Detection of *Staphylococcus Aureus* and Their Toxin Genes Inhabit On The Scorpions Surface

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Abstract

Introduction:

The transmission of infectious agents by arthropods is of particular importance. Every year, many people are bitten by scorpions around the world. *Staphylococcus aureus* is of the most important infectious bacteria. This study aimed to investigate the distribution of *S. aureus* in scorpion specimens and the presence of some toxin genes in these species.

Methods

The fauna of scorpions in the Kuhdasht region was studied for one year. Then, *S. aureus* was identified on the body surface of scorpions by biochemical and molecular methods, and the presence of *Sea*, *Seb*, *Sec*, *Sed*, *See*, *Pvl*, *Tsst1*, *Eta*, *Etb*, and *mecA* genes was examined by the PCR method. The pattern of antibiotic resistance was determined by the antibiogram method. MRSA isolates were identified using genotypic and phenotypic methods.

Results

Of 75 studied scorpion specimens, *Hottentotta saulcyi* was the most abundant species. Sixteen (21.3%) isolates of *S. aureus* were identified from all samples. The highest and lowest antibiotic resistance levels belonged to penicillin and clindamycin, respectively. MRSA was observed in 50% of the isolates. Thirteen out of 16 isolates possessed at least one of the toxin genes.

Discussion

Due to the presence of *S. aureus* on the body surface of scorpions, it should always be expected that an infection may occur after the bite. Moreover, the presence of toxin genes in the studied isolates showed that infection with these bacteria would seriously threaten one's health.

Introduction

Arthropods are a large branch of invertebrates that encompass organisms such as Gnathostomata, Arachnids, Crustaceans, etc. A group of arthropods are harmless to humans and also play an important role in the biological cycle. Some groups are harmful to humans and can cause numerous injuries through bites, stings, or other toxic and inflammatory secretions, in addition to transmitting various pathogens (Leitner et al. 2015). Some arthropods can only injure the site of the bite, and in some cases, bacterial contamination of the mouthpieces of these arthropods can cause secondary infections. Many pathogens are transmitted by arthropods. Examples include plague, Crimean Congo haemorrhagic Fever, and Dengue Fever, which are transmitted by fleas, ticks, and *Aedes* mosquitoes, respectively (Organization 2014).

Scorpion bites are one of the most important health and medical problems and, in addition to the resultant anxiety and concern; the problem incurs high treatment costs and poses many people to the risk of death every year. Therefore, the development of infection after arthropod bites is a special medically important issue (Ahmadi et al. 2020).

Staphylococcus aureus is a gram-positive and facultative anaerobic cocci, which is medically the most important species in the genus *Staphylococcus*. This bacterial strain can cause a wide range of simple skin infections (such as

pimples, boils, scabs, stys, and abscesses) to dangerous diseases, such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, and septicemia. *S. aureus* is one of the five most common causes of nosocomial infections, in particular postoperative wound infections (Khan et al. 2017; Lee et al. 2018). Toxins of this bacterium play an important role in its pathogenesis. Recent studies, for instance, have shown an association between PVL (Panton Valentine Leukocidin) and methicillin-resistant strains of *S. aureus*, or the toxic shock syndrome toxin-1 gene produces a protein that causes systemic disease (Hennekinne et al. 2012; Oliveira et al. 2018; Kim 2019). The Exfoliative toxin is another important toxin of this bacterium that can cause complications such as staphylococcal scaly skin syndrome. Staphylococcal enterotoxins are a class of superantigens and are also involved in food poisoning (Hennekinne et al. 2012; Oliveira et al. 2018; Kim 2019). On the other hand, infections caused by methicillin-resistant *S. aureus* (MRSA) have turned into a global concern. The presence of the *mecA* gene is the cause of drug resistance in MRSA (Goering et al. 2019). Therefore, it is of paramount importance to identify these important isolates in scorpions. The prevalence of *S. aureus* in scorpions and the identification of pathogenic genes in isolated bacteria have not been studied up to now.

This study aimed to investigate the fauna of scorpions in Kuhdasht city, the distribution of *S. aureus* in surface of scorpions, the frequency of *Sea*, *Seb*, *Sec*, *Sed*, *See*, *Pvl*, *Tsst1*, *Eta*, *Etb*, and *mecA* genes in *S. aureus* isolates, and the frequency of MRSA in these isolates.

