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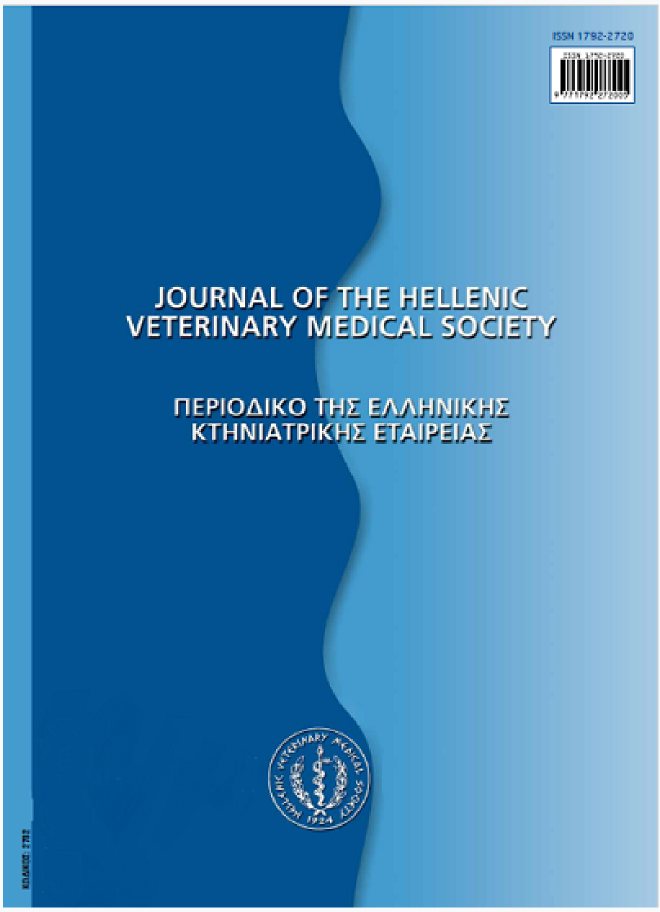

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Research article
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Genomic identification of Toxic shock syndrome producing and methicillin resistant *Staphylococcus aureus* strains in human and sheep isolates

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ABSTRACT. Disease-associated *Staphylococcus aureus* strains often promote infections by producing potent protein toxins such as toxic shock syndrome toxin (TSST). The mecA gene allows a bacterium to be resistant to antibiotics such as methicillin, penicillin and other penicillin-like antibiotics. The aim of this study was to determine the prevalence of *Staphylococcus aureus* strains producing these two genes. In this study, within 110 cases isolated in Chaharmahal and Bakhtiari province, *Staphylococcus aureus* was isolated by microbiological methods. Then PCR was done for 66 samples to identify the mecA and TSST-1 genes. The results showed within 30 samples of human skin infection 18 cases (60%) were MRSA and 5 samples (16.66%) were positive for TSST-1 gene. Within 36 samples of ewe subacute mastitis 10 samples (27.77%) and 5 (13.88%) had mecA and TSST-1 genes respectively. Therefore the prevalence of methicillin resistance and toxic shock syndrome producing *Staphylococcus aureus* isolates was significant in Chaharmahal and Bakhtiari. Due to the presence of these isolates in Iran and their threatening role in public health, more attention for their monitoring and treatment is essential.

Keywords: *Staphylococcus aureus*, Toxic shock syndrome, methicillin resistance, human, sheep.

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INTRODUCTION

Staphylococcus aureus is the most common species of *Staphylococcus* to cause *Staph* infections. *S. aureus* can cause some illnesses, from minor skin infections, such as pimples (Tuncer et al., 2009), impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis. *S. aureus* can successfully cause such different illnesses due to a combination of nasal carriage and bacterial immunoevasive strategies (Kluytmans et al., 1997; Cole et al., 2001). It can infect skin, soft tissue, respiratory, bone, joint, endovascular or wound. It is one of the most common causes of hospital-acquired infections and is one of the cause of postsurgical wound infections (AL-Ruaily et al., 2011)

Strains of *Staphylococcus aureus* can produce some extracellular protein toxins and enzymes, including enterotoxins, toxic shock syndrome toxin 1 (TSST-1), exfoliative toxin (ET), hemolysins, and coagulase (Iandolo, 1989).

