ATYPICAL STAPHYLOCOCCUS AUREUS AS AN AETIOLOGICAL AGENT OF MASTITIS IN COWS

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Abstract

The aim of the study was to characterise the 493 tube-coagulase-negative Staphylococcus sp. strains isolated from the udder quarters of milking dairy cows in one herd with a high bulk milk somatic cell count (SCC). The isolates were examined phenotypically by cultural features, Gram stain, catalase, coagulase, and API biochemical tests, and genotypically by PCR for chosen genes. About 10% of the strains were examined by the disc diffusion method for their sensitivity to chosen antibiotics. All the analysed strains showed a α, β, or α+β zone of haemolysis on plates with 5% sheep blood agar, and they were Gram-positive cocci in clusters, catalase positive, API 20 Staph and slide coagulase test positive, but tube coagulase test negative. All the isolates from the 206 examined expressed the nuc gene, 97.4% of the 196 examined were positive for the coa gene, and 20.1% (from the 323 examined) had the blaZ gene. In addition, the 268 examined strains were negative for the mecA gene. The strains were highly susceptible in vitro to oxacillin and amoxicillin/clavulanic acid (100%), and penicillin, cloxacillin, cephalexin, cefoperazon, as well as bacitracine (more than 90%), and highly resistant to neomycin, streptomycin or tetracycline (more than 70%). The SCC in 21.4% of the premilk samples from the quarters infected with these atypical Staph. aureus was under 100,000/ml. In the remaining quarters, the SCC ranged from 101,000/ml to 2,000,000/ml, or even more. In conclusion, mastitis in cows can be caused by atypical coagulase tube negative strains of Staphylococcus aureus.

Key words: dairy cows, mastitis, atypical Staphylococcus aureus.

Mastitis remains the most frequent and most costly disease in dairy cows (13, 18, 37). Staphylococci, apart from streptococci and coliforms, make up, at present, the main etiological agents of udder inflammations (3, 11, 13, 24-26). One of the typical features that distinguishes the more pathogenic Staphylococcus strains from the less pathogenic is the ability to produce free coagulase and bound coagulase (clumping factor), by analysed isolates (5, 9). This feature is also considered one of the virulence factors (7, 44). Strains that produce coagulase (coagulase-positive staphylococci; CPS) are simply called Staphylococcus aureus. Staph. intermedius is also coagulase-positive, as are some strains of Staph. hyicus. However, in a review of clinical papers, only the tube-coagulase test that detects the free coagulase is reported as being the first criterion that differentiates Staph. aureus from other species of staphylococci (5, 14, 24-26, 33, 39).

Staph. aureus is a predominant aetiological agent in both subclinical and clinical forms of udder inflammations. Infections and inflammations caused by Staph. aureus (the major mastitis pathogen) are the most difficult to treat and control (1, 2, 33, 38). The role of coagulase-negative staphylococci (CNS) that belong to minor mastitis pathogens has clearly increased during the last few years (8, 19, 32, 36, 40, 45). Among CNS, Staph. chromogenes, Staph. hyicus, Staph. epidermidis, Staph. simulans, Staph. warneri, Staph. xylosus, and Staph. sciu are the most frequently isolated from mastitis (5, 19, 33, 35). Changes in milk connected with CNS are less intensive compared to those caused by coagulase-positive staphylococci (25, 31).

The purpose of the study was to characterise the free coagulase-negative Staphylococcus sp. strains isolated from the milk of cows with subclinically inflamed mammary glands in one herd.

Material and Methods

The examined staphylococci (493 strains) were isolated from the milk of subclinically and clinically inflamed udders in one herd (600 cows) with a bulk milk somatic cell count (BMSCC) higher than 400,000/ml. The quarter milk (inflamed secretion) samples were collected aseptically by the scientific personnel of the Department from 468 lactating cows. After a clinical examination of the udders and milk, the teats were cleaned, dipped in an approved disinfectant, and then the ends of teats were once more disinfected with alcohol...
swabs, and allowed to dry. The first few streams were discarded, and then 2–4 ml of secretion were collected in sterile tubes. The samples were cooled and immediately transported to the laboratory. The bacteriological examinations were performed according to the commonly accepted rules (22), and the somatic cell count (SCC) were measured with Fossomatic 90. The characteristic free coagulase-negative, clumping factor positive Staphylococcus sp. were then examined for nuc, coa, mec A, and bla Z genes by conventional PCR (4, 10, 15, 17, 20).

The antimicrobial sensitivity of the isolated staphylococci was tested by the disk diffusion method and performed according to the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards) guidelines in Mueller-Hinton agar. The following antibacterial agents (Oxoid) were used: oxacillin (ox; 1 µg), penicillin (P; 10 i.u.), amoxicillin with clavulanic acid (Amc; 30 µg), cloxacillin (Ob; 5 µg), cefoperazone (Cfp; 30 µg), cephalaxin (Cl; 30 µg), tetracycline (Te; 30 µg), neomycin (N; 30 µg), streptomycin (S; 10 µg), and bacitracin (B; 10 i.u). Staph. aureus ATCC 25923 was the control strain. The interpretation of the test results: sensitive (S), intermediate sensitive (I) and resistant (R), were based on CLSI criteria.

**Results**

Atypical Staph. aureus were isolated from 493 (27%) quarters of 264 (56.4%) cows. Apart from these bacteria, infections were also caused by coagulase-negative staphylococci (non-haemolytic, free coagulase-negative and clumping factor-negative) (19.1% of quarters), Streptococcus sp. (1.3%), coliform bacteria (0.7%), and Cor. bovis (1.0%). The remaining quarters (47.8%) were bacteriologically negative, and 3.2% of quarter samples were contaminated. The analysed strains (Table 1) showed a α, β or α+β zone of haemolysis on plates with 5% sheep blood agar; they were Gram-positive cocci in clusters, catalase positive, API 20 Staph and slide coagulase test positive, but tube coagulase test negative. All the isolates from the 206 examined expressed the nuc gene, 97.4% of the 196 examined were positive for the coa gene, and 20.1% (from the 323 examined) had the blaZ gene. In addition, 268 examined strains were negative for the mecA gene.

