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Phenotypic and Genotypic Characterization of Antibiotic Resistant *Staphylococcus aureus* (MRSA) Isolates from Patients in Ahvaz city, Iran.

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ABSTRACT

There is an increasing concern about methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the community. This study aimed to evaluate the rate of *S. aureus* colonization in outpatients and inpatients as the primary endpoint, and also to study genetic and phenotypic characteristics of methicillin resistance. 230 samples of patients were collected through 2013-2014. Microbiological and biochemical tests were done to detect the methicillin-resistant *Staphylococcus aureus*. Dick Diffusion method was done to identify the antibiotic resistance of the isolates. PCR assay was used for presence of resistance *mecA* gene. Totally 50 *Staphylococcus aureus* were detected, the least resistance was found for Vancomicin (2%) and the most resistance was found for Methicillin, Penicillin and Ampicillin(100%). The resistance to Erythromycin, Ciprofloxacin, Clindamycin and Gentamicin were 48%, 34%, 34%, 34% respectively. The results of PCR showed that 49 isolates (98%) contain *mecA* gene and only 1 isolate (2%) not harbour mecA gene. In comparison between phenotypic and genotypic of the isolates revealed that phenotypic resistance to methicillin will cover by mecA gene. Our results revealed that 98% isolates harbor mecA genes and more resistant to methicillin-related mecA genes.

Keywords: methicillin resistance - Staphylococcus aureus - mecA gene-PCR

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INTRODUCTION

There is worldwide concern about the appearance and rise of bacterial resistance to commonly used antibiotics. In this regard program for monitoring resistance have been implemented in many countries [1-3]. *Staphylococcus aureus* is one the most important factor of infections in hospitals which its antibiotic resistance daily increased. This bacteria is a human and animal pathogen involved in multiple disease processes including skin and soft tissue infections, sepsis, osteomyelitis and pneumonia and wound [4]. Because of its intrinsic virulence, its ability to cause a diverse array of life-threatening infections, and its capacity to adapt to different environmental conditions, this organism constitutes a serious public health burden, not only in the hospital environment, but also in the community setting. Methicillin-resistant *Staphylococcus aureus* (MRSA) was first detected in Europe in 1961, soon after the introduction of methicillin.

Methicillin resistance is mediated by PBP-2a, a penicillin-binding protein encoded by the mecA gene that allows the organism to grow and divide in the presence of methicillin and other betalactam antibiotics. The mecA gene is located on a mobile genetic element known as the staphylococcal chromosome cassette (SCC mec) [5]. Community-acquired (CA) MRSA is most often associated with skin and soft tissue infections in young, healthy individuals with no recent health care exposure and Most CA-MRSA strains are sensitive to none beta-lactam antibiotics[6].

CA-MRSA infections have been observed with increasing frequency among patients in hospital settings, because patients who acquire CA-MRSA strains in the community may require hospitalization and subsequently transmit such strains to other hospitalized patients [7]. MRSA also can cause severe invasive diseases, such as necrotizing fasciitis, wound infections, otitis media and otitis externa, urinary tract infection and endocarditis [6].

The detection of MRSA carriers is important for the prevention and follow-up of these infections. In addition to hospital-associated MRSA infections, community-acquired infections caused by MRSA are of an increasing concern [8]. The emerging problem of MRSA colonization in food producing animals and the links with human infection have an impact both on food production and on health of people that work with animals or work in hospitals with possible risks of disease for the general population.

According to the latest reports more than 90 percent of golden *staphylococcus* strains produce beta-lactamase and therefore they are resistant to penicillin [9]. The first meticillin resistant strain of *staphylococcus aureus* was recognized in 1961 in Europe and now this organism is a high-concerned pathogen due to its increasing resistance to beta-lactame and vancomycin antibiotic groups in the world [10]. Meticillin-resistant *staphylococcus aureus* is a pathogene in causing disease and death in Iran and world [11-12]. Therefore, it seems necessary to investigate their resistance to antibiotics which are used in antibiogram test in every clinical laboratory. This study was conducted to baseline profile of antimicrobial resistance of *Staphylococcus aureus* (MRSA) isolated from patients in Ahvaz- Iran and identify the resistance gene mecA, also comparison between phenotypic and genotypic characterization of antibiotic resistant in the isolates.

