



Molecular and Virulence Characteristics of Methicillin-Resistant *Staphylococcus aureus* Bacteria Recovered From Hospital Cockroaches

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Received 2019 October 01; Revised 2020 January 01; Accepted 2020 January 11.

Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most imperative cause of nosocomial infections. Cockroaches are the routine insects accountable for the spread of resistant bacterial strains, exclusively MRSA.

Objectives: The current survey aimed to appraise the frequency of Pantone-Valentine leucocidin (PVL) and Staphylococcal cassette chromosome *mec* (*SCCmec*) in MRSA bacteria recovered from hospital cockroaches.

Methods: Thirty-six MRSA isolates were recovered from the external washing samples of American and German hospital cockroaches. Bacteria were subjected to the PCR amplification of *SCCmec* types and the *PVL* gene.

Results: The *SCCmec* types III (44.44%), I (27.77%), and II (16.66%) were the most frequent types among MRSA bacteria. The frequency of *SCCmec* types IVa, IVd, and V was 2.77%, 2.77%, and 5.55%, respectively. The *SCCmec* types IVb and IVc were not detected in the assessed samples. Twelve out of 36 (33.33%) MRSA isolates harbored the *PVL* gene. The frequency of the *PVL* gene was 35.71% and 25%, respectively, among MRSA bacteria recovered from *Periplaneta americana* and *Blattella germanica* hospital cockroaches.

Conclusions: The current research is an initial description of *SCCmec* types and the *PVL* gene among MRSA bacteria recovered from hospital cockroaches. High frequency of *SCCmec* types I, II, and III and moderate-to-low frequency of the *PVL* gene signify the occurrence of health care associated-MRSA.

Keywords: Methicillin-Resistant *Staphylococcus aureus*, Antibiotic Resistance, Molecular Characters, Hospital Cockroaches

1. Background

Cockroaches are one of the predominant insects in residential environments including health care settings and hospitals. They have been tremendously fruitful in misusing the spaces within human habitation. Cockroaches are recognized for survival, transmission, and spread of hazardous microbial pathogens (1, 2). Two of the most imperative and mutual cockroach species originated in Iranian hospitals are American (*Periplaneta americana*) and German cockroaches (*Blattella germanica*). *Periplaneta americana* is larger and typically has a glossy reddish-brown color (1, 2). Both of them are acknowledged as the reservoirs of pathogenic bacteria, predominantly *Staphylococcus aureus* (3, 4).

Staphylococcus aureus usually exists in the nose, respiratory tract, and the skin (5). It is accountable for the outbreaks of nosocomial and community-acquired infections including wound and burn infections, urinary tract infections (UTIs), respiratory tract infections (RTIs), blood

and soft tissues infections, food-borne diseases, and food poisoning (5). *Staphylococcus aureus* is habitually resistant toward numerous types of antibiotic agents (6-9). Some of them are resistant toward methicillin antibiotics, which are named as methicillin-resistant *S. aureus* (MRSA). Methicillin-resistant *S. aureus* bacteria have a boosted irrefutable standing owing to their substantial resistance toward various types of antibiotics, leading to their higher pathogenicity (6-9).

Staphylococcal chromosomal cassette *mec* (*SCCmec*) is a genetic division of the MRSA bacteria accompanying with the *mecA* gene and is responsible for virulent characteristics (10). It is predominantly originated in MRSA bacteria of the hospital environment. It is characteristically grouped into types I, II, III, IV, and V rendering to *ccr* and *mec* alleles (10). The *SCCmec* type IV is further grouped into a, b, c, and d types (10). The pathogenicity of MRSA bacteria correspondingly relies on abundant surface antigens and extracellular proteins. MRSA strains are mainly produce leukocidal toxins, which suggests that Pantone-Valentine leuco-

cidin (PVL) is one of the most imperative virulence factors with substantial contribution to the pathogenicity of diseases caused by MRSA bacteria (11).

2. Objectives

The current survey was performed to measure the frequency of *SCCmec* types and the *PVL* gene among MRSA bacteria recovered from external washing samples of *P. americana* and *B. germanica* hospital cockroaches.

