Prevalence of Staphylococcal Cassette Chromosome *mec* and Panton-Valentine Leukocidin Gene Amongst the Methicillin-resistant *Staphylococcus aureus* Strains Isolated From Fowl Meat

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Abstract
Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered to be one of the most important causes of foodborne diseases.
Objective: The current examination was performed to examine the distribution of staphylococcal cassette chromosome *mec* (*SCCmec*) and Panton-Valentine leukocidin (*PVL*) gene amongst the MRSA strains isolated from raw fowl meat samples.
Materials and Methods: A total of 240 fowl meat samples were collected and cultured. MRSA strains were identified using cefoxitin and oxacillin susceptibility tests. DNA samples extracted from the MRSA strains were subjected to polymerase chain reaction (PCR) for detection of *SCCmec* and *PVL* gene.
Results: Twenty-two out of 240 (9.16%) raw fowl meat samples were positive for *S. aureus* strains. Twelve out of 22 *S. aureus* strains (54.54%) were determined as MRSA strains. The incidence of MRSA strains in raw chicken, turkey, quail, and ostrich meat samples was 66.66%, 50%, 50%, and 33.33%, respectively. The incidence of *SCCmec* IVa, *SCCmec* IVd, and *SCCmec* V was 50%, 8.33% and 41.66%, respectively. The applied method failed to detect *SCCmec* types I, II, III, IVb, and IVc. The incidence of the *PVL* gene amongst the MRSA strains was 75%.
Conclusion: The presence of *SCCmec* IV and *SCCmec* V and *PVL* gene revealed occurrence of community-associated MRSA (CA-MRSA) in fowl meat samples. Further studies are required to find additional epidemiological aspects of the MRSA strains in fowl meat samples.

Background

Fowl meat is among the world's most nutritious foods. It contains adequate portions of proteins, minerals, vitamins and edible fats essential for human health. However, the consumption of contaminated fowl meat can cause several dangerous disorders such as food poisoning. Fowl meat can easily be contaminated by hand manipulation through inspection in the slaughterhouses. Bacterial contamination of fowl meat can occur during different stages of meat inspection in the abattoir and also through their transport and sale.

Staphylococcus aureus is a Gram-positive, cocci-shaped and catalase positive bacterium frequently found in the respiratory tract and on the skin.[1,2] It is responsible for hospital and community-acquired infections and foodborne diseases.[1,2] The outbreak of diverse kinds of gastrointestinal diseases recognized by nausea, abdominal cramps, vomiting, weakness, and diarrhea and toxic

shock syndrome is attributed to foodborne *S. aureus*.[1,2] Recently, methicillin-resistant *S. aureus* (MRSA) has developed an emerging issue in foodstuffs and hospitals.[3,4] It has been recognized that around 50% of *S. aureus* strains were considered to be methicillin resistant.[5-7] American survey revealed a yearly estimation of 96 000 cases of MRSA infections with approximately 21% mortality rate.[8] The staphylococcal chromosomal cassette *mec* (*SCCmec*) is a genetic element of the MRSA strains linked to the *mecA* gene and is responsible for virulence characteristics of bacteria.[9] *SCCmec* elements are typically divided into types I, II, III, IV, and V with regard to the pattern of the *ccr* and *mec* alleles.[9] *SCCmec* IV is additionally classified into a, b, c and d subdivisions.[9] Pathogenicity of *S. aureus* strains depends on the presence of numerous surface antigens and extracellular proteins. The recurrent retrieval of MRSA produces leukocidal toxins. This proposes that the Panton-Valentine leukocidin (*PVL*) is one of the most

important virulence factors in the pathogenicity of diseases caused by MRSA strains.[10] Fowl meat can easily be contaminated with the MRSA during different stages of meat inspection. Therefore, it is essential to assess the microbial quality of fowl meat particularly for the presence of MRSA.

Objective

There is insufficient information on the incidence and molecular characteristics of the MRSA strains in fowl meat. Consequently, the present examination was performed to determine the incidence rate and frequency of *SCCmec* types and *PVL* gene amongst the MRSA strains isolated from raw ostrich, chicken, quail, and turkey meat samples.