Toxic shock syndrome toxin (TSST) is a super antigen with a size of 22KDa produced by 5 to 25% of *Staphylococcus aureus* isolates. It causes toxic shock syndrome (TSS) by stimulating the release of interleukin-1, interleukin-2 and tumor necrosis factor. Mainly, the toxin is not produced by bacteria growing in the blood; rather, it is produced at the local site of an infection, and then enters the blood stream. *S. aureus* isolates producing TSST-1 cause the toxic shock syndrome of humans and animals (Schlievert, 1993).

The increase and emergence of *S. aureus* strains resistant to the antibiotic methicillin (MRSA strains), particularly in nosocomial settings has been reported (Haley et al., 1982). The intrinsic resistance to these antibiotics is attributed to the presence of mecA, that encodes for a protein with the size of 78-kDa called penicillin binding protein 2a. The mecA gene is a gene found in bacterial cells. mecA allows a bacterium to be resistant to antibiotics such as methicillin, penicillin and other penicillin-like antibiotics (Hartman and Tomasz, 1984; Utsui and Yokota, 1985).

The methods most commonly are used for the detection of staphylococcal toxins include immunodiffusion, agglutination, radioimmunoassay, and enzyme-linked immunosorbent assay (Johnson et al., 1991). Among the techniques used to identify toxin genotypes, DNA-DNA hybridization and PCR have been established to be very successful and reliable (Johnson et al., 1991). There are several reports describing the use of multiplex PCR for detection of *Staphylococcus aureus* strains (Zambardi et al., 1994; Vannuffel et al., 1995; Salisbury et al., 1997; Schmitz et al., 1997). In this report, we detected the presence of two staphylococcal genes using 66 isolates of *S. aureus* which were first characterized with microbiological tests by using individual primers. We conclude that the prevalence of *S. aureus* strains with methicillin resistance and toxic shock syndrome genes in this area was significant but further studies are needed to determine the exact prevalence of *S. aureus* strains that are positive in phenotype and genotype for these toxins.

MATERIALS AND METHODS

Sampling

110 cases from two different groups (55 samples of human skin infections and 55 ewe subacute mastitis cases) were collected in Chaharmahal and Bakhtiari province.

Screening for subclinical cases was performed immediately before the collection of milk samples for the microbiological diagnosis of mastitis by the California Mastitis Test (CMT) according to the technique of Schalm and Noorlander (1957). Samples were also collected for somatic cell count (SCC) into flasks containing bronopol for counting in an electronic Somacount 300 (Bentley Instruments®) Mammary glands with a positive reaction in the CMT or SCC > 3.0×10^5 cells/mL milk (McDougall et al., 2001) and that were bacteriologically positive were classified as subclinical mastitis.

The samples were transported to laboratory of microbiology and were stored at -20 until testing.

A complete history of recurrences due to failure of previous treatments, severity of skin lesions and the number of involved quarters was obtained and recorded.

Microbiological methods

Microbiological tests which include Re-cultivation, bio-chemical tests and coagulase were performed. The human skin lesions and sheep milk and skin lesions from each case were streaked for isolation onto mannitol salt agar plates (PML Microbiologicals, Mississauga, Ontario, Canada). The plates were incubated at 36°C for 48 h in 5% CO2. After incubation, the plates were examined for the characteristic morphology of *S. aureus*, and suspicious colonies were subcultured onto tryptic soy agar plates with 5% sheep blood (PML Microbiologicals) and incubated for 24 h. Gram stain, catalase, and coagulase tests were performed to confirm the identification of *S. aureus*.

