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Detection of Methicillin Resistant Staphylococcus aureus by phenotypic & genotypic method from clinical samples in a tertiary care hospital

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ABSTRACT

INTRODUCTION: Methicillin-resistant Staphylococcus aureus (MRSA) is an important human pathogen associated with nosocomial and community-acquired infections.[1] The mecA gene is considered to be one of the important virulence factors of S. aureus responsible for the acquisition of resistance to methicillin.[2] The main aim of this study was to investigate the prevalence, pattern of antibiotic susceptibility and mecA gene.[3]

METHODS: A total of 110 MRSA isolates with mecA gene were isolated from 545 isolates. Antibiotic susceptibility testing (AST) was performed by the Kirby-Bauer disc diffusion method using cefoxitin and genotypic testing of the mecA gene by PCR.

RESULTS: Of 545 isolates, 110MRSA isolates with mecA gene were detected and were multiple drug resistant (MDR). In AST, gentamicin and amikacin were found to be the least effective, while vancomycin was the most effective. The prevalence of the mecA gene was 20.18% (110/545).

CONCLUSION: This study concludes that PCR is highly sensitive for the detection of the mecA gene.

KEYWORDS: MRSA, cefoxitin, MDR, PCR

INTRODUCTION

Methicillin-resistant S. aureus (MRSA) is a major cause of nosocomial and community-acquired infections and continues to cause a variety of clinical syndromes worldwide.1,2 A major concern now is the spread of MRSA in the community, possibly due to out- of-hospital antibiotic pressure and transfer out of the hospital setting. Community-acquired MRSA strains (CA-MRSA) differ from healthcare-associated MRSA (HAMRSA) in that they are more commonly acquired from skin and soft tissue infections and can cause severe pneumonia in otherwise healthy individuals.3

MATERIAL & METHODS

Collection of samples: In the study, samples such as urine, blood, sputum, pus excretions and pus were collected from the patients and brought to the laboratory for further processing.

Sample processing/identification tests/biochemical tests: The grown colonies were identified by morphology, Gram stain and biochemical tests. S. aureus was confirmed by the following tests: yellow

stained colonies on mannitol salt agar, catalase positive, slide and tube coagulase positive, showed beta hemolysis on blood agar, DNase producing.

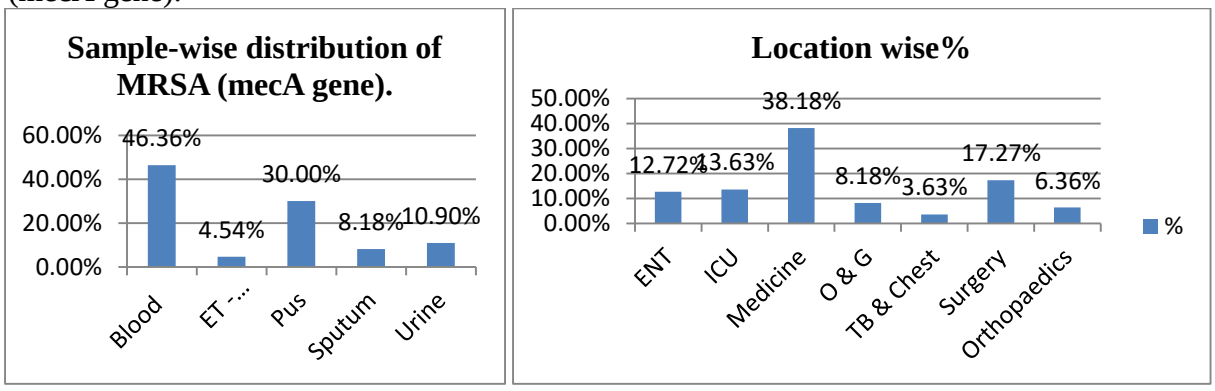
Antibiotic susceptibility testing (AST): The antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion technique according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The suspension concentration of the test organism was adjusted to 0.5 McFarland standards. The lawn culture was grown on a Mueller–Hinton agar plate. Antibiotic discs were placed over the lawn culture and the plate was incubated aerobically at 37°C for 24 hours. Finally, the plate was analysed for the zone of inhibition and interpreted according to CLSI guidelines.

