Int J BioMed Public Health. 2018; 1(2):71-75
10.22631/ijbmph.2018.116735.1014

International Journal
of
BioMedicine and Public Health
http://www.ijbmph.com

Original Article

Study of antibiotic resistance pattern in methicillin-resistant *Staphylococcus aureus* isolated from clinical samples of hospitals in Tabriz – Iran

Abolfazl Jafari-Sales[1]*, Farhad Farhadi[2], Mehdi Ezdiyadi[2], Davood Tarbiat-Nazloo[1]

ARTICLE INFO

Article History:
Received 25 January 2018
Revised 5 February 2018
Accepted 6 February 2018
Published online 27 April 2018

Keywords:
Staphylococcus aureus;
Antibiotic resistance;
Methicillin-resistant

[1]*Department of Microbiology, Kazeroon branch, Islamic Azad University, Kazeroon, Iran;*
[2]*Department of Research and Development, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran.*
Correspondence:
Abolfazl Jafari-Sales. Department of Microbiology, Kazeroon branch, Islamic Azad University, Kazeroon, Iran
a.jafari_1392@yahoo.com

ABSTRACT

Introduction: Methicillin-resistant *Staphylococcus aureus (S. aureus)* is commonly a cause of nosocomial infections. The increase in infection rates caused by this bacteria in developing countries has led to many problems. The aim of this study is to determine the antibiotic resistance pattern in methicillin-resistant *S. aureus* strains isolated from clinical specimens.
Methods: In this cross-sectional study, 256 isolates of *S. aureus* were collected from Tabriz hospitals and treatment centers. The isolates were identified by standard laboratory methods and cultured in a specific environment. Identification of Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains was made through phenotypic method. In order to evaluate antibiotic susceptibility patterns of strains, a disk diffusion method based on CLSI protocol was also performed. Data was analyzed by Chi-square test and SPSS 16 software.
Results: Out of 256 examined samples, 197 (76.95%) of them were methicillin-resistant *Staphylococcus aureus*. The phenotypic evaluation of the antibiotic resistance pattern of methicillin-resistant *S. aureus* showed that the highest resistance was for 100% penicillin antibiotics, 94.22% co-amoxiclav and 81.22% gentamicin antibiotics and the lowest resistance was observed as chloramphenicol (16.75%). There was no significant relationship between age, sex, and MRSA infections (P> 0.05).
Conclusion: High resistance *S. aureus* to penicillin, co-amoxiclav, gentamicin and also the high frequency of isolation of MRSA of hospital studied samples are remarkable. The present study demonstrates the need for continuous monitoring of antimicrobial susceptibility in *S. aureus* in order to determine the optimal drug regimens.

Introduction

Staphylococcus species are easily grown in many culture and are also metabolically active. *S. aureus* has become one of the most important health problems worldwide due to the potential of pathogenicity and increasing resistance to antimicrobials. These bacteria is resistant to penicillin by producing beta-lactamase enzymes. Resistance to methicillin, indicating resistance to all penicillinase-resistant penicillins and cephalosporins (1,2). This opportunistic pathogen expresses a variety of virulence factors, such as adhesions, enzymes, and toxins. In the early 1940s and before the administration of penicillin, for the treatment of Staphylococcal infections, mortality from *S. aureus* infection was 80% after two years of using penicillin, the first case of resistance was observed in the hospital and subsequently penicillin resistance was gradually developed in the community, as in 1960, about 80% of *S. aureus* strains were resistant to penicillin. This bacteria is

one of the main causes of hospital infections worldwide, which is easily resistant to many common antibiotics. Infections caused by these bacteria, despite antibiotic treatment, have severe complications, so prevention of infections caused by this bacterium and the rooting of its distribution centers in hospitals is essential. *S. aureus* causes a wide range of diseases including endocarditis, osteomyelitis, pneumonia, and toxic shock syndrome. regarding to the above, the increased resistance of staphylococci to other antibiotics such as erythromycin, tetracycline and even strains of vancomycin-intermediate *S. aureus* (VISA) or vancomycin-resistant *S. aureus* (VRSA) caused continuous effort to find new antimicrobials (3,4).