DNA isolation

Total DNA was isolated from 5 ml of brain heart infusion broth culture grown overnight for all the bacterial strains used in the study. The DNA isolation method was a modification of the protocol by Doyle and Doyle (1990) (Chapaval et al., 2008). A total of 2.5ml from a 5ml overnight culture in BHI were centrifuged at 33000 x g for 30 sec. The supernatant was discarded and the pellet was re-suspended in 700 µl extraction buffer (1.4M NaCl; 100mM Tris-HCl [pH 8.0]; 200mM EDTA [pH 8.0], 40%PVP (polyvinylpyrrolidone); 2% CTAB (cetyltrimethylammonium bromide), 20mg/ml Proteinase K; 0.2% β-Mercaptoethanol). The tube was incubated at 65°C for 30min with occasional mixing at every 10min. Then, 650µl chloroform-isoamyl alcohol (24:1) was added and the solution was centrifuged at 33,000 x g for 7min. The upper aqueous phase was transferred to a 1.5-ml tube and 200 µl extraction buffer without proteinase K was added. The solution was gently mixed and 650µl chloroform-isoamyl alcohol (24:1) was added. The tube was centrifuged at 33,000 x g for 7min after which the upper aqueous phase was transferred for a fresh tube. Chloroform-isoamyl alcohol (24:1) extractions were performed twice using 650µl of the chemicals. The DNA was precipitated by adding an equal volume of isopropanol at room temperature. The solution was mixed and centrifuged at 33,000 x g for 7min. The isopropanol was removed and the pellet was washed twice with 70µl 70% ethanol. The DNA pellet was air-dried and re-suspended in 40µl TE buffer (10mM Tris-HCl [pH 8.0]; 1mM EDTA [pH 8.0] + 10µgml RNAse) and incubated at 37 °C for 30 min. D concentration was determined as micrograms per milliliter according to A260 values. Template DNA in amounts ranging from 10 to 1,000 ng was used in the study.

PCR

PCR test was carried out using the primers designed for TSST-1 and mecA genes. The sequences of primers were as follows: TSST-1 F: ACCCCTGTTCCCTTATCATC-3', TSST-1 R: 5'TTTTCAGTATTTGTAACGCC -3' and mecA F: ACTGCTATCCACCCTCAAAC -3', mecA R: 5'-CTGGTGAAGTTGTAATCTGG -3'. The PCR thermal cycle programs were consisted of denaturation at 94 º C for 5 min followed by 35 cycles at 94 º C for 45 s, 45 º C (TSST-1) or 47 º C (mecA) for 45 s and 72 º C for 45 s, followed by a final extension at 72 º C for 5 minutes. The negative and positive controls (ATCC: 25923) were used in each test. The PCR products were visualized after electrophoresis in 1.3% agarose by staining with ethidium bromide and compared to DNA markers (100 base pair ladder, Fermentas). Then the PCR products were sequenced in an automated fluorescent dideoxy sequencing system (Bioneer, Korea).

Sequencing

Two PCR positive samples in a volume of 50 ml were sent to Bioneer Company for sequencing.

RESULTS

In this study, 110 cases from two different groups (55 samples of human skin infections and 55 ewe

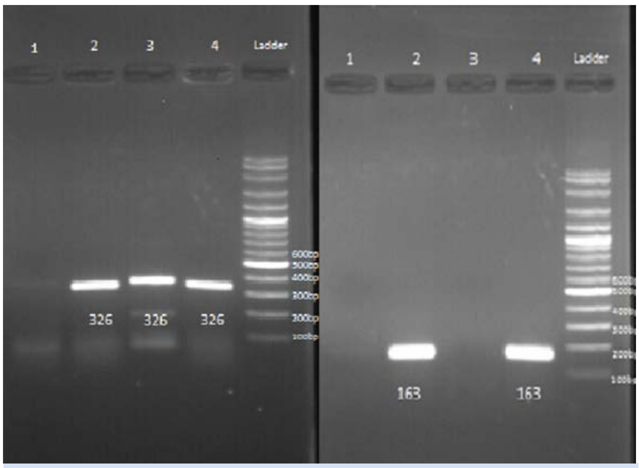

Figure 1: Amplification TSST-1 gene (Right), Amplification of mecA gene (Left)

Right to left: Gene ruler (100bp), 4: positive control for TSST-1, 2&3: TSST-1 band (326bp), 1: negative control for TSST-1, Gene ruler (100bp), 4: positive control for mecA, 2: mecA band (163bp), 1: negative control for mecA.

acute mastitis cases) were isolated in Chaharmahal and Bakhtiari province. *Staphylococcus aureus* was isolated from the samples by microbiological methods. Then PCR was done for 66 samples to identify the existence of TSST-1 and mecA genes. PCR reaction was performed to amplify a 326 bp fragment of TSST-1 gene and a 163 bp fragment of the mecA gene (Figure 1).

