Identification of Antibiotic Resistance Patterns of Methicillin-Resistant Staphylococcus Aureus Isolates from Patients in Selected Hospitals in Isfahan

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ABSTRACT

Background and Objective: Staphylococcus aureus is one of the important factors causing nosocomial infections. The emergence of antibiotic-resistant Staphylococcus strains to Methicillin and other antibiotics has brought about several problems in treatment of the infections caused by Staphylococcus strains. The aim of this study is to identify antibiotic resistance patterns of Methicillin-resistant Staphylococcus aureus isolates from patients in selected hospitals in Isfahan.

Materials and Methods: In this descriptive cross-sectional study, a total of 300 samples were isolated from hospital patients in Isfahan. Clinical strains were investigated phenotypical characterization like Gram stain, catalase, coagulase and carbohydrate fermentation to identify SA, then disk diffusion test was performed on SA based on CLSI to isolate MRSA, in addition antibiotic sensibility pattern obtained using Oxacillin, Tetracycline, Clindamycin, Rifampin, Ampicillin, Ciprofloxacin, Gentamicin, Cotrimoxazole, Vancomycin. The PCR was performed for the detection of the mecA gene in all the MRSA isolates.

Results: In this study, 210 (70%) samples in a total of 300 isolates of Staphylococcus aureus were Methicillin-resistant. Evaluation and assessment of antibiotic resistance in MRSA isolates showed the greatest resistance to Oxacillin (100%), Tetracycline (97%), Clindamycin (92%), Rifampin (75%), Ampicillin (70%), respectively, while the lowest levels of resistance were observed to antibiotics Ciprofloxacin (61%), Gentamicin (50%), Cotrimoxazole (34%), and Vancomycin (0%), respectively. The implication of this high resistance is that Methicillin-resistant Staphylococcus aureus infections should be treated with more precaution and not with Penicillin and other ineffective antibiotics. The presence of mecA gene in all isolates was confirmed by PCR.

Conclusion: In this study, the spectrum of antibiotic resistance in MRSA isolates is similar to other studies. The Effectiveness of Tetracycline, Rifampin and Clindamycin is still very low on MRSA samples.

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Introduction:

Staphylococcus aureus is one of the main pathogens causing community and hospital acquired infections. The bacteria are often found on the skin and mucous membranes, and also are the most important type of Micrococcaceae families in terms of morbidity. These bacteria can cause a wide range diseases from soft tissue infections, abscesses, septic arthritis, necrotizing fasciitis to the incidence of food poisoning in person.(1)

Emergence of antibiotic resistance in these bacteria has begun since 1941. The first strains of Methicillin-resistant Staphylococcus aureus appeared in England in 1961. The strains of Methicillin-resistant Staphylococcus aureus (MRSA) get
resistant to Methicillin through the acquisition of mecA gene. This gene is transmitted by Staphylococcal cassette chromosome (SCCmec). Acquiring this gene, MRSA strains are capable to code a new protein called PBP2a. This new protein is less likely to bind to beta-lactam antibiotics. (2) 25% to 50% of healthy people are carriers of Staphylococcus aureus. (3) Some people have this bacterium in their nose and these play a significant role in the spread of bacteria into the environment. Prevention of infection can reduce antibiotic resistance. Removing bacteria from nasal carriers, especially hospital workers, will reduce the spread of large amounts of bacteria in the environment. (4) Now the emergence of MRSA strains is increasing all around the world. Using of newer antibiotics, the incidence of new resistance cases of these drugs is observed. On the other hand the emergence of strains resistant to Vancomycin (VRSA), increases the risk of severe infections by Staphylococcus aureus. The emergence of multidrug-resistant strains of Staphylococcus aureus increases the need for continuous antibiotic resistance review. (5) In this study, 300 Staphylococcus aureus isolates that were collected in 2012 among two hospitals of choice in Isfahan have been studied in terms of Methicillin resistance and other antibiotic resistance.

Methods

300 samples of S. aureus were collected through Shariati and Sadooghi hospitals during 2012. Samples included of bacteriemia 24.5 % (74 samples), urine samples 36% (108 samples), samples from abscesses and wounds 21.4% (64 samples) and samples from the lungs 18.1% (54 samples). Samples were transferred to the laboratory on Blood agar medium. After the tests, to preserve samples for a longer period of time, the Brain Heart Infusion Broth medium containing 15% glycerol was used. The samples were kept in -20°C. (6)

Determining susceptibility to Methicillin with Kirby-Bauer agar screening method: For detection of Methicillin-resistant strains, agar-screening method was used. Twenty-four-hour culture of bacteria were used for this test. According to the CLSI standard rule concentration of 8 mg/ml Oxacillin have been considered as a criterion for identification of Methicillin-resistant strains in the Agar Dilution Method. Sigma Oxacillin powder (Australia) was prepared. Required amount of Oxacillin powder calculates and was added to the Müller-Hinton Agar medium. At the end, 4% NaCl was added to the medium. (6-8)

