ISMB TUMS

Frequency and Antimicrobial Resistance Patterns of Methicillin-Resistant *Staphylococcus aureus* in Tehran

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ARTICLE INFO

Article type:
Original Article

Article history:
Received: 13 Aug 2013
Revised: 04 Sep 2013
Accepted: 28 Oct 2013

Keywords:
Methicillin-Resistant *Staphylococcus aureus*
Microbial Sensitivity Tests
Vancomycin

ABSTRACT

Background: Methicillin-Resistant *Staphylococcus aureus* (MRSA) is the one of most commonly isolated organisms from clinical samples which can cause life-threatening infections. The emergence and spread of antibiotic resistance makes the treatment of these infections more complicated. In this study, we aimed to determine the patterns of antibiotic resistance among MRSA isolates from Tehran, Iran.

Methods: From December 2012 to April 2014, 120 clinical samples were collected. MRSA was identified by cefoxitin disc diffusion. Antimicrobial susceptibility testing was performed on MRSA isolates for eight other antibiotics by disc diffusion method according to CLSI (2013) recommendations. Also, the minimum inhibitory concentration (MIC) was determined for vancomycin by MIC test strips.

Results: According to disc diffusion, 60 (50%) isolates showed resistance to cefoxitin. Among these isolates, the rate of resistance to nitrofurantoin, vancomycin, teicoplanin, doxycycline, trimethoprim, erythromycin, clindamycin, and ciprofloxacin were 0%, 0%, 0%, 28.3%, 28.3%, 58.3%, 63.3%, and 70%, respectively. All isolates were susceptible to vancomycin according to disc diffusion and MIC.

Conclusion: Compared to other reports from Iran, our study indicated a moderate rate for MRSA. However, the rates of resistance to generally prescribed antibiotics in these isolates were high. In this situation, it is recommended to monitor the antibiotic resistance in these hospitals.

- ***Please cite this paper as:*** Hassanzadeh S, Pourmand MR, Hadadi A, Nourijeylani K, Yousefi M, Mashhadi R, Askari E. Frequency and Antimicrobial Resistance Patterns of Methicillin-Resistant *Staphylococcus aureus* in Tehran. *J Med Bacteriol.* 2013; **2** (3, 4): pp. 41-46.

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Introduction

Staphylococcus aureus is a major human pathogen responsible for a range of infections from mild folliculitis to more severe, life-threatening septicemia (1). It is also carried persistently or intermittently by at least half of the human population (2). About 1% of all admitted patients are infected with this pathogen in the U.S. hospitals (3). Patients admitted to high risk wards such as ICU, burn unit, surgical post-operative and dermatology wards are more likely to be infected (4).

Treatment of infections caused by this organism has become difficult due to increase in resistance to methicillin (5). Methicillin-Resistant *Staphylococcus aureus* (MRSA) has spread both hospital and community settings (6). MRSA strains usually express an altered penicillin binding protein (PBP_{2a}) encoded by the *mecA* gene located in the chromosome (6, 7). MRSA is well-known for its widespread resistance to antibiotics. They are not only resistant to penicillins and cephalosporins but also often demonstrate resistance to a wide range of antibiotics that are generally used in hospitals (8, 9).

In many areas the rates of MRSA isolates have been reported about 50-73% (7, 10-12). Therefore, it is necessary to know the prevalence of MRSA for choosing appropriate antibiotic therapy. Recently, many studies have reported an increase in the prevalence of multidrug-resistant MRSA with rates ranging from 30-80% (13, 14). The present study indicates frequency and antimicrobial resistance patterns of MRSA in Tehran, Iran.

Material and Methods

Isolation and identification of S. aureus

120 *S. aureus* isolates were collected from clinical samples of three hospitals affiliated with Tehran University of Medical Sciences (Shariati, Sina and Emam Khomeini hospitals). Isolates were obtained from different clinical specimens including blood culture, wound swabs, urine and sputum. Samples were identified and confirmed by Gram staining and conventional biochemical tests (15).

Antibiotic susceptibility testing

Methicillin resistance isolates were recognized by Kirby-Bauer disc diffusion method using cefoxitin (30 µg, MAST) discs in Müeller-Hinton agar (MHA) supplemented with 2% NaCl. For MRSA isolates, the antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method against these antibiotics: clindamycin (2 µg), ciprofloxacin (5 µg), erythromycin (15 µg), trimethoprim (30 µg), vancomycin (30 µg), teicoplanin (30 µg), doxycycline (30 µg), and nitrofurantoin (300 µg). All antibiotic discs were purchased from MAST Company (United Kingdom). Suspension equivalent to 0.5 McFarland were cultured on MHA then incubated at 35°C for 18-24 h. The zone inhibition was measured and interpreted according to clinical and laboratory standards institute (CLSI) guidelines (16).

