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ORIGINAL ARTICLE

Prevalence of Enterotoxin Genes in Poultry *Staphylococcus aureus* Isolates

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ABSTRACT
Staphylococcus aureus is an important opportunist that can cause superficial to life-threatening illnesses in humans and a variety of animal species. Staphylococcal enterotoxin has an important role in food poisoning in human. Staphylococcal enterotoxin A is the most commonly reported enterotoxin in staphylococcal food poisoning. This study revealed the presence of enterotoxin (like) genes (sea, seb, sec, sed, see, seg, seh, sei, selj, selk, sell, selm, seln, selo, selp, selq and selu) in 100 poultry S. aureus isolates that were collected from 165 healthy flocks immediately before slaughtered in slaughter houses from Iran and Belgium. All the isolates were confirmed as Staphylococcus aureus by standard biochemical and molecular test. Ten out of 100 isolates were confirmed as MRSA belonging to the animal-associated clone ST398. 25 of the isolates carried sea. Fifty seven percent of the isolates were positive for the five staphylococcal enterotoxin genes, seg, sei, selm, seln and selo (egc cluster). All isolates were negative for other genes that we have screened in this study. All MRSA isolates were negative for all the genes. Our data indicated that poultry S. aureus isolates can possess superantigen genes and consumption of the contaminated carcasses with these bacteria may be induce food poisoning in humans. In comparison, no relevant significant differences between the frequency of the genes encoding entrotoxins in the groups of S. aureus isolates from Iran and Belgium could be found.
Key words: *Staphylococcus aureus,* poultry, enterotoxin, superantigen, MRSA

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INTRODUCTION

Staphylococcus aureus is an important food born pathogen in humans [1-2]. It belongs to the normal flora found on the skin and mucous membranes of mammals and birds. In humans it is a major pathogen that causes a wide variety of diseases such as life-threatening toxic shock syndrome and food poisoning [3]. In chickens, staphylococcal infections are a worldwide problem causing dermatitis, osteomyelitis, arthritis, synovitis and septicemia. Economic losses are due to lameness, mortality, decreased weight gain, decreased egg production and condemnation of carcasses at the slaughterhouse [4-6].

The ability of *S. aureus* to cause disease is thought to be due to a combination of virulence factors, such as toxins, cell surface-associated adhesins and secreted exoproteins [3]. Nearly all *S. aureus* strains produce a group of extracellular protein toxins, including the so-called superantigens [7]. The superantigens are a group of structural and biologically related proteins containing staphylococcal enterotoxins (SEs), enterotoxin-like proteins (SEls) (those toxins that cannot induce emesis after oral administration in a primate model or that have not been tested). These toxins cause food poisoning and several allergic and autoimmune diseases. In all the genes encoding staphylococcal enterotoxin, there is an often difference in the number of mobile genetic elements therein. May be due to extraordinarily high resistance to proteolytic enzymes of staphylococcal enterotoxin A (SEA), this enterotoxin alone or together with other SEs/ SEls is the most commonly reported in staphylococcal food poisoning [8-11]. In humans, after ingestion of food contaminated with SEs, staphylococcal food poisoning symptoms may appear after a few hours, depending on susceptibility and toxic dose ingested. The symptoms include nausea, vomiting, abdominal cramps which are usually followed by diarrhea [12].

In poultry, the contribution of these staphylococcal virulence factors to pathogenicity is currently not known [13]. The aims of this study were to determine the presence of well-known and more recently described superantigen genes in poultry *S. aureus* isolates. Therefore, *S. aureus* isolates that were

collected from poultry were screened for genes encoding staphylococcal enterotoxin (like) genes (*sea, seb, sec, sed, see, seg, seh, sei, selj, selk, sell, selm, seln, selo, selp, selq and selu*).

