



Characterization of Virulence Factors and Prophage Profiles of Methicillin-Resistant *Staphylococcus aureus* Strains Isolated from a Referral Hospital in Tehran, Iran

Fateh Rahimi^{1*} and Leili Shokoohizadeh²

¹Department of Microbiology, Faculty of Sciences, University of Isfahan, Isfahan, Iran

²Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

*Corresponding author: Department of Microbiology, Faculty of Sciences, University of Isfahan, Hezar-Jireb St., Isfahan, Iran. Tel: +98-3137932250, Fax: +98-3137932456, Email: fateh.rahimi@hotmail.com

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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) has been considered as an important pathogen with a variety of virulence factors in communities and hospitals worldwide.

Objectives: In this study, we focused on the detection of different virulence factors and enterotoxin genes of MRSA strains isolated from a referral hospital in Tehran, Iran. Moreover, the presence of different prophage types was studied.

Methods: A total of 491 MRSA strains were isolated during three years from a referral hospital in Tehran. The staphylococcal enterotoxin (*sea-seq*) and *pvl*, *hly*, *sak*, *eta* and *tst* genes were detected. A multiplex-polymerase chain reaction (PCR) assay was used for prophage typing of MRSA isolates.

Results: Totally, 11 enterotoxin and 5 virulence factor genes were detected in MRSA strains. The *sea*, *sek*, *seq*, and *hly* genes were present in all the MRSA and other enterotoxin genes. *sel*, *seg*, *sem*, *sei*, *sen*, *seo*, *sec* and *sep* were detected in 32.8%, 20.3%, 12.6%, 8.3%, 4.1%, 2.6%, 1.6% and 0.4% of the strains, respectively. A total of 93%, 81%, 15.9% and 5.7% of the strains harbored the *sak*, *eta*, *tst* and *pvl* genes, respectively. SGF, SGFa and SGFb prophage type genes were detected in 100% of the MRSA strains, and four different prophage patterns were identified among the strains.

Conclusions: The presence of different prophage-encoded virulence factors among MRSA strains enable MRSA to produce a broad range of diseases, indicating MRSA strains as a potential threat to patients' health.

Keywords: Methicillin-Resistant *Staphylococcus aureus*, Virulence Factors, Prophage Typing, Enterotoxins

1. Background

Staphylococcus aureus and especially methicillin-resistant *S. aureus* (MRSA) is a major human and animal pathogen that causes both nosocomial and community-acquired (CA) infections. *S. aureus* is responsible for a variety of diseases, from mild skin infections such as abscesses and postsurgical wound infections to severe life-threatening respiratory infections such as sinusitis (1, 2). During the years, these bacteria have been known as one of the most common causes of hospital-acquired (HA) infections, which can acquire resistance to a variety of antibiotics such as oxacillin and vancomycin that enable them to be present and persist in hospitals and communities in different geographical regions worldwide (3, 4).

Pathogenic strains of *S. aureus* often promote in-

fections by producing exotoxins such as enterotoxins, staphylokinase (SAK), toxic shock syndrome toxin-1 (TSST-1) β -lysin, capsular polysaccharides, lipase, exfoliative toxin, and Panton-Valentine leukocidin (PVL), as well as expressing cell-surface proteins such as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) (2, 5). Most of the *S. aureus* virulence factors are encoded by accessory genetic elements, including plasmids, prophages, and pathogenicity islands or by genes located next to the staphylococcal cassette chromosome (SCC) implicated in methicillin resistance (1, 6).

Prophages are widespread among MRSA isolates and are responsible to encode and disseminate potent staphylococcal virulence factors that convert non-virulent isolates to virulent ones, indicating their key role in virulence and pathogenesis. Although staphylococcal prophages have been studied extensively (6-8), nowadays they are

used to type MRSA isolates (7, 9, 10).

2. Objectives

We have previously reported the prevalence, *SCCmec* types, antibiotic resistance patterns and clonal dissemination of 491 MRSA strains in a referral hospital in Tehran, Iran (3), but in the present study using the same bacterial isolates, we investigated the relationship between virulence factors and the presence of different prophage types in MRSA strains.

3. Methods

3.1. Sampling and Identification of MRSA

We have previously described the sampling procedure. Briefly, during three years from July 2011 to September 2014, 2103 suspected strains were isolated from clinical samples of inpatients and outpatients in a referral hospital and its clinic in Tehran, Iran, which were identified as *S. aureus* using species-specific primers for *nucA* gene. All the 491 MRSA strains were confirmed according to their resistance to oxacillin (1 µg) and presence of *mecA* gene (3). In this study, all the *S. aureus* strains were tested for resistance to cefoxitin (30 µg) as recommended by the Clinical and Laboratory Standard Institute (CLSI) (11).

