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Research paper

Molecular epidemiology of staphylococcus aureus isolated from the patients, personnel and hospital environments in Sanandaj (Iran)

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Abstract

The aim of this study was to determine the prevalence and characteristics of methicillin-resistant Staphylococcus aureus (MRSA) isolates from the patients, personnel and environments of a hospital in Sanandaj. During this study, 30 MRSA isolates were collected and analyzed with using spa typing method. Out of the 30 S. aureus isolates, 28 (93.33%) was methicillin resistant (MRSA), 16 *spa* types (t030, t711, t002, t1556, t325, t267, t1358, t230 , t2422, t969, t1814, t037, t300, t044, t180, and t5598) were found in different wards of the hospital. The most frequent were t030 (17.2 %), t711 (13.7 %). Our data demonstrated that the overall, resistances to erythromycin, gentamycin, and ciprofloxacin, were most frequent (87.5, 81.25, and 62.5 respectively).

Keywords: *Methicillin-Resistant Staphylococcus Aureus (MRSA); Meca; Spa Typing.*

1. Introduction

S. aureus is a major nosocomial pathogen that causes a range of diseases, including endocarditis, osteomyelitis, pneumonia, toxic-shock syndrome, food poisoning, and, soft-tissue infections [1], [2].In 1961, 2 years after the introduction of methicillin, S. aureus developed methicillin-resistance due to the acquisition of the mecA gene.The mecA gene, is located on a mobile genomic island, that is called staphylococcal cassette chromosome mec (SCCmec) [3].During the last decades, diverse typing methods,have been used for monitoring S. aureus spread.PFGE has been the gold-standard method for distinguishing different MRSA strains in order to monitor their spread. However, PFGE is a technically demanding and a time-consuming method [4], [5].MLST, based on the sequence polymorphism of approx. 500-bp long fragments of seven housekeeping genes was designed to study the S. aureus population genetic structure. However, sequencing of several genes is time-consuming and costly [5], [6].Spa typing is a reliable tool for typing S. aureus and has become the most popular MRSA typing method.The method is based on sequencing of the polymorphic X region of the protein A gene (spa), present in nearly all S. aureus strains.The X region comprises of a variable number of typically 24 bp repeats flanked by well conserved regions [6-8].Many studies have evaluated the usefulness of spa typing for diverse epidemiological purposes and confirmed its ease of use, speed, high discriminatory power, reproducibility and typeability, and the portability of results.Spatyping is suitable for computerized analysis, and at least two commercial software packages are currently available for spa typing.The central spa server is organized by the SeqNet.org typing network, which currently includes 45 laboratories from 25 European countries[3-5], [9], [10].In this study we investigated the molecular epidemiology of MRSA strains in different wards of the hospital with Spa typing method.

2. Materials and methods

2.1. Sample collecting

This study included 30 S. aureus isolates collected from different hospital wards, between 2014and2015 in Kurdistan University of medical sciences. All isolates were previously identified as S. aureusby a standard microbiological procedure[11]Isolates were incubated at 37℃ for 24h on blood agar and single colonies were tested with tube and slide coagulase, catalase, DNase tests, and growth on Mannitol salt agar. To confirm the identity with the species, the nuc gene was amplified by a PCR-based method, using the primers 5'AGTTCAGCAAATGCATCACA-3',5'- ACGCAA GCC TTG ACG AAC TAA AGC-3' [12].

2.2. PCR amplification of mecA

DNA template was prepared purified and stored until needed at -20℃[13].PCR amplification of the mecA gene was performed to confirm MRSA.PCR was performed with 2µl extracted template DNA, 2µl mecA Primers5'TCCAGATTACAACTTCACCAGG-3',5'- CCACTTCATATCTTGTAACG -3'[14] and 11µL of master mix(polymerase Taq enzyme,$Mgcl_2$, dNTP, $SO_4(NH_4)_2$,TrisHCI, Tween – 20) and10µl deionized water in a final volume of 25µL.The thermal cycling program was as follows: initial denaturation (5 min at 94℃); followed by 30 cycles of denaturation (60 sec at 94℃), annealing (60 sec at 55℃),and extension (60 sec at 72℃); and a single extension (7min at 72℃).

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2.3. Antimicrobial susceptibility test

The Kirby-Bauer agar disk diffusion method was used to determine the susceptibility patterns of the S. aureus isolates, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. The antibiotics tested were as follows: ciprofloxacin, erythromycin, and Gentamicin.S. aureusATCC 33591was used as the control strain.