The results showed that within 30 cases of human skin lesions 5 cases had TSST-1 gene (16.66%) and 18 cases had mecA gene (60%). Within 36 samples of ewe subacute mastitis 5 samples had TST-1 gene (13.88%) and 10 samples had mecA gene (27.77%). After sequencing, the BLAST of the read sequences, confirmed the presence of *S.aureus*- TST-1 and mecA gene fragments in the positive samples (Figures 2&3).

The relationship between some variables and having TST-1 and mecA genes was evaluated with statistical tests. Using Chi-Square test, there weren't any statistical relationships between having these two genes and recurrences due to failure of previous treatments, severity of skin lesions and the number of involved quarters ($P>0.05$). The results of gene expression within groups studied are presented in the tables.1-3.

DISCUSSION

The control of antibiotic resistant and toxigenic Staphylococcus infections is very difficult. The phenotypic and genotypic classification of these *Staphylococcus aureus* strains will be very beneficial in the diagnosis and better control of them. For any health care system in any society is very necessary and is essential that important nosocomial pathogens correctly be identified and the exact pattern of antibiotic resistance being determined. With such efforts effective prevention and treatment strategies against these pathogens can be used. Regional studies aimed at obtaining information about the strains of Staphylococcus, as well as their antibiotic resistance could help physicians to select suitable treatment guidelines.

Staphylococcus aureus is an opportunistic pathogen that under favorable conditions causes infection in humans and animals (Balaban and Rasooly, 2000). Different strains of this organism may produce toxins and virulence factors (Ertas et al., 2001).

We used a PCR-based diagnostic protocol to detect TSST-1 and the mecA genes in DNA extracted from human and sheep isolates of *S. aureus*. Individual primers were used to identify these Staphylococcus genes. The PCR primers were shown to be very specific, reliable, and very efficient for detection of all these two genes. There have been few well-documented cases of TSS in Iran (Varmazyar-najafi et al., 2016). It is unclear whether the small number of reported TSS cases is due to the failure to recognize the disease, underreporting of the disease, or it is because of microbiologic or immunologic reasons.

In recent decades antibiotic resistance is considered as a major public health problem and because of improper and abundant use of antibiotics, the prevalence of antibiotic-resistant strains is increasing rapidly (Najera-sanchez et al., 2003; Orwin et al., 2003; Hososaka et al., 2007). Because animals are one of the human's food sources, their infection with dangerous organisms may cause disease in human through consumption of contaminated food or contact with infected animals. The uncontrolled use of some medications, especially antibiotics has led to development of antibiotic resistance bacterial strains and if such bacteria spread to human beings, cause serious and dangerous diseases those common antibiotics may fail for their treatment (Khoei et al., 2014).

Nowadays methicillin is one of the most commonly used antibiotics for the treatment of nosocomial infections caused by *Staphylococcus aureus* strains. Unfortunately, the rapid emergence of strains resistant to methicillin causes this antibiotic lost their effectiveness for their treatment. So epidemiological studies to understanding the prevalence of this *Staphylococcus aureus* strains seems necessary (Dinges et al., 2000; Deurenberg et al., 2005; Zamani et al., 2007; Sajith Khan et al., 2012). Methicillin-resistant strains of *Staphylococcus aureus* have mecA gene or MIC of 4 μg / ml or more (Hososaka et al., 2007).

Increasing antibiotic resistance is a concern and should always be monitored. Methicillin resistance is independent of beta-lactamase production and is due to the presence of the mecA gene with the length of 1.2 kb. The prevalence of this type of resistance varies in different areas and different times (Gilbert and Humphrey, 1998; Enright et al., 2002; Japoni et al., 2004).

Query ID	lcl|Query_244509	**Database Name**	nr
Description	tst_PCR_tst_F	**Description**	Nucleotide collection (nt)
Molecule type	nucleic acid	**Program**	BLASTN 2.6.1+
Query Length	289		

Staphylococcus aureus gene for toxic shock syndrome toxin, complete cds, strain: TSST	274	274	56%	9e-70	97%	LC075482.1
Staphylococcus aureus strain MRSA NN 353 Toxic Shock Syndrome toxin (TssT) gene, partial cds	237	237	44%	1e-58	100%	KT124627.1
Staphylococcus aureus strain MRSA NN 226 Toxic Shock Syndrome toxin (TssT) gene, partial cds	231	231	43%	5e-57	100%	KT124628.1

