Toxic bovine mastitis caused by *Staphylococcus aureus* in twin cows

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**Summary**

In this report, we describe two cases of bovine toxic mastitis associated with *S. aureus* and we provide DNA microarray based characterization data of the strain causing the disease. Both cows had recently calved and suffered from anorexia, pyrexia, and an elevated heart rate. In both animals, at least one mammary gland was swollen, hardened, sensitive to touch, and produced brownish or bloody secretions. The clinical state of the animals deteriorated quickly and both cows had to be euthanized within 48 hours after presentation. The *S. aureus* strain, which was isolated from the mastitis milk of both cows, was assigned to *spa* type t267, *agr* type I, capsule type 5 and CC97, a clonal complex recently identified as the evolutionary origin of two emerging clones of human epidemic community-associated methicillin-resistant *S. aureus*. The strain did not harbour any genes conferring resistance to antimicrobial agents and we did not detect any genes coding for enterotoxins, toxic shock syndrome toxin, or exfoliative toxins. Taking into consideration that twin cows were affected by this rare disease, we suggest that host factors may play a crucial role in toxic mastitis associated with *S. aureus*.

**Introduction**

Bovine mastitis represents a major challenge to the worldwide dairy industry and causes severe economic losses, arising not only from decreased milk production, but also from costs of treatment, premature culling, and death. *Staphylococcus (S.) aureus* can lead to subclinical, acute or chronic mastitis, and has recently been associated with the rare symptom complex of “toxic mastitis” in cattle, which is characterized by a combination of mastitis symptoms and clinical signs of toxemia including lethargy, tachycardia, anorexia, pyrexia or hypothermia, as well as muscle weakness (Andrews et al., 2004; Menzies et al., 2000; Bleul et al., 2006). However, previous studies investigating bovine toxic mastitis mainly focus on coliforms, as *S. aureus* causes only 3 – 17% of toxic
mastitis cases (Menzies et al., 2000; Menzies et al., 2003; Bleul et al., 2006). Thus, to date, information on bovine toxic mastitis caused by *S. aureus* is extremely scarce and pathogenesis is unclear. In this report, we describe two cases of *S. aureus* toxic mastitis that occurred in twin cows and we provide characterization data of the strain that caused the disease. Our objective was to (1) provide data on the clinical course of toxic *S. aureus* mastitis and (2) collect information on the genetic traits of an *S. aureus* strain able to cause toxic mastitis in order to identify potential pathophysiological mechanisms.

**Case 1 and 2**

The two cases of toxic mastitis occurred in 6-year old Simmental twin cows that originated from the same farm and had recently calved (48 h and 24 h). The cows were presented to the Veterinary Ambulatory Clinic (University of Zurich) on December 3rd, 2013, and January 20th, 2014, respectively. Cow1 was presented due to fever and anorexia and had been in the previous lactation due to clinical *S. aureus* mastitis following anudder lesion (quarter B) caused by traumatic insult. Cow2 was in sternal recumbency and its skin was cold. Both animals showed anorexia, pyrexia with a rectal temperature of 41.5 °C and 40.0 °C, an elevated heart rate (120 bpm; 112 bpm), and an elevated respiratory rate (40 breaths/ min; 44 breaths/ min). Cow1 exhibited a rectal temperature of 38.2 °C, reddened mucous membranes with a capillary refill time of 2 to 3 seconds. Examination of the digestive system revealed reduced ruminal motility in cow1 and no ruminal motility in cow2. Swinging and percussion auscultation were negative on both sides. The consistency of the feces equaled a Skidmore score of 3 (Skidmore et al., 1996). A gynecological exam showed vaginal discharge of clear mucus. There were no signs of retained fetal membranes. In both cows, examination of the mammary gland revealed painfull swelling and hardening in at least one quarter, with grossly changed secretion. In cow1, quarter B was affected with thickened brownish secretions that contained some necrotic tissue. In cow 2, quarter C and D were affected and showed bloody and watery secretion. The clinical exams resulted in no further pathological findings. The preliminary diagnoses were toxic mastitis or hypocalcemia, as the clinical exam did not suggest toxic metritis, right displaced abomasum, ileus, or salmonellosis.