3.2. DNA Extraction

DNAs of all the MRSA isolates were extracted using QI-Aamp DNA Mini Kit (QIAGEN, Hilden, Germany). The DNA concentration of each isolate was measured by the Nanodrop ND-1000.

3.3. Prophage Typing

A multiplex-PCR assay consisting of specific primers for SGA, SGB, SGF, SGD and SGL prophage serogroups and SGFa and SGFb as two prophage subtypes was employed for the prophage typing of MRSA strains as described previously (10).

3.4. Detection of Virulence Genes

PCR reactions and specific primers for genes encoding ETA and ETB (exfoliative toxin A and B) (12), TSST (13), PVL (14), SAK (15) and beta-haemolysin (HLB) (15) were employed to test the presence of different virulence factor genes among MRSA strains. The PCR cycles and conditions were the same as published previously.

Moreover, a wide range of staphylococcal enterotoxin genes (*sea-seq*) were detected using specific primers in separate PCR reactions according to the protocols reported previously (16).

4. Results

4.1. Prevalence of MRSA

Among all the 2103 *S. aureus* isolates tested, 491 (23.3%) strains showed resistance to cefoxitin and were identified as MRSA. In a previous publication, we also showed that all these 491 strains were resistant to oxacillin and harbored *mecA* gene, which confirmed them as MRSA strains and were analyzed further.

4.2. Prophage Types and Prophage Patterns

Results of prophage typing of MRSA strains revealed that except for SGD prophage types, all types of prophages were detected in this study and all the strains contained at least one prophage serogroup (SGF) and two subgroups (SGFa and SGFb) (Table 1). Amongst all the four different prophage patterns detected, prophage pattern 3 consisting of SGB, SGF, SGFa and SGFb prophage serogroups was the predominant one (81%; n = 398), and SGA and SGL prophage types were only detected in *pvl*-positive CA-MRSA strains.

4.3. Prevalence of Virulence Genes

4.3.1. Enterotoxins

Amplification of staphylococcal enterotoxin genes revealed the presence of 11 enterotoxin genes among MRSA strains, in which all (100%) the strains were positive for *sea*, *sek* and *seq* genes (Table 2). Moreover, *sel* and *seg* genes were detected in 32.8% and 20.3% of the MRSA strains, respectively, and the prevalence of *sem*, *sei* and *sen* genes was limited to 12.6%, 8.3% and 4.1% of the strains, respectively. On the other hand, none of the MRSA tested was found positive for genes encoding enterotoxins B, D, E and H, and the frequency of *sep*, *sec* and *seo* genes varied from 0.4% to 2.6%.

According to the results of enterotoxin genes detection, 17 different patterns of enterotoxin were detected among the strains (Table 2), in which enterotoxin pattern 1 consisting of *sea*, *sek* and *seq* genes was the predominant pattern and was detected in 54% (n = 265) of the strains, followed by pattern 5 (n = 98; 20%). In addition, enterotoxin pattern 10, which harbored all the detected genes, except for *sep* gene, had the lowest frequency and was found in 3 (0.6%) strains.

4.3.2. Virulence Factor Genes

The *hly* gene was detected in 100% of MRSA strains, and 93% (n = 457) of the strains were positive for *sak* gene encoding SAK. Moreover, none of the MRSA tested was positive for *etb* gene, and *eta* (exfoliative toxins A) gene was present in 81% (n = 398) of the strains. *tst* and *pvl* genes had the lowest frequencies compared to other virulence factors

Table 1. Prophage Types and Prophage Patterns of Methicillin-Resistant *Staphylococcus aureus* Strains

Pattern	SGA	SGB	SGF	SGFa	SGFb	SGL	No. (%)
1	+	+	+	+	+	+	6 (1)
2	+	-	+	+	+	+	22 (5)
3	-	+	+	+	+	-	398 (81)
4	-	-	+	+	+	-	65 (13)

Table 2. The Frequency and Enterotoxin Patterns of Methicillin-Resistant *Staphylococcus aureus* Strains^a

Pattern	SEA	SEC	SEG	SEI	SEK	SEL	SEM	SEN	SEO	SEP	SEQ	No. (%)
1	+	-	-	-	+	-	-	-	-	-	+	265 (54)
2	+	-	-	-	+	-	-	-	-	+	+	2 (0.4)
3	+	-	-	-	+	-	-	+	-	-	+	3 (0.6)
4	+	-	-	-	+	-	+	-	-	-	+	12 (2.4)
5	+	-	-	-	+	+	-	-	-	-	+	98 (20)
6	+	-	-	-	+	-	+	+	-	-	+	2 (0.4)
7	+	-	+	-	+	-	+	+	-	-	+	2 (0.4)
8	+	-	+	-	+	-	+	-	-	-	+	33 (6.7)
9	+	-	+	+	+	-	-	-	-	-	+	4 (0.8)
10	+	+	+	+	+	+	+	+	+	-	+	3 (0.6)
11	+	-	+	+	+	+	+	+	+	-	+	3 (0.6)
12	+	-	+	+	+	-	+	+	+	-	+	7 (1.4)
13	+	+	+	+	+	+	-	-	-	-	+	3 (0.6)
14	+	+	-	-	+	+	-	-	-	-	+	2 (0.4)
15	+	-	-	+	+	+	-	-	-	-	+	7 (1.4)
16	+	-	+	+	+	+	-	-	-	-	+	14 (2.8)
17	+	-	+	-	+	+	-	-	-	-	+	31 (6.3)
N	491	8	100	41	491	161	62	20	13	2	491	
%	100	1.6	20.3	8.3	100	32.8	12.6	4.1	2.6	0.4	100	