Staphylococcus aureus gene for toxic shock syndrome toxin, complete cds, strain: TSST
Sequence ID: **LC075482.1** Length: 1600 Number of Matches: 1
Range 1: 625 to 786

Score	**Expect**	**Identities**	**Gaps**	**Strand**	**Frame**
274 bits(148)	9e-70()	159/164(97%)	2/164(1%)	Plus/Plus	

Features:

```
Query  126  GATGGCAGCATCAGCCTTATAATTTTTCCGAGTCCTTATTATAGCCCTGCTTTTACaaaa  185
            ||| ||||||| ||| | |||||||||||||||| |||||||||||||||||||||||||
Sbjct  625  GAT-GCAGCAT-AGCTTGATAATTTTTCCGAGTCATTATTATAGCCCTGCTTTTACAAAA  682

Query  186  ggggaaaaagttgacttaaacacaaaaagaactaaaaaaaGCCAACATACTAGCGAAGGA  245
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  683  GGGGAAAAAGTTGACTTAAACACAAAAAGAACTAAAAAAAGCCAACATACTAGCGAAGGA  742

Query  246  ACTTATATCCATTTCCAAATAAGTGGCGTTACAAATACTGAAAA  289
            ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  743  ACTTATATCCATTTCCAAATAAGTGGCGTTACAAATACTGAAAA  786
```

Staphylococcus aureus strain MRSA NN 353 Toxic Shock Syndrome toxin (TssT) gene, partial cds
Sequence ID: **KT124627.1** Length: 316 Number of Matches: 1
Range 1: 1 to 128

Score	**Expect**	**Identities**	**Gaps**	**Strand**	**Frame**
237 bits(128)	1e-58()	128/128(100%)	0/128(0%)	Plus/Plus	

Features:

```
Query  162  TATTATAGCCCTGCTTTTACaaaaggggaaaaagttgacttaaacacaaaaagaactaaa  221
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TATTATAGCCCTGCTTTTACAAAAGGGGAAAAAGTTGACTTAAACACAAAAAGAACTAAA  60

Query  222  aaaaGCCAACATACTAGCGAAGGAACTTATATCCATTTCCAAATAAGTGGCGTTACAAAT  281
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   AAAAGCCAACATACTAGCGAAGGAACTTATATCCATTTCCAAATAAGTGGCGTTACAAAT  120

Query  282  ACTGAAAA  289
            ||||||||
Sbjct  121  ACTGAAAA  128
```

Staphylococcus aureus strain MRSA NN 226 Toxic Shock Syndrome toxin (TssT) gene, partial cds
Sequence ID: **KT124628.1** Length: 314 Number of Matches: 1
Range 1: 1 to 125

Score	**Expect**	**Identities**	**Gaps**	**Strand**	**Frame**
231 bits(125)	5e-57()	125/125(100%)	0/125(0%)	Plus/Plus	

Features:

```
Query  165  TATAGCCCTGCTTTTACaaaaggggaaaaagttgacttaaacacaaaaagaactaaaaaa  224
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TATAGCCCTGCTTTTACAAAAGGGGAAAAAGTTGACTTAAACACAAAAAGAACTAAAAAA  60

Query  225  aGCCAACATACTAGCGAAGGAACTTATATCCATTTCCAAATAAGTGGCGTTACAAATACT  284
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   AGCCAACATACTAGCGAAGGAACTTATATCCATTTCCAAATAAGTGGCGTTACAAATACT  120

Query  285  GAAAA  289
            |||||
Sbjct  121  GAAAA  125
```

Figure 2: Sequencing of a S. aureus-tst-1 PCR product: There is 97- 100% identity between PCR product sequence and S.aureus tst-1 sequences published in Genebank.

