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Evaluation of Antimicrobial Resistance and Immune Evasion Cluster Genes in Clinical Methicillin-Resistant Staphylococcus aureus (MRSA) Isolates from Khuzestan Province, Iran

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ABSTRACT

Aims: Methicillin-resistant *Staphylococcus aureus* (MRSA) is recognized as an important health problem worldwide. To counteract the human innate immunity, *S. aureus* produces a number of immune evasion clusters (IEC) including staphylokinase (SAK), staphylococcal enterotoxin P (SEP), staphylococcal enterotoxin A (SEA), staphylococcal complement inhibitor (SCIN), and chemotaxis inhibitory protein (CHIP), encoded by *sak*, *sep*, *sea*, *scn*, and *chp* genes, respectively. These genes are carried by β-haemolysin-converting bacteriophages. The present study was conducted to determine the IEC phage types and antibiotic resistance patterns in 145 clinical MRSA isolates from Khuzestan Province, Iran.

Materials & Methods: All the isolates were investigated by disc diffusion method and PCR assay for *sak*, *sep*, *sea*, *scn*, and *chp* genes.

Findings: The assessment of antibiotic resistance showed the highest resistance rate towards penicillin (97.25%), followed by methicillin (95.8%), ceftazidime (81.4%), erythromycin (71.8%), clindamycin (61.4%), ciprofloxacin (60.7%), gentamycin (56%), imipenem (56.55%), and vancomycin (0%), respectively. Also, the frequency of IEC types was as follows: Type A, 4.8%; Type B, 9%; Type C, 13.1%; Type D, 12.4%; Type E, 27.6%; Type F, 1.4%; Type G, 0.7%; and Type H, 6.9%. On the other hand, 24.1% of the isolates showed no IEC type.

Conclusion: The findings showed that IEC-carrying bacteriophages were highly prevalent among the MRSA strains, resulting in the adaptation and counteraction of bacteria with the human immune system. Therefore, understanding the role of IEC in bacteria virulence can improve our knowledge about the evolution, vaccination, and treatment of *S. aureus* infection.

Keywords: Immune evasion cluster; Methicillin-resistant *Staphylococcus aureus*; Phage typing; Antibiotic resistance

CITATION LINKS

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Introduction

Staphylococcus aureus as an opportunistic pathogen can colonize the skin and nasopharynx of healthy individuals. This bacterium is responsible for various human diseases, including bacteremia, skin infections, toxic shock syndrome, necrotizing pneumonia, osteomyelitis, and endocarditis [1, 2, 3]. Evidence suggests that *S. aureus* has a remarkable potential to acquire antimicrobial resistance. The first case of resistance to methicillin was reported in 1961 [3]. *mecA* gene located on a mobile genetic element was found to be responsible for … resistance; this gene is also the cause of resistance to other β-lactam antibiotics. Methicillin-resistant *S. aureus* (MRSA) infection is still a major global concern due to the difficulty in its treatment, particularly when the isolates acquire multidrug resistance (MDR) elements, especially against the vancomycin [4, 5]. In this regard, Yu and collogues reported that MRSA isolates harbor more virulence genes than other isolates [6].