3. Methods

3.1. Bacteria and Further Identification

We recovered 36 MRSA isolates from external washing samples of hospital cockroaches (12). Cockroaches were obtained from private hospitals of Tehran Province, Iran, using standard traps (13). After immobilization by freezing (0°C for 5 min), the species of cockroaches were identified under a dissecting microscope according to the method by Harwood and James (14). Methicillin-resistant *S. aureus* bacteria were recovered from *P. americana* and *B. germanica* hospital cockroaches based on a previously described procedure (13). Methicillin-resistant *S. aureus* bacteria were confirmed using cefoxitin (30 µg) and oxacillin (1 µg) susceptibility tests. Examinations were done based on the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (15).

3.2. DNA Extraction

Methicillin-resistant *S. aureus* bacteria cultured on the tryptic soy broth (Merck, Germany) medium were analyzed by the DNA extraction kit (Thermo Fisher Scientific, Germany) following the manufacturer's guidelines. Purity, quality, and quantity of DNA were assessed using the previously described procedures (6, 12).

3.3. PCR-Based Detection of *SCCmec* Types and *PVL* Gene

Table 1 exemplifies the PCR circumstances applied for the identification of *SCCmec* types and the *PVL* gene (16, 17). A programmable DNA thermo-cycler (Eppendorf Mastercycler 5330, Germany) was used for this purpose. Amplified samples were appraised by electrophoresis based on a previously described technique (16, 17).

3.4. Statistical Analysis

Statistical analysis was done using SPSS 21.0 Numerical software (SPSS Inc., Chicago, IL, USA). The chi-square test and Fisher's exact two-tailed test were applied to assess any significant relationship between the frequency of *SCCmec* types and the *PVL* gene among MRSA bacteria. A P value of < 0.05 was considered the significance level.

4. Results

4.1. *SCCmec* Types

Figure 1 signifies the results of the gel electrophoresis of the *SCCmec* types found in MRSA bacteria. Table 2 shows the frequency of *SCCmec* types and the *PVL* gene among MRSA bacteria. We observed that *SCCmec* III (44.44%), I (27.77%), and II (16.66%) were the most frequent types among MRSA bacteria. There were no *SCCmec* types IVb and IVc among MRSA bacteria. The frequency of *SCCmec* type I was 28.57% and 25%, type II was 17.85% and 12.50%, and type IIIs was 42.85% and 50% among *P. americana* and *B. germanica* hospital cockroaches, respectively. Statistical significant difference was found between kind of samples and frequency of *SCCmec* types ($P < 0.05$).

4.2. *PVL* Gene Frequency

Figure 2 signifies the results of the gel electrophoresis of the *PVL* gene in MRSA bacteria. Twelve out of 36 (33.33%) MRSA bacteria harbored the *PVL* gene. The frequency of the *PVL* gene was 35.71% and 25% among MRSA bacteria isolated from *P. americana* and *B. germanica* hospital cockroaches, respectively. Statistical significant difference was found between d of samples and frequency of the *PVL* gene ($P < 0.05$).

5. Discussion

Cockroaches are regarded as the sources of diverse types of antibiotic-resistant bacteria. They correspondingly can be the vectors for the transmission of numerous diseases into human inhabitants, predominantly in public places such as hospitals (18). The results of the current survey signified that MRSA bacteria recovered from hospital cockroach samples harbored the *PVL* gene and various types of *SCCmec* units. Methicillin-resistant *S. aureus* bacteria harbored a high frequency of *SCCmec* types III, I, and II. Methicillin-resistant *S. aureus* is usually subcategorized into healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA). Epidemiological surveys show that HA-MRSA bacteria harbor *SCCmec* I, II, or III while CA-MRSA bacteria harbor *SCCmec* types IV or V (19). Furthermore, HA-MRSA bacteria have a lower frequency of the *PVL* gene than CA-MRSA bacteria (19). Consequently, the majority of MRSA bacteria recovered from external washing samples of hospital cockroaches were HA-MRSA.