BLAST Results

Job title: mecA_PCR_mecA_F

RID	B936YGM901R (Expires on 03-01 03:32 am)		
Query ID	lcl|Query_142319	**Database Name**	nr
Description	mecA_PCR_mecA_F	**Description**	Nucleotide collection (nt)
Molecule type	nucleic acid	**Program**	BLASTN 2.6.1+
Query Length	145		

Staphylococcus aureus strain MRSA19 penicillin binding protein 2a-like (mecA) gene, partial sequence	235	235	87%	2e-58	100%	GQ146438.1
Staphylococcus aureus strain MRSA17 penicillin binding protein 2a-like (mecA) gene, partial sequence	235	235	87%	2e-58	100%	GQ146439.1

Staphylococcus aureus strain MRSA19 penicillin binding protein 2a-like (mecA) gene, partial sequence
Sequence ID: **GQ146438.1** Length: 138 Number of Matches: 1

See 1 more title(s)
Range 1: 12 to 138

Score	Expect	Identities	Gaps	Strand	Frame
235 bits(127)	2e-58()	127/127(100%)	0/127(0%)	Plus/Plus	

Features:

```
Query  9    CCTTGTAGCACACCTTCATATGACGTCTATCCATTTATGTATGGCATGAGTAACGAAGAA  68
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  12   CCTTGTAGCACACCTTCATATGACGTCTATCCATTTATGTATGGCATGAGTAACGAAGAA  71

Query  69   TATAATAAATTAACCGAAGATAAAAAAGAACCTCTGCTCAACAAGTTCCAGATTACAACT  128
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  72   TATAATAAATTAACCGAAGATAAAAAAGAACCTCTGCTCAACAAGTTCCAGATTACAACT  131

Query  129  TCACCAG  135
            |||||||
Sbjct  132  TCACCAG  138
```

Staphylococcus aureus strain MRSA17 penicillin binding protein 2a-like (mecA) gene, partial sequence
Sequence ID: **GQ146439.1** Length: 138 Number of Matches: 1
Range 1: 12 to 138

Score	Expect	Identities	Gaps	Strand	Frame
235 bits(127)	2e-58()	127/127(100%)	0/127(0%)	Plus/Plus	

Features:

```
Query  9    CCTTGTAGCACACCTTCATATGACGTCTATCCATTTATGTATGGCATGAGTAACGAAGAA  68
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  12   CCTTGTAGCACACCTTCATATGACGTCTATCCATTTATGTATGGCATGAGTAACGAAGAA  71

Query  69   TATAATAAATTAACCGAAGATAAAAAAGAACCTCTGCTCAACAAGTTCCAGATTACAACT  128
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  72   TATAATAAATTAACCGAAGATAAAAAAGAACCTCTGCTCAACAAGTTCCAGATTACAACT  131

Query  129  TCACCAG  135
            |||||||
Sbjct  132  TCACCAG  138
```

Figure 3: Sequencing of a S. aureus-mecA PCR product: There is 97-100% identity between PCR product sequence and S.aureus- mecA sequences published in Genebank.

Table 1:The TST-1 and MecA positivity situation based on the number of involved quarters

the number of involved quarters	1	2	3	4
TST-1 positive	1	1	1	2
TST-1 negative	8	8	8	7
mecA positive	1	2	2	5
mecA negative	6	6	6	8

Table 2:The TST-1 and MecA positivity situation based on the number of recurrences

the number of recurrences	1	2	3	4
TST-1 positive	0	2	2	1
TST-1 negative	4	14	7	6
mecA positive	1	4	3	2
mecA negative	4	11	5	6

Table 3:The TST-1 and MecA positivity situation based on the severity of skin lesions

Severity of skin lesions	Mild	Moderate	Severe
TST-1 positive	1	2	2
TST-1 negative	9	12	10
mecA positive	3	3	4
mecA negative	6	7	7

In this study the findings about MRSA obtained by PCR. Of 30 isolates of *S. aureus* from human skin infection, 18 samples (60%) and of 36 *S. aureus* isolates from ewes subacute mastitis 10 samples (27.77%) were positive for mecA gene. Totally of 66 isolates of *S. aureus*, 28 samples (41.8%) carried mecA gene. In the study performed by Becker et al. in Germany (2003) of 219 isolates of *Staphylococcus aureus*, 40 samples (18.2%) carried tst gene (Becker et al., 2003). In a similar study in Canada, Mehrotra et al. detected tst gene in 15% of *Staphylococcus aureus* isolates (Mehrotra et al., 1996). Deurenberg et al. in the Netherlands (2005) investigated the presence of tst gene in 51 isolates of methicillin-resistant *Staphylococcus aureus* strains. 24% of apparently healthy and 14% of hospitalized people was reported that were positive for this gene (Deurenberg et al., 2005).