Generally, the virulence factors of *S. aureus* include toxins, adhesions, immune evasion proteins, and pore-forming proteins [1, 2]. The first-line defense against the bacterial infections is innate immunity [5]. To evade this part of the immune system, *S. aureus* expresses some small proteins [7]. One of these human-specific immune modulators is the staphylococcal complement inhibitor (SCIN). SCIN is a C3 convertase inhibitor, which prevents the generation of C3b component and opsonophagocytosis of *S. aureus* by neutrophils [8, 9]. The chemotaxis inhibitory protein of *S. aureus* (CHIPS), as another immune-modulating protein, specifically blocks phagocyte activation by C5a through binding to C5a receptor (C5aR) and formylated peptide receptor (FPR)[8, 10]. Staphylococcal enterotoxin A (SEA) is a superantigen, which can regulate the operation of chemokine receptors on monocytes, such as CCR1, CCR2, and CCR5 [8, 9]. Another superantigen, which limits the host's innate immune response to *S. aureus* antigens, is staphylococcal enterotoxin P (SEP). SEP also prevents bacterial elimination [3]. SEA and SEP can cause foodborne poisoning; however, strains harboring these enterotoxins are often methicillin-sensitive [11]. On the other hand, staphylokinase (SAK) secreted by *S. aureus* modulates the innate immune system through anti-opsonic activities and defensins destruction [8]. SAK converts plasminogen to plasmin and causes the cleavage of important opsonic molecules, including IgG and C3b; therefore, phagocytosis of staphylococci by neutrophils is prevented [9]. SCIN, CHIPS, SEA, SEP, and, SAK are encoded by *scn*, *chp*, *sea*, *sep*, and *sak* genes, respectively to form an immune evasion cluster (IEC) [8]. So far, eight IEC variants have been identified, which are designated as A to H [8, 9]. *scn* gene is common among the all variants. Except for Variant H, other variants harbor a combinations of *scn* gene with one or more other genes, including *chp*, *sak*, *sea*, and *sep* genes [1]. IECs are carried by bacteriophages and integrated with the β-hemolysin-encoding genes. Consequently, these bacteriophages are known as β-hemolysin-converting phages [9]. Evidence shows that these phages are found in more than 90% of the clinical *S. aureus* isolates from humans; however, not all genes are present on a given bacteriophage, and gene combinations are diverse [3, 12].

Objectives: The aim of this study was to characterize MRSA isolates, based on the antibiotic resistance pattern and β-hemolysin-converting phages. Our findings can help design therapeutic strategies and preventive measures against the infection transmission.

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Materials and Methods

Sample collection and characterization: The isolates were collected from the clinical samples of patients, admitted to the affiliated hospitals of Ahvaz Jundishapur University of Medical Sciences from April 2015 to December 2015. The clinical samples were collected from wounds (N: 67), urine (N: 16), and blood samples (N: 23); respiratory infections (N: 19); abscesses (N: 9); and catheters (N: 11). Conventional biochemical methods were used for the detection of *S. aureus* isolates, including Gram staining, DNase, catalase, furazolidone (100 µg) susceptibility testing, bacitracin (0.04 units) resistance tests, slide and tube coagulase tests, and mannitol fermentation on mannitol salt agar.

Detection of MRSA isolates: According to the Clinical & Laboratory Standards Institute (CLSI) guidelines for the detection of MRSA isolates, resistance to cefoxitin (30 µg) was investigated on Mueller-Hinton Agar (MHA) [13]. Then the resistant isolates were confirmed as MRSA with respect to the presence of *mecA* gene using the PCR method, as described previously [14].

Antimicrobial susceptibility testing: The isolates susceptibility to antibiotics was examined by disk diffusion method according to the CLSI guidelines (13). The antibiotics disks included imipenem (30 µg), clindamycin (2 µg), gentamicin (10 µg), methicillin (5 µg), erythromycin (15 µg), penicillin (10 units), ciprofloxacin (5µg), and ceftazidime (30 µg). The vancomycin-resistant *S. aureus* (VRSA) isolates were screened by culturing on the brain-heart infusion (BHI) agar using 6 µg/ml of vancomycin [15].

DNA extraction: The strains were cultured on the BHI broth overnight at 37 °C. Then chromosomal DNA was extracted using the Gram-positive bacteria DNA extraction kit (SinaClon; Iran) based on the manufacturer's instructions.

IEC profiling: The complement of IEC genes was determined by the PCR assay to investigate the phage types of the isolates. The primers and conditions used for the detection of *chp*, *sak*, *sea*, *sep*, and *scn* genes were previously described by van Wamel *et al.* (2006) (Table 1) [8]. The isolates typing scheme was from A to H according to the studies done by van Wamel *et al.* (2006) and Price *et al*. (2012) (Table 2) [8, 16].