Materials and Methods

Samples

From April to October 2018, a total of 240 diverse kinds of raw fowl meat samples including chicken (n=50), turkey (n=60), quail (n=70) and ostrich (n=60) were randomly collected from the shopping centers of the Tehran province, Iran. The samples were carefully transported to the laboratory in a refrigerator. All meat samples revealed typical physical properties such as odor and color.

Staphylococcus aureus Isolation and Identification

Twenty-five grams of each fowl meat sample were inoculated into 225 mL of buffered peptone water (PW, Merck, Germany) and subjected to Stomacher (Bagmixer 400W, Interscience, Saint-Nom, France) for around 2 minutes. Then, 5 mL of enriched PW media was inoculated into 50 mL Trypticase soy broth (TSB, Merck, Germany) supplemented with sodium pyruvate (1%) and NaCl (10%) and incubated for about 18 hours at 35°C. A loopful of the previous media was transferred to Baird-Parker agar supplemented with egg yolk tellurite emulsion (Merck, Germany) and then incubated for about 24 hours at 37°C. Dark shiny colonies bounded by clear regions were identified using biochemical examinations such as catalase, coagulase and urease activities, oxidase test, resistance to bacitracin (0.04 U), glucose O/F test, nitrate reduction, phosphatase, deoxyribonuclease (DNase, Merck, Germany) test, Voges–Proskauer (Merck, Germany) test and carbohydrate (xylose, sucrose, trehalose and maltose, fructose, lactose, mannose) fermentation tests.[11-15]

Identification of MRSA Strains

Susceptibility patterns of *S. aureus* bacteria were examined against cefoxitin (30 μg) and oxacillin (1 μg) disks (Himedia, India). The examinations were completed according to the procedures of the Clinical and Laboratory Standards Institute (CLSI).[16]

DNA Extraction

MRSA strains were sub-cultured on TSB media (Merck, Germany) and incubated at 37°C for about 48 hours. DNA was extracted from MRSA strains using the DNA extraction kit (Thermo Fisher Scientific, Germany) based on the manufacturer's guidelines. The extracted DNA was enumerated (NanoDrop, Thermo Scientific, Waltham, MA, USA), its purity was determined (A260/A280), and its concentration was adjusted to 50 ng/μL. The quality of DNA was examined using 2% agarose gel electrophoresis.

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

Table 1 characterizes the sequence of primers and conditions of polymerase chain reaction (PCR) performed for detection of *SCCmec* types and *PVL* gene.[17,18] A programmable DNA thermo-cycler (Eppendorf Mastercycler 5330, Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) was applied for this purpose. The amplified samples were confirmed by electrophoresis based on a technique developed previously.[17,18]

Statistical Analysis

Statistical analysis was performed using the SPSS software version 21.0 (SPSS Inc., Chicago, IL, USA). Moreover, chi-square test and Fisher exact two-tailed test were applied. A P value less than 0.05 was considered statistically significant.

Results

Incidence of *Staphylococcus aureus* and MRSA Strains

A total of 260 raw fowl meat samples were examined for the presence and molecular characterization of MRSA strains. Table 2 characterizes the incidence of *S. aureus* and MRSA strains in different kinds of raw fowl meat samples. Twenty-two out of 240 (9.16%) raw fowl meat samples were positive for *S. aureus* strains. Raw chicken meat (18%) samples had the maximum incidence of *S. aureus*, while raw ostrich meat (5%) had the minimum. Statistically significant difference was found between kinds of samples and the incidence of *S. aureus* ($P < 0.05$). Twelve out of 22 *S. aureus* strains (54.54%) were determined as MRSA strains. The incidence of MRSA strains in raw chicken, turkey, quail and ostrich meat samples was 66.66%, 50%, 50%, and 33.33%, respectively. Statistically significant difference was found amongst kinds of samples and the incidence of MRSA strains ($P < 0.05$).

Distribution of *SCCmec* Types

Table 3 characterizes the distribution of *SCCmec* types and *PVL* gene amongst the MRSA strains isolated from samples of fowl meat. MRSA strains isolated from fowl meat samples only harbored *SCCmec IVa* (50%), *SCCmec IVd* (8.33%) and *SCCmec V* (41.66%). However, there were no positive results for *SCCmec* types I, II, III, IVb, and IVc.