MATERIALS AND METHODS

Bacterial isolates

S. aureus isolates were collected from the nose and cloaca of healthy chickens from 39 different flocks in Belgium and five different flocks In Ilam, Iran after sampling 165 randomly selected industrial broiler farms. 81 isolates from Belgium and 19 isolates from Iran were isolated. In each flock five chickens were randomly sampled in their nose and cloaca with sterile cotton swab. The samples were inoculated on Columbia agar supplemented with sheep blood, colistin, and nalidixic acid (CNA; Oxoid, Basingstoke, United Kingdom) and incubated over the night at 37ºC. Isolates were identified as *S. aureus* by colony morphology, standard biochemical methods and growth on modified Baird-Parker medium [14]. PCR amplification of the *femA* gene, which has been reported to be specific for *S. aureus* [15], was performed to confirm the identification of *S. aureus* [16].

Ten of the isolates from Belgium have been characterized before as MRSA strains belonging to the zoonotically important animal-associated clone ST398 [17].

DNA extraction

For DNA extraction a single colony of bacteria was suspended in 20 µl lysis bufer (0.25% SDS, 0.05 N NaOH). After heating at 95˚C for 5 minutes samples were centrifuged briefly at 16000 g at room temperature. Then diluted by adding 180 µl distilled water. Another centrifugation for 5 minutes at 16000 g was performed to remove the cell debris. Supernatants were frozen at -20ºC until further use.

PCR assay

PCR tests were done for the detection of the superantigen genes. Each 30 µl PCR mixture contained 3 mM $MgCl_2$, 2.5 U Taq DNA polymerase, 200 µM of dNTP, 200 pmol of both primers and 3µl DNA sample. Amplification of DNA was performed with a DNA thermal cycler (Biometra, Gottingen, Germany). For detection of *sea-see* and *seh* the positive control were kindly provided by Helle Daugaard Larsen [18]. *S. aureus* strain A900322 was used as a positive control for detection *seg, sei, selm, seln, selo, selp* and *sej*. For *selq* and *sell S.aureus* strain HT2005 0018 were used as positive control [19]. These strains were provided by Michèle Bes of the Centre National de Référence des Toxémies staphylococciques (France). For *selu* KH454 and for *selk* DV70 were used as positive controls [20]. 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

A total of 100 *S. aureus* isolates were screened for the genes mentioned above by PCR. 71% of the isolates, at least positive for one enterotoxin (like) gene. In 57 of the *S. aureus* isolates, five staphylococcal enterotoxin genes *seg*, *sei*, *selm*, *seln* and *selo* (*egc* cluster) were detected. Nineteen of the isolates were positive for *sea*. Genes encoding SEB, SEC, SED, SEE, SHE, SEJ, SELK, SELK, SELP, SELQ and SELU were absent in this groups of *S. aureus* isolates. Five out of 100 *S. aureus* isolates were positive for both *sea* and *egc* cluster genes. Statistically no significant differences between the frequency of the genes encoding enterotoxin that we were looking in this study between the isolates from Belgium and Iran were founded. The MRSA isolates were negative for all the genes that were screened.

DISCUSSION

In this study two groups of *S. aureus* isolates from poultry were screened for the presence of genes encoding staphylococcal enterotoxin (like) genes. Nineteen *S. aureus* isolates were isolated from Iran and eighty- one isolates from Belgium. When the frequency of the genes encoding entrotoxins is compared between two groups of *S. aureus* isolates, no relevant significant differences could be found.

57 % of the isolates carried the genes *seg, sei, selm, seln and selo* which form together the so-called *egc* cluster and 19% of them contained the *sea* gene. Our data in this study indicate that poultry *S. aureus* isolates could possess the genes encoding staphylococcal enterotoxin.

Table1. Primers used in this study.