S. aureus, the second leading cause of nosocomial infections after *Escherichia coli*, has therefore undergone significant changes in the pattern of antibiotic susceptibility over the past years (5). Methicillin-resistant *S. aureus* (MRSA) is one of the most threatening microorganisms for global human health (6-7). The first one was in England in 1961, after the detection of *S. aureus* infection and the resistance of this bacterium to the treatment and the MRSA strains were also defined (8). Factors such as long-term hospital stay, unusual use of antibiotics, non-compliance with individual and collective health, failure to know how to use antibiotics before coming to the hospital can be the prerequisites for the emergence of MRSA. Today, the only selected antibiotic for treat infections caused by MRSA strains is glycopeptide antibiotics such as vancomycin, of course, studies have shown that the resistance to vancomycin is low at the low levels due to the acquisition of the van gene from the enterococci and also from the change in the structure of the walls of these bacteria (9). Recent studies have estimated that 8-12% of hospitalized patients suffer from side effects of infection. Identification and treatment of colonized individuals can reduce the incidence of MRSA (10). The transmission of this strain is usually through direct contact and often through hands contacts. Infectious wastes are active and people remain infectious for a long time (11). The first stage in the pathogenesis of *S. aureus* is colonization. Asymptomatic colonized individuals are a source of human illness (12). The purpose of this study is to identify and determine the antibiotic resistance pattern of methicillin-resistant isolates from patients of hospitals and treatment centers of Tabriz during 2016-2017.

Methods

In this cross-sectional study, 256 isolates of *S. aureus* from outpatients and admissions wards of Tabriz hospitals and treatment centers were collected during a span of October 2016 to October 2017. Samples included blood, urine and other specimens (phlegm and abscess). A questionnaire was also prepared for all patients and the required information (age, sex, location of infection) was recorded according to ethical charter. Initially, the strains of *S. aureus* were identified using biochemical tests (Gram stain, catalase, coagulase slide, tubular coagulase and DNase, and mannitol fermentation on Mannitol Salt Agar (MSA). Sensitivity test was performed by Kirby-Bauer disk diffusion method (2,5) to obtain resistance to methicillin and antimicrobial susceptibility and antibiotic resistance patterns of isolated samples from patients. Antibiotic disks used in this test include: penicillin (10 µg), co-amoxiclav (10 µg), erythromycin (15 µg), gentamycin (10 µg), chloramphenicol (30 µg), cefotaxime (30 µg), vancomycin (30 µg), oxacillin (1 µg) was the Iranian Padtan Teb Medicine Company. For this purpose, the disks were sterilized with proper distance from each other on the Muller Hinton Agar medium and then incubated for 24 hours at 37 ° C. Then the diameter of the inhibition zone of bacterial growth was measured and compared with the standard table. Finally, the results were expressed as sensitive, semi-sensitive, and resistant (13) based on the diameter of the growth halo around the discs. Plates were incubated within 15 minutes after applying the disks at 37° C for 24 hours. The diameter of the growth inhibition zones around each disk was also measured to the nearest whole mm using sliding scale. An inhibition zone diameter of ≤ 10 mm around the oxacillin disc was considered as resistance; 11–12 mm indicated intermediate, and ≥ 13 mm was considered sensitive (14-15). The resistance of the strains to oxacillin actually represents the resistance to beta-lactam antibiotics, such as penicillins, methicillin and cephalosporin.

Table 1. Antibiotic resistance of *S. aureus* isolates by type of sample

Antibiotics	Clinical samples				Total
	Urine	Wound	Blood cultures	Others	
Penicillin	118 (100%)	55 (100 %)	22 (100%)	2 (100 %)	197 (100 %)
Gentamicin	111 (94.07%)	37 (67.28 %)	10 (45.45%)	2 (100 %)	160 (81.22 %)
Co-amoxiclav	109 (92.37%)	55 (100 %)	22 (100%)	1(50 %)	187 (94.92 %)
Chloramphenicol	12 (10.17%)	14(25.45 %)	5 (22.73%)	2(100 %)	33 (16.75 %)
Erythromycin	80 (67.80%)	41(74.54 %)	15 (68.18%)	0(0 %)	136 (69.06 %)
Cefotaxime	87 (73.72%)	39(70.90 %)	18 (81.81%)	2(100 %)	146 (74.11 %)

S.aureus ATCC 33592 (MRSA) was used as a standard strain. The results were analyzed using SPSS (version 16) and chi square test. In all cases, P <0.05 was considered significant.

