

SCCmec Types in Methicillin-Resistant *Staphylococcus aureus* Strains of Various Types of Milk

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Research Article

Abstract

Background: Methicillin-resistant *Staphylococcus aureus* is a major cause of food-borne diseases with impact of antibiotic resistance. Milk and dairy products are considered as sources of MRSA. The current research was done to study the prevalence of SCCmec factors in the MRSA recovered from raw milk in Iran.

Methods and findings: Two-hundred milk samples were collected and immediately cultured. MRSA strains were isolated and subjected to PCR amplification. Sixty out of 200 (30%) raw milk samples were positive for MRSA. Bovine had the highest (20%) prevalence of MRSA, while caprine had the lowest (15%). Statistically substantial variance was found between the prevalence of MRSA and type of samples ($P < 0.05$). SCCmec IVa (60%), SCCmec V (50%) and SCCmec IVb (25%) were the most frequently detected. Bovine and caprine milk samples had the highest and the most variable prevalence of SCCmec types.

Conclusion: MRSA are an important risk factor in dairy farms. Therefore, further studies are needed to find strategies for control of the presence of *S. aureus* and especially MRSA in dairy products.

Keywords: Prevalence; Methicillin-resistant *Staphylococcus aureus*; SCCmec types; Raw milk.

1. Introduction

Staphylococcus aureus (*S. aureus*) is a Gram-positive and coccial bacterium which is associated with nosocomial and community-acquired infections all-around the world [1]. It is also an important foodborne pathogen involved in severe gastrointestinal disorders. It has been assessed that the *S. aureus* is a causative agent for 245,000 foodborne illnesses per year in the United States [2]. Raw milk is one of the most common sources of Staphylococcal foodborne diseases [3,4]. Staphylococcal foodborne infections are primarily related to the emergence of antibiotic resistance [5,6]. The bacterium has the

highest levels of resistant against methicillin and other types of beta-lactams antimicrobial agents [5-7]. It has been documented that about 50% of strains of this bacterium were methicillin-resistant *S. aureus* (MRSA) [5-7]. Earlier research which was accompanied on the United States showed an annual estimate of 94,000 MRSA infections with nearly 20% mortality rate [8].

MecA is a gene responsible for resistance against methicillin. It is a 21 to 67 kb component with a specific location of the staphylococcal chromosomal cassette *mec* (SCCmec) [9]. SCCmec recognized by the attendance of indispensable indicators, i.e., *mec* and *ccr* genes. SCCmec components are typically divided into five types of I, II, III, IV and V (IVa, IVb, IVc and IVd) [9,10].

Unfortunately, basic principles of good hygiene have not been observed in most Iranian milking halls. Therefore, the present study was performed to investigate the prevalence of MRSA in raw milk samples and also distribution of SCCmec types in bacterial isolates.

2. Materials and Methods

2.1 Samples collection and MRSA identification

From April to September 2014, a total of 200 raw milk samples including bovine (n=80), ovine (n=60) and caprine (n=60) were purchased from supermarkets of various parts of Tehran, Iran. Milk samples had normal physical characteristics. Samples were purchased under sterile hygienic conditions and were transferred to the laboratory at 4°C in a cooler with ice packs.

Isolation of MRSA strains from the 25 ml of milk samples was performed using the Mueller Hinton broth (Merck, Germany) cultures according to the method described by Dormaneh et al. [11].

2.2 DNA extraction

MRSA isolates were sub-cultured on Tryptic Soy Broth (Merck, Germany) and then incubated at 37°C for 24 h. Genomic DNA was extracted from

the bacterial colonies using the DNA extraction and purification kit (Fermentas, Germany) according to the manufacturer's instruction. Concentration of extracted DNA samples was determined using the spectrophotometer at 260 nm (SQ 2800, Unico, USA) [12].

2.3 Detection of SCCmec profiles

Table 1 represents the sequence of primers and PCR conditions applied for amplification of SCCmec alleles [13]. DNA thermo-cycler (Eppendorf Mastercycler, Germany) was applied in all PCR amplifications. Totally, 10 microliters of PCR products were committed on a 2% agarose gel consisting of 0.5 mg/ml of CYBR Green (Fermentas, Germany) at 90 V for 20-30 min. Products were interpreted using ultraviolet illumination. *S. aureus* ATCC 6538 and sterile distilled water were applied as positive and negative controls, respectively.

2.4 Statistics

Data recovered from all tests were subjected to SPSS/19.0 software and $P < 0.05$ was determined as a significance level.

3. Results

Table 2 showed the incidence of MRSA in raw milk samples. Results revealed that 60 out of 200 (30%) raw milk samples were contaminated with MRSA. Bovine had the highest (20%), while caprine had the lowest (15%) prevalence of MRSA. Statistically significant difference was seen between the incidence of MRSA and type of samples ($P < 0.05$).

Table 3 shows the incidence of SCCmec alleles in MRSA strains recovered from raw milk samples. The most prevalent alleles of SCCmec were SCCmec IVa (60%), SCCmec V (50%) and SCCmec IVb (25%). Bovine and caprine milk samples had the highest prevalence of SCCmec types. Statistical differences were seen between the incidence of SCCmec IVa and SCCmec III ($P = 0.018$) and SCCmec V and SCCmec II ($P = 0.021$). Significant variance was seen between the type of milk samples and presence of SCCmec alleles ($P < 0.05$).

4. Discussion

Findings of the current research represented that the MRSA had a high (30%) prevalence in various

Table 1. Oligonucleotide primers used for detection of SCCmec types of the MRSA strains isolated from raw milk samples.