Identification of methicillin resistance: Methicillin-resistant S. aureus (MRSA) was identified using Cefoxitin (30 µg) plates. The plates were incubated at 37°C for an incubation period of 24 hours. The zone of inhibition (ZOI) diameter of growth was recorded and interpreted as susceptible or resistant based on the CLSI guideline. S. aureus isolates were classified as methicillin resistant if the ZOI with the cefoxitin disc was >22 mm, growth on hichrome agar and genotypic examination of the mecA gene by PCR.

Molecular method for the detection of the mecA gene: Isolates were subjected to DNA extraction using (Himedia Mumbai) followed by amplification of mecA gene by PCR using primers mecA F (5'-GTG AAG ATA TACCAAGTG ATT-3'); mecA R (5'-ATG CGCTATAGATTGAAAGGAT-3') synthesised by thermocycler. Add 15µl of the master mix to each PCR tube that has been labelled. Pipette up and down to thoroughly mix each sample tube with 10 µl of the DNA eluate from the extraction process. As a negative control, 10 µl of PCR water equivalent to the above was used. After the PCR tubes were capped, they were placed on the rotor of the PCR machine. PCR master mix (2x-Himedia) 12.5 µl, 2 Molecular Grade Water 7.5 µl, 3 Template 3 µl, 4 Forward Primer (10pM) 1 µl, 5 Reverse Primer (10pM) 1, total volume 25 µl. Known primers were used to detect the presence of the mecA gene. 43 Initial heating: 94°C for 4 minutes Denaturation: 94°C for 30 seconds Annealing: 53°C for 30 seconds Extension: 72°C for 1 minute Final elongation: 73°C for 4 minutes Cooling: 4°C until removal of the tubes. The results of the PCR experiments were analysed by gel electrophoresis, in which the amplified products were separated. After PCR amplification, the products were separated on a 1.5 % agarose gel with ethidium bromide (0.5 µg/ml) to determine their identity. A total of 10 µl of the PCR product was added to each well. An additional 10 µl of a 100 bp DNA ladder was added to the last well. The dish was filled with 1x TBE buffer and exposed to electrophoresis at 110 volts and 90 amps for 30 minutes at room temperature. Bands were visualised using a BIO-RAD GEL DOC set equipped with a UV/VISIBLE transilluminator.

RESULT

A total of 545 MRSA strains were isolated from various clinical samples and analysed for MRSA using the cefoxitin diffusion method. Among those of the people tested, 110 (20.18%) were found to be infected with MRSA (mecA gene).



(1) **(2)**

Figure 1: Sample distribution of MRSA (mecA gene). Figure 2: Location-based distribution of MRSA (mecA gene).

The distribution of MRSA (mecA gene) among different clinical specimens was highest in blood (46.36%), followed by pus (30%), urine (10.9%), sputum (8.18%) and lowest in ET secretion (4.54%).

Most MRSA were isolated in the medical department (38.18%), followed by surgery (17.27%), intensive care (13.63%), ENT (12.72%), O & G (8.18%), orthopaedics (6.36%) and least by the tuberculosis and chest department (3.63%).

Of the total 110 MRSA isolates (mecA gene), the highest proportion was obtained from male patients (59%), while the lowest proportion was from female patients (41%). Moreover, males in the age group of 11 to 20 and 31 to 40 years were more frequently infected (18.46%), while females in the age group of 31 to 40 years were more frequently infected.

It was determined that 110 (20.18%) patients were infected with MRSA (mecA gene). Amines (81.81%). The least common was erythromycin (12.72%), followed by azithromycin (15.45%), rifampicin (32.72%), gentamicin and amikacin (33.63%).) The antibiotic pattern of the isolates showed the highest resistance to erythromycin (80%), followed by azithromycin (77.27%), rifampicin (66.36%), amikacin and gentamicin (65.45%) and Trimethoprim (68.18%). No resistance was reported to vancomycin and the least resistant drugs were teicoplanin (8.18%) and linezolid (16.36%).

