *et al*. 2011) [18] In our study out of 100 cefoxitin resistant strain 89 were mec A positive and 11 were mec A negative. It is somewhat similar to studies Bhutia *et al*. 2012 [19].

In our study out of 100 cefoxitin resistant strain 89 were mec a positive and 11 were mec a negative. It is somewhat similar to studies. (Jain A *et al*. 2008) [20]

False positivity results in the MERSA detection needless use of reserve second-line drugs like vancomycin and linezolid. This leads to more drug resistance and increases in health-care costs. False negative reports of MRSA lead to treatment failure, and increased nosocomial and community spread of this deadly microbe. It is always advisable to combine two methods, one with high sensitivity and the other with high specificity.

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