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ORIGINAL: Evaluation of Antibiotic Resistance Pattern and *mecA* Gene Frequency in Methicillin-Resistant *Staphylococcus aureus* Strains Collected from Clinical Specimens in Marand Hospital and Treatment Centers, Iran

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ABSTRACT

Introduction: *Staphylococcus aureus* causes a wide range of infections and as a multivalent pathogen is one of the causative agents of nosocomial and community infections. Therefore, the aim of this study was to identify and determine the pattern of antibiotic resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from patients in hospitals and medical centers in Marand city and also to evaluate the presence of *mecA* gene.

Material and Methods: In this cross-sectional descriptive study, 385 samples of *S. aureus* were collected from different clinical samples of patients in hospitals and medical centers of Marand city. *S. aureus* was identified using standard biochemical methods. Methicillin resistance was determined by disk diffusion method in the presence of oxacillin and cefoxitin. The pattern of antibiotic resistance of the strains was determined by disk diffusion method and according to CLSI recommendation and also PCR method was used to evaluate the frequency of MecA gene.

Results: In the present study, out of 385 samples of *S. aureus*, 215 (55.84%) samples were methicillin resistant. PCR results for *mecA* gene showed that 110 samples had *mecA* gene. The highest antibiotic resistance was observed against penicillin (100%) and erythromycin (83.63%). Most MRSA were isolated from urine and wound samples.

Conclusion: The results of this study indicate the prevalence of methicillin-resistant species and also the increase in antibiotic resistance of MRSA to various antibiotics. Therefore, in order to prevent increased resistance to other antibiotics, it is recommended to avoid inappropriate use of antibiotics.

Introduction

Staphylococcus aureus has the potential to cause skin infections and surgical wounds that are easily grown in many culture media and are metabolically active and, as a multivalent pathogen, cause disease in hospitals and on the surface (1). Due to its potential pathogenesis and increasing resistance to antimicrobial drugs, it is one of

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the major health problems in the world. This bacterium is resistant to penicillin due to the production of the enzyme beta-lactamase. Methicillin resistance indicates resistance to all penicillinase-resistant penicillins and cephalosporins (2, 3). Due to the structure of the genome of this bacterium, resistant and pathogenic strains are spreading. *S. aureus* covers a wide range of nosocomial and community infections (4). *S. aureus* causes a wide range of diseases including endocarditis, osteomyelitis, pneumonia, and toxic shock syndrome. Due to the above and the increasing resistance of Staphylococcus to other antibiotics such as Erythromycin, Tetracycline and even strains with relative resistance to vancomycin (VISA) or resistant to vancomycin (VRSA), which caused Make continuous efforts to find new antimicrobial drugs (3). *S. aureus* is the second leading cause of nosocomial infections after *Escherichia coli*, so the geographical area has undergone significant changes in the pattern of antibiotic susceptibility over the past years (5, 6). Antibiotic resistance in this bacterium is carried through chromosomes and plasmids, which increases with the indiscriminate use of antibiotics (7). Methicillin-resistant Staphylococcus aureus (MRSA) emerged in the early 1980s as one of the leading causes of nosocomial infections (8). Methicillin is resistant to the enzyme's penicillinase. This resistance is due to the production of a specific penicillin-binding protein called PBP2a (Penicillin Binding Protein 2a), which has a very weak binding affinity to beta-lactam antibiotics (9, 10). PBP2a is encoded by the *mecA* gene and transfected with the large Staphylococcal cassette chromosome (SCCmec) into the chromosome of resistant strains (11). Strains containing this gene are also resistant to many other antibiotics (multidrug-resistance) which, in addition to causing problems in the treatment of the disease, cause colonization and spread in the hospital environment and spread to other patients (12). Recent studies estimate that 12-8% of hospitalized patients suffer from the side effects of this infection. Identification and treatment of colonized individuals can reduce the incidence of MRSA (13). *S. aureus* strains, in addition to the antibiotic methicillin, may be resistant to other antibiotics such as macrolides, lincosamides, beta-lactams, fluoroquinolones, streptogr-amins, and aminoglycosides (14, 15). Early diagnosis and isolation of these patients can prevent the spread of MRSA strains in the hospital environment and medical staff. Therefore, the aim of this study was to identify and determine the pattern of antibiotic resistance of MRSA isolated from patients in hospitals and medical centers in Marand city and also to investigate the presence of *mecA* gene.

