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ORIGINAL

Staphylococcal cassette chromosome *mec* in the *Staphylococcus aureus* isolated from retail meat

Cromosoma de cassette estafilococo mec en el Staphylococcus aureus aislado de carne de venta al por menor

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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are among the most emerging causes of hospital- and community-acquired infections. Retail meat is considered as one of its sources. The present survey was performed to assess the distribution of Staphylococcal cassette chromosome *mec* (*SCCmec*) amongst MRSA isolates of retail meat.
Methods: A total of 28 MRSA isolates of retail meat samples were assessed to distribute the *SCCmec* type. MRSA. Isolates were confirmed using the biochemical tests and cefoxitin and oxacillin susceptibility tests. PCR was used to detect *SCCmec* types amongst the MRSA isolates.
Results: A total of 7 different *SCCmec* types were detected in the MRSA bacteria isolated from retail meat samples. *SCCmec* type V (46.4%) had the highest distribution amongst examined MRSA isolates, while SCCmec type I, IVb, and IVc (7.1%) had the lowest. There were no positive results for the *SCCmec* type II. Statistically, a significant difference was obtained between the source of MRSA isolation and *SCCmec* distribution (P <0.05).
Conclusion: As most isolates harboured *SCCmec* types IV and V, they originated from the community and called community-associated MRSA (CA-MRSA). Thus, the role of retail meat as a source of CA-MRSA was determined in this survey.

Keywords: *Staphylococcus aureus*, SCCmec, PCR, retail meat.

Resumen

Antecedentes: Las cepas de *Staphylococcus aureus* resistente a la meticilina (SARM) se encuentran entre las causas más emergentes de infecciones hospitalarias y comunitarias. La carne al por menor se considera una de sus fuentes. El presente estudio se realizó para evaluar la distribución del cromosoma de cassette estafilocócico mec (*SCCmec*) entre los aislados de SARM de la carne al por menor.
Métodos: Se evaluó un total de 28 aislados de SARM de muestras de carne al por menor para distribuir el tipo de *SCCmec*. MRSA. Los aislados se confirmaron mediante las pruebas bioquímicas y de susceptibilidad a la cefoxitina y la oxacilina. Se utilizó la PCR para detectar los tipos de *SCCmec* entre los aislados de SARM.
Resultados: Se detectaron un total de 7 tipos diferentes de *SCCmec* en las bacterias MRSA aisladas de las muestras de carne del comercio minorista. El tipo V de *SCCmec* (46,4%) fue el más distribuido entre los aislados de SARM examinados, mientras que los tipos I, IVb y IVc de *SCCmec* (7,1%) fueron los más bajos. No hubo resultados positivos para el *SCCmec* tipo II. Estadísticamente, se obtuvo una diferencia significativa entre la fuente de aislamiento de SARM y la distribución de *SCCmec* (P <0,05).
Conclusiones: Dado que la mayoría de los aislamientos albergaban *SCCmec* tipo IV y V, se originaron en la comunidad y se denominaron SARM asociados a la comunidad (CA-MRSA). Así pues, en este estudio se determinó el papel de la carne al por menor como fuente de SARM comunitario.

Palabras clave: *Staphylococcus aureus*, SCCmec, PCR, carne al por menor.

Introduction

Staphylococcus aureus (*S. aureus*), a Gram-positive and catalase-positive bacterium, is an important cause of foodborne diseases characterized by weakness, nausea, vomiting, abdominal cramps and toxic shock syndrome[1]. Foods with animal origins, particularly raw meat samples, are considered reservoirs of the bacterium[2]. Foodborne diseases caused by *S. aureus* are complicated owing to the high pathogenicity of bacterium and the emergence of antibiotic resistance[3]. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are among the most important causative agent of nosocomial infections and complicated foodborne diseases[4]. It is responsible for around 100,000 nosocomial infection cases with about 20-30% mortality rates in the USA[5]. Diseases caused by this bacterium are mostly resistant to antibiotic therapy and complicated owing to higher hospitalization procedure and loads of therapeutic charges[6].

MRSA strains have a small Staphylococcal cassette chromosome *mec* (*SCCmec*), preventing phagocytosis and indirect cellular immunity and producing an enzyme that inactivates most penicillin and methicillin-based therapies[7,8]. *SCCmec* elements are typically classified into types I, II, III, IV, and V concerning the pattern of the *ccr* and *mec* alleles. SCCmec IV is additionally classified into a, b, c and d subdivisions[9]. Assessment of the SCCmec profile of the MRSA bacteria may show their origins and severity of diseases they can occur.

Regarding the important role of meat as a reservoir of MRSAS strains, the present survey was performed to assess the distribution of SCCmec types amongst MRSA bacteria isolated from retail meat samples in Iran.

Materials and methods

Ethical consideration

The survey was confirmed by the Ethical Council of Research of the Department of Food Hygiene, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.