Determination of Minimum Inhibitory Concentration (MIC) for Vancomycin

MIC test strips (Liofilchem, Italy) were used to determine the lowest concentration of vancomycin required to inhibit the growth

of MRSA. Suspension equivalent to 0.5 McFarland were cultured on MHA then incubated at 35°C for 18-24 h. Reading was done by two researchers according to manufacturer's instructions (16).

S. aureus ATCC 8325-4 was used as control methicillin-sensitive strain and *S. aureus* COL as control a methicillin-resistant strain and provided from urology research center.

Results

By cefoxitin disc diffusion method, 60 (50%) isolates were found to be MRSA. All MRSA strains were highly resistant to ciprofloxacin (70%), clindamycin (63.3%), and erythromycin (58.3%). These isolates were moderately resistant to doxycycline (28.3%), trimethoprim (28.3%), and teicoplanin (16.7% as intermediate resistance). All (100%) the strains were sensitive to vancomycin and nitrofurantoin. The details of antimicrobial susceptibility pattern are shown in Table 1.

Also all isolates were susceptible to vancomycin according to MIC results (MIC50 and MIC90 were 0.5 and 0.75 µg/dL, respectively) *(Table 2).*

Table 1. Results of antimicrobial susceptibility testing on MRSA isolates (n = 60) in this study

Antibiotic	Resistant (%)	Intermediate (%)	Susceptible (%)
Vancomycin	0 (0)	0 (0)	60 (100)
Nitrofurantoin	0 (0)	0 (0)	60 (100)
Teicoplanin	0 (0)	10 (16.7)	50 (83.3)
Trimethoprim	17 (28.3)	1 (1.7)	42 (70)
Doxycycline	17 (28.3)	26 (43.3)	17 (28.3)
Erythromycin	35 (58.3)	0 (0)	25 (41.7)
Clindamycin	38 (63.3)	1 (1.7)	21 (35)
Ciprofloxacin	42 (70)	6 (10)	12 (20)

MRSA: Methicillin-Resistant *Staphylococcus aureus*

Table 2. MIC of vancomycin for MRSA isolates in this study

MIC (µg/dL)	No. of isolates (%)
0.125	17 (28.3)
0.25	10 (16.6)
0.5	15 (25)
0.75	12 (20)
1	6 (10)

MRSA: Methicillin-Resistant *Staphylococcus aureus*; MIC: Minimum Inhibitory Concentration

Discussion

During the past five decades, MRSA has spread worldwide. For example, the reported rate of MRSA in the United States, Portugal, Italy and India is 25-50%, 54%, 43-58% and 40%, respectively (8, 17). In this study, 50% of the isolates were MRSA which is in the range of other reports from Tehran (28.88-90%, mean: 52.7%) (11, 18-21).

Due to alternating pattern of antibiotic resistance in *S. aureus*, it suggested to conduct periodical surveillance of antimicrobial resistance every 3 to 4 years (19, 20). In the current study, we found a high rate of resistance to ciprofloxacin, clindamycin and erythromycin. In the study conducted by Rahimi *et al*, the rate of resistance to methicillin, ciprofloxacin, erythromycin, clindamycin, nitrofurantoin and vancomycin

was 30%, 95%, 93%, 75%, 2% and 0%, respectively (22). In the study of Farhadian A *et al*, resistance rate for methicillin, erythromycin, clindamycin, ciprofloxacin and vancomycin was 46%, 42%, 33%, 29% and 0%, respectively (23). Also, Moghadam *et al*. reported resistance rate for oxacillin, ciprofloxacin, clindamycin, erythromycin and vancomycin as 50%, 44.5%, 33.7%, 35% and 0%, respectively (12). Compared to these studies, our findings suggested vancomycin and nitrofurantoin as the most effective antibiotics against MRSA isolates. This can be partially explained by low consumption of these antibiotics in the hospitals of this study (22).

Vancomycin previously considered the drug of choice for treatment of MRSA infections. The appearance and spread of vancomycin resistance is a real public health threat (24). Previously, some studies in Iran reported a high prevalence (7%) of vancomycin-resistant *S. aureus* (VRSA) (25). However, more reliable diagnostic methods are required for detection of VRSA in laboratories as recommended by CLSI. The standard methods for MIC detection should be used for screening of vancomycin resistance (16). The number of VRSA reported worldwide and in Iran seems to be limited (24). However, recent emergence of VRSA in the community is a serious public health concern (26).

In conclusion, compared to other reports from Iran, our study indicated a moderate rate for MRSA. However, the rates of resistance to generally prescribed antibiotics in these isolates were high. In this situation, precise and continuous surveillance is recommended to monitor the antibiotic resistance in these hospitals.

Acknowledgment

None declared.

Conflict of Interest

None declared conflicts of interest.

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