Methods

Sample collection

In this cross-sectional descriptive study, 385 samples of *S. aureus* were isolated from various clinical specimens such as urine (170 samples), blood (104 samples), sputum (62 samples), tracheal exudate (34 samples), and wound (15 samples). Biochemical methods (hot staining, catalase, slide coagulase, tubular coagulase and DNase and mannitol fermentation) were used for identification. All strains were numbered and stored at -70 ° C for experiments. A questionnaire was prepared for all patients and the required information (age, sex, location of infection) was recorded in accordance with the ethical charter (2).

Antibiotic resistance pattern

Pattern of antibiotic resistance on Mueller Hinton Agar medium (Merck, Germany) and using disk diffusion method for antibiotics (padtanteb Iran) Amoxiclav (20 µg), Erythromycin (5 µg), Gentamicin (30 µg) Ciprofloxacin (5 µg), cefotaxime (30 µg), penicillin (10 µg), chloramphenicol (30 µg), and ampicillin (300 µg) as recommended by Clinical and Laboratory Standards Institute (CLSI) (3, 5). For this purpose, the discs were placed on the Mueller Hinton Agar medium at a suitable distance from each other. Then it was incubated for 24 hours at 37 ° C. Then the diameter of the bacterial growth inhibition

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Table 1. Primers used in this study

Gene	PCR product size	Reference
mec A F: AAAATCGATGGTAAAGGTTGGC *mec A* R: AGTTCTGCAGTACCGGATTTGC	532 bp	27

zone was measured and compared with the standard table. Finally, based on the diameter of the growth inhibition zone around the discs, the results were expressed as sensitive, intermediate, resistant (16).

Determination of MRSA

Disc diffusion method on agar was used to determine MRSA. Discs of oxacillin (1 µg) and cefoxitin (30 µg) on Mueller Hinton Agar medium (containing 4% NaCl) were used for phenotypic detection of MRSA isolates. The resistance of these strains to oxacillin and cefoxitin actually indicates resistance to beta-lactam antibiotics such as penicillin, methicillin and cephalosporins (17, 18). *S. aureus* ATCC 33592 (MRSA) was used as the standard strain.

Genotypic evaluation of strains

DNA extraction of bacterial strains was performed by Invitek Strateg Business kit (made in Canada). A specific primer pair was used to detect the *mecA* gene (***Table 1***). PCR reaction with a final volume of 50 µl containing 10 picomoles from each primer, 0.2 µmol dNTP, 1 microliter DNA template, 2.5 micromoles MgCl2, 10 microliters 10X PCR buffer and 1.25 units of Polymerase Taq DNA enzyme (all consumables were made by Sinagen Iran). Thermal cycler device program contains 5 minutes of initial denaturation at 94 ° C and then 30 cycles with temperature conditions of 1 minute and Denaturation at 94 ° C, Annealing at 55 ° C for 30 seconds, Extension/elongation at temperature 72 ° C for 30 seconds and finally the final Extension/elongation at 72 ° C for 10 minutes. The PCR product was then evaluated on 1% agarose gel by electrophoresis and the gel containing PCR products was placed in a tank containing ethidium bromide for 15 to 20 minutes after the end of the electrophoresis period and then the bands were observed under UV light. Finally, it was photographed and printed. *S. aureus* ATCC 33591 with *mecA* gene was used as a positive control. The results were analyzed using SPSS software (version 19) and chi square test. In all cases, $p < 0.05$ was considered significant (19).