The possible reason for the higher frequency of HA-MRSA bacteria in the examined samples is that external washing samples of hospital cockroaches contained MRSA bacteria of the hospital environment, which were carried

Table 1. Target Genes and Oligonucleotide Primers Used for Detection of SCCmec Types and PVL Gene in MRSA Bacteria Recovered From Hospital Cockroaches (17, 18)

Target Gene	Primer Sequence (5' - 3')	PCR Product, bp	PCR Program, s	PCR Volume (50 µL)
SCCmec I	F: GCTTAAAGAGTGCCT-TACAGG	613	1 cycle: 93°C, 7 min. 10 cycles: 93°C C, 55 s; 64 °C, 50 s; 72°C, 2 min. 25 cycles: 94°C, 45 s; 55°C C, 45 s; °C, 2 min. 1 cycle: 72°C, 10 min	5 µL PCR buffer 10 ×; 2 mM MgCl ₂ ; 150 µM dNTP (fermentas); 0.75 µM of each primers F & R; 1.5 U Taq DNA polymerase (fermentas); 3 µL DNA template
	R: GTTCTCTCATAGTATGACGTCC			
SCCmec II	F: CGTTGAAGATGATGAAGCG	398		
	R: CGAAATCAATGGTTAATGGACC			
SCCmec III	F: CCATATTGTGACGATGCG	280		
	R: CCTTAGTTGTCGTAACAGATCG			
SCCmec IVa	F: GCCTTATCGAAGAAACCG	776		
	R: CTACTCTTCTGAAAAGCGTCG			
SCCmec IVb	F: TCTGGAATTACTTCAGTGC	493		
	R: AAACAATATTGCTCTCCCTC			
SCCmec IVc	F: ACAATATTGTATTATCGGA-GAGC	200		
	R: TTGGTATGAGTATTGCTGG			
SCCmec IVd	F: CTCAAAATACGACCCCAAT-ACA	881		
	R: TGCTCCAGTAATTGCTAAAG			
SCCmec V	F: GAACATTGTACTTAAAT-GAGCG	325		
	R: TGAAAGTTGTACCCTTGACACC			
PVL	F: ATCATTAGTAAATGTCG-GACATGATCCA	433	1 cycle: 94°C, 5 min. 30 cycle: 94°C, 30 s; 55°C, 30 s; 72°C, 30 s. 1 cycle: 72°C, 5 min	5 µL PCR buffer 10 ×; 2 mM MgCl ₂ ; 150 µM dNTP (fermentas); 0.75 µM of each primers F & R; 1.5 U Taq DNA polymerase (fermentas); 3 µL DNA template
	R: GCATCAASTGTATTGGACAT-GATCCA			

Table 2. Frequency of SCCmec Types and PVL Gene Among MRSA Bacteria Recovered From External Washing Samples of Hospital Cockroaches^a

Samples (Number of Positive for MRSA)	Number of Positive Samples for Each SCCmec Type (%)								Number of Positive Samples for PVL Gene (%)
	I	II	III	IV				V	
				a	b	c	d		
<i>P. americana</i> (n = 28)	8 (28.57)	5 (17.85)	12 (42.85)	1 (3.57)	-	-	1 (3.57)	1 (3.57)	10 (35.71)
<i>B. germanica</i> (n = 8)	2 (25)	1 (12.50)	4 (50)	-	-	-	-	1 (12.50)	2 (25)
Total (n = 36)	10 (27.77)	6 (16.66)	16 (44.44)	1 (2.77)	-	-	1 (2.77)	2 (5.55)	12 (33.33)

^aValues are expressed as No. (%).

owing to the contact of the exterior parts of the cockroach's bodies with the sources of infections existed in the hospitals. The previously available data convey that the majority of PVL-positive *S. aureus* bacteria are related to soft tissue and skin infections (20). Consequently, PVL-positive MRSA bacteria recovered from hospital cockroaches may originate from the cases of soft tissue infections in hospitals. Methicillin-resistant *S. aureus* has been presented as one of the most predominant pathogenic bacteria recovered from *B. germanica* and *P. americana* cockroaches (3, 4, 21-23). Nevertheless, the isolation of the SCCmec types and

the PVL gene among MRSA bacteria recovered from hospital cockroaches was not scrutinized beforehand.