Bacterial strains

From May to October 2018, a total of 28 MRSA bacteria were isolated from retail meat samples collected from Iran. Isolates were confirmed another time using some biochemical tests, including Gram staining, hemolytic activity on sheep blood agar (Merck, Germany), catalase activity, coagulated test (rabbit plasma), oxidase test, glucose O/F test, resistance to bacitracin (0.04 U), mannitol fermentation on Mannitol salt agar (Merck, Germany), urease activity, nitrate reduction, phosphatase, deoxyribonuclease (DNase, Merck, Germany) test, Voges-Proskauer (Merck, Germany) test and carbohydrate (xylose, trehalose, sucrose, and maltose, fructose, lactose, mannose) fermentation tests[10].

MRSA identification

Isolates were confirmed as MRSA bacteria using cefoxitin and oxacillin susceptibility testing[11-14].

DNA extraction and quality assessment

MRSA isolates were sub-cultured on TSB media (Merck, Germany) and incubated for 48 h at 37 oC. According to the manufacturer's instruction, genomic DNA was extracted from the bacterial colonies using the DNA extraction kit (Thermo Fisher Scientific, St. Leon-Rot, Germany)[15, 16]. Purity (A260/A280) and concentration of extracted DNA were then checked (NanoDrop, Thermo Scientific, Waltham, MA, USA)[17-22]. The quality of extracted DNA was assessed using electrophoresis of DNA on a 2% agarose gel stained with ethidium bromide (0.5 µg/mL) (Thermo Fisher Scientific, St. Leon-Rot, Germany)[23-25].

Polymerase Chain Reaction (PCR)-based detection of SCCmec types

Table I disclosed the PCR protocol used for SCCmec types detection[26, 27]. A programmable DNA thermo-cycler (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) was used in

Table I: PCR protocol used for detection of SCCmec types [26, 27].

Target gene	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR volume (50µL)
SCCmec I	F: GCTTTAAAGAGTGTCGTTACAGG R: GTTCTCTCATAGTATGACGTCC	613	**1 cycle:** 93°C ---------- 7 min **10 cycles:** 93°C ---------- 55 s 64°C ---------- 50 s 72°C ---------- 2 min **25 cycles:** 94°C ---------- 45 s 55°C ---------- 45 s 72°C ---------- 2 min **1 cycle:** 72°C ---------- 10 min	5 µL PCR buffer 10X 1.5 mM $MgCl_2$ 200 µM dNTP (Thermo Fisher Scientific, St. Leon-Rot, Germany) 0.5 µM of each primers F & R 1.25 U Taq DNA polymerase (Thermo Fisher Scientific, St. Leon-Rot, Germany) 2.5 µL DNA template
SCCmec II	F: CGTTGAAGATGATGAAGCG R: CGAAATCAATGGTTAATGGACC	398		
SCCmec III	F: CCATATTGTGTACGATGCG R: CCTTAGTTGTCGTAACAGATCG	280		
SCCmec IVa	R: GCCTTATTCGAAGAAACCG R: CTACTCTTCTGAAAAGCGTCG	776		
SCCmec IVb	F: TCTGGAATTACTTCAGCTGC R: AAACAATATTGCTCTCCCTC	493		
SCCmec IVc	R: ACAATATTTGTATTATCGGAGAGC R: TTGGTATGAGGTATTGCTGG	200		
SCCmec IVd	F: CTCAAAATACGGACCCCAATACA R: TGCTCCAGTAATTGCTAAAG	881		
SCCmec V	F: GAACATTGTTACTTAAATGAGCG R: TGAAAGTTGTACCCTTGACACC	325		

all PCR reactions[28,29]. Amplified samples were analyzed by electrophoresis (120 V/208 mA) in 2.5% agarose gel. The gel was stained with 0.1% ethidium bromide (0.4 µg/ml). The UVI doc gel documentation systems (Grade GB004, Jencons PLC, London, UK) were applied to analyze images[30-32].

Statistical analysis

Statistical analysis was done using the SPSS 21.0 statistical software (SPSS Inc., Chicago, IL, USA). Chi-square test and Fisher's exact two-tailed test were used to assess any significant relationship between collected data[33]. P-value <0.05 was considered as a significant statistical level[34].

Results

A total of 28 MRSA isolates of retail meat samples were assessed for the distribution of the *SCCmec* type. MRSA. **Table II** shows the *SCCmec* profile of the MRSA bacteria isolated from retail meat samples. Seven different *SCCmec* types were detected in the MRSA bacteria isolated from retail meat samples. *SCCmec* type V (46.4%) had the highest distribution amongst examined MRSA isolates, while SCCmec type I, IVb, and IVc (7.1%) had the lowest. There were no positive results for the *SCCmec* type II. Statistically, a significant difference was obtained between the source of MRSA isolation and *SCCmec* distribution (P <0.05).