Results

385 samples of *S. aureus* from different clinical samples such as 170 samples (44.16%) from urine, 104 samples (27.01%) from blood, 62 samples from sputum (16.10 samples), 34 samples from tracheal exudate (8.83 samples), and 15 samples Were isolated from wounds (3.90 samples). There were 264 samples from the outpatient ward (68.57%) and 121 samples from the inpatient ward (1.43%). The mean age of patients was 49.4 ± 27 ranging from a minimum of 10 months to a maximum of 60 years. 198 samples (51.43%) were for men and 187 samples (48.57%) were for women. 98 patients (25.45%) had a history of antibiotic use. A total of 215 (55.84%) of the 385 samples were methicillin resistant. There was no statistically significant difference in the distribution of MRSA between age groups ($p > 0.05$). 115 (53.49%) men and 100 (46.51%) women were methicillin resistant. There was no statistically significant difference in the distribution of MRSA between men and women ($p > 0.05$). 145 urine samples (67.44%), 42 wound samples (19.53%), 17 blood samples (7.91%) and 11 sputum samples (5.12%) were methicillin resistant. There was no statistically significant difference between the frequency of MRSA and the type of sample ($p > 0.05$). PCR results for *mecA* gene showed that 110 samples had *mecA* gene (***Figure 1***). The frequency of *mecA* gene in clinical samples was 76 (69.1%) urine samples, 20

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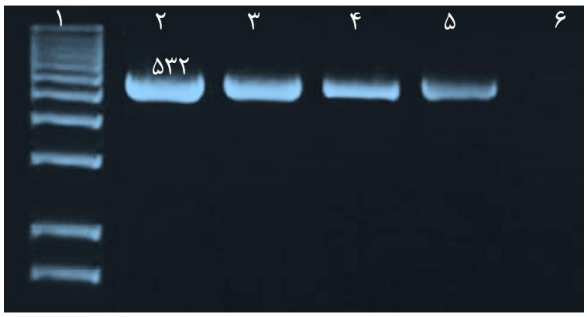

Figure 1. Electrophoresis of PCR product of *mecA* gene
1: 100 bp marker, 2: positive control, 3-5: *mecA* gene isolates, 6: negative control

(18.18%) wound samples, 9 (8.18%) blood samples and 5 (4.54%) sputum samples. Comparison of PCR results with disk diffusion method showed that 99 isolates (90%) also had oxacillin disk resistant *mecA* gene. Antibiogram results of antibiotics showed that the highest resistance to penicillin is 100% and erythromycin is 83.63% (***Figure 2***). There was no statistically significant difference between the site of infection and resistance to antibiotics (p> 0.05).