Borbon-Esquer et al. (24) conveyed that of 102 MRSA bacteria recovered from hospitalized children in Mexico, 97 (95%) harbored SCCmec type II, 5 (5%) harbored SCCmec type Iva, and all (100%) of them were PVL-negative. Momtaz and Hafezi (25) conveyed that SCCmec type III (24.52%) had the highest frequency among MRSA bacteria recovered from clinical infections. They signified that the frequency of the PVL gene was 40.90%. Higher frequency of SCCmec types I, II, and III was also reported from India (26), Saudi

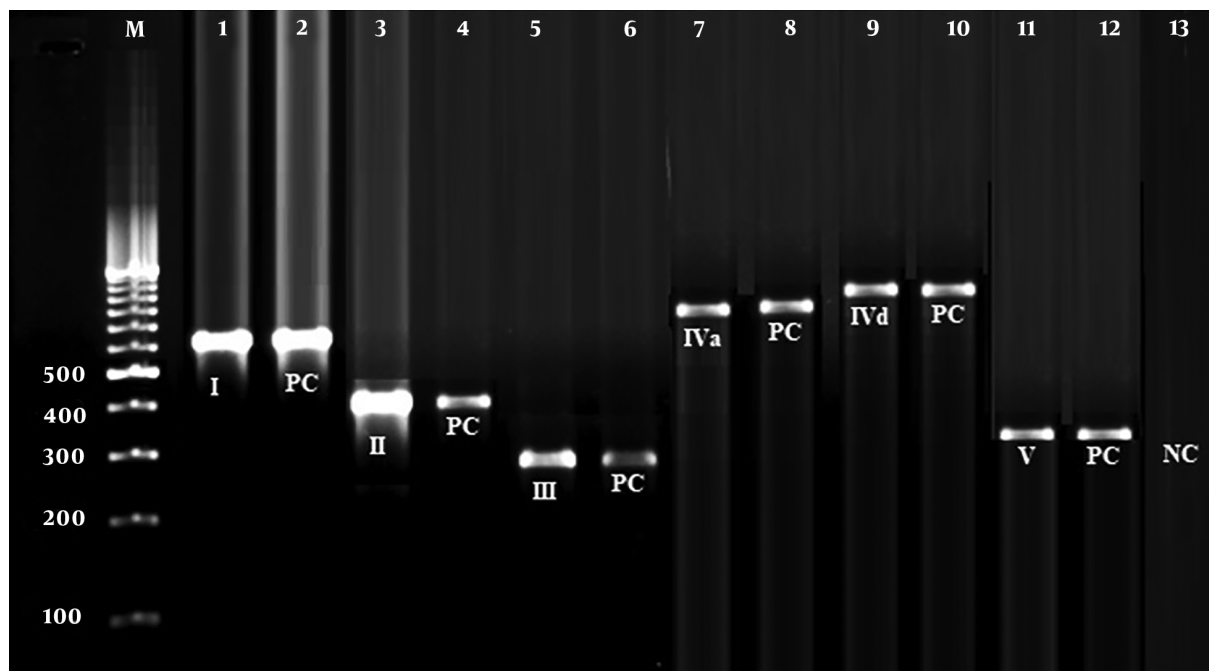


Figure 1. Outcomes of the gel electrophoresis of the *SCCmec* kinds perceived in the MRSA bacteria

