Detection of *mecA*, *eta*, *etb* and *tst*-1 genes from staphylococcal isolates

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Abstract

Introduction: *Staphylococcus aureus* (*S. aureus*) is a bacterium found on the skin and hair of people and animals. *S. aureus* could product some extracellular protein.
Materials and methods: One hundred and fifty *S. aureus* isolates that were collected from different resources were screened for the *mecA*, *tst*-1, *eta* and *etb* genes by PCR. 50 isolates were selected from human staphylococcal isolates and 100 from animal staphylococcal isolates.
Results: Ten out of the 50 human *S. aureus* isolates and 5 out of 50 *S. aureus* isolates from milk cow were just positive for *mecA* but none of the poultry *S. aureus* isolates were positive for *mecA*. All of the isolates were negative for the *eta*, *etb*, *tst*-1.
Conclusion: The results in this study indicate that the prevalence of *mecA* in human staphylococcal isolates is higher than prevalence of this gene in poultry staphylococcal isolates in Ilam- Iran but for the other genes there is no difference. Detection of *mecA* in cow milk could be a pose for public health hazards.

Keywords: *Staphylococcus aureus*, *eta*, *etb*, *tst*-1, *mecA*

Introduction

Staphylococcus aureus (S. aureus) is a type of coccal bacterium commonly found on the skin and hair as well as in the noses and throats of people and animals. These bacteria are present in up to 25 percent of healthy people and are even more common among those with skin, eye, nose, or throat infections (1). In animal mastitis, dermatitis, osteomyelitis, arthritis, synovitis and septicemia due *S. sureus* are described (2-4). This bacterium can be founded in the environmental and can survive for long periods of time. Several biotypes isolated from different hosts have been described within the species *S. aureus* (5).
Staphylococci produce α-, ß- and δ-haemolysins, a group of active molecules, which may damage cells, as well as enzymes such as coagulase, nuclease, hyaluronidase and clumping factor. All these agents are involved in the destructive and inflammatory effects of staphylococcal infections. When the bacterium enters the circulatory system, it has a high affinity for collagen-rich surfaces, such as the articular surface of joints, and synovial sheaths located around joints and tendons. Staphylococci also tend to localize in the growth plate of actively growing bones. In cows *S. aureus* is one of the most important for mastitis.
A group of the exteracellular protein toxins such as superantigenes produce by *S.aureus* strains (6). The superantigens are a group of structural and biologically related proteins containing staphylococcal enterotoxins (SE), enterotoxin-like

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proteins and toxic shock syndrome toxin-1(TSST-1). The extracellular protein is coded by different genes. The gene *tst*-1 was subsequently identified as the major causative agent of toxic shock syndrome. Toxic shock syndrome is an infection caused by *S. aureus* and *Streptococcus pyogenes* bacteria, with most cases related to *S. aureus* (7).

Exfoliative toxin A and exfoliative toxin B consist of 242 and 246 amino acids and have a molecular weight of 26,950 and 27,274 daltons, respectively. Exfoliative toxin A (ETA) and exfoliative toxin B (ETB) are responsible for most human cases of staphylococcal scalded-skin syndrome (SSSS) (8-10). Also there is some reports that staphylococcal enterotoxins or TSST-1 are more frequently associated with staphylococcal scarlet fever (SSF) than exfoliative toxins. SSSS refers to a spectrum of blistering skin diseases caused by *S. aureus* exfoliative toxins, which usually affect newborns and young children, however, it may also be seen in adults with compromised renal function, and SSF is one of the staphylococcal toxin-mediated syndromes (11- 14)

The modification of the staphylococcal target is the way for these bacteria to become resistant to all β-lactams antibiotics, including the β-lactamase stable penicillins (such as methicillin and oxacillin) and cephalosporins. This type of resistance is due the *mecA* gene, which is imported into its genome by acquisition of a mobile genetic element called staphylococcal cassette chromosome mec (SCCmec). The first clinical MRSA isolates were reported as early as 1961, just one year after the launch of methicillin. During the past 20 years MRSA strains have become a major cause of hospital-acquired infections in humans and isolation of these strains from animals has been reported with an increasing frequency. The prevalence of hospital-acquired MRSA strains (HA-MRSA) in the world is very different, from <1% in the north to >40% in the south and the west of Europe and to 54.5% in the United States (15).

As *S. aureus* could pass from milk and poultry meat to human, if they carry the gene that mentioned above could be an alert for public health.

In this study a groups of *S. aureus* isolates that they were collected from cow milk and poultry carcasses were screened for genes encoding the exfoliative toxins (ETA and ETB), shock syndrome toxin-1 (TSST-1) and *mecA*.

Materials and methods

Bacterial isolates and culture media: A selective medium that can be used for detection of staphylococci is columbia agar containing 5% sheep or ox blood as well as 15 mg nalidixic acid and 10 mg colistinsulphate (Oxoid supplement SR 70) per liter of medium. These antimicrobial agents inhibit Gram-negative bacteria. Other specific media for staphylococci are modified Baird-Parker and Mannitol salt agar (16). Colonies usually are round, smooth, raised and glistening and appear after 24 hours incubation at 37°C. S. aureus is able to grow at a wide range of temperatures (7 °C to 48.5 °C with an optimum of 30 to 37°C), pH (4.2 to 9.3, with an optimum of 7 to 7.5) and sodium chloride concentrations (up to 15% NaCl). *S. aureus* colonies show haemolysis and typical white to yellow colour on blood agar. Alpha and delta haemolysin can make a clear zone around the colonies on blood agar .

