

International 58th Meat Industry Conference “Meat Safety and Quality: Where it goes?”

Characteristics of enterotoxigenic coagulase positive staphylococci isolated from bovine milk in cases of subclinical mastitis

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Abstract

Coagulase positive staphylococci are the most common cause of bovine subclinical mastitis. The goal of this study was to investigate if isolates from milk in cases of mastitis synthesize enterotoxins and express resistance to methicillin. A total of 50 strains of coagulase positive staphylococci were tested using immunoassay ELFA technique. Ten isolates were enterotoxin positive and RealTime PCR was used to identify genes encoding enterotoxins and methicillin resistance. One isolate carried the enterotoxin B encoding gene, but none were positive for the methicillin resistance gene. All isolates were confirmed as *Staphylococcus aureus* biochemically and by PCR.

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Peer-review under responsibility of scientific committee of The 58th International Meat Industry Conference (MeatCon2015)

Keywords: *Staphylococcus aureus*; mastitis; enterotoxins

1. Introduction

Coagulase positive staphylococci are the contemporary world's most common causative agents of subclinical mastitis in dairy cows due to numerous virulence factors such as pigments, hemolysins and mobile genetic elements

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encoding antimicrobial resistance. These factors are usually expressed simultaneously since this renders staphylococci to be more adaptable to conditions in the mammary gland and in harsh environmental conditions. There were numerous reports that some strains of these microorganisms isolated from bulk milk containers or inflamed mammary gland are capable of enterotoxin SEA-SEE synthesis and, as such, could be hazards in dairy products made of raw milk^{8,9,11,12}. In subclinical mastitis, there are no visible alterations of milk, so hence this condition often fails to be detected. Counts of *Staphylococcus aureus* in contaminated milk range from a couple of hundred per ml to 10⁵ cfu/ml and if milk is not properly stored, counts exponentially rise, as does the risk of food intoxication^{5,9}.

Enterotoxin-producing strains of *S. aureus* originating from cows suffering from mastitis most frequently synthesize enterotoxin C. It is considered that there is correlation between an increase of somatic cell count and presence of *sec* gene in pathogenesis of mastitis. Moreover, it has been demonstrated that methicillin resistant *S. aureus* are often involved in enterotoxin production^{1,11}. Methicillin resistant staphylococci are reservoirs of genes responsible for resistance and once within the food chain, pose a risk to human health.

The aim of this study was to investigate if coagulase positive staphylococci isolated in cases of subclinical mastitis occurring in large-scale dairy farming supplying a market of about 3 million people produce enterotoxins, to define phenotype characteristics of these isolates, and to determine if any isolates carry the gene responsible for methicillin resistance.

2. Materials and methods

A total of 50 strains of coagulase positive staphylococci were previously collected in cases of subclinical mastitis. Primary identification of isolates has been performed on the basis of color of pigment, hemolysis on blood agar, catalase reaction, rabbit plasma coagulation and by biochemical profiling using API Staph20 kit (Biomerieux, France).

Molecular confirmation encompassed multiplex PCR technique aimed to detect presence of 16S rRNA (marker of *Staphylococcus* spp.), *nuc* gene (marker of *S. aureus*) and *mecA* gene (marker of methicillin resistance). Following primer were used: 16S sense 5'-GTGCCAGCAGCCGCGGTAA-3' and 16S antisense 5'-AGACCCGGAACGTATTCAC-3', *nuc* sense 5'-TCAGCAAATGCATCACAAACAG-3' and *nuc* antisense 5'-CGTAAATGCACTTGCTTCAGG-3', *mecA* sense 5'-GGGATCATAGCGTCATTATTC-3' and *mecA* antisense 5'-AACGATTGTGACACGATAGCC-3'. ATCC 43300 *Staphylococcus aureus* was used as MRSA positive control.

Capability of synthesis of enterotoxins SEA-SEE was tested using a fluorescent immunoenzyme assay (miniVidas, Biomerieux, France) while specific gene (in this case *sea*, *seb* and *sed*) identification was carried out using RealTime PCR (data on primers not shown).

3. Results and discussion

A total of 10 strains (20%) were demonstrated to produce staphylococcal enterotoxins. Their biochemical and genetic characteristics are shown in Table 1.

We opted to test for the presence of *sea*, *seb* and *sed* genes, since enterotoxins encoded by these genes were found to be most frequent in dairy products. We managed to identify a solitary strain possessing *seb* gene, while the other nine were neither *sea* nor *sed*. After intensive scrutiny, we strongly believed these must carry *sec* genes. Our findings were in accordance with results obtained by several other authors^{9,11,12} who established percentages of enterotoxin producing strains among clinical strains in range from 22.1% to 25.5%, although prevalences of 11% to 77.3% could be demonstrated occasionally.

The majority of authors claimed that enterotoxin C would be the single most dominant enterotoxin found in milk and some dairy products contaminated by strains of *S. aureus*^{1,2,7,10,12} in cases of subclinical mastitis. Jorgensen *et al.* also reported that other classical enterotoxins or their respective genes (SEA, SEB, SED and SEE) would be rarely detected in clinical strains⁹.

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