**Treatment**

A milk sample was collected from the affected quarters for microbiological examination and the mammary gland was hand-stripped and flushed using 0.9 % sodium chloride solution. Both cows were treated using Cloxacoli intramammary injectors containing a combination of cloxacillin and colistin twice daily (Virbac AG, Glattbrugg, Switzerland) and flunixin meglumine (2.2 mg/kg Flunixinim i.v., Dr. E. Graeub AG, Berne, Switzerland). Isotonic sodium chloride solution supplemented with glucose (9 g/L NaCl + 50 g/L glucose) was administered intravenously (40 L/24 h) through an indwelling catheter. In addition, cow 1 received marbofloxacin (2 mg/ kg 10 % Marbo, Vetoquinol AG, Ittigen, Switzerland) and cow2 received vitamin E + Selen (15 mg sodium selenite, 375 mg α-tocopheryl acetate, s.c., Tocoselenit, Dr. E. Graeub AG, Berne, Switzerland) and 500 mL Calciamyl 40-MP (3.13 g calcium gluconate and borogluconate, 0.55 g magnesium and 1.42 g phosphorus as magnesium hypophosphite per 100 mL, Dr. E. Graeub AG, Berne, Switzerland) intravenously. The state of both animals deteriorated quickly. On the following day, the cows showed decreased rectal temperatures (39.5 °C; 38.3 °C) and respiratory rates (36 breaths/min; 40 breaths/min). Although therapy was continued, the swelling of the affected mammary glands increased. As cow2 was found in lateral recumbency and exhibited a heart rate of 230 bpm, it was euthanized. In cow1, the parenchyma of gland B was profoundly hardened and the gland produced watery secretion. Its state further deteriorated over night. Cow1 was found in lateral recumbency and showed anorexia and grinding of teeth. The animal exhibited a rectal temperature of 38.2 °C, reddened mucous membranes, a heart rate of 120 bpm and a respiratory rate of 40 breaths/min. The cow was euthanized due to a poor prognosis.

**Bacterial isolation and genotyping**

The milk samples were plated onto Brolacin agar (Merck, Zug, Switzerland), as well as 5 % sheep blood agar, which yielded white colonies exhibiting double zone hemolysis suggestive of *S. aureus*. The putative *S. aureus* isolates from both cows were finally confirmed using the species-specific markers of the DNA microarray Genotyping Kit (Alere Technologies GmbH, Jena, Germany). For the microarray based genotyping, cells were lysed and DNA was extracted using the *S. aureus* Genotyping Kit and the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturers’ instructions. Nanodrop ND-1000 UV/Vis spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) was used to determine the concentration of nucleic acids. The DNA microarray enabled detection of the absence or presence of 333 genes and their allelic variants including species markers and virulence genes, as well as genes conferring resistance to antimicrobial agents. Microarray results also allowed for assignment of the strain to a clonal complex (Monecke et al., 2008).

We performed *spa* typing as previously described (Wattigner et al., 2012). The polymorphic X region of the *spa* gene was determined using PCR amplification, PCR purification, and subsequent sequencing. The *spa* type was
assigned using the spa-server (http://spa.ridom.de/) as described elsewhere (Harmsen and Claus, 2003).

Results

*S. aureus* was isolated from mastitis milk samples of both cows. The isolates were assigned to *spa* type t267 and CC97. The DNA microarray allows not only for identification of virulence genes, but also for the detection of a vast range of resistance genes, including *bla*Z and *mec*A involved in beta lactam and methicillin resistance, respectively. For SA1608, we did not detect any genes conferring resistance to antimicrobial agents. SA1608 belonged to *agr* type I and capsule type 5. It exhibited various genes encoding hemolysins, leukocidins, adhesion factors, and proteases (Tab. 1). In contrast, no genes of the immune evasion cluster were detected such as *sak* (encoding staphylokinase), *scn* (encoding the staphylococcal complement inhibitor), and *chp* (encoding the chemotaxis inhibitory protein). The strain exhibited neither enterotoxin genes involved in food poisoning, nor genes encoding superantigens associated with the toxic shock syndrome (*tst*) or the staphylococcal scalded skin syndrome (*eta*/*eta*/*eta*/).