2.4. Spa typing

Spa gene typing was conducted according to the procedure described by Ridom GmbH (http://www.ridom.de).PCR was performed with 4µL extracted template DNA, 3 µL Spaprimers5'-TAAAGACGATCCTTCGGTGAGC -3',5'- CAGCAGTAGTGCCGTTTGCTT -3' [15],and 25µl master mix(polymerase Taq enzyme,$Mgcl_2$,dNTP, $SO_4(NH_4)_2$,TrisHCI, Tween – 20) and 18µl deionized water in a final volume of 50 µl. The thermal cycling program was as follows: initial denaturation (5 min at 94℃); followed by 35 cycles of denaturation (45 sec at 94℃), annealing (60 sec at 58℃), and extension (60 sec at 72℃); and a single extension (10min at 72℃).Amplified PCR products were visualized by agarose gel electrophoresis in a 2% agarose gel at 100 V for 40 min and sequenced at Macrogen Inc. (Seoul, Korea).Data were analyzed using the Spa Server database (http://SpaServer.ridom.de).

3. Results and discussion

In overall, 30 strains of S. aureus have been isolated from different wards of the hospital such as Pediatric, Surgery, Emergency, Surgery, Internal ,Infection,PICU(pediatric PICU(pediatric ICU) ,Orthopedic, Cardiac, and ICU. Of the 30 S. aureus isolates, 27 (93.33%) were methicillin-resistant (MRSA). Looking at the frequency of resistance to antimicrobial agents of the strains (Table 1) revealed that the resistances to erythromycin, Gentamycin, and Ciprofloxacin were most frequent (87.5, 81.25, and 62.5%) respectively. The spa types were assigned by sequencing analysis of PCR products of the spa gene (Table 2). Sixteen spa types (t030, t711, t002, t1556, t325, t267, t1358, t230, t2422, t969, t1814, t037, t300, t044, t180 and t5598) were found different wards of the hospitals. The most frequent were t030 (17.2 %), t711 (13.7 %).

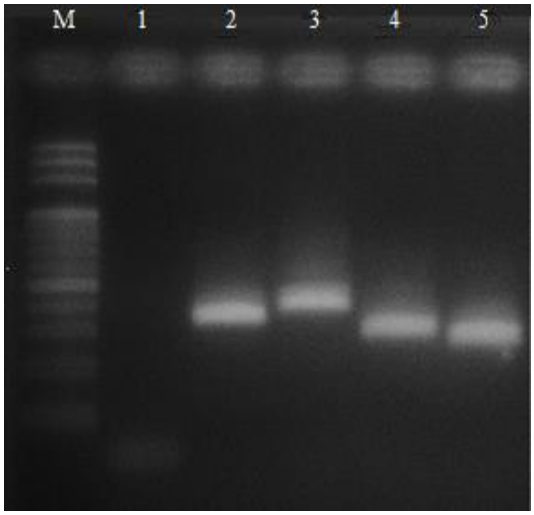

Fig. 1: An Ethidium Bromide-Stained Agarose Gel Electrophoresis Showingamplicons of X Region of the Spa Gene of S. Aureus Strains.Line M: Marker, Molecular Weight (100-1000 Bp) Line 1: Strain of Negative Control, Without Spa Gene Line 2: Strain of Positive Gene (ATCC 33591, S. Aureus), with Spa Gene Lines 3, 4, 5: Positive Samples with Spa Gene (200-600 Bp).

Table 1: Frequency of Resistance to Antimicrobial Agents among MRSA Isolates

Antibiotics	S [n (%)]	I [n (%)]	R [n (%)]
G	1(3.34)	5(16.66)	24(80)
E	1(3.34)	3(10)	26(86.66)
Ci	5(16.66)	5(16.66)	20(66.66)

G= Gentamycin, E= Erythromycin, Ci= Ciprofloxacin, S= Susceptible I= Intermediate, R= Resistance.

Table 2: Antibiotic Resistance Profiles and Molecular Typing of S.Aureus Isolates

No. of isolates	Sample	Wards	Antibiotic resistance	Spa type	Repeats	mecA
1	Trachea	ICU	E	t030	15-12-16-02-24-24	+
2	Blood	Pediatric	E-Ci-Ge	t030	15-12-16-02-24-24	+
3	Urine	Infection	E-Ci	t030	15-12-16-02-24-24	+
4	Trachea	Internal	E- Ge	t030	15-12-16-02-24-24	+
5	Nasal swab	PICU	E-Ci	t030	15-12-16-02-24-24	+
6	Tourniquet	Surgery	E-Ci-Ge	t711	04-21-17-34-24-34-22-25	_
7	Patient bed	Surgery	E-Ci-Ge	t711	04-21-17-34-24-34-22-25	+
8	Personnel	Surgery	E	t711	04-21-17-34-24-34-22-25	+
9	Blood	ICU	E-Ci-Ge	t711	04-21-17-34-24-34-22-25	+
10	CSF	Surgery	E-Ci-Ge	t002	26-23-17-34-17-20-17-12-17-16	+
11	CSF	Pediatric	Ge	t002	26-23-17-34-17-20-17-12-17-16	+
12	Patient bed	Pediatric	E	t1556	26-23-13-23-31-05-17-25-17-25-16-75-28	+
13	Trolley	Pediatric	E -Ge	t1556	26-23-13-23-31-05-17-25-17-25-16-75-28	+
14	Urine	ICU	Sensitive to all	t325	07-12-21-17-34-13-34-34-33-34	_
15	Secretion	PICU	Ge	t325	07-12-21-17-34-13-34-34-33-34	+
16	Urine	ICU	E-Ci-Ge	t267	07-23-12-21-17-34-34-34-33-34	+
17	Abscess	Orthopedic	E-Ci-Ge	t267	07-23-12-21-17-34-34-34-33-34	+
18	Blood	ICU	E-Ci-Ge	t1358	26-23-13-23-31-29-17-25-17-25-28	+
19	Nasal swab	Operation	E-Ci-Ge	t1358	26-23-13-23-31-29-17-25-17-25-28	+