Discussion

Several infectious diseases cause important mortality and morbidities in recent years[35-38]. Most of them are resistant to diverse classes of antimicrobial agents[39-42]. MRSA bacteria are emerging and antibiotic-resistant causes of diverse kinds of nosocomial and community-associated infections[43]. Methicillin resistance is one of the most important features of antibiotic resistance in the *S. aureus* strains. In the 1960s, as soon as methicillin was introduced, methicillin-resistant strains of *S. aureus* appeared. These bacteria were also resistant to all available penicillins and other beta-lactam antibiotics. Before 1990, MRSA strains were confined to healthcare centres and hospitals and were called Healthcare-Associated MRSA (HA-MRAS). Gradually, however, there were reports of MRSA disease in people who had no contact with healthcare centres and hospitals, and these new strains were called Community-Associated MRSA (CA-MRSA)[44,45]. The differences between the two groups are related to genotypic, epidemiological, clinical characteristics and the range of infections they cause. Reports have shown that HA-MRSA strains usually cause internal infections, pneumonia, bloodstream infections, and surgical site infections, but CA-MRSA strains usually cause soft tissue infections, skin, sores, boils, and severe cases of sepsis. HA-MRSA strains usually carry large *SCCmec* tapes, i.e. III, *SCCmec* I, *SCCmec* II and *SCCmec* III. These strains contain the *mecA* gene. Studies have shown that HA-MRSA strains are usually resistant to non-beta-lactam antibiotics (such as penicillins, cephalosporins, carbapenems, and macrolides) and rarely carry the Panton-Valentine Leukocidin (*PVL*) gene. In contrast, CA-MRSA strains have smaller *SCCmec* types, i.e. *SCCmec* IV and *SCCmec* V, are less resistant to non-beta-lactam antibiotics and often carry the *PVL* gene. In general, CA-MRSA antibiotic susceptibility is higher than HA-MRSA strains[46,47]. Studies have shown that all infections that are treated on an outpatient basis and those hospitalized for less than 48 hours are all caused by CA-MRSA. In contrast, if a person is hospitalized for more than 48 hours, the staph infection is caused by HA-MRSA. Studies have shown that the *SCCmec* IV cassette is strongly associated with infectious strains in patients with no risk factors for HA-MRSA[48]. Due to the higher frequency of *SCCmec* IV and V types in the present survey, it seems that most of the MRSA strains isolated in the present study belong to CA-MRSA strains. However, confirmation of this issue requires more information.

Some researches have been conducted in this field. Saadati et al. (2019)[49] reported that the MRSA strains isolated from fowl meat samples only harboured *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. Hanson et al. (2011)[50] stated that the *SCCmec* types IV and V were the most commonly detected amongst the MRSA isolates of meat samples. Wu et al. (2019)[51] reported that the *SCCmec* types III, IV and V were the most commonly detected amongst the *S. aureus* bacteria isolated from meat and meat products (bacon/

Table II: *SCCmec* profile of the MRSA bacteria isolated from retail meat samples.

Retail meat samples	N. MRSA isolates	N. isolates harboured each SCCmec (%)							
		I	II	III	IVa	IVb	IVc	IVd	V
Raw bovine	13	-	-	1 (7.6)	5 (38.4)	-	1 (7.6)	1 (7.6)	6 (46.1)
Raw ovine	9	1 (11.1)	-	1 (11.1)	4 ()	2 (22.2)	1 (11.1)	1 (11.1)	4 (44.4)
Raw caprine	6	1 (16.6)	-	2 (33.3)	3 (50.0)	-	-	1 (16.6)	3 (50.0)
Total	28	2 (7.14)	-	4 (14.2)	12 (42.8)	2 (7.1)	2 (7.1)	3 (10.7)	13 (46.4)

sausage, poultry, pork, mutton and beef) in China. A similar distribution of the *SCCmec* types amongst the MRSA isolates was also reported from Japan[52], Iran[53], and India[54].

The present survey was a preliminary survey on the distribution of *SCCmec* types in the MRSA bacteria isolated from retail meat samples. It is limited to the absence of *PVL* gene detection and antimicrobial resistance assessment of MRSA strains. Additionally,m absence of the sequencing analysis is another important limitation of the present research.

Conclusion

In conclusion, high distribution of *SCCmec* types, particularly types IV and V was reported in this survey. Because the frequency of *SCCmec* types in this sample was such that the isolated strains are related to CA-MRSA, meat samples can be used as sources of survival and transmission of CA-MRSA. Complete cooking of meat before consumption, observing scientific and ethical principles in prescribing antibiotics and using meat-based foods, especially from reputable brands, restaurants and factories, can prevent serious food poisoning and the survival and spread of MRSA at Prevent community level.

Interests conflict

The researchers declare that they have no conflict of interest.

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