Najjar pirayeh et al. in 2010 showed the prevalence of MRSA isolates was 48% (Varmazyar-najafi et al., 2016). As well as in the study which was performed in Shiraz (2005) the prevalence of MRSA was reported to be 38% (Hososaka et al., 2007). These results are consistent with the findings obtained in the present study. In this study, 41.8% of samples was mecA positive, which is in accordance with frequencies reported from Iran (Zeinali et al., 2011), India (Sajith Khan et al., 2012) and Turkey (Tuncer et al., 2009). In the study performed by AL-Ruaily and his colleagues in 2002 only 13% of strains were positive for mecA gene (AL-Ruaily et al., 2011), as well as the research was performed by Goud et al. in 2011 that isolated *S.aureus* from 22.5% of healthy individuals and 16.6% of these isolates were positive for mecA gene (Goud et al., 2011).

Identification of methicillin-resistant *Staphylococcus aureus* is sometimes complex due to heterogeneous expression of resistance gene and the influence of other variables such as PH, temperature and salt concentration (de Carvalho et al., 2009).The ability of *Staphylococcus aureus* strains to cause different diseases depends on producing several different types of extracellular toxins. The majority of *S. aureus* strains isolated from patients with symptoms of toxic shock syndrome produce a toxin called toxic shock syndrome toxin -1 (Mehrotra et al., 1996) which is a super antigen (Orwin et al., 2003).

In many countries, scientists have studied TSS as a health problem. TSS has been documented to occur worldwide. Colonization with *S. aureus* is generally highest (20 to 30%) in the nose or oropharynx while vaginal colonization with *S. aureus* has been determined to be lower (10% to 20%) in the United States, Europe, and Asia. However there is variation in incidence reports of TSST-1 producing strains of *S. aureus* in different countries (Parsonnet et al., 2008).

Risk factors for the staphylococcal type include the use of very absorbent tampons and skin lesions (Low, 2013). In other hand, *S. aureus* which caused mastitis can potentially produce staphylococcal toxic shocksyndrome toxin-1 (Dastmalchi Saei et al., 2013). Therefore in this study the sampling was performed using skin lesions absorbent tampons were used for them and mastitis cases.

Given the importance of the detection of tst gene, Johnson et al. (1991) showed that the use of PCR to identify tst genes is better than other methods, such as immunological assays. It is more sensitive, faster and less expensive (Mehrotra et al., 1996). Therefore, this method was used to identify *S.aureus* genes in this study. In the present study, of 36 *S. aureus* isolates from samples of human skin infection 5 cases (13.5%) and of 30 *S. aureus* isolates from samples of ewe subacute mastitis 5 cases (16.66%) were positive for tst gene. Totally, of 66 *S. aureus* isolates, 10 samples (15%) had tst gene.

In the study performed by Parsonnet et al. (2008), 159 *S. aureus* isolates were studied. 14 strains (9%) were tst positive and of 12 toxigenic strains, 2 strains were methicillin-resistant (Parsonnet et al., 2008). In this study of 10 tst positive isolates, 3 strains (30%) were methicillin-resistant. Of 28 MRSA isolates, 3 strains (10.7 %) had tst gene. Hoseini Alfatemi et al. (2014) performed a study in Shiraz to identify the profile of some virulence genes including: sea, seb, sed, tst, eta, etb, LuKS/F-PV, hla and hld in methicillin-resistant *S. aureus* strains by PCR technique. The frequency of the tst gene was 10.95% (Hoseini Alfatemi et al., 2014).

Nemati et al. (2015) in Ilam analyzed *S. aureus* isolates that were collected from different resources. Samples were screened for the mecA, tst-1, eta and etb genes by PCR. 50 isolates were selected from human Staphylococcus isolates and 100 from animal Staphylococcus isolates. Ten out of the 50 human *S. aureus* isolates and 5 out of 50 *S. aureus* isolates from cow milk were just positive for mecA. None of the poultry *S. aureus* isolates were positive for mecA. All of the isolates were negative for the eta, etb and tst-1 (Nemati et al., 2015).