MATERIAL AND METHODS

Experimental

A total of 230 clinical samples (urine, wound, abscess ...) were collected from patients in Golestan, Razi and Aria hospital of Ahvaz, during December 2013- January 2014. The questionnaire was prepared by the team containing information like sex, age, *etc*, and was filled by patients. 50 isolates were totally resistant to meticillin, penicillin and ampicillin.

Manitol Salt Agar (Merck, Germany) and DNAase agar (Merck, Germany), were used to detect *Staphylococcus aureus*. samples was cultured and incubated for 24 h at 37 ^{o}C. Morphological and biochemical identification (Gram staining, oxidase negative, Katalase positive, Coagulase positive) was used to confirm the *Staphylococcus aureus*. Antimicrobial susceptibility test was carried out by the disk diffusion method according to the recommendations reported by the Clinical and Laboratory Standard Institute (CLSI), (Table1) [13]. The presence of gene associated with resistance to Meticilin (mecA) were determined by PCR. Total DNA of the isolates were extracted using Genomic DNA purification kit (Viogene, Hungary). The isolated DNA was

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resuspended in 50 µl of Tris-EDTA (TE) buffer at *p*H 8. PCR was performed using 1 primer sets (Cinagen, Iran) described by Sambrook and et al [14], for identification antibiotic resistance gene. PCR method was performed as described previously, has showed in (Table2). PCR reactions were performed in a total volume of 25 µl, including 1.5ml $MgCl_2$, 50mM KCl, 10mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 1 µl of each dNTP (Fermentas), 1 µl primers, 0.2 µl of Taq DNA polymerase (Fermentas), and 2 µl of DNA. Amplification reactions were carried out using a DNA thermo-cycler (Bio- Rad) as follows: Five min at 94 °C, 35 cycles each consisting of 30 Second at 94 °C, 30 s at 56 °C and 1 min at 72 °C, followed by a final extension step of 5 min at 72 °C. Amplified samples were analyzed by electrophoresis in 1% agarose gel and stained by ethidium bromide. A DNA molecular weight marker with 100 bp increments (100 bp DNA ladder, Fermentas) was used as a size standard. DNA was extracted from *Staphylococcus aureus* (Obtained from Department of Microbiology, Faculty of Med, Tehran University) using Genomic DNA purification kit (Viogene, Hungary) and used as template for standard control in PCR.

Table 1: Zone size for antimicrobial disk

Antimicrobial Agent	abbreviation	contain per disk	R*	I**	S***
Penicillin	P	10 µg	≤28	------	≥29
Methicillin	ME	10 µg	≤30	------	≥32
Ciprofloxacin	Cp	5 µg	≤15	16-20	≥21
Ampicillin	AM	10 µg	≤13	14-16	≥17
Clindamycin	CC	2 µg	≤14	15-20	≥21
Erythromycin	E	15 µg	≤13	14-22	≥23
Gentamicin	GM	10 µg	≤12	13-14	≥15
Vancomycin	V	-----	≤16	4-8	≥2

Table (2): Primer sequences and expected sizes of product

Target gene	Sequence(5´→3´)	Amplicon length	Annealing T (°C)
mecA-F	AATTCCACATTG TTTGGGTCTAA	300	*56*
mecA -R	GTA GAA ATG ACT GAA CGT CCG ATA A		

RESULT AND DISCUSSION

Fiffty strains were confirmed as *Staphylococcus aureus* by biochemical and microbial tests were detected (32samples from Golestan hospital, 18samples from Aria hospital and Razi clinic). The results of antibiotic resistance tests showed that the minimal resistance was for Vacomycine (2%) and the maximal resistance was founding for Methicillin, Penicillin and Ampicillin (100%). The resistance to Erythromycin, Ciprofloxacin, Clindamycin and Gentamicin were 48%, 34%, 34%, 34% respectively (Table 3). The results of PCR test showed that 49 isolates contain *mecA* (98%) *,* Only 1 of those cases lacking the gene (2%). The comparison between phenotypic and genotypic of the isolates revealed that *mecA* gene have the most contributions in incidence resistance to methicillin.