Bacterial DNA was extracted using Phenol–Chloroform Method: 500 ml physiologic serum was poured into a 1.5 ml microtube and bacteria which grown on 24-hour in Brain Heart Infusion Agar Medium, is transferred to it. Centrifuging microtube for 5 minutes, at a speed of 8000 RPM. Discarded supernatant liquid and adding 500 ml Lysis Buffer to the Microtube. Heating microtube in water bath for 20 minutes at 100° C, Then 500 ml of phenol added to microtube and centrifuged for 20 minutes at 12,000 RPM. The supernatant liquid is transferred to a new microtube. 500 ml of Chloroform added to and centrifuged for 15 minutes at 8000 RPM. The supernatant liquid is transferred to a new microtube. One milliliter of cold 96% ethanol and 150 ml of three molar potassium chloride solution are added to Microtube. Centrifuging microtube for 20 minutes, at 12000 RPM, and at 4° C. Finally the DNA is precipitated at the bottom of the Microtube. The supernatant throw it away. 500 ml microtube and add 70% alcohol for 5 minutes at a speed of 14,000 RPM at 4° C, centrifuged. Throwing alcohol away. Repeating this step three times. After removing the alcohol in the last stage, heat the Microtube using a Hot Plate for 30 min at 45° C. After completing this step, DNA is visible as a white spot on the bottom of the microtube. Next, 50 ml of distilled water was added to microtube and heating for 20 minutes at 65° C so far the DNA be solved in distilled water. Then keep the microtube at -20° C. (7)

Performing PCR tests to detect the mecA gene: For the detection of the mecA gene, two primers F and R were used with the sequences 5’ TGGCTATCGTGCACATCG 3’ and 5’ CTGGAACTTGGTGGACAGAG 3’, respectively. A compound used for PCR contained 0.5 microliter of each primers, 2.5 ml 10X buffer, 0.4 microliter DNTP, 0.6 microliter MgCl2, 0.3 microliter Taq polymerase enzyme, 5 microliters of each sample DNA the final volume with distilled water reached up to 25 ml. (1) The thermocycler machine program has started with a step of 5 min at 94° C for initial melting stage, then 35 cycles consisting of 15 seconds at 94° C for melting stage, 15 seconds at 58° to stage annealing and 20 seconds at 72° C for an extension period continued. A final extension step of 5 min and a temperature of 72° C was also considered. (1) Product length was 310 base pairs and to view the product we used electrophoresis techniques with a concentration of 1% agarose gel.

Measuring antibiotic resistance through Kirby-Bauer disk diffusion method: Bacterial suspensions were prepared by twenty-four hour bacteria culture to be used in this test. Bacterial concentration was prepared equivalent to 0.5 McFarland and was inoculated on Müller-Hinton agar medium. The Antibiogram discs, Ciprofloxacin, Cotrimoxazole, Tetracycline, Ampicillin, Vancomycin, Gentamicin, Oxacillin, Clindamycin, Rifampin, produced by Hi Media Company (America), were used for this test. Disks were qualified through the strain ATCC 25923 standard control. Then were incubated at 35° C for 24 hours, and the results measured under manufacturer standard. (2)
Results:

Susceptibility determination to Oxacillin by mean of agar screen method: Using this method, it was found that 210 strains out of 300 strains isolated in this study are resistant to a minimum concentration of 8 micrograms per liter to Oxacillin.

Results of PCR tests for detection of gene mecA: 210 isolates were resistant to Oxacillin in terms of having the gene mecA; they were examined by PCR method. All the strains were mecA positive.

The results of antibiotic resistance measurement through the Kirby-Bauer disk diffusion method: using this method to assess antibiotic resistance in Methicillin-resistant isolates to another antibiotic. Evaluation of antibiotic-resistant isolates indicates which the most of the isolates resist to antibiotics Oxacillin (100%), Tetracycline (97%), Clindamycin (92%), Rifampin (75%), Ampicillin (70%) and antibiotics Ciprofloxacin (61%), Gentamicin (50%), Cotrimoxazole (24%) and Vancomycin (0%), with less resistance.

Discussion

Staphylococcus aureus is one of the most important human pathogen. These bacteria are turning to be a major cause of nosocomial infections all around the world. This bacterium causes a broad spectrum of diseases, ranging from superficial skin infections to severe invasive infections, including septicemia, pneumonia, endocarditis and deep skin abscess. The death rate can increase up to 35%. (9)

Bacterial virulence factors enable bacteria to evade the host immune system and can have toxic effects on the host. These factors include cell surface components (protein A and proteins bind to collagen) and various external proteins. Before the use of penicillin to treat infections caused by the bacteria Staphylococcus aureus, septicemia caused a lot of deaths in patients. Penicillin-resistant strains of Staphylococcus aureus (PRSA) has emerged after the use of antibiotics in the 1950s and 1960s. (1)

From then on the treatment of infections caused by Staphylococcus aureus was done by beta-lactamase-resistant penicillins such as Methicillin and Oxacillin. In these bacteria, transfer of mecA gene in Methicillin-sensitive strains by Staphylococal Cassette Chromosome (SCCmec), resulting in the production of an altered penicillin-binding protein called PBP2a. PBP2a production causes resistance to Methicillin and Oxacillin, and the emergence of MRSA strains since the 1980s. (6)

Methicillin-resistant Staphylococcus aureus strains isolated from hospital (HA-MRSA) and community (CA-MRSA) are different either in source of isolated or the SCCmec fragment length and patterns of resistance to other antibiotics. Primarily in community MRSA strains isolates from skin infections, while in the hospital, mostly it causes pneumonia, bacteremia and wound infections after surgery. (2)

Due to the prevalence of strains resistant to Methicillin and other antibiotics on the one hand, and the emergence of Vancomycin-resistant strains of Staphylococcus aureus (VRSA) on the other hand, ongoing control of Staphylococcus aureus resistance to various antibiotics always be treasured.

