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Bovine *Staphylococcus aureus*: Subtyping, evolution, and zoonotic transfer

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ABSTRACT

Staphylococcus aureus is globally one of the most important pathogens causing contagious mastitis in cattle. Previous studies using ribosomal spacer (RS)-PCR, however, demonstrated in Swiss cows that *Staph. aureus* isolated from bovine intramammary infections are genetically heterogeneous, with *Staph. aureus* genotype B (GTB) and GTC being the most prominent genotypes. Furthermore, *Staph. aureus* GTB was found to be contagious, whereas *Staph. aureus* GTC and all the remaining genotypes were involved in individual cow disease. In addition to RS-PCR, other methods for subtyping *Staph. aureus* are known, including *spa* typing and multilocus sequence typing (MLST). They are based on sequencing the *spa* and various housekeeping genes, respectively. The aim of the present study was to compare the 3 analytic methods using 456 strains of *Staph. aureus* isolated from milk of bovine intramammary infections and bulk tanks obtained from 12 European countries. Furthermore, the phylogeny of animal *Staph. aureus* was inferred and the zoonotic transfer of *Staph. aureus* between cattle and humans was studied. The analyzed strains could be grouped into 6 genotypic clusters, with CLB, CLC, and CLR being the most prominent ones. Comparing the 3 subtyping methods, RS-PCR showed the highest resolution, followed by *spa* typing and MLST. We found associations among the methods but in many cases they were unsatisfactory except for CLB and CLC. Cluster CLB was positive for clonal complex (CC)8 in 99% of the cases and typically positive for t2953; it is the cattle-adapted form of CC8. Cluster CLC was always positive for t529 and typically positive for CC705. For CLR and the remaining subtypes, links among the 3 methods were generally poor. Bovine *Staph. aureus* is highly clonal and a few clones predominate. Animal *Staph. aureus* always evolve from human strains, such that every human strain may be the ancestor of a novel animal-adapted strain. The zoonotic transfer of IMI- and milk-associated strains of *Staph. aureus* between cattle and humans seems to be very limited and different hosts are not considered as a source for mutual, spontaneous infections. Spillover events, however, may happen.

Key words: *Staphylococcus aureus*, bovine intramammary infection, subtyping, phylogeny, zoonotic transfer

INTRODUCTION

Staphylococcus aureus is known as an important pathogen responsible for contagious IMI in cattle worldwide (Sears and McCarthy, 2003). It is of central interest for the dairy industry and veterinary medicine in Switzerland, as it is responsible for massive economic loss in Switzerland (Heiniger et al., 2014).

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Intensive investigations were carried out in Switzerland in the last years to subtype *Staph. aureus* involved in bovine mastitis (Fournier et al., 2008; Graber et al., 2009). Fournier et al. (2008) developed a genotyping method based on amplifying the 16S-23S rRNA intergenic spacer region by PCR (ribosomal spacer-PCR, **RS-PCR**), which showed the associations of genotype with virulence gene pattern and clinical properties of the disease. In the first instance, 17 different genotypes were found and further characterized for their virulence gene patterns (Fournier et al., 2008). *Staphylococcus aureus* genotype B (**GTB**) and GTC were the most frequent genotypes found in bovine milk (80%), but only GTB was contagious, with cow prevalences of up to 87% within herds, whereas GTC showed noncontagious behavior, infecting only single cows in a herd and single quarters of the udder (Fournier et al., 2008; Graber et al., 2009). *Staphylococcus aureus* GTB is characterized by the presence of the enterotoxin genes *sea*, *sed*, and *sej* and a SNP within the *lukE* gene (**lukEB**). In contrast, *Staph. aureus* GTC typically carries the enterotoxin genes *sec*, *seg*, *sei*, and *tst* (coding for toxic shock toxin-1) and is lukEB-negative (Fournier et al., 2008). All the other genotypes (**GTOG**) associated with Swiss mastitic dairy milk were rare, and their virulence gene patterns were inconsistent except that all were *sea*-negative (Fournier et al., 2008).

In humans, *Staph. aureus* is a well-known pathogen, responsible for wound infections and septicemia; it is, as a multiply resistant pathogen, an important and increasing problem in hospital environments and individual patients (Bannermann and Peacock, 2007). In terms of food safety, *Staph. aureus* is capable of producing a variety of enterotoxins that cause severe vomiting and diarrhea in humans (Hennekinne et al., 2012; Hummerjohann et al., 2014).

Staphylococcus aureus has been extensively studied and various typing methods have been described for subtyping this pathogen (Rabello et al., 2007; Hwang et al., 2010; van den Borne et al., 2010; Sakwinska et al., 2011). Two common methods are *spa* typing (Harmsen et al., 2003) and multilocus sequence typing (**MLST**; Enright et al., 2000). The former is based on DNA sequencing of the variable spacer region of the staphylococcal *spa* gene, whereas the latter requires the sequencing of 7 housekeeping genes. Other methods include pulsed-field gel electrophoresis (Bardiau et al., 2013; Lundberg et al., 2014) or binary typing (Zadoks et al., 2000). These analytic methods are useful to gain further insight into the pathogenesis of bovine mastitis caused by *Staph. aureus* but, because of their low throughput and high cost, they are less appropriate for large clinical investigations and routine analyses in veterinary medicine. Furthermore, the method is linked to the within-herd epidemiological and pathogenic properties of the different subtypes.

In the present study including 12 European countries, we compared the 3 analytic methods RS-PCR, *spa* typing, and MLST for subtyping 456 strains of *Staph. aureus* isolated from milk of bovine IMI and bulk tanks. Furthermore, we deciphered the phylogeny of animal *Staph. aureus* and studied the zoonotic transfer of *Staph. aureus* between cattle and humans.

MATERIALS AND METHODS

Strains

In total, 456 bovine strains of *Staph. aureus* originating from 12 European countries were examined (Table 1). They were reused from the companion study by Cosandey et al. (2016). The strains had been stored in skim milk at −20°C and were recultured on blood agar (bioMérieux Suisse s.a., Geneva, Switzerland) by plating a loopful of bacteria on the agar and incubating it at 37°C for 24 h. As described by Cosandey et al. (2016), the isolates had originated from aseptically collected milk samples from single quarters (**SQM**) with subclinical IMI, or from bulk tank milk (**BTM**) samples obtained according to the guidelines of the National Mastitis Council (NMC, 1999), or they had been taken from the authors' bacterial culture collections (**BC**). The BC strains had all been obtained from milk of cows with subclinical IMI using aseptic sample collection.

The BTM and SQM samples were selected from herds where IMI caused by *Staph. aureus* had been observed in the past year. The countries and regions delivering BTM and SQM samples are listed in Table 1. Among these countries, the cows of the herds to be sampled were selected according to different criteria including, at maximum, all lactating cows of a herd or, at minimum, the quarters positive by California Mastitis Test. Regardless of the selection criterion, however, SQM samples were collected aseptically. Cows with clinical mastitis and those under antimicrobial treatment were excluded. The SQM and BTM samples originated from 231 farms. The Swiss strains were taken from the authors' strain collection and included a random subset of *Staph. aureus* GTB (n = 39) and GTC (n = 32). For the other Swiss genotypes, all available strains of the strain collection were used (n = 15).

Genotypes, *spa* types, sequence types, and clonal complexes (**CC**) were considered associated with IMI if the isolates had been obtained from aseptically taken milk samples as in the case the SQM pools or if they had been taken from the authors' strain collections (BC). An additional subset of 7 Swiss strains was used