Table (3): E. coli isolated from diarrheal patients

Antimicrobial Agent	)S(	)I(	)R(
Penicillin	-	-	100
Methicillin	-	-	100
Ciprofloxacin	62	4	34
Ampicillin	-	-	100
Clindamycin	66	-	34
Erythromycin	52	-	48
Gentamicin	66		34
Vancomycin	98	-	2

This study provides prevalence and antibiotic resistance of *Staphylococcus aureus* (MRSA) isolates from patients suffered from wound infections, urinary tract infection, sepsis and Purulent infections in Ahvaz, southwest of Iran. Although antibiotic therapy is not recommended in the most infections with *Staphylococcus*

aureus and many cases are self-cure but it is necessary in some conditions. Our findings showed that *Staphylococcus aureus* was the major cause of human infections in this area. It is in agreement with previous studies in Iran [14-15]. Resistance to beta-lactame antibiotics is common and doctors do not recommend indiscriminate these antibiotics. Unfortunately the large number of isolates was resistant to the first line of antibiotics. A lot of researches have been run in recent years on detection, identification, and molecular characterization of antibiotic resistance genes, which has led to a more accurate assessment of the role of these bacteria in human disease. The results of this study showed the most prevalent resistances were found to Methicillin, Penicillin and Ampicillin, which are widely prescribed orally in routine treatment of outpatients suffered from some infections such as urinary tract infections in developing countries. High resistance to these drugs might be spread by selective pressure of antibiotics in resistant bacteria. Resistant bacteria can transmit these abilities to other bacteria specially *Staphylococcus aureus*. Significantly resistance to Vacomycine (2%) was not shown. In our study, about 98% of isolated *Staphylococcus aureus* carried *mecA* gene, which is in consistent with result of antibiogram on these isolates in disc diffusion method. These findings suggest that we should reassess the currently use culture media in disk diffusion method in medical diagnostic laboratories. These results are close to the results of Rahimi Alang, et al (2010) study. They reported the frequency of *staphylococcus aureus* in personnel of Golestan Hospital was 24% and meticillin resistance was 3% among them. In their study the most resistance was observed to penicillin. In addition, all the strains were susceptible to vancomycin. The results of their study were compatible with the present study [16].

CONCLUTION

It seems that the wide spread and improper use of antibiotics has led to an increasing rate of antimicrobial resistance [17]. Our results revealed that 98% isolates harbor mecA genes and more resistant to methicillin-related mecA genes. This study did not assess the contribution of certain potential exposures to antibiotics and antibiotic resistant bacteria remarked on elsewhere: antibiotic use by household members, day care attendance, exposure to contaminated water sources, contact with animal microflora. We have not investigated the prevalence of different mechanisms underlying the resistant phenotypes presented here. Irregular prescription of antibiotics, selection of inappropriate antibiotic and lack of susceptibility testing led to increased resistanse antibiotical in human.

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REFERENCES

[1] Aarestrup FM. J Vet Pub Heal 2004; 51: 380-388.
[2] Cizman M. Int J Antimicro Agents 2003; 21: 297-307.
[3] Li D, Liu B, Chen M. J Microb Methods 2010; 82: 71-77.
[4] Crago B, Ferrato C, Drews SJ, Svenson LW, Tyrrell G .Food Micro 2012; 32: 202-205.
[5] Kreiswirth B, Kornblum J, Arbeit R, Eisner W, Maslow JN, McGeer A. J Science1993; 259:227-30.
[6] Diep B, Chambers H, Graber C, Szumowski JD, Miller LG, Han LL. AnnIntern Med 2008;148:249-57.
[7] Otter J, French G. Lancet Infect Dis 2006; 6:753.
[8] Witte W, Cuny C, Klare I, Nübel U, Strommenger B. Inter J of Med Micro 2008; 298: 365-377.
[9] Pinho MG, de Lencastre H, Tomasz A. Proc Natl Acad Sci U S A 2001; 98:10886– 10891.
[10] Holden MT, Feil EJ, Lindsay JA, Peacock SJ, Day NP. Proc Natl Acad Sci U S A 2004; 101:9786–9791.
[11] Shehab el-din SA, et al. Egypt J Plast 2003; 27:1-10.
[12] Sheldon L Kaplan. Uptodate 2007; 15: 3.
[13] Plaunt TR, Shryock C, Thornsberry RD. approved standard document M31-A, vol. 19. CLSI 1999.
[14] Sambrook J, Russell D. 3th ed. New York, Cold Spring Harbor laboratory press, Molecular cloning, a laboratory Manual 2001; PP: 8.4-8.5.
[15] Rahimi-Alang S, Asmar M, Cheraghali F, Yazarlou S, Amini A, Shakeri F.J Med Zahedan 2010; 13:17-22.
[16] Tavakoli A,Yazdani R,Bokaeian M. Tabib-E-Shargh 2001;1(3):1-7.
[17] Mool J, Chansiripornchai P. Thai J Vet Med 2010; 40: 311-315.