Table 1) Names and sequences of primers used in this study

Primer name	Sequence (5'→3')	References
sak	AGGCGATGACGCGAGTTAT GCGCTTGGATCTAATTCAAC	8
scn	AGCACAAGCTT GCCAACATCG TTAATATTTACTTTTTAGTGC	8
chp	TTTACTTTTGAACCGTTTCCTAC CGTCCTGAATTCTTAGTATGCATATTCATTAG	8
sep	AATCATAACCAACCGAATCA TCATAATGGAAGTGCTATAA	8
sea	AGATCATTCGTGGTATAACG TTAACCGAAGGTTCTGTAGA	8
mecA	TCCAGATTACAACTTCACCAGG CCACTTCATATCTTGTAACG	14

Table 2) Different IEC phage types and their incidence found among the MRSA isolates

Phage type	Genes	Number (%)
A	*sea, sak, chp, scn*	7 (4.8)
B	*sak, chp, scn*	13 (9)
C	*chp, scn*	19 (13.1)
D	*sea, sak, scn*	18 (12.4)
E	*sak, scn*	40 (27.6)
F	*sep , sak, chp, scn*	2 (1.4)
G	*sep , sak, scn*	1 (0.7)
H	*scn*	10 (6.9)
None	*sep* or *sak* or *chp* or *sea* or none	35 (24.1)
Total		145 (100)

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Ethics Statement: All the study stages complied with the Declaration of Helsinki. Also, oral consents were obtained from all the participants for the participation in this study.

Statistical analysis: Fisher's test and Chi-square test were used for the comparison of data. For all statistical analyses, SPSS software (v.22.0) was used. P-values less than 0.05 were considered as statistically significant.

Findings

Clinical features of *S. aureus* isolates: Out of 145 MRSA isolates, 67 (46.2%) isolates belonged to the female participants, while 78 (53.8%) strains were isolated from male participants (P> 0.05). The strains were mostly isolated from wound (46.2%), followed by blood (15.9%), respiratory infection (13.1%), urine (11%), catheter (7.6%), and abscess (6.2%) samples.

Antimicrobial susceptibility profiles of *S. aureus* isolates: All the isolates were assessed for susceptibility to 10 antibiotics. The antimicrobial resistance patterns of the strains are listed in Table 3. All the investigated isolates were cefoxitin-resistant and *mecA* gene positive. Among the isolates, the highest resistance rate was recorded towards penicillin (97.25%), followed by methicillin (95.8%), ceftazidime (81.4%), and erythromycin (71.8%). None of the isolates were resistant to vancomycin. On the other hand, MDR (≥ 3 classes) was recorded in 95.5% of the isolates.

IEC genes and phage typing of the isolates: Among the IEC genes, *scn* gene was the most prevalent (110; 75.8%), followed by *sak* (87; 60%), *chp* (42; 28.9%), *sea* (27; 18.6%), and *sep* (4; 2.75%) genes. Eight types (A-H) were previously defined for different combinations of IEC genes, harbored by β-hemolysin-converting bacteriophage. The predominant IEC scheme was Type E. Overall, 35 isolates showed no IEC type and contained none or only one gene. The details of the detected types are presented in Table 3.

Table 3) Antibiotic resistance patterns of MRSA isolates

Antimicrobial agent	Sensitive	Intermediate	Resistant
	N (%)	N (%)	N (%)
P	4 (2.75)	0 (0)	141 (97.25)
V	135 (93.1)	10 (6.9)	0 (0)
CP	51 (35.2)	6 (4.1)	88 (60.7)
E	32 (22)	9 (6.2)	104 (71.8)
CC	53 (36.5)	3 (2.1)	89 (61.4)
GM	61 (42)	3 (2)	81 (56)
CAZ	11 (7.6)	16 (11)	118 (81.4)
IPM	59 (40.7)	4 (2.75)	82 (56.55)
Me	4 (2.8)	2 (1.4)	139 (95.8)
Fox	0 (0)	0 (0)	145 (100)

P: penicillin; V: vancomycin; CP: ciprofloxacin; E: erythromycin; CC: clindamycin; GM: gentamicin; CAZ: ceftazidime; IMP: imipenem; Me: methicillin; and Fox: cefoxitin

