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Carriage frequency, diversity and methicillin resistance of *Staphylococcus aureus* in Danish small ruminants

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ABSTRACT

The ecology of *Staphylococcus aureus* in animals has recently gained attention by the research community due to the emergence of livestock-associated methicillin-resistant strains (MRSA). We investigated carriage frequency and clonal diversity of *S. aureus* in 179 sheep and 17 goats in Denmark using *spa* typing and MLST. *S. aureus* was detected in 74 sheep (41%) and 11 goats (64%). The isolates belonged to 26 *spa* types (including six novel *spa* types) and 12 STs (including three novel STs). The most common lineage was ST133, which was found in 65% sheep and 55% goats. MRSA was found in three animals and two of them harboured *mecC* and corresponded to the same lineage (ST130, t843) previously reported in *mecC*-associated human MRSA infections in Denmark. The remaining MRSA isolate belonged to ST398 but its recovery in sheep could be a consequence of cross contamination due to contact with pigs. This study provides novel data about the occurrence of *S. aureus* in small ruminants, revealing high carriage frequency and diversity in these animals. The finding of *mecC* in ovine ST130 isolates suggests that sheep may be a reservoir of this new emerging MRSA clone of suspected animal origin. Inclusion of sheep in national MRSA surveillance programmes in animals is advisable in view of this finding.

1. Introduction

Different *Staphylococcus aureus* lineages show selective affinity towards specific animal hosts. This host specificity was already described in the 70's and 80's using phenotypic tests (Devriese, 1984; Devriese and Oeding, 1976) and more recent studies have confirmed this host specificity (Price et al., 2012; Smith et al., 2005; Sung et al., 2008; van Leeuwen et al., 2005; Vautor et al., 2009) using molecular methods such as multi locus sequence typing (MLST). Clonal complexes (CCs) consisting of related sequence types (STs), have been associated with specific hosts. For example ST5 is predominant among poultry isolates; ST398, ST9 and ST433 among porcine isolates; and CC97 and CC50 among bovine isolates (Delgado et al., 2011; Hasman et al., 2010; Smith et al., 2005). CC133 and ST522 have been found to be the main responsible for mastitis in sheep and goats (Jørgensen et al., 2005; Porrero et al., 2012) but limited information is available on *S. aureus* healthy carriage frequency and diversity in these hosts.

Despite host specificity, human infections with animal associated strains have been increasingly observed. A special public health concern is the transmission of methicillin-resistant *S. aureus* (MRSA) from animals to people in close contact with animals such as pig farmers (Smith et al., 2009), horse personnel (Weese et al., 2005), dog owners (Cefai et al., 1994) and veterinary staff (Hanselman et al., 2006; Seguin et al., 1999). Recently, a novel homolog of the methicillin resistance determinant *mecA* (*mecC*) has been found among human MRSA isolates

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in the United Kingdom (UK), Denmark and Germany (Cuny et al., 2011; García-Álvarez et al., 2011; Shore et al., 2011). Methicillin-resistant *mecC*-positive ST130 has also been isolated frombulk milk in the UK (García-Álvarez et al., 2011) and more recently two clinical cases have been linked to contact to bovine and ovine carriage in Denmark (Paterson et al., 2012; Petersen et al., 2012), suggesting that this new type of MRSA may originate from livestock. The objective of this study was to investigate carriage frequency, diversity and methicillin resistance of *S. aureus* in small ruminants in Denmark.

2. Methods

2.1. Sampling

From September 2011 to February 2012, 179 sheep and 17 goats originating from 41 farms were sampled by introducing a sterile cotton swab in the oral and nasal cavities. Swabs were collected from 81 living animals on farms and from 115 dead animals at the slaughterhouse, shortly after beheading. Samples originated from the island of Bornholm ($n = 20$), the island of Zealand ($n = 20$), Jutland peninsula ($n = 34$) and the island of Lolland and surrounding islands ($n = 122$).

2.2. Isolation and identification of S. aureus

Swabs were enriched in 5 mL Mueller–Hinton broth with 6.5% of NaCl. After 18 h incubation at 37 °C, 10 μL of enrichment were plated on mannitol-salt agar, calf-blood agar (introduced after screening of the first 68 animals to facilitate *S. aureus* detection) and *Brilliance*™ MRSA 2 agar (Oxoid, UK) and incubated overnight. Presumptive *S. aureus* colonies were subcultured and confirmed by *spa* PCR (Harmsen et al., 2003). Presumptive MRSA colonies, displaying the characteristic blue colour on *Brilliance*™ MRSA 2 agar were confirmed by PCR amplification of *mecA* or *mecC* (García-Álvarez et al., 2011; Zhang et al., 2004) using the following conditions: denaturation for four min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at 55 °C and 60 s at 72 °C, and a final extension at 72 °C for 10 min.

2.3. spa and multi locus sequence typing (MLST)

All isolates were *spa* typed (Harmsen et al., 2003). Grouping analysis of *spa* types was performed using the Based Upon Repeat Pattern (BURP), Ridom Staphtype Software. Software settings were a cut-off value of 4 for clustering and exclusion of types with less than 5 repeats (Mellmann et al., 2007).

MLST was performed on16 strains selected by BURP analysis (Enright et al., 2000) and PCR products were sequenced using BigDye XTerminator® Purification Kit and a 3130xl Genetic Analyzer (Applied Biosystems, Life technologies). The sequences were analysed using the MLST module of CLC Main Workbench version 4.9. Sequence types (STs) were assigned to clonal complexes (CCs) using the eBURST algorithm (Feil et al., 2004).

2.4. Database search for human infections with genotypes found in small ruminants

The *S. aureus* populations from sheep and goats were compared to human clinical isolates by searching for the obtained ovine *spa* types in the national databases of human MRSA and *S. aureus* bacteraemia (SAB) isolates curated at SSI, DK. These databases contain *spa* types of all human MRSA ($n = 5534$) and *S. aureus* bacteraemia ($n = 7954$) cases in Denmark since 2007.

3. Results

S. aureus was isolated from 85 (43%) of the 196 nasal swabs tested, including 74 (41%) and 11 (65%) samples from sheep and goats, respectively. Thirty-eight isolates originated from living animals and 47 from post-mortem samples, yielding to 28positive farms out of the 41 farms represented in the study population. *S. aureus* was recovered in 48% and 40% of the samples taken from living and dead animals, respectively. Only three (1.5%)sheep of the 196