Methods

Sample collection

Scorpion specimens were collected from different locations of Kuhdasht city since Mar. 21, 2018 to Mar. 21, 2019. Since scorpions are mainly night-active and their cuticles glow under ultraviolet (UV) light, scorpions were searched by a UV lamp at night. The observed specimens were taken from the tail area by scorpion pliers, placed in a separate sterile plastic container, and transferred to the laboratory. The sampling date, the collector name, weather conditions and environmental factors at the time of sampling, station name, geographical location (GPS), and altitude were recorded for each sample.

Identification of scorpions

The scorpions were identified by a stereomicroscope (Olympus SZ-CTV) using the identification keys of Farzanpay (Farzanpay 1987), Dehghani (Dehghani 2006), Navidpour (Navidpour et al. 2011), and Kovarik (Kovářik 2007) and then studied phenometrically. The patterns of morphological differences between the studied scorpions specimens in the registered stations of Kuhdasht city (Table 1) based on measurable traits were examined. The studied populations were separated from each other in the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) tree (Figure 1). The applied techniques used were based on standardized methods.

Staphylococcus aureus isolation

In the laboratory, normal saline swabs were gently mopped on the surface of the body and telson of scorpion samples and cultured on Blood Agar and Nutrient Agar media. The bacteria were purified, stained by Gram staining, and then *S. aureus* was initially identified using biochemical tests, namely catalase, coagulase, blood agar hemolysis, DNase production, and mannitol salt agar (Ghaznavi-Rad and Ekrami 2018). All the employed equipment was sterile. This step was performed separately for each sample.

DNA extraction

The boiling method was used to extract DNA. First, two to three colonies of the bacterium were transferred to a microtube containing 300 µl of DNase/RNase-Free distilled water. Then, the lids of the microtubes were closed completely with parafilm and placed in boiling water for 15 min. Next, the suspension was centrifuged (10000 g, 5 min), the DNA-containing supernatant was transferred to clean sterile microtubes, and kept at -20 °C for further experiments (Kazemnia et al. 2014).

Molecular identification of bacteria

PCR method was used for accurate and final identification of *S. aureus* isolates. At this stage, the isolates were identified using specific primers, namely 5'-GCGATTGATGGTGATACGGTT-3' (forward) and 5'-AGCCAAGCCTTGACGAAGTAAAGC-3' (reverse). The final volume (25µl) of the reaction included 3 µl of DNA template, 0.7 µl of each primer (10 pM), 0.5 µl of dNTPs (25 µM), 0.2 µl of Smart *Taq*DNA polymerase, 1 µl of MgCl₂ (2 mM), 2.5 µl of 10X *Taq* buffer (Amersham, Pharmacia Biotech), and 16.4 µl of distilled water. Finally, all PCR products were electrophoresed on the gel (Brakstad et al. 1992). Sequencing was performed for all 16 PCR products. These sequences were recorded with accession numbers MZ816748 to MZ816763 in GenBank.

Detection of toxin genes

The *Sea*, *Seb*, *Sec*, *Sed*, *See*, *Pvl*, *Tsst1*, *Eta*, and *Etb* toxin genes in *S. aureus* were identified by the PCR method with primers used in previous studies (Japoni-Nejad et al. 2013; Leke et al. 2017). To detect MRSA isolates by the genotypic method, the *mecA* gene was examined in all isolates by PCR. The final volume (25 µl) of the reaction was similar to the previous step. Finally, all PCR products were electrophoresed on the gel.

Antibiotic resistance pattern and identification of MRSA isolates

The antibiotic resistance pattern in isolates was determined on Müller-Hinton agar medium by the disk diffusion method (Kirby-Bauer) according to the Clinical and Laboratory Standards Institute (CLSI). Antibiotics used were penicillin (10 units), cefoxitin (30 µg), gentamicin (10 µg), tobramycin (10 µg), azithromycin (15 µg), tetracycline (30 µg), ciprofloxacin (30 µg), clindamycin (2 µg), trimethoprim (5 µg), and rifampin (5 µg) (Weinstein et al. 2019). The phenotypes of MRSA isolates were detected by the disk diffusion method using the cefoxitin disk (30 µg).

Drawing a phylogenetic tree and Statistical Analysis

The MVSP (Multivariate Statistical Package) software was used to draw a phylogenetic tree and classify scorpions. Statistical analyses were done by SPSS (Statistical Package for the Social Sciences) software at a significance level of 0.05.