For identification of *S. aureus*, several additional tests such as oxidase, catalase and coagulase are used. *S. aureus* is oxidase-negative and catalase and coagulase -positive. The coagulase test has been the most widely used method for the definitive identification of *S. aureus*.

For this study fifty *S. aureus* isolates from poultry carcasses, fifty *S. aureus* from cow milk and fifty *S. aureus* isolates from students (25 isolates) and staffs (25 isolates) in Ilam University were selected.

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The isolates from poultry were collected after sampling 75 different industrial farms in slaughter house. For collecting these bacteria ten samples were taken from the inside of the carcasses with a sterile cotton swab from each flock. The samples from cow milk were collected after sampling of 30 farms (10 samples from one farm). 10 milliliter milk were taken from one cow and transport to microbiology laboratory as soon as possible. The isolates from human were collected from 60 student and 60 staffs in Ilam University. The samples were taken from nose with sterile cotton swab.

The entire sample biochemically characterized as *S. aureus* by the standard biochemical methods (16).

PCR assay and DNA extraction: For DNA extraction, one colony suspended in 20 µllysis buffer (0.25% SDS, 0.05 N NaOH) and heated at 95˚C for 5 minutes. The sample was centrifuged briefly at 16000 g at room temperature. After adding 180 µl distilled water and centrifugation for 5 minutes at 16000 g, these sample preparations were stored at -20˚C and the supernatant was used as the DNA extract. For *mecA* the positive control was used as the same Nemati et al (17) used .

For other gene the positive control strains were kindly provided by HelleDaugaard Larsen (18). Primers used in the PCR assays, as well as expected amplicon sizes and the references, are shown in Table 1.

After amplification, 5 µl amplicon was mixed with 3 µl sample buffer (50% glycerol, 1 mM cresolred) and electrophoresis was performed. After electrophoresis, gels were visualized under UV light and photographed. The Gene Ruler™ 100 bp DNA Ladder Plus (MBI Fermentas, St. Leon-Rot, Germany) was used as a DNA size marker.

Results

Fifty *S. aureus* were isolated from humans originating from nose derived from 60 student and 60 staffs at Ilam University. Fifty *S. aureus* were isolated from 300 milk samples and fifty *S. aureus* were derived from 750 samples from poultry carcasses over 5 months at slaughter house.

A total of 150 *S. aureus* isolates were screened for the *mecA*, *tst*-1, *eta* and *etb* genes. Ten out of 50 *S. aureus* isolates from human were positive for *mecA*. Also five out of the 50 *S. aureus* isolates from cow milk were positive for *mecA*. That means these isolates are MRSA. The isolates were negative for *tst*-1, *eta* and *etb*.

Table1. Primers used in this study.

Gene targeted	Primer sequence	Amplicon size (bp)	Reference
tst	5′ACCCCTGTTCCCTTATCATC3′ 5′TTTTCAGTATTTGTAACGCC3′	326	21
eta	5′GCAGGTGTTGATTTAGCATT3′ 5′AGATGTCCCTATTTTTGCTG3′	93	21
etb	5′ACAAGCAAAAGAATACAGCG3′ 5′GTTTTTGGCTGCTTCTCTTG3′	226	21
mecA	5′GAAAAAAAGGCTTAGAACGCCTC3′ 5′GAAGATCTTTTCCGTTTTCAGC3′	150	23

Discussion

Staphylococcus aureus is both a successful colonizer and an important pathogen in humans and animals that is flexible, adaptable, and multifaceted in its interaction with the surrounding. In animal it can be involved in some problems such as mastitis, bumble foot and arthritis and is most commonly isolated from the joints, tendon sheaths and bone. The bacterium can be present on normal skin and can be isolated from the environment.

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It may thus contaminate foods as a result of processed carcasses. The contamination of the poultry carcasses and milk by *S. aureus* is important because it can survive, colonize, and persist at various processing stages in commercial processing plants. Enterotoxin-producing *S. aureus* is one of the most common causes of food-borne human illness throughout the world (19).

Toxic shock syndrome and exfoliative toxin are two toxins can cause wide variety of diseases in human. In this and previous study all the *S. aureus* isolates were negative for the genes encoding these toxins. The prevalence of the genes encoding enterotoxin in staphylococcal isolates was different. Smyth et al (20) in Northern Ireland, 86.7% of the isolates contained genes egc cluster.

The *mecA* gene encodes an altered penicillin binding protein (PBP) 2a with very low affinity for β-lactam antibiotics (21) and the bacteria which harbor this gene are named methicillin-resistant S. aureus (MRSA). Nemati et al (17) found 10 out of 81 poultry *S. aureus* that were positive for *mecA* that is not agreement with this study that all poultry *S. aureus* isolates were negative for this gene.

In this study three groups of *S. aureus* isolates from poultry, milk cow and human were screened for detection of *mecA*, *tst*-1, *eta* and *etb*. The results showed that none of the *S. aureus* isolates carried *eta*, *etb* and *tst*-1 genes but 10 human *S. aureus* and cow milk *S. aureus* isolates carried *mecA*. The absence of the gene encoding toxic shock syndrome and exfoliative toxin in theses staphylococcal isolates indicates that these genes cannot be held responsible for the diseases that may be induction with these toxins in human, otherwise, too many *S. aureus* isolates from animal has to be screened for these genes.

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