DISCUSSION

The distribution of MRSA (mecA gene) in various clinical sites is highest in blood (46.36%), followed by saliva (30%), urine (10.9%), sputum (8.18%). %), the least amount of ET secretion (4.54%). Other Indian studies by Puthiya Purajil Preeja et al., showed maximum MRSA isolated from pus 84.09% and blood 13.6% .[4] Various other studies reported from Myanmar by Pan Ei Soe et al., having similar result for high prevalence from blood 52%.[5] Tsering et al., from Sikkim showed prevalence in Sputum 56.52%, Blood 50%, Urine 45.83%, throat swab 41% and Pus 27.05%.[6] Tiwari et al., from Bhuneswar reported in Pus 45%, Urine 20.05%.[7] Khan et al., from Lucknow reported in pus 24%, blood 4.29%, Urine 43.71%, Sputum 11.14%.[8] Arora et al., from Punjab reported in pus 51.2%, blood 31.06%, urine 10.08%, sputum 0.02%.[3] Several studies published by Pan Ei Soe et al in Myanmar have similar results with a high rate of blood 52%. 50%, urine 45.83%, throat swab 41%, pus 27.05%. ascites 24%, blood 4.29%, urine 43.71%, sputum 11.14%, blood 4.29%, urine 43.71%, sputum 11.14%.[12] In people reported from Mangaluru, pus was 27.07%, blood was 22.22%, urine was 42.8%, sputum was 29.4.[9] Kaur et al reported from Pune, pus was 13.56%, blood was 5.56%, urine was 5.32%, 69%. In the INSAR group, MRSA was isolated from pus in 40% of patients, followed by blood in 48% and urine in 52%.[11] Gynecology and obstetrics (8.18%), orthopedics (6.36%), tuberculosis and chest (3.63%) were the least common specialties.[10] A study by Pan Ei Soe et al. 49% were found in internal medicine, 38% in surgery, 50% in pediatrics, 74% in intensive care, 66% in dermatology and 51% in emergency departments.[12] Among these, the highest rate of recovery was in male patients (59%) and the lowest rate was in female patients (41%). In addition, the infection rate was higher in males aged 11-20 and 31-40 (18.46%), while the infection rate was higher in females aged 31-40 (33.33%). investigated and found that MRSA isolates were more common in male patients (70.6%) than in female patients (29.4%), and male patients accounted for 70.6% of the total isolations. According to research by Sajina Dhungel et al., MRSA isolates were found to be more prevalent among male patients (70.6%) than female patients (29.4%), with male patients accounting for 70.6 % of all isolates.[13] Patients over the age of 60 had the highest proportion of MRSA isolates (35.4%), followed by patients between the ages of 46 and 60 (29.4%)[14] is higher than that of women (17.0%), the highest number of MRSA is detected in the 35-44 age group (30.8%), followed by the 45-44 age group. Over 64 years of age (22.2%), 15-24 years of age (17.4%), 1-14 years of age (14.6%), 25-34 years of age (10.5%).[15] Among the samples, vancomycin (100%) showed sensitivity, followed by teicoplanin (90.9%) and linezolid (81.81%). The least common is erythromycin (12.72%), followed by azithromycin (15.45%), rifampicin (32.72%), gentamicin and amikacin (33.63%). The antibiotic pattern of the isolates showed the highest resistance to erythromycin (80%), followed by azithromycin (77.27%), rifampicin (66.36%), amikacin and gentamicin (65.45%), and Trimethoprim (68.18%). Vancomycin was also not reported, the least resistant drugs were teicoplanin (8.18%) and linezolid (16.36%).[16] According to previous studies, most of the isolates showed high resistance to cotrimoxazole (63%) and ciprofloxacin (57.8%).[17] Kaur et al. Among resistant MRSA isolates, gentamicin, ciprofloxacin, moxifloxacin and erythromycin are 100% resistant, clindamycin is 97.22%, vancomycin and teicoplanin are 100% sensitive, followed by linezolid with 97.22%.[10] Erythromycin (81.25%) refused.[18] However, in 2006, Rajaduraipandi et al. reported that the prevalence of linezolid-

resistant S. aureus in the southern Indian state of Tamil Nadu was 2.4%.[19] In addition, Tool et al. The incidence of linezolid resistance in orthopedic patients is reported to be 24%, which leads to nosocomial infections and excessive use of antibiotics.[18]

CONCLUSION

MecA gene was detected positively by PCR, which is a sensitive method. This is followed by teicoplanin with 100% and linezolid with 81.81%. Erythromycin (12.72%) is the highest, followed by azithromycin (15.45%), rifampicin (32.72%), gentamicin and amikacin (33.63%). PCR is a more sensitive method for detecting the mecA gene than phenotypic methods such as HiChrome and Cefixitin Disc Diffusion.

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