Results

Identification of scorpions and phylogenetic tree

A total of 75 scorpions were collected from the registered stations in Kuhdasht city. The caught scorpions were examined for 17 measurable traits (Table 1). Phenometric study included *Hottentotta saulcyi* (42), *Scorpio maurus* (16), *Hemiscorpius lepturus* (10), *Compsobuthus matthiesseni* (5), *Androctonus crassicaud* (1), and *Orthochirus iran* (1) species, respectively. *H. saulcyi* (56%) was the least abundant and *A. crassicaud* and *O. iran* (1.33%) had the highest abundance. UPGMA results showed the presence of two branches in the tree. In the smaller branch, *H. saulcyi* was isolated from the other species. This branch is divided into two sub-branches. Payastan station was separated from other populations of this species. The second sub-branch was divided into two groups, the smaller group

consisting of Qibla and South Kuhdasht stations. The larger group was divided into two subgroups, where Kashmahoor and Graboo Kuhdasht stations were assigned to two separate groups. The larger branch consisted of two sub-branches. The smaller subdivision was subdivided into two groups, where *Scorpio maurus* of Payastan station (No. 4) was separated from the rest and formed a smaller group. In the larger group, *H. lepturus* in the Grub station (No. 5) was separated from the others, and *S. maurus* in the Grub, Qibla, and Kashmahoor stations was placed in the larger subgroup. This subgroup was subdivided into two parts, and Grubb station was separated from the other two stations. The larger subdivision was also divided into two groups. The larger group was divided into two subgroups, where the *O. iranusi* formed a smaller subgroup in the North Kuhdasht station and was separated from the populations of *C. matthiesseni*. The larger subgroup was divided into two parts, with a smaller population in South Kuhdasht. The larger section was divided into two sub-sections, the smaller sub-section consisting of the Payastan station and the larger sub-section comprising Grub and Kashmahour stations. The smaller group consisted of two subgroups, of which *H. lepturus* in Keshmahoor was a smaller subgroup. The larger subgroup was subdivided into two parts, where *S. maurus* was the smaller part in the North Kuhdasht station, and *H. lepturus* of Payastan and *S. maurus* of South Kuhdasht stations formed the larger part.

Staphylococcus aureus isolation and molecular detection

Based on molecular and biochemical characteristics, 16 (21.3%) isolates of *S. aureus* were identified out of 75 studied scorpion specimens. The detected isolates were respectively *S. maurus* (7, 43.75%), *H. saulcyi* (5, 31.2%), and *A. crassicaud*, *H. lepturus*, *C. matthiesseni*, and *O. iranusi* each with 1 (6.25%) isolate. The lowest and highest species containing *S. aureus* were respectively *A. crassicaud*, *H. lepturus*, *C. matthiesseni*, and *O. iranusi* each with 1 (6.25%) species and *S. maurus* (7 samples, 43.75%).

Antibiotic Resistance Pattern And Identification Of Mrsa Isolates

Examination of antibiotic resistance pattern of the studied isolates showed that the highest and lowest levels of antibiotic resistance belonged to penicillin and clindamycin, respectively. Of the 16 studied isolates, two isolates were resistant to all tested antibiotics. Given that 9 isolates were resistant to Cefoxitin, 9 isolates of MRSA were identified phenotypically (Fig. 2).

Detection of toxin and mecA genes

Of the 16 isolates of *S. aureus*, three (18.75%) isolates had none of the toxin genes studied, while the other isolates possessed at least one of the toxin genes. On the other hand, *sed*, *sec*, and *pvl* genes were observed in none of the isolates. With a frequency of 50%, the *mecA* gene was identified in two samples of *H. saulcyi*, one sample of *O. iranusi*, and five samples of *S. maurus*. In other words, 8 MRSA isolates were identified genotypically. Since eight out of nine isolates were considered MRSA by the phenotypic method and had a positive PCR test of the *mecA* gene, one false positive sample was detected in the phenotypic method. The results indicated that the two species, *A. crassicaud* and *H. lepturus*, contained none of the studied toxin genes (Table 2).