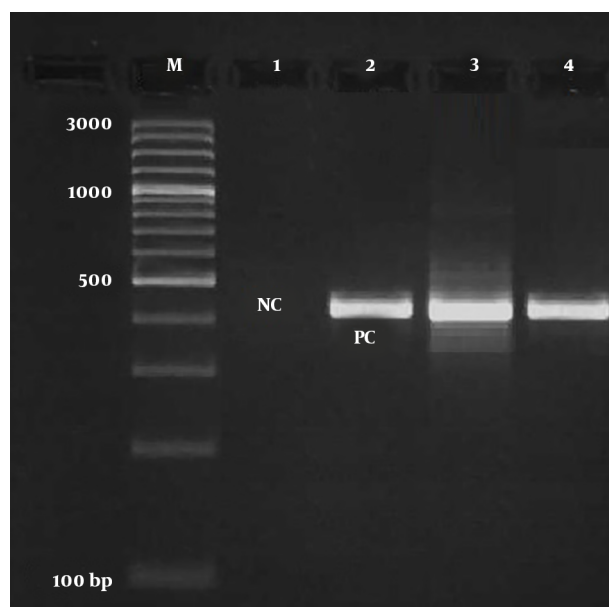


Figure 2. Outcomes of the gel electrophoresis of the *PVL* gene perceived in the MRSA bacteria

Arabia (27), and Brazil (28). Goudarzi et al. (29) indicated that diverse *SCCmec* types including *SCCmec* type III (38.9%), II (31.1%), IV (28.9%), and I (1.1%) were obtained from MRSA

bacteria recovered from the cases of UTIs in Iran.

We showed that only 33.33% of the MRSA bacteria harbored the *PVL* gene. The *PVL* gene is one of the main exotoxins of MRSA. The occurrence of the *PVL* gene in MRSA bacteria recovered from clinical infections was also reported beforehand (30, 31). The occurrence of the *PVL* gene among *SCCmec* types I, II, and III bacteria was also conveyed by Lima et al. (32) and Glikman et al. (33). Nevertheless, *PVL*-positive isolates were not discovered in *SCCmec* types I, II, and III bacteria in previous research (34, 35). Consequently, it can be concluded that the existence of the *PVL* gene is not an explanatory factor for CA-MRSA, as it may be existed in HA-MRSA or maybe absent from CA-MRSA.

It is known that *SCCmec* types I, II, and III are present in HA-MRSA strains and *SCCmec* types IV and V are present in CA-MRSA strains. The studied cockroaches could move freely inside and outside the hospital environment. Thus, they could carry both HA-MRSA (from the hospital environment) and CA-MRSA (from outside the hospital environment) strains as reported in our survey. Thus, it is not surprising that both CA-MRSA and HA-MRSA strains were found in the studied samples. Additionally, MRSA bacteria isolated from *B. germanica* and *P. americana* cockroaches harbored various *SCCmec* types. This may be probably because of differences in the type of feeding, lifestyle, and crossing paths and diverse living locations of the two cock-

roach species inside or outside the hospital environment.

5.1. Conclusions

The current survey is an initial description of the identification of *SCCmec* types and the *PVL* gene among MRSA bacteria recovered from external washing samples of hospital cockroaches. High frequency of *SCCmec* types I, II, and III and comparatively low frequency of the *PVL* gene characterize the occurrence of HA-MRSA bacteria in *B. germanica* and *P. americana* hospital cockroaches. This finding discloses an imperative public health hazard concerning the attendance of HA-MRSA bacteria in hospital cockroaches. The results showed that *B. germanica* and *P. americana* hospital cockroaches are the reservoirs of MRSA bacteria in the hospital environment. Further surveys are mandatory to gain supplementary information about the epidemiological share of hospital cockroaches in the survival and transmission of MRSA bacteria.

Footnotes

Authors' Contribution: Zohreh Abdolmaleki and Farhad Safarpour Dehkordi did study design, PCR genetic alignment, writing, and drafting of the manuscript. Zohreh Mashak did sample collection, culture-based identification, and statistical analysis.

Conflict of Interests: The authors state no conflict of interest in the publication of the current paper.

Ethical Approval: The study was accepted by the Ethics Board of the Faculty of Veterinary Medicine, Karaj Branch, Islamic Azad University, Karaj, Iran (agreement reference no.: 1159706100005).

Funding/Support: This work was supported by the Faculty of Veterinary Medicine, Islamic Azad University, Karaj Branch, Karaj, Iran (grant no.: 1159706100005).

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