Table 1. PCR Properties Applied for Detection of *SCCmec* Types and *PVL* Gene in the MRSA Strains Isolated from Fowl Meat

Purpose gene	Oligonucleotides (5'-3')	Size (bp)	Temperature	Volume (50 µL)
SCCmec I	F: GCTTTAAAGAGTGTCGTTACAGG R: GTTCTCTCATAGTATGACGTCC	613		
SCCmec II	F: CGTTGAAGATGATGAAGCG R: CGAAATCAATGGTTAATGGACC	398	1 cycle: 93°C ------------ 7 min.	
SCCmec III	F: CCATATTGTGTACGATGCG R: CCTTAGTTGTCGTAACAGATCG	280	10 cycles: 93°C ------------ 55 s	5 µL PCR buffer 10X
SCCmec IVa	F: GCCTTATTCGAAGAAACCG R: CTACTCTTCTGAAAAGCGTCG	776	64°C ------------ 50 s 72°C ------------ 2 min	2 mM Mgcl$_2$ 150 µM dNTP (Fermentas)
SCCmec IVb	F: TCTGGAATTACTTCAGCTGC R: AAACAATATTGCTCTCCCTC	493	25 cycles: 94°C ------------ 45 s	0.75 µM of each primers F & R 1.5 U Taq DNA polymerase
SCCmec IVc	F: ACAATATTTGTATTATCGGAGAGC R: TTGGTATGAGGTATTGCTGG	200	55°C ------------ 45 s 72°C ------------ 2 min	(Fermentas) 3 µL DNA template
SCCmec IVd	F: CTCAAAATACGGACCCCAATACA R: TGCTCCAGTAATTGCTAAAG	881	1 cycle: 72°C ------------ 10 min	
SCCmec V	F: GAACATTGTTACTTAAATGAGCG R: TGAAAGTTGTACCCTTGACACC	325		
PVL	F: ATCATTAGGTAAAATGTCTGGACATGATCCA R: GCATCAASTGTATTGGACATGATCCA	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 10X 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

Table 2. Incidence of *Staphylococcus aureus* and MRSA Strains Amongst Different Kinds of Raw Fowl Meat Samples

Kinds of Raw Meat Samples	No. of Samples Collected	No. of Samples Positive for S. aureus (%)	No. of Samples Positive for MRSA (%)
Chicken	50	9 (18)	6 (66.66)
Turkey	60	6 (10)	3 (50)
Quail	70	4 (5.71)	2 (50)
Ostrich	60	3 (5)	1 (33.33)
Total	240	22 (9.16)	12 (54.54)

Table 3. Distribution of *SCCmec* Types and *PVL* Gene Amongst the MRSA Strains Isolated from Samples of the Fowl Meat

Samples (No. of Samples Positive for MRSA)	No. of Positive Samples for Each *SCCmec* Type (%)								Number of Samples Positive for *PVL* Gene (%)
	I	II	III	IV				V	
				a	b	c	d		
Chicken (6)	-	-	-	3 (50)	-	-	1 (16.66)	2 (33.33)	4 (66.66)
Turkey (3)	-	-	-	1 (33.33)	-	-	-	2 (66.66)	2 (66.66)
Quail (2)	-	-	-	1 (50)	-	-	-	1 (50)	2 (100)
Ostrich (1)	-	-	-	1 (100)	-	-	-	-	1 (100)
Total (12)	-	-	-	6 (50)	-	-	1 (8.33)	5 (41.66)	9 (75)

Distribution of the *PVL* Gene

The incidence of the *PVL* gene amongst the MRSA strains was 75%. Statistically significant difference was found amongst kinds of samples and incidence of *SCCmec* types ($P < 0.05$).

Discussion

Up to know, a large number of investigations have described the isolation of MRSA strains from livestock and derived foods (raw, undercooked, ready to eat and even cooked foods), as well as from the professionals working in livestock husbandry and the food production chain settings.[17-21] Thus, from the epidemiological perspective, it is necessary to assess the prevalence and molecular characteristics of MRSA strains recovered from fowl meat as a highly consumed foodstuff.