Gene targeted	Primer sequence	Amplicon size (bp)	Reference
sea	5' GGT TAT CAA TGT GCG GGT GG 3' 5' CGG CAC TTT TTT CTC TTC GG 3'	102	16
seb	5' GTA TGG TGG TGT AAC TGA GC 3' 5' CCA AAT AGT GAC GAG TTA GG 3'	164	16
sec	5' AGA TGA AGT AGT TGA TGT GTA TGG 3' 5' CAC ACT TTT AGA ATC AAC CG 3'	451	16
sed	5' CCA ATA ATA GGA GAA AAT AAA AG 3' 5' ATT GGT ATT TTT TTT CGT TC 3'	278	16
see	5' TAC CAA TTA ACT TGT GGA TAG AC 3' 5' CTC TTT GCA CCT TAC CGC 3'	170	32
seg	5' AAT TAT GTG AAT GCT CAA CCC GAT C 3' 5' AAA CTT ATA TGG AAC AAA AGG TAC TAG TTC 3'	642	19
seh	5' CAA TCA CAT CAT ATG CGA AAG CAG 3' 5' CAT CTA CCC AAA CAT TAG CAC C 3'	375	19
sei	5' CTC AAG GTG ATA TTG GTG TAG G 3' 5' AAA AAA CTT ACA GGC AGT CCA TCT C 3'	576	19
sej	5' CAT CAG AAC TGT TGT TCC GCT AG 3' 5' CTG AAT TTT ACC ATC AAA GGT AC 3'	142	30
selk	5' ATG GCG GAG TCA CAG CTA CT 3' 5' TGC CGT TAT GTC CAT AAA TGT T 3'	197	30
sell	5' CAC CAG AAT CAC ACC GCT TA 3' 5' TCC CCT TAT CAA AAC CGC TAT 3'	410	30
selm	5' CTA TTA ATC TTT GGG TTA ATG GAG AAC 3' 5' TTC AGT TTC GAC AGT TTT GTT GTC AT 3'	325	31
seln	5' ACG TGG CAA TTA GAC GAG TC 3' 5' GAT TGA TCT TGA TTA TGA G 3'	475	31
selo	5' GAG AGT TTG TGT AAG AAG TCA AGT G 3' 5' GAT TCT TTA TGC TCC GAA TGA GAA 3'	556	3
selp	5' CTG AAT TGC AGG GAA CTG CT 3' 5' ATT GGC GGT GTC TTT TGA AC 3'	187	30
selq	5' GAA CCT GAA AAG CTT CAA GGA 3' 5' ATT CGC CAA CGT AAT TCC AC 3'	209	30
selu	5' TAA AAT AAA TGG CTC TAA AAT TGA TGG 3' 5' ATC CGC TGA AAA ATA GCA TTG AT 3'	142	35

In human *S. aureus* isolates, *egc* cluster genes are frequent found in commensal strains isolates [21-23] this is agreement with our data as all of the isolates in this study were collected from healthy chicken. In studies of human *S. aureus* strains, the *egc* genes also appeared to be the most prevalent superantigen genes [24-25]. Of the poultry isolates studied by Smyth et al [3] in Northern Ireland, 86.7% contained these genes. It must be stated however that in the latter study, only fifteen poultry strains were included.
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 [3,12,13,26]. These findings are in agreement with our study for classical SEs (*seb-see*) but *sea* was found more frequently in our isolates.
The consumption of foods containing sufficient of one or more preformed enterotoxin could induce staphylococcal food poisoning (SFP). SEA is the most common toxin associated with food poisoning concerning to staphylococcal enterotoxin [8,27]. The fact that 19 % of the isolates investigated in the present study contained the *sea* gene, may thus be important from the public health point of view.
The MRSA isolates in this study were negative for all the superantigen genes that were screened [17]. This finding is not in agreement with the occurrence of these genes among MRSA isolates in other studies [28-29]. These were studies on clinical isolates however, whilst the animal-associated ST 398 MRSA strains that were investigated here, were not causing any problems to the chickens. It has been shown that the presence of enterotoxin genes can differ within a certain clone of *S. aureus*, as they are on mobile genetic elements [25]. However, the fact that these genes appear to be absent in the poultry strains of the animal-associated MRSA ST398, makes them unsuitable for subtyping these strains for epidemiological reasons.

In conclusion, according to our data, poultry can carry *S. aureus* that are likely to be enterotoxigenic. All of the isolates were collected from cloaca and nose in chickens immediately before slaughter, thus contamination of poultry carcasses is not unlikely and might pose a public health hazard.

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