Discussion

Our study demonstrated that scorpions identified in these areas belonged to three families: *Hemiscorpiidae*, *Scorpionidae*, and *Butiidae*. In a study conducted by Nemato-allahi *et al.* in Aligudarz city, *Hutentota salsi* had the highest distribution in different stations, which is consistent with our study (Nematollahi *et al.* 2020). Akbari *et al.* conducted a study in Kohgeluyeh and Boyer Ahmad and Chaharmahal and Bakhtiari provinces and found that the *Butiidae* family had the highest species diversity with six genera. They caught only one genus, *Hemiscorpius*, from the *Hemiscorpiidae* family, which is in line with the present study due to almost similar geographical conditions to

Lorestan province (Akbari et al. 2001). It can generally be concluded that scorpions of the Butiidae family are the most abundant scorpions in the region and *H. salsi* is the dominant species in these areas. It should be noted that *H. salsi*, which is one of the most dangerous scorpions in Iran, and *Hemiscorpius lepturus*, which is one of the most dangerous scorpions and causes the death of many scorpion bites in Iran, comprised respectively 55 and 13% of scorpion species caught in Kuhdasht city, which can be a warning to the health officials of the province.

More importantly, although *S. maurus* is less dangerous to humans than the other species caught in this study, health workers should take diagnostic tests for *S. aureus* in people bitten by scorpions as this study proved that this bacterium is present on the body of these scorpions. It has now been proven that many bacteria live on the surface or inside the body of arthropods, acting as carriers of numerous pathogens that cause important human and animal diseases. These carriers play an important role in the transmission of bacteria to other organisms, including mammals. *S. aureus* is one of the pathogenic bacteria that have been confirmed to be present in association with some arthropods (Fagan et al. 2003; Nayduch et al. 2013; Abraham et al. 2017).

The presence of some genes enables this bacterium to become more pathogenic and even cause death in some cases. No study has so far investigated the distribution of this bacterium in scorpions and the presence of the *Sea*, *Seb*, *Sec*, *Sed*, *See*, *See*, *Pvl*, *Tsst1*, *Eta*, *Etb*, and *mecA* genes in these species, making it impossible to compare the data of this study with similar studies. Furthermore, our results can provide a new perspective on the complications after scorpion bites. In other words, post-bite complications caused by scorpion were not considered previously to be possibly related to infection caused by *S. aureus* and its produced toxins. Depending on the type of scorpion bites, the bacterium can easily enter the tissue if the scorpion's body surface or telson is infected with the bacterium. In this study, 16 (21.3%) out of 75 scorpion samples contained this bacterium, which shows the presence of bacteria on the scorpion's body surface and telson and the importance of the study of bacterial infections after scorpion bites. Unfortunately, 11 (68.75%) out of the 16 studied isolates carried at least one of the toxin genes.

On the other hand, one of the current problems faced by humans is the presence of antibiotic-resistant bacteria (Organization 2017). In this study, a relatively high pattern of antibiotic resistance was observed in the isolates, and two isolates were resistant to all tested antibiotics; therefore, infection by these bacteria after scorpion bites can be extremely dangerous for the infected person. The two isolates were isolated from *S. maurus* in regions with more human communities; hence these antibiotic-resistant isolates might have been transferred to animals in the surrounding environment by human activities. MRSA is currently one of the major therapeutic challenges. MRSA isolates are divided into three groups: healthcare-associated MRSA (HA-MRSA), community-associated MRSA (CA-MRSA), and livestock-associated MRSA (LA-MRSA) (Junnila et al. 2020). Rapid identification and treatment of MRSA infections are the most important procedures to prevent the spread of infection and reduce the risk of mortality in patients (Blot et al. 2002). This is because the *mecA* gene is reportedly not found in MRSA strains; hence the molecular methods that identify the *mecA* gene are the gold standard methods (Wallet et al. 1996; Brown et al. 2005).

In this study, therefore, the identification of MRSA isolates by phenotypic and genotypic methods was very important because the exact and definite frequency of these isolates was determined here. According to our results, a significant frequency of 50% was obtained for MRSA isolates in scorpion communities carrying *S. aureus*. The higher frequency of MRSA isolates in *S. maurus*, which were more closely related to human communities, could probably result from the acquisition of these bacteria from humans and animals in contact with humans. In other words, these isolates probably originate from CA-MRSA and LA-MRSA, which in turn can indicate the role of humans in changing the pattern of bacterial distribution in the environment.