^a Enterotoxins B, D, E, H, R, S and T were not detected.

and were detected in 15.9% (n = 78) and 5.7% (n = 28) of the MRSA strains tested. The presence of *pvl* gene was only limited to CA-MRSA strains.

5. Discussion

In this study, we described the prevalence of different prophage types among MRSA strains isolated from a referral hospital in Tehran, Iran, during three years. We have reported previously that prophage typing could be a useful method for typing of MRSA strains (7, 9, 17). Our findings revealed that all the prophage types, except for SGD, were present among the strains and SGF prophage type was the predominant one, which is consistent with previous reports during 2012 - 2016 in Iran (7, 9, 10, 16, 18). On

the other hand, four prophage patterns were reported in this study, which is similar to previous reports from Iran, in which prophage pattern 3 was reported as the dominant pattern. Dominance of some prophage patterns in the current and previous studies further suggests the circulation of nearly the same MRSA clones in hospitals and communities. The *pvl* gene was only detected in SGA prophage-positive CA-MRSA strains, which is in line with previous reports (10, 17, 19).

In the present study, 11 different staphylococcal enterotoxins and five virulence genes were detected among all the MRSA strains. These results indicated that there was no special relationship between clinical samples and types of virulence factors. Unlike the previous study (16), we could detect different enterotoxins and virulence factor genes

among the isolates. The frequency of *sea*, *sek*, *seq* and *h1b* genes was higher than that in other reports from Iran and other countries (18, 20-23). Moreover, the enterotoxin pattern 1 consisting of enterotoxin A, K and Q was the most frequent enterotoxin pattern. Different prevalence rates of enterotoxin genes have been reported worldwide (16, 18, 24-29). The discrepancy in the prevalence of different genes is most likely due to the origin of the isolates and the genetic structure of each isolate. The lower prevalence of enterotoxin genes (*sea*, *seb*, *seg*, *sei* and *sej*) among MRSA isolates was reported in the Czech Republic (30). The low prevalence of some enterotoxin genes such as *seg*, *sei*, *sem*, *sen* and *seo* (so-called *egc*) in the present study could be due to the theory that indicated these enterotoxin genes are more frequent among commensal strains compared to pathogenic ones (31).

Exfoliative toxin is a causative agent of staphylococcal scalded skin syndrome (SSSS), and documents showed that there were no significant differences in the prevalence of this toxin among MRSA and methicillin-sensitive *S. aureus* (MSSA) strains. Sila et al. revealed that 3% of MSSA strains carried the *eta* gene, whereas 10% of MRSA were *eta*-positive (30). In the present study, we could not find any relationship between the presence of *eta* gene and patients' age, but it has been shown previously that exfoliative toxin is more frequent among children compared to adults (32).

ETA is an SGB prophage-encoded virulence factor and was present among all MRSA strains harboring this prophage type. SAK is a 16-kDa prophage-encoded protein, which acts as a fibrin-specific activator of human plasminogen produced by certain *S. aureus* strains, which indicates the proteolytic activity of MRSA strains (33). We found that 93% of MRSA strains isolated from different clinical samples such as urine, wound, sputum and cerebrospinal fluid (CSF) were positive for *sak* gene, which is higher than the previous report from Iran (18). Moreover, the *tst* gene was found in 16% of the MRSA strains. Although different frequencies of *tst* gene have been reported previously (20, 34-37), our finding in the present study is significantly higher than other reports.

In conclusion, our findings illustrated the presence of highly virulent MRSA strains in a referral hospital in Tehran. The increasing rate of virulence factors may be due to the study of higher number of MRSA strains or the presence of more virulent strains and higher risk patients in hospitals. The presence of different bacteriophages encoding virulence factors among MRSA strains enables them to produce a broad spectrum of diseases, which highlights the potential threat to patients. Bacteriophage typing and the identification of different prophage types could be useful for the prediction of such virulence factors.

Footnotes

Authors' Contribution: Fateh Rahimi researched, supervised and developed the study concept, design and critical revision of the manuscript; Leili Shokoohizadeh researched and prepared the draft of the manuscript.

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