Thung and colleagues conducted a study in 2009, the status of antibiotic resistance in MRSA strains isolated from a teaching hospital in Malaysia, were as follows: 100% resistance to penicillin and Oxacillin, resistance to Cotrimoxazole 73%, 47% resistance to Tetracycline, Erythromycin resistance was 92%, 76% resistance to Gentamicin, Ciprofloxacin resistance by 94%, resistance to Rifampin 12%, 18% resistance to Clindamycin. This study was performed on 66 isolates of MRSA that did not show any resistance to Vancomycin. (10)

In another study in 2010 by Ionescu and colleagues have been done on Romania, 36% of strains have been MRSA. This study was performed on 56 isolates of MRSA that resisted to all antibiotics beta-lactam group. Resistance to Tetracycline 99%, 50% to Erythromycin, Rifampin 69%, 63% to Gentamicin, Ciprofloxacin 58% and 5% resistance to Cotrimoxazole has been reported. None of the strains have shown resistance to Clindamycin and Vancomycin. (11)

In this study, 300 samples were examined, including a sample of blood 24.5% (74 samples), urine samples, 36% (108 samples), samples from abscesses and wounds 21.4% (64 samples) and samples of lung 18.1% (54 samples). 210 isolates (70%) out of 300 cases of Staphylococcus aureus was identified resistant to Methicillin. In this study analysis of antibiotic resistance of MRSA isolates showed the greatest resistance to antibiotics Oxacillin (100%), Tetracycline (97%), Clindamycin (92%), Rifampin (75%), and Ampicillin (70%). While, there is less resistance to antibiotics Ciprofloxacin (61%), Gentamicin (50%), Cotrimoxazole (34%) and Vancomycin (0%).

In this study, three methods were used to confirm Methicillin-resistant strains. Agar screening method is suggested as the best phenotypic methods for the detection of MRSA strains by CLSI guidelines. PCR method was used to identify gene mecA, which has a major role in resistance to Methicillin. Disk diffusion method was used for the detection of Oxacillin resistance.

Kaleen and colleagues in a study in 2010 on 139 strains of MRSA in Pakistan used Oxacillin disk diffusion method for measuring antibiotic resistance. The results were as follows: 6% of the strains were resistant to Cotrimoxazole, 93% of the strains were resistant to Chloramphenicol, and Tetracycline-resistant strains 64%, Fluoroquinolone 38% and 22% resistant to macrolide-resistant. Results of this study demonstrate the similarity of antibiotic resistance in MRSA strains in Iran and Pakistan. (12)

In a survey by Japoni and colleagues at Namazi Hospital has been done on 356 strains of Staphylococcus aureus, 156 Methicillin-resistant strains have been identified. To assess the antibiotic resistance of MRSA strains to different antibiotics by dilution and
E-test MIC assay methods used. In this study, 6.9% of the strains resistant to Rifampin, 8.64% of strains were resistant to Cotrimoxazole, 3.67% of strains were resistant to Clindamycin, 8.78% of strains were resistant to Tetracycline, 2.76% of strains were resistant to Ciprofloxacin and 91% of strains were resistant to Gentamicin.(13)

Rezazadeh and colleagues in a study of 80 strains of MRSA have done in Arak. 5.80% of strains were resistant to Tetracycline and 7.85% of the strains were resistant to Ciprofloxacin. (9)

In a study by Rahimi et al, 396 Methicillin-resistant S.aureus isolates collected from hospitals in Tehran and in terms of resistance to other antibiotics have been studied. In this study, the method for identifying MRSA strains Dilution agar, E-test and PCR for mecA gene identification is used. In this study, 100% of strains were resistant to Oxacillin, 77% of strains were resistant to Clindamycin, 79% of the isolates were resistant to Tetracycline, 92% of strains were resistant to Ciprofloxacin, 51% of strains were resistant to Cotrimoxazole, and 60% of strains were resistant to Gentamicin. (3)

Conclusion:

Reduced susceptibility of Staphylococcus aureus strains to antibiotics, increasing need to introduce appropriate treatment protocols for the treatment of infections caused by bacteria. In a study by Jain and colleagues in 2014 has been done in America. With the implementation of appropriate protocols, transfer rate of MRSA strains in ICU in seven years has decreased by 85%. (14) Using this protocol, the control, management and measurement of infection in different hospitals and clinics can reduce the rate of drug resistance in bacteria.

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