The present study presents the first results in the world and our findings confirm the possibility of infection with *A. aureus* after the scorpion bite. This, therefore, alarms that the possibility of infection by *A. aureus* should be considered

after the scorpion bite based on the appearance of the lesion, and the necessary procedures should be taken in this regard. Similar studies are also recommended in other parts of the world to better determine the distribution of this bacterium and the genes studied in scorpions.

Declarations

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Declaration of competing interest

The authors declare that there is no conflict of interest.

Authors contributions

Mehdi Moradikhan: samples collection, performed experimental analysis and wrote the paper. **Alireza Shayestehfar:** research design, data collection and edited the paper. **Majid Komijani:** research design, data analysis and edited the paper.

Consent to Participate

The authors confirm that the manuscript has been read and approved by all named authors and that all of us have approved the order of authors listed in the manuscript.

Consent to Publish

All authors agree to publish the data of this paper.

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Tables

Table 1
Information of collected species

Location	Latitude (N°)	Longitude (E°)	ASL	TAC	Season								Density (%)
					Spring		Summer		Autumn		Winter		
					T	H	T	H	T	H	T	H	
South Kuhdasht	33.4333	47.6167	1172	Forest	19	19	19	17	17	18	18	19	b: 9.09, d: 9.09, f: 5.55
Northern Kuhdasht	33.6833	47.4667	1237	Forest	53	52	52	53	57	55	56	53	d: 5.45, e: 100, f: 16.66
Keshmahor	33.4500	47.4500	1334	Pasture and Forest	29	29	30	26	26	27	28	29	b: 36.36, c: 7.69, d: 7.27, f: 5.55
Paye Astan	33.4375	47.2821	1262	Shrub	27	23	24	24	27	27	28	28	b: 18.18, c: 7.69, d: 30.9, f: 11.11
Garab	33.4741	47.2374	1015	Shrub	14	15	15	13	13	14	13	14	b: 9.09, c: 30.76, d: 14.54, f: 38.88
Gheble	33.5530	47.1310	1192	Shrub	57	54	53	53	58	56	57	57	a:100 b: 9.09, c: 15.38, d: 20, f: 5.55
Tange Ab Barik	33.4849	47.0221	971	Forest	7	7	8	6	6	7	6	7	b: 18.18, c: 38.46, d: 12.72, f: 16.66
ASL: Above Sea Level; TAC: Type of Area Coverage; T: Temperature, Centigrade; H: Humidity, Percentage; a: <i>Androctonus crassicauda</i> ; b: <i>Compsobuthus matthiesseni</i> ; c: <i>Hemiscorpius lepturus</i> ; d: <i>Hottentotta saulcyi</i> ; e: <i>Orthochirus iranus</i> ; f: <i>Scorpio maurus</i>													

Table 2
Distribution of toxin genes according to source of bacterial isolation

Species	Toxin genes								
	<i>Tsst1</i>	<i>See</i>	<i>Sed</i>	<i>Sec</i>	<i>Seb</i>	<i>Sea</i>	<i>Pvl</i>	<i>Etb</i>	<i>Eta</i>
<i>C. matthiesseni</i>	-	-	-	-	-	-	-	-	+
<i>H. lepturus</i>	-	-	-	-	-	-	-	-	-
<i>H. saulcyi</i>	+	+	-	-	-	+	-	-	+
<i>H. saulcyi</i>	-	-	-	-	-	-	-	-	-
<i>H. saulcyi</i>	-	-	-	-	-	-	-	-	-
<i>H. saulcyi</i>	-	-	-	-	-	-	-	-	+
<i>H. saulcyi</i>	-	-	-	-	-	-	-	+	-
<i>O. iranous</i>	-	-	-	-	-	-	-	+	-
<i>S. maurus</i>	+	+	-	-	+	-	-	-	-
<i>S. maurus</i>	+	+	-	-	+	+	-	-	+
<i>S. maurus</i>	+	+	-	-	-	-	-	-	+
<i>S. maurus</i>	+	-	-	-	+	-	-	-	+
<i>S. maurus</i>	-	-	-	-	-	+	-	+	-
<i>S. maurus</i>	-	-	-	-	-	-	-	-	-
<i>S. maurus</i>	-	-	-	-	-	-	-	+	-
<i>A. crassicaud</i>	-	-	-	-	-	-	-	-	-

Figures

Figure 1

The tree obtained from morphological data in six different species of scorpions caught from registered stations in Kuhdasht city

Figure 2

Antibiotic resistance pattern of isolates