

Research Article:

The *spa* Typing and Characterization of Nasal Carriage Methicillin-resistant *Staphylococcus aureus* Isolates From Healthy Children

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ABSTRACT

Background: The Methicillin-resistant *Staphylococcus aureus* (MRSA) strains cause wide spectrum infections in hospitals and are responsible for the majority of the community-acquired infections. The emergence of community-acquired MRSA in patients without risk factors represents a high risk for public health.

Objectives: In this study, we aimed to molecular typing the nasal carriage MRSA strains harbored *mecA* gene isolated from healthy children living in the North of Iran and also to determine the pattern of vancomycin susceptibility in these isolates.

Methods: The MRSA strains were identified using standard microbiological and molecular procedures. The antibiotic susceptibility test was performed according to the clinical and laboratory standards institute. The MRSA strains were characterized by *spa* typing.

Results: In total, 14 different patterns of *spa* genes were obtained from MRSA isolates in this study, which included t790 (23.07%), t2962 (15.38%), t267 (15.38%), t159 (7.69%) and t701, t094, t1816, t11332, t937, t2135, t180, t160 [1 (3.84%) isolate per each type]. The rate of resistant isolates to cefoxitin, cefalotin, clindamycin, cefazolin, co-amoxiclav, co-trimoxazole, and cephalixin antibiotics were 69.23%, 100%, 46.15%, 96.15%, 100%, 3.48%, and 100%, respectively. In total, six (23.07%) revealed vancomycin-intermediate resistant phenotype and five (19.23%) isolates revealed vancomycin-resistant *S. aureus* phenotype.

Conclusions: This study showed the different variants of MRSA strains based on molecular typing, among healthy children. Continuous surveillance studies to monitor MRSA should be encouraged to gain a better understanding of the circulating MRSA strains and prevent community spread of this pathogen.

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1. Introduction

Staphylococcus aureus, as an important cause of community-acquired and nosocomial infections, has remained a significant health problem across the world (1-4). Methicillin-resistant *Staphylococcus aureus* (MRSA) was first recognized in the early 1960s, shortly after the introduction of methicillin, a narrow spectrum semi-synthetic penicillin in clinical practice, through the acquisition of the *mecA* gene. This gene encodes for a modified form of penicillin-binding protein, which has a lower affinity for all beta-lactam antibiotics (3-5). The MRSA strains are responsible for a wide spectrum of clinical diseases, ranging from superficial infection of the skin and soft tissue to severe and life-threatening diseases such as pneumonia, sepsis, osteomyelitis, endocarditis, and toxic shock syndrome (1, 2).

After the emergence of MRSA in the clinical practice, in the late 1980s, vancomycin became the first choice for treatment of MRSA infections in the world. Shortly after that, *S. aureus* isolates with reduced susceptibility to teicoplanin, a structural relative of vancomycin, were reported in Europe in 1990. Subsequently, the emergence of Vancomycin-intermediate resistant (VISA) and Vancomycin-resistant *S. aureus* (VRSA) isolates were reported in 1997 and 2002, respectively. Since then, the increased rate of VRSA was reported from all over the world. The option for treatment of MRSA-induced infections and clinical symptoms are limited. Therefore, MRSA has remained a significant threat to human health globally (6-12).

Molecular typing of MRSA is an essential tool in the surveillance of healthcare-associated infections. Rapid identification of the strains will help control and prevention of MRSA, causing community-acquired or nosocomial infections. There are several molecular techniques for MRSA typing such as Multilocus Sequence Typing (MLST), Pulsed-field Gel Electrophoresis (PFGE), Staphylococcal Cassette Chromosome *mec* (SCC*mec*) typing and Staphylococcal Protein A (*spa*) typing (1). These methods are accurate and time-consuming. Thus, due to laborious character and difficulties in comparing data between laboratories and the requirement for inter-laboratory standardization, *spa* typing method, which is based on assessment of short sequence repeats of hypervariable X region in the *spa* gene, exhibits excellent discriminatory power and has become a useful typing tool, with cheaper procedure, and standardized nomenclature (7).

Furthermore, *spa* typing allows data comparison between clinical laboratories at the international and na-

tional levels (13, 14). The prevalence of different *spa* types among *S. aureus* isolates varies in different areas around the world (9). Although several studies have reported the *spa* typing and characterization of MRSA in patients in healthcare settings, this subject has not been investigated in healthy children in the North of Iran. The present study aimed at molecular typing via *spa* typing and characterization of nasal carriage methicillin-resistant *Staphylococcus aureus* isolates from healthy children living in the North of Iran.