Target genes	Primer sequence (5'-3')	Size of product (bp)
SCCmec I	F: GCTTTAAAGAGTGTCTGTTACAGG R: GTTCTCTCATAGTATGACGTCC	613
SCCmec II	F: CGTTGAAGATGATGAAGCG R: CGAAATCAATGGTTAATGGACC	398
SCCmec III	F: CCATATTGTGTACGATGCG R: CCTTAGTTGTCGTAACAGATCG	280
SCCmec IVa	F: GCCTTATTCGAAGAAACCG R: CTA CTCTTCTGAAAAGCGTCCG	776
SCCmec IVb	F: TCTGGAATTACTTCAGCTGC R: AAACAATATTGCTCTCCCTC	493
SCCmec IVc	F: ACAATATTTGTATTATCGGAGAGC R: TTGGTATGAGGTATTGCTGG	200
SCCmec IVd	F: CTCAAATACGGACCCCAATACA R: TGCTCCAGTAATTGCTAAAG	881
SCCmec V	F: GAACATTGTTACTTAAATGAGCG R: TGAAAGTTGTACCCTTGACACC	325

Table 2. Total distribution of MRSA in various types of raw milk samples.

Types of samples	No. samples collected	No. MRSA (%)
Bovine	80	40 (20)
Ovine	60	11 (18.33)
Caprine	60	9 (15)
Total	200	60 (30)

Table 3. Prevalence of SCCmec types in the MRSA strains isolated from various types of raw milk samples.

Type of samples (No. positive)	Prevalence of SCCmec types (%)							
	SCCmec I	SCCmec II	SCCmec III	SCCmec IVa	SCCmec IVb	SCCmec IVc	SCCmec IVd	SCCmec V
Bovine (40)	1 (2.50)	2 (5)	3 (7.50)	28 (70)	10 (25)	6 (15)	2 (5)	24 (60)
Ovine (11)	-	-	1 (9.09)	3 (27.27)	2 (18.18)	-	-	2 (18.18)
Caprine (9)	-	1 (11.11)	1 (11.11)	5 (55.55)	3 (33.33)	1 (11.11)	-	4 (44.44)
Total (60)	1 (1.66)	3 (5)	5 (8.33)	36 (60)	15 (25)	7 (11.66)	2 (3.33)	30 (50)

types of milk samples. As far as we recognize, there were rare available information about the incidence of MRSA in milk in Iran. High prevalence of MRSA in raw milk samples of our investigation showed an important public health issue regarding the consumption of raw milk. Higher prevalence of MRSA in the bovine milk samples is maybe due to the higher population of cow in the milking process. Therefore, contamination can easily be transmitted from infective cows to others. Additional cause for the high occurrence of MRSA strains in the milk samples of our study is the pollution of dishes and containers using for maintenance and also transporting of milk. In addition, lack of the maintenance of milk in low temperature is another risk factor which may lead to the growth and proliferation of MRSA. The possibility of transmission of dangerous MRSA strains from an infected hand and respiratory nasal secretions of staffs of the milking halls to milk is another risk factor which should not be overlooked. Unfortunately, Iranian ranchers are using from traditional milking or hand milking which cause transmission of dangerous pathogens from hands to the milk.

Several investigations have been performed in this field al-around the world. Riva et al. [14] reported that 35 *S. aureus* strains were isolated from 383 raw milk samples (9.13%) of Milan (northern Italy) which was lower than our findings. They indicated that the incidence of MRSA strains were 1.82%. Haran et al. [15] showed that herd prevalence of MSSA was 84%, while MRSA herd prevalence was 4% which was lower than our findings. In a research which was performed by Mirzaei et al. [16], a total of 200 Iranian raw milk samples were screened for the prevalence of MRSA. They showed that the incidence of coagulase positive *S. aureus* in raw and pasteurized milk and also traditional butter and cheese samples were 44%, 8%, 18% and 24%, respectively. The prevalence of MRSA in raw milk, pasteurized milk and traditional butter samples were 82%, 50% and 22%, respectively. High presence of MRSA strains in bovine milk has been reported from various countries including Germany, Italy, Turkey and England [17-20].

We found that the MRSA strains harbored the high and variable prevalence of *SCCmec* alleles which is an additional pathogenic factor for infections. Totally, *SCCmec IVa*, *SCCmec V* and *SCCmec IVb* were the most prevalent alleles in our findings. Community Acquired MRSA (CA-MRSA) are mainly considered by *SCCmec IV* and *V* alleles, while Healthcare (H) A-MRSA are recognized by *SCCmec I*, *II* and *III* alleles [21]. This topic revealed that the MRSA strains of this research project were mainly associated with CA-MRSA. In the other hand, detection of *SCCmec* types *IV* and *V* suggested the emergence of CA-MRSA strains in this geographical area and occurrence of *SCCmec I* and *II* alleles indicated a possible transmission of MRSA from humans to animals. High prevalence of *SCCmec* types *IV* and *V* were also detected by Nemeghaire et al. [22], Haran et al. [23] and Havaei et al. [24].

5. Conclusion

In summary, an overall incidence of 30% and a relatively great occurrence of *SCCmec* alleles in the MRSA strains in milk samples pose a possible community health threat regarding Iranian raw milk samples. Using from hygienic conditions for the milking process, application of industrial devices for milking, maintenance of milk in a refrigerator and finally well boiling of milk before consumption and even using from pasteurized milk are key points which may reduce the risk of milk-borne MRSA. Regular and authorized prescription of antibiotics can reduce the occurrence of resistance *S. aureus* strains.

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