Discussion

The results of the toxic *S. aureus* mastitis described in this report are highly consistent with the results of a study from Northern Ireland investigating bovine toxic mastitis in general. Menzies et al. showed that toxic mastitis cases occur preferably from November to March (84%) and within 4 days of calving (29%) (Menzies et al., 2000). The isolates were assigned to *spa* type t267. While strains of this *spa* type have been detected among bovine mastitis strains in Switzerland (Johler et al., 2011), no cases of toxic mastitis were associated with these isolates. The isolates were also assigned to CC97, a clonal complex detected amongst 16–21% of *S. aureus* strains isolated from bovine mastitis milk in Switzerland (Johler et al., 2011; Moser et al., 2013). Bovine *S. aureus* strains of CC97 were recently identified as the evolutionary origin of two emerging clones of human epidemic community-associated methicillin-resistant *S. aureus* (CA-MRSA) (Spoor et al., 2013). Acquisition of mobile genetic elements such as plasmids, phages, and *S. aureus* pathogenicity islands is common among *S. aureus*. It was suggested that after the host jump to humans, the bovine methicillin sensitive CC97 strains acquired various genes encoding virulence factors and antimicrobial resistance genes that facilitate their global spread amongst the human population (Spoor et al., 2013). As the isolates exhibited identical *spa* types and clonal complexes, and highly similar microarray profiles, they were identified as the same *S. aureus* strain (SA1608).

In the DNA microarray analysis, we did not detect any genes in SA1608 that confer resistance to antimicrobial agents, consistent with studies reporting only very low rates of bovine *S. aureus* CC97 strains exhibiting resistance to antibiotic agents (Moser et al., 2013; Spoor et al., 2013). SA1608 exhibited various genes encoding hemolysins, leukocidins, adhesion factors, and proteases that are common among bovine *S. aureus* in Switzerland (Johler et al., 2011; Moser et al., 2013). We also detected several genes coding for virulence factors for which a ruminant host-specific activity was demonstrated, including the lukM/F leukotoxin or the van Willebrand factor binding protein (Guinane et al., 2010; Viana et al., 2010). In contrast, no genes of the immune evasion cluster were detected such as *sak* (encoding staphylokinase), *scn* (encoding the staphylococcal complement inhibitor), and *chp* (encoding the chemotaxis inhibitory protein). This is consistent with the fact that these genes typically are associated with immune evasion in the human and not in the bovine host (van Wamel et al., 2006). Interestingly, the strain exhibited neither enterotoxin genes involved in food poisoning, nor genes encoding superantigens associated with the toxic shock syndrome (*tst*) or the staphylococcal scalded skin syndrome (*eta*/*eta*/*eta*/).

### Table 1: DNA microarray results for selected virulence genes detected in *S. aureus* strain SA1608.

<table>
<thead>
<tr>
<th>Group</th>
<th>Gene/Probe</th>
<th>Virulence factor/Function</th>
</tr>
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<tbody>
<tr>
<td>Hemolysins &amp; leukocidins</td>
<td><em>hla</em></td>
<td>alpha hemolysin (cytotoxin)</td>
</tr>
<tr>
<td></td>
<td><em>hlb</em></td>
<td>beta hemolysin (cytotoxin)</td>
</tr>
<tr>
<td></td>
<td><em>hlgA, lukS</em></td>
<td>gamma hemolysin (cytotoxin)</td>
</tr>
<tr>
<td></td>
<td><em>hld</em></td>
<td>delta hemolysin (cytotoxin)</td>
</tr>
<tr>
<td></td>
<td><em>lukM/F</em></td>
<td>bovine leukocidin (cytotoxin)</td>
</tr>
<tr>
<td></td>
<td><em>lukD/E</em></td>
<td>leukocidin (cytotoxin)</td>
</tr>
<tr>
<td>Proteases</td>
<td><em>aur</em></td>
<td>aurolysin (complement inhibitor)</td>
</tr>
<tr>
<td></td>
<td><em>spA/A/E</em></td>
<td>serine protease-like proteins A/B/E (exhibit sequence homology to epidermolytic toxins)</td>
</tr>
<tr>
<td></td>
<td><em>sspA</em></td>
<td>glutamylendopeptidase (V8 protease)</td>
</tr>
<tr>
<td></td>
<td><em>sspP/B</em></td>
<td>staphopain A/B protease</td>
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References


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