2. Methods

2.1. Isolation and identification of Methicillin-resistant *S. aureus*, vancomycin-intermediate resistant, and vancomycin-resistant *S. aureus* strains

In this descriptive study, 26 non-duplicate MRSA isolates were recovered from healthy children aged 6-12 years living in the North of Iran. These isolates were identified as *S. aureus* methicillin-resistant, as we described previously (4). Identification of these isolates was performed according to the standard microbiological procedures (morphology, Gram stain, catalase test, coagulase test, mannitol salt agar fermentation, and molecular procedures). Antibiotic susceptibility test was performed by the Kirby-Bauer method according to the Clinical and Laboratory Standards Institute (CLSI) guideline and antibiotic disks, including cefoxitin, cefalotin, clindamycin, cefazolin, co-amoxiclav, co-trimoxazole, and cephalexin (15).

For detection of MRSA strains, oxacillin screen agar was used. *Staphylococcus* strains were cultured on Muller Hinton agar containing 4% NaCl, and six mg/L oxacillin and then incubated for 24 hours (4, 12). Genomic DNA was extracted by the boiling method. Then for confirmation of MRSA strains, the isolates were subjected to Multiplex PCR assay to detect *spa* and *mecA* genes, and the previously described protocol was adjusted (4). For detection of VISA and VRSA isolates, the minimum inhibitory concentration of vancomycin by microdilution broth method according to CLSI standards was performed. VISA was defined when *S. aureus* strains exhibited increased resistance to vancomycin (MIC =4-8µg/mL), and VRSA as *S. aureus* isolates showed complete resistance to vancomycin (MIC ≥16µg/mL) (15, 16).

2.2. The *spa* typing of MRSA strains

Spa type is a repeated sequence comprising 24 repeated nucleotides (eight codons). To do typing, the short Sequence Repeats (SSR) of the polymorphic X

region of the protein A gene (*spa*) from MRSA isolates were amplified with the following primers: *spa*-1113F 5' – TAAAGACGATCCTTCGGTGAGC –3' and *spa*-1514R 5' –C AGCAGTAGTGCCGTTTGCTT –3'. PCR reactions were performed in 25µL final volumes containing 2µL of purified DNA, 0.5µL of each primer, 12.5µL of 2 x Green PCR Master Mix and 8.5µL of distilled water. The PCR amplification conditions for *spa* primer were as follows: the initial denaturation at 94°C for five minutes, and next 30 cycles consisting of a denaturation step at 94°C for 30 seconds, annealing at 59°C for one minute, extension at 72°C for one minute, as well as a final extension step at 72°C for ten minutes, and storage at 4°C at the end. *S. aureus* ATCC 25923 contains *spa* gene and considered as the positive control.

Amplified products were directly sequenced for both strands using the PCR primers by Bioneer Company (Seoul, South Korea). Sequences were then checked for the quality and trimmed by CLC Genomics version 12.0.2 (Qiagen, Germany). The *spa* typing was per-

formed by the module implicated inside the BioNumerics software version 7.6.3 (Applied Maths, Belgium). The sequences were submitted to Genbank under the accession number MK443456-MK443471. Finally, the type ability of this study was calculated as 87.1% with the method described previously (17).

3. Results

A total of 26 healthy boys with the Mean±SD age of 8.84±3.36 year (age range: 6 to 12 year), nasal carrying MRSA, enrolled in this study. All MRSA strains isolated from these children contained the *mecA* gene. The rate of resistant isolates to ceftioxin, cefalotin, clindamycin, cefazolin, co-amoxiclav, co-trimoxazole, and cephalixin were 69.23%, 100%, 46.15%, 96.15%, 100%, 3.48%, and 100%, respectively. Table 1 presents the in vitro susceptibilities of vancomycin against MRSA isolates. Totally six (23.07%) and five (19.23%) isolates were VISA and VRSA, respectively.

Table 1. In vitro susceptibilities of vancomycin against Methicillin resistant *Staphylococcus aureus* isolates

Number of Isolates	Vancomycin Concentration (µg/mL)	≤0.125	0.25	0.5	1	2	4	8	16	32	>64	MIC Range (µg/mL)	Mode (µg/mL)	Number of VISA Isolates	Number of VRSA Isolates
26	Number of isolates with MIC (µg/mL)	3	1	4	4	3	5	1	1	2	2	(0.125-64)	4	6	5

VRSA: Vancomycin-resistant *S. aureus*; VISA: Vancomycin Intermediate Resistant *S. aureus*

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Table 2. Molecular characterization of MRSA strains with *spa* typing method

No.	Spa Type	Repeat Succession	Number of Isolates
1	t790	26-23-13-23-31-29-17-25-17-25-16-28	6
2	t267	07-23-12-21-17-34-34-33-34	4
3	t2962	26-23-13-23-25-28	4
4	t159	14-44-13-12-17-17-23-18-17	2
5	t701	11-10-21-17-34-24-34-22-25-25	1
6	t094	07-23-12-34-34-12-12-23	1
7	t1816	07-12-21-17-34-13-34-34-33-34	1
8	t11332	04-21-12-41-486-17-12-12-17	1
9	t937	08-16-34-24-34-34-17-17	1
10	t2135	08-16-02-16-34-13-17-34-16	1
11	t180	09-02-16-34-34-34-17-34-16-34	1
12	t160	07-23-21-24-33-22-17	1
13 and 14	New types identified in this study, and submitted in the <i>spa</i> server (www.spaserver.ridom.de)	23-13-23-31-29-17-25-17-25-16-28 And 08-16-02-16-02-31-25-17-17-24-24	2