Results

Of the 256 samples of *S. aureus*, 168 (65.63%) belonged to men and 88 (34.37%) belong to women. 141 (55.08%) samples from the urine, 69 (26.96%) samples from the wound, 39 (15.23%) samples of blood and 7 (2.73%) were isolated from other specimens (Sputum and Abscesses). 186 (72.66%) of samples were collected from hospitalized sector and 70 (27.34%) of them were collected from outpatients .The mean age of patients was 37.4±27 and variation range was between 10 months to 75 years old. Of the 256 samples examined, 197 (76.95%) were methicillin resistant. There was no significant difference in the distribution of MRSA between age groups (P>0.05). 119 (60.4%) of men and 78 (39.6%) of women were methicillin-resistant. There was no significant difference in the distribution of MRSA between male and female groups (P>0.05). 118 samples (59.89) were samples of urine, 55 (27.92%) samples from the wound, 22 (11.17%) samples from the blood and 2 (1.02%) samples from other samples (Sputum and Abscesses) were resistant to methicillin. No statistically significant differences were found regarding frequency of MRSA in Sample type (P>0.05). The highest resistance to penicillin is 100%, co-amoxiclav is 94.92% and gentamicin is 81.22% (Table 1). All isolates of MRSA (100%) were sensitive to vancomycin. There was no statistically significant difference between the place of infection and resistance to antibiotics (P>0.05).

Discussion

In this study, the resistance rate of *S. aureus* was high in comparison to penicillin, co-amoxiclav and gentamicin resistance. In this study, 76.95% of isolated *S. aureus* specimens were resistant to methicillin, which was more than that reported by Zamani *et al.* (16), Moradi *et al.* (17), Javani *et al.* (18), Haghgoo *et al.* (17), Wang *et al.* (20), Moosavian *et al.* (21). In some studies, in Saudi Arabia and Kuwait, this value was reported to be 33% and 32% respectively (22-23). Rezazadeh *et al.* showed that 80 samples in sum of 100 isolates of *S. aureus* were methicillin-resistant. The highest rate of resistance was observed for penicillin antibiotics (100%), tetracycline (88.5%), levofloxacin (85.7%) and ciprofloxacin (85.7%) respectively, while the lowest levels of resistance were observed to be antibiotics chloramphenicol (5.70% respectively (24). The findings of the present study are consistent. Parhizgari and *et al.*, from 180 strains of *S. aureus* which showed that 59 strains (37.2%) were resistant to methicillin. Resistance to methicillin-resistant *S. aureus* strains including: chloramphenicol (3.38%), rifampin (45.76%), norfloxacin (89.83%), gentamicin (89.83%), ciprofloxacin, (91.52%), azithromycin, (88.13%), cotrimoxazole (86.44%) and all isolates strains were sensitive to vancomycin and nitrofurantoin (25), which is consistent with the results of this study. Askari *et al.* showed that 50.9% of the isolates of *S. aureus* were resistant to methicillin. The highest antibiotic resistance in MRSA strains was related to penicillin (96.6%), erythromycin (45%) and ciprofloxacin (36.6%) (26), The frequency of MRSA is not consistent with the results of this study. This inconsistency differs while number of samples and place of the study matters. Vaez *et al.* (2009) showed that 121 strains showed that 104 strains (85.9%) were resistant to methicillin. The prevalence of methicillin-resistant strains in urine samples and ulcers were 36.5% (38 samples) and 24.7% (25 samples) was more than other clinical

specimens. The highest rate of resistance was observed for 100% to penicillin, 97.6% to co-amoxyclav, 71.4% to cephotaxime and64.3% to Erythromycin respectively (27) which is consistent with the results of this study. Zamani *et al.* (2005) Showed 70 samples of *S. aureus*, in which 50% of the samples were MRSA. The antibiotic pattern of this methicillin-resistant *S.aureus* showed that penicillin resistance (100%) and cloxacillin (91.4%) were higher (16), these results are consistent with the present study. Moradi *et al.* (2011) reported the highest susceptibility to 96.2% vancomycin and 88.2% chloramphenicol (17). Sensitivity to vancomycin in this study was 100% which was more sensitive than Akanbi *et al.* (28) and Ghassemian *et al.* (29) studies in number. These results are also consistent with the present study. In our study, there was no significant relationship between age, sex, and MRSA infections. In the study of Mohraz *et al.*, There were no significant relationships between sex and MRSA-induced infection (30).

Conclusion

The results showed that chloramphenicol is highly effective against methicillin-resistant strains besides the prevalence of *S. aureus* strains to methicillin had a high number in this study, and the recent study indicated an increased resistance to methicillin-resistant *S. aureus* compare with other antibiotics, and it is a serious warning for the treatment of *S. aureus* infection in the region. Therefore, in order to prevent the increase of resistance to other antibiotics, it is necessary to avoid the administration of uncontrolled and unnecessary antibiotics available.

Ethical disclosure

In this study tests that threatened the health of individuals were not used.

Acknowledgements

Thanks to the assistance of the Islamic Azad University of Ahar, Iran (Microbiology Laboratory)

Authors' contribution

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding/Support

None declared

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