The current research was performed to determine the distribution of *SCCmec* types and *PVL* gene amongst the MRSA strains isolated from fowl meat samples. The total incidence of MRSA strains was 5%, which was significant. Furthermore, our results verified significant relationship amongst kinds of raw fowl meat and the incidence of MRSA. A lower incidence of MRSA strains was found in researches conducted in Japan,[19] Korea,[20] Italy[21] and the Netherlands.[22] The incidence of MRSA strains in meat in Brazil was 21.72%.[23] Additionally, the incidence of MRSA strains in meat in Turkey,[24] Egypt,[25] Germany,[26] and Denmark[27] was 30%, 40.80%, 71.50% and 52.00%, respectively. The role of meat as a reservoir of MRSA strains was also determined in Australia,[28] the United Kingdom[29] and the United States.[30] Our findings revealed that chicken and, in some cases, turkey may be the sources of MRSA strains. On the contrary, quail and especially ostrich were not permanent sources of MRSA strains. Different incidence rates of *S. aureus* strains in fowl meat samples may be due to the differences existed in the type of nutrition, method of slaughtering and amount of activated water (AW) of fowl meats. A high incidence of bacteria is probably due to the lack of preventive measures or disorganization to avoid contamination with *S. aureus* from the start point of the process, at the abattoirs, where the fowl skin and mucous membranes are frequently contaminated, whether it is naturally or as a result of cross-contamination with infected carcasses or even staff.[11-14]

Moreover, the current research revealed a high incidence of *SCCmec* types IVa, IVd and V and *PVL* gene in the MRSA strains. The high incidence of *PVL* gene in the MRSA strains represents a major health hazard to the public regarding the pathogenic nature of this gene. The *PVL* gene is one of the main *S. aureus* exotoxins. Previously published data reported that majority of *PVL*-positive *S. aureus* strains were related to soft tissue and skin infections.[31] Thus, *PVL*-positive MRSA may originate from infected staff and meat inspectors in slaughterhouses. The presence of the *PVL* gene amongst the *S. aureus* strains recovered from food samples has been reported previously.[32,33] Recent epidemiological surveys revealed that diseases caused by *PVL*-positive MRSA strains are more severe with poor prognosis for a longer period of time and mainly do not have satisfactory response to therapeutic agents due to the occurrence of antibiotic resistance.[32,33] *PVL*-positive MRSA strains were also detected in different types of human clinical infections, particularly wound, burn, pulmonary, urinary tract and gastrointestinal infections.[32,33]

MRSA strains harbored only *SCCmec* types IV and V. MRSA is often sub-categorized as healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA). Epidemiological studies revealed that CA-MRSA strains harbor *SCCmec* IV or *V*, while HA-MRSA strains harbor *SCCmec I*, *II* or *III*.[34] Therefore, majority of MRSA strains recovered from raw fowl meat samples of the current examination were CA-MRSA. A high incidence of *SCCmec* type *IV* in beef and pork samples was also reported by Jackson et al.[35] Vossenkuhl et al[36] stated that most of the MRSA strains of turkey meat samples harbored *SCCmec* V (58–72%) and IVa (20%-28%), which was similar to our results. The high incidence of *SCCmec* IV and V in foods with animal origin has also been reported previously.[37-39] Therefore, raw meat samples may be the sources of *CA*-MRSA with high incidence of *SCCmec* IV and V. In keeping with a high prevalence of MRSA strains in studied samples, the high prevalence of other foodborne pathogens has also been reported previously in different kinds of Iranian food samples.[40-46]

Conclusion

In conclusion, we recognized a high incidence of *SCCmec* types and *PVL* gene in the MRSA strains recovered from raw fowl meat samples. The majority of MRSA strains recovered from chicken meat samples harbored *PVL* gene and *SCCmec* types. Moreover, raw fowl meat samples were the main sources of CA-MRSA with a higher incidence of *SCCmec* IV and V kinds. Thus, fowl meat samples are considered as potential sources of CA-MRSA with a high incidence of the *PVL* gene. The simultaneous presence of *PVL* gene and *SCCmec* types IV and V in the MRSA strains represents a health hazard to the public considering the consumption of raw or undercooked fowl meat. The current examination is the first survey of the characterization of *SCCmec* types and *PVL* gene amongst the MRSA strains recovered from turkey, quail and ostrich meat samples. Moreover, multi-population-based studies should be conducted to gain additional comprehensive information on the incidence and frequency of MRSA in different Iranian populations.

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