The characteristics of the SCC in infected milk samples is presented in Table 2.

The examined atypical Staph. aureus were mostly isolated from quarters with a SCC higher than 100,000, and in more than 30% of infected quarters the SCC exceeded 500,000/ml. Macroscopic changes in milk were observed in seven (1.4%) infected quarters. The average SCC in the quarters infected with these staphylococci was 618.3 ±1,254.8/ml. The SCC in the quarters infected with CNS also ranged between 100,000 or less and 5,000,000 and more, but a CNS of more than 500,000 was determined in only 9.5% of quarters. The average SCC in quarters infected with CNS was 414.4 ±1,495.3/ml.

All analysed the strains (Fig. 1) were in vitro sensitive to oxacillin, amoxicillin/clavulanic acid, cephalaxin, and bacitracin. With the exception of 1.7% of these, the isolates were also sensitive to cloxacillin, cephalaxin, and cefoperazone. They were resistant mostly to neomycin, streptomycin and tetracycline.

**Table 1**

<p>| Characteristics of the free coagulase-negative Staph. aureus strains |
|---------------------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Examined features</th>
<th>Number of examined strains</th>
<th>Positive strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemolysis</td>
<td>493</td>
<td>493</td>
</tr>
<tr>
<td>Free coagulase (tube coagulase test)</td>
<td>493</td>
<td>0</td>
</tr>
<tr>
<td>Clumping factor (slight coagulase test)</td>
<td>493</td>
<td>493</td>
</tr>
<tr>
<td>coa gene</td>
<td>196</td>
<td>191</td>
</tr>
<tr>
<td>nuc gene</td>
<td>206</td>
<td>206</td>
</tr>
<tr>
<td>mec A gene</td>
<td>268</td>
<td>0</td>
</tr>
<tr>
<td>blaZ gene</td>
<td>323</td>
<td>65</td>
</tr>
</tbody>
</table>

**Table 2**

Somatic cell count in quarter premilk of cows with mastitis caused by atypical Staph. aureus

<table>
<thead>
<tr>
<th>Infected quarters</th>
<th>Somatic cell count (x 10^3/ml)</th>
<th>Changes in milk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 100</td>
<td>101 - 300</td>
</tr>
<tr>
<td></td>
<td>301 - 500</td>
<td>501 - 1,000</td>
</tr>
<tr>
<td></td>
<td>1,001 - 2,000</td>
<td>2,001 - 5,000</td>
</tr>
<tr>
<td></td>
<td>changes in</td>
<td>milk</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>21.4</td>
</tr>
<tr>
<td></td>
<td>493 = 100%</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>30.6</td>
</tr>
<tr>
<td></td>
<td>87</td>
<td>17.9</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1.4</td>
</tr>
</tbody>
</table>
Discussion

Atypical free coagulate-negative staphylococci were the main cause of subclinical mastitis in the farm, causing problems with the production of an acceptable quality of milk. All the analysed strains were positive for haemolysins and clumping factor, all harboured the nuc gene, and 97.4% had the coa gene. These features are characteristic for Staph. aureus (5, 9). However, the strains were negative in the tube-coagulase test, and then such strains are called atypical Staph. aureus. The atypical Staph. aureus strains were frequently isolated from ill people (28, 29, 43, 44). Only some authors have reported an atypical Staph. aureus isolated from mastitis in cows. Laevens et al. (21) isolated the haemolytic positive, clumping factor negative, but heat resistant deoxyribonuclease positive strain from mastitic milk in a dairy herd. This strain infected from 7.5% to 17.7% of quarters. Additionally, Fox et al. (8) identified a coagulate-negative variant of Staph. aureus that was isolated from aseptically collected milk samples from 25 cows with subclinical mastitis in a herd of 250 dairy cows. Coagulase-negative strains that amplified the coa gene were most recently found in dairy products in Brazil (41). Some of these atypical strains harboured the enterotoxin genes (41).

The analysed atypical strains caused a more intensive increase in SCC if compared to the subclinical inflammations caused by CNS in the same farm. This concurs with our earlier results (25), and with the data of other authors (6, 30). For example Djabri et al. (6) noted on average 357,000 cells in milk from quarters infected with Staph. aureus vs. 138,000/ml in those infected with other staphylococci.

The sensitivity of the atypical Staph. aureus to antibiotics was very high. The analysed staphylococci were more susceptible in vitro if compared to earlier results (23, 27). On the other hand, de Oliveira et al. (30) have determined the MIC concentration for Staph. aureus strains from 11 countries, and they have stated that the overall level of resistance was generally low for all antimicrobial agents that are currently available commercially to treat bovine mastitis, tested regardless of country. However, it should be added that coagulate-positive staphylococci were more resistant to penicillin, ampicillin, amoxicillin, cloxacillin, cefoperazone, tetracycline, lincomycin, and neomycin than CNS strains (23, 27). Watts and Salmon (42) have emphasised the need to identify MRSA because these strains are resistant to all compounds currently approved for the treatment of bovine mastitis. Atypical Staph. aureus did not express the mec A gene, and all were sensitive to oxacillin.

In conclusion, clumping factor should be routinely examined apart from the tube coagulase test, because mastitis in cows can be caused by atypical coagulate tube negative strains of Staph. aureus. However, the most precise method would be the identification of the coa and nuc genes by PCR.

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References