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The typing of all isolates yielded 14 spa types as following: t790 (23.07%), t2962 (15.38%), t267 (15.38%), t159 (7.69%) and t701, t094, t1816, t11332, t937, t2135, t180, t160 (1 [3.84%] isolate per each type) (Table 2). An interesting finding of this study was the emergence of two new spa types found with the Ridom Spa Server that was submitted in the server (www.spaserver.ridom.de).

4. Discussion

The MRSA strains produce various clinical manifestations in the hospitals and the community (18-22). This pathogen can adapt to a variety of conditions, and successful clones can result in epidemic or pandemic infections (7).

In this study, we characterized the MRSA strains isolated from the nose of healthy children. Although children have higher persistent carriage rates than adults, the rate of nasal carrying *S. aureus* varies depending on age, from approximately 45% during the first eight weeks to 21% by six months. Nevertheless, there is a transition from persistent carriage to intermittent or no carriage states during adolescence (18). Generally, nasal carriers of MRSA have an increased risk of acquiring infection with this pathogen. In our study, we found five MRSA strains harbored *mecA* gene, which were also VRSA by the phenotypical method. Our findings are alarming for the presence of vancomycin-resistant MRSA strains among healthy children, which should be addressed in future studies with larger sample sizes. Many Iranian laboratories do not follow a specific guideline for reporting and confirming VRSA, so the prevalence of VRSA strains is not known in our country. Askari et al. in a systematic review reported 24 VRSA isolates from Iran up to September 2012 (11).

Vancomycin is the primary antibiotic for the treatment of serious infections caused by MRSA strains. However, VRSA is defined as an isolate with a minimum inhibitory concentration of vancomycin greater than or equal to 16 µg/mL based on the determination by broth microdilution method and there are some reports regarding the genotype-negative phenotype-positive VRSA within resistance range (13, 16). Further studies are needed to advance identification of VISA and VRSA isolates, which in turn can be used to assess better the prevalence of these isolates, as well as to facilitate the development of optimal treatments (16). Although new therapies such as phage therapy have been suggested for the treatment of MRSA infection and this new therapeutic option has a promising future as an alternative or a supple-

ment to antibiotic treatments of infection due to MRSA strains, antibiotic therapy is still the first choice (23-25).

Nowadays, typing and analysis of MRSA strains have become a routine procedure in many countries and are being used as a tool to show the relationship between strains and clones. Although many MRSA genotypes and clonal clusters have been identified in different geographic regions, certain gene clones are predominant in some regions (26-30). For example, in the current study and among 26 MRSA isolates, we found 14 different genotypes. Asadollahi et al. in a comprehensive review reported that The Netherlands has the most diverse range of spa types (34 types), followed by China (22 types), Germany (16 types), UK (15 types), Spain (11 types), Sweden (10 types), Iran (8 types), France (7 types), and Switzerland (6 types). In our study, we detected that the spa type t790 (in six isolates) was predominant, and t2962 (in four isolates), t267 (in four isolates), t159 (in two isolates) and t701, t094, t1816, t11332, t937, t2135, t180, t160 (in one isolate) were in the next lines.

Asadollahi et al. reported that the spa types t008 and t002 are the most frequently repeated spa types in 16 countries. The following most frequently repeated spa types were t037 (12 countries), t044 (11 countries), t084 (8 countries), t012 and t127 (7 countries), t041 (6 countries), and t019, t011, t034, t355, t189, t304 (5 countries) (7). In Asia, t030 was the dominant type located in China, while in Iran, this type reported as the fifth most common spa type (7).

Fourteen spa types among 26 MRSA strains of nasal carriage *S. aureus* are caused by different variants of the spa genes. Detection of the molecular characteristics of *S. aureus* isolates is important for distinguishing the relations between isolates, and consequently, the implementation of appropriate infection control measures. Conclusion of this study showed the different variants of MRSA strains among healthy Iranian children. Moreover, the characterized isolates with reduced sensitivity to vancomycin are of concern. Continuous surveillance studies to monitor MRSA strains and the health education in school should be encouraged to gain a better understanding of the circulating MRSA and to prevent community-spread of this pathogen.

Ethical Considerations

Compliance with ethical guidelines

In line with the principles of research ethics, written informed consent was obtained from the parents of the

children. This study was approved by the Ethics Committee of Qaemshahr Branch, Azad University of Qaemshahr (378. ID Code: 10730548952006).

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Authors' contributions

All authors contributed in preparing this article.

Conflicts of interest

The authors declared no conflict of interest.

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