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Prevalence of genes encoding exfoliative toxins, toxic shock syndrome toxin-1 among poultry *Staphylococcus aureus* isolates

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ABSTRACT

Staphylococcus aureus is a major pathogen in humans and animals. Part of its pathogenicity is due to the production of extracellular protein toxins, called superantigens. In this study a groups of S. aureus isolates in Iran and Belgium from poultry were screened for genes encoding the exfoliative toxins (ETA and ETB), shock syndrome toxin-1 (TSST-1). Fifty S. aureus isolates from 20 poultry farms in Iran and Eighty-one isolates from 39 different industrial farms in Belgium were isolated by the standard biochemical methods. Ten of the isolates in Belgium have been characterized before as Meticillin Resistance Staphylococcus aureus (MRSA). These isolates were screened for the genes encoding eta, etb, tst by PCR test. In none of these isolates, toxin gene sequences were amplified. These results indicate that superantigens encoded by genes that are detectable with the PCR tests used here, are not involved in poultry.

Key words: *Staphylococcus aureus*, poultry, exfoliative toxins, shock syndrome toxin-1

INTRODUCTION

S. aureus is an important pathogen in human and animals (1). In poultry, dermatitis, osteomyelitis, arthritis, synovitis and septicemia due *S. sureus* are described (2-4). *S. aureus* belongs to the normal flora found on the skin and mucous membranes of mammals and birds. This bacterium can be disseminated in the host's environmental and can survive for long periods of time. Several biotypes isolated from different hosts have been described within the species *S. aureus* (5).

Nearly all of the *S.aureus* strains produce a group of the exteracellular protein toxins, such as hemolysins, coagulase, superantigens (6-7). The superantigens are a group of structural and biologically related proteins containing staphylococcal enterotoxins (SE), enterotoxin-like proteins (those toxins that can not be induce emesis after oral administration in a primate model or they are not tested) and toxic shock syndrome toxin-1(TSST-1).

The gene *tst*-1, which is present on the bacterial chromosome within a 15.2 kb mobile genetic element, was subsequently identified as the major causative agent of toxic shock syndrome. Toxic shock syndrome is an acute and

potentially fatal illness that is characterized by high fever, diffuse erythematous rash, desquamation of the skin 1 or 2 weeks after onset (if not fatal before this time), hypotension and involvement of three or more organ systems (7-8). Exfoliative toxin A (ETA) and exfoliative toxin B (ETB) are responsible for most human cases of staphylococcal scalded-skin syndrome (SSSS) (9-10). On the other hand, some studies have reported that staphylococcal enterotoxins or TSST-1 are more frequently associated with staphylococcal scarlet fever (SSF) than exfoliative toxins (11-13). 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. It has been hypothesized that SSF is a mild manifestation of SSSS. SSSS is estimated to have 3% mortality for children and approximately 50% for adults (9, 14-15).

There is an ongoing debate as to whether the exfoliative toxins are superantigens or not (16-20).

As *S. aureus* could pass from poultry to human, if they carry the gene encoding exfoliative toxins (ETA and ETB) and shock syndrome toxin-1 (TSST-1) could be an alert for public health.

In our knowledge it has not been reported too much paper about the prevalence of these genes in poultry so in this study two groups of *S. aureus* isolates that they were collected from nose and cloace of poultry before they slaughtered in slaughter house were screened for genes encoding the exfoliative toxins (ETA and ETB), shock syndrome toxin-1 (TSST-1).

MATERIALS AND METHODS

Bacterial isolates and culture media

Fifty *S. aureus* isolates from 20 industrial poultry flocks in Iran and Eighty-one *S. aureus* isolates from 39 different industrial farms in Belgium were tested. These isolates were taken from different industrial farms immediately before they slaughtered. For collecting these bacteria ten samples were taken from the nose and cloace with a sterile cotton swab from each flock and biochemically characterized as *S.aureus* by the standard biochemical methods (Baird parker (21), Vogues Proskauer test, Coagulase tube test). Also **a**mplification of the *fem*A gene, which has been reported to be specific for *S.aureus* (22-23), was used as a control to confirm of diagnosis of *S. aureus*. Ten of the isolates have been characterized before as Meticillin Resistance *Staphylococcus Aureus* (MRSA) strains belonging to the animal-associated clone ST398 (24).

PCR assay and DNA extraction

DNA was carried out from a suspension of single colony in 20 µl lysis bufer (0.25% SDS, 0.05 N NaOH). Then they were heated at 95˚C for 5 minutes and 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.

Positive control strains for *eta*, *etb*, *tst*, were kindly provided by Helle Daugaard Larsen (25). As an internal positive control to confirm the quality of the extraction and the absence of PCR reaction, primers specifically designated to amplify *femA* were used. 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

All of the isolates were tested by PCR for the genes encoding the exfoliative toxins (ETA and ETB), shock syndrome toxin-1 (TSST-1). In none of the *S. aureus* isolates genes encoding staphylococcal exotoxin that we mentioned above were amplified by PCR.

DISCUSSION

Poultry can be carrier of *S. aureus* and the isolates are likely to be enterotoxigenic. Of the poultry isolates studied by Smyth et al (26) in Northern Ireland, 86.7% of the isolates contained genes *seg, sei, selm, seln and selo* which form together the so-called *egc* cluster. In most other studies concerning poultry *S. aureus* isolates, genes encoding the classical SEs (*sea-see*) are absent or occur in less than 3% of the tested isolates (26-29). In this study a groups of *S. aureus* isolates from poultry in Belgium and Iran were screened for prevalence of gene encoding exfoliative toxins and shock syndrome toxin-1. Our results showed that none of the *S. aureus* isolates in this study carried *eta*, *etb* and *tst1* genes. According to different studies poultry can be presumed to contain *S. aureus* and the isolates are likely to be enterotoxigenic. These isolates were collected from cloace and nose in chickens in slaughter house immediately before they slaughtered and it can be important for raw poultry carcasses.

In conclusion, the absence of *eta*, *etb* and *tst1* gens detectable with PCR tests in the *S. aureus* isolates from poultry in different countries indicates that these genes cannot be held responsible for the diseases that may be induction with exfoliative toxins (ETA and ETB) and shock syndrome toxin-1 (TSST-1) in human, otherwise, too many *S. aureus* isolates from poultry has to be screened for these genes.

Table1. Primers used in this study

Gene targeted	Primer sequence	Amplicon size (bp)	Reference
tst	5’ ACC CCT GTT CCC TTA TCA TC 3’ 5’ TTT TCA GTA TTT GTA ACG CC 3’	326	23
eta	5’ GCA GGT GTT GAT TTA GCA TT 3’ 5’ AGA TGT CCC TAT TTT TGC TG 3’	93	23
etb	5’ ACA AGC AAA AGA ATA CAG CG 3’ 5’ GTT TTT GGC TGC TTC TCT TG 3’	226	23

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