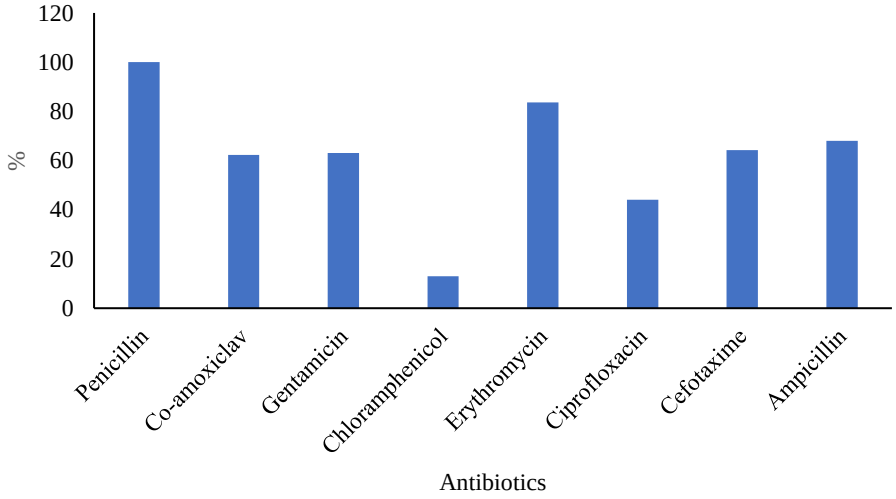

Figure 2. Antibiotic resistance of *S. aureus* isolated in disc diffusion test

Discussion

Antibiotic resistance has become a global problem, with drug inefficiencies for a variety of infections increasing mortality and costly health care costs. That is why researchers around the world are looking for an effective solution to this problem. Staphylococci are one of the groups of bacteria that have shown high resistance to antibiotics in recent years, and one of the most important reasons for this resistance is the inappropriate use of antibiotics. In this study, the pattern of antibiotic resistance and the frequency of *mec A* gene in *S. aureus* isolates were investigated by PCR. In the present study, 55.84% of the samples were resistant to methicillin. This amount is similar to the study of Peerayeh et al. (20) and less than the study of Jafari-Sales (21) and more than the study of Stanley et al. (22). Also, the highest resistance to penicillin (100%) and erythromycin (83.63%) and on the other hand, resistance to other antibiotics in the study area is increasing rapidly. In Jafari-sales study, out of 100 samples, 75 samples were resistant to methicillin, which, as in the present article, had the highest antibiotic resistance in MRSA strains to penicillin (100%) (21). In the study of Rahimpour Hesari et al., Out of 50 isolates of *S. aureus*, 34 were resistant to methicillin and the highest antibiotic resistance was related to penicillin (98%) and the lowest to vancomycin (0%) followed by chloramphenicol (8%) (23). In the study of Mahdiyoun et al., 174 isolates of MRSA were identified, with the highest resistance to erythromycin (85.1%), followed by clindamycin (77.6%) and the lowest resistance to co-trimoxazole (24.7%). Also, all

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isolates were sensitive to vancomycin. In this study, using PCR technique, all isolates were examined for *mec A* gene and finally all isolates had *mecA* gene (24). In the study of Nourbakhsh et al., from 110 isolates of *S. aureus*, the highest antibiotic resistance was reported to methicillin (90.2%) and then ciprofloxacin (89.5%). Also, according to the data of this study, there is a significant relationship in terms of the prevalence of antibiotic resistance with gender and age, so that the highest percentage of patients is in the age range of 61-70 (19). However, in the present article, there was no statistically significant difference between age and gender. In the study conducted by Jafari-Sales, there was no statistically significant difference between age group and gender (21). The results of this study showed that 110 samples (28.57%) had *mecA* gene, which is approximately equal to (25) with the study of Kadkhoda et al. (30%) and less than the study of Mohammad Jani et al. (35%) (26), Shokri et al. (57.77%) (27), Jafari et al. (68%) (21). In a study by Zamani et al., Out of 70 isolates of *S. aureus*, 35 (50%) showed the methicillin resistance gene, but in the study of antibiotic resistance pattern by disk diffusion agar method, only 22 cases (31.4%) showed methicillin resistance (28). The results of Askari et al. study showed that 50.9% isolates were resistant to methicillin by disk diffusion method and (58.8%) by PCR method (*mec A* gene) (29). In the present study, comparing the results of PCR and disk diffusion, it was found that out of 100 isolates with *mecA* gene, 99 isolates were also resistant to oxacillin disk. This is consistent with the findings of Koupahi et al. (30) and stanly et al. (22).

Conclusion

The results of this study indicate an increase in the resistance of MRSA strains to various antibiotics. Resistance to other antibiotics is also increasing in the study area. As a result, this study identified the correct and timely identification of MRSA strains as well as the non-use of inappropriate antibiotics and the administration of appropriate antibiotics according to the pattern of antibiotic resistance of *S. aureus*, to prevent resistance to other antibiotics.

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