20	Ventilator	ICU	E -Ge	t230	08-16-02-16-34	+
21	Nasopharynx swab	ICU	E-Ci-Ge	t230	08-16-02-16-34	+
22	Blood	Infant	E -Ge	t2422	07-23-12-34-38-12-12-23-02-12-23	+
23	Urine	Cardiac	E-Ci-Ge	t969	15-12-02-24-24	+
24	Urine	Internal	E-Ci-Ge	t1814	07-12-21-17-34-34-34-33-34	+
25	Urine	Internal	E-Ci-Ge	t037	15-12-16-02-25-17-24	+
26	Patient bed	Emergency	E-Ci-Ge	t300	01-12-16-16-02-16-02-25-24-24	+
27	Nasal swab	ICU	E-Ci-Ge	t180	09-02-16-34-34-34-17-34-16-34	+
28	Blood	Emergency	E-Ci-Ge	t5598	08-16-34-24-34-17	+
29	Incubator	Surgery	E-Ci-Ge	t044	07-23-12-34-34-33-34	+
30	CSF	Internal	E-Ci-Ge	NT	NT	+

G: Gentamicin, Ci: Ciprofloxacin, E: Erythromycin, NT: Not Typeability

The increasing incidence of infections caused by methicillin-resistant Staphylococcus aureus (MRSA), prevention of infection and spread of bacteria in hospitals to focus detection is essential. MRSA isolates to have appeared and become the most common cause of nosocomial infections in Iran [16]. In this study, the prevalence rate of MRSA was 93.33%. High rate of MRSA (Hospital-acquired/associated MRSA infections) are reported in East Asia, especially in Sri Lanka (86.5%), South Korea (77.6%), Vietnam (74.1%), Taiwan (65.0%), Thailand (57.0%) and Hong Kong (56.8%). In 2010, 26 European countries reported MRSA rate from 0% in Denmark, Estonia, The Netherlands, Norway and Sweden to 55.6% in Malta [17], [18]. The difference at the resistance rate of S.aureus to antibiotics in different countries or hospitals is due to the differences in policies patient treatment or hospital health control. Different methods of cultivation and isolation, antibiotic susceptibility assay, geographical differences and the population of the study also cause variation in previous findings. In addition, inappropriate prescription antibiotics in non-bacterial infections, non-full course of treatment as well as easy availability of antibiotics help to emergence and spread of multi-drug resistance strains[19].We used spa-typing since it has several advantages in terms of speed, ease of use, ease of interpretation and database creation for MRSA strain typing[20].The The most frequently encountered MRSA spa type among our strains was t030, probably the most widespread MRSA in Iranian hospitals [21]. Studies have shown that multidrug-resistant strains Spa type t030 and it is becoming resistant to many antibiotics [22], [23]. however, Countries of origin spa-type t030 has been shown in , Austria, Bulgaria, China, Croatia, Cyprus, Czech Republic, Denmark, France, Germany, Iran, Lebanon, Macedonia, Netherlands, Norway, Romania, South Africa, Spain, Sweden, Switzerland, Turkey. Overall, the present study suggests a recirculation movement of S. aureus strains between the patients, personnel and hospital environment in a hospital .The spread of antimicrobial to MRSA clones with specific genotypes within patients and personnel is a particular cause of concern and could have important implications for the implementation of effective infection control strategies and measures. In the current study, spa typing method showed 96.66% type ability we suggest the use of additional typing methods such as PFGE, BURP and MLST to overcome the limitation of a single locus-based molecular typing (spa typing).

4. Conclusions

This study showed a high rate of methicillin resistance among isolates S. aureus. Therefore, the correct selection of appropriate antibiotics to treat infections caused by S. aureus and prevention of resistance to antibiotics effective against most strains is important. Use of spa typing in this study might be a used as appropriate tool with other molecular methods in classification of this microorganism. Thus, the continuous review of the pattern of resistance and genetic characteristics of these organisms areimportant in order to controlling infections acquired from the hospital and community.

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