Arfatahery et al. (2016) performed a research with the aim of characterization of toxin genes and antimicrobial susceptibility of *Staphylococcus aureus* isolates in fishery products in Iran. The results indicated that 34% of fish and shrimp samples were contaminated with *S. aureus*, and 23.8% of these isolates were mec-A positive. 3.9% were positive for tst-1 gene (Arfatahery et al., 2016). Varmazyar najafi et al. (2016) detected methicillin- resistance gene in *Staphylococcus aureus* isolated from traditional white cheese in Iran. 19 samples (31.67%) were contaminated with *S. aureus*. Three out of 19 *S. aureus* isolates (15.7%) were phenotypically resistant to methicillin (disk diffusion), while 4 (21.05%) of them were genotypically confirmed as MRSA strains (Varmazyar najafi et al., 2016). Khoei et al. (2015) studied antibiotic resistance pattern and frequency of mecA gene in *Staphylococcus aureus* isolated from Tabriz. 44.5% isolates were confirmed as MRSA by cefoxitin disc screening test and 53.3% isolates by showing the presence of mecA gene (Khoei et al., 2014).

The difference between cities in Iran and between different countries is most likely a reflection of differences in lifestyle and health practices. People in Chaharmahal and Bakhtiari province who described themselves as living in traditional conditions were more likely to have positive results than those who had assumed a better and more hygienic lifestyle. These results, taken together, suggest that the development of TSST-1 producing *S. aureus* strains in Chaharmahal and Bakhtiari province is large and probably a function of environmental and genetic factors.

Staphylococcus aureus infections are among the most important hospital- acquired infections. Enterotoxins and toxic shock syndrome toxin -1 secreted by *S.aureus* are some of important virulence factors and PTSAgs which have profound effects on their hosts.

S. aureus is a versatile microorganism that causes infection in different hosts. Moreover, this bacterium is one of the most important pathogens in the etiology of infectious mastitis in cows, goats, and sheep, causing chronic infection of the mammary tissue that is difficult to treat (Ai-res-de-Souza et al., 2007).

The infections caused by *Staphylococcus aureus* strains that are resistant to antibiotics (mainly hospital-acquired infections) and TSST-1-producing strains are growing in many countries. The results of this study showed there was a high prevalence of tst and mecA genes in the studied samples which may indicate that these strains are circulating in the community. These strains may be as reservoirs for transmission of antibiotic resistance genes to the other strains and this could endanger public health. We characterized the Staphylococcus isolates for the production of TSST-1 and methicillin resistance.

The virulence profile of *S. aureus* strains, suggest that virulence factors spread among flocks and successfully establish an infection in the mammary gland of sheep, causing mastitis. The tst gene is located on a pathogenicity island, and can be transferred from one bacterium to another. The presence of this gene in one strain suggests that it probably acquired this virulence gene from other staphylococci, rendering it more virulent (McDougall et al., 2001; Schalm et al., 1957).

Staphylococcus aureus is a commensal and pathogen of several mammalian species, particularly humans and cattle. Animal lineages are closely related to human lineages and only a handful of genes or gene combinations may be responsible for host specificity. That's the point that people who work in veterinary farms or laboratories' or every work that deals with the animal and people that use unhealthy milk and milk products are at more risk (Sung et al., 2008).

The use of PCR assay help to provide valuable information required for appropriate treatment and control during outbreaks of *S. aureus* infections and diseases. It is important to recognize that this technique only will identify strains harboring the toxin genes and is independent of the expression and secretion of the toxin. To verify toxin production by any given isolate, time- and labor-intensive immunological methods may be used to detect the excreted toxins. Considering the low cost and much shorter time required to detect the genes of *S. aureus* by PCR, we believe this to be a powerful tool for studying the genotypes of staphylococcus isolates. This procedure was specially developed to fit into the daily work pattern of a routine clinical laboratory, since genotypic detection of drug resistance and the presence of toxin genes is becoming an important component of the diagnostic inventory of such laboratories.

CONCLUSION

The results of this study showed that more likely abundant use of antibiotics causes a high prevalence of MRSA strains in Chaharmahal and Bakhtiari province. Furthermore, these results, suggest that the development of TSST-1 producing *S. aureus* strains in Chaharmahal and Bakhtiari province is large and probably a function of environmental and genetic factors. Therefore presentation of programs and rules for the controlled use of antibiotics and the application of new methods in identifying new strains of *S. aureus* seems necessary.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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