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Discussion

S. aureus is an important human and livestock pathogen, which can cause a broad spectrum of diseases [7]. The rapid growing resistance of this bacterium to antibiotics, such as methicillin and other β-lactam antibiotics, imposes challenges to the infection treatment. The prevalence of MRSA is higher in Iran than in neighboring countries in the Middle East (except for Iraq) [15, 17]. Evidence suggests that the frequency of some virulence genes in MRSA isolates is higher than in methicillin-susceptible *S. aureus* (MSSA) strains; this can be the reason for great diversity and variability in these isolates [2, 18, 19]. In MRSA isolates, the IEC genes carried by β-hemolysin-converting bacteriophages, facilitate nasal colonization and adaptation of *S. aureus* isolates to their human host [9, 20, 21]; in addition, propagation and intracellular survival of bacteria increase in the nose [9]. However, different IEC types are usually carried by bacteriophages. It should be noted that bacteria usually tend to lose their bacteriophages, which is because of variable frequency of IEC genes among the *S. aureus* isolates [22].

The antimicrobial susceptibility test of MRSA isolates indicated that penicillin, methicillin, ceftazidime, erythromycin, clindamycin, and ciprofloxacin are not effective empirical options in the treatment of *S. aureus* infections. Also, resistance to these antimicrobial drugs has been reported in other studies from Iran; therefore, empirical treatment of MRSA-infected patients with these antibiotics may not be effective [15, 23, 24]. Nevertheless, vancomycin can be an effective antibiotic against MRSA, as all the tested isolates were susceptible to this antimicrobial drug. This finding is in agreement with the finding of other studies conducted in Iran [15, 25]. In the present study, more than 95% of the *S. aureus* isolates were MDR, indicating the widespread antimicrobial resistance in *S. aureus* isolates and the need for continuous monitoring the antibacterial resistance development in MRSA isolates.

In the present study, 75.9% of MRSA isolates contained an IEC-carrying bacteriophage, which was slightly lower than that reported in other studies [8, 9]. However, lower prevalence rate was reported in another study [1], which is presumably related to the animal origin of these isolates. In another study conducted in Iran, the prevalence of IEC-carrying bacteriophages among the clinical *S. aureus* isolates was lower than that reported in the present study [15]. In addition, several differences were observed in the prevalence of phage types in the clinical MRSA isolates, compared to those reported in some previous studies. The predominant IEC variant was Type E, while in other studies, human infecting isolates mostly belonged to Type B [3, 8, 9, 15]. These findings suggest the presence of variations in IEC types, found in the isolates of different geographical regions; this stems from the fact that IEC is a dynamic DNA element, which can successfully spread among the *S. aureus* isolates, resulting in the adaptation and counteraction to the human host [8].

Although in this study, prophage integration was found in approximately 76% of the human clinical isolates, the prevalence of individual genes was varied, which is due to the fact that all IEC genes are not transferred by a given bacteriophage. Similar to a study by van Wamel *et al*. (2006), the most frequent genes were *scn*, *sak*, and *chp* genes, respectively [8]. Nonetheless, the prevalence of *chp* gene was varied considerably between the different studies, indicating the reduced prevalence of phage Type B and increased prevalence of phage Type E.

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Conclusion

In conclusion, based on the reported antimicrobial susceptibility testing results, vancomycin can be considered as an effective empirical option for the treatment of MRSA infections. The IEC-carrying bacteriophages are highly prevalent among the MRSA strains and varied in different geographical regions. This reveals the successful and continuous spread of IEC through the clinical *S. aureus* population. Therefore, IEC genes can act as the potential targets for the vaccination or treatment of *S. aureus* infections.

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Ethical Permissions: The study was approved by the research committee of Shahid Chamran University of Ahvaz.

Conflicts of interest: The authors declare that they have no competing interest.

Authors' contribution: SER and SA participated in search for subjects and data, also performance of the research. SER conducted the research. SER and HM analyzed and interpreted the data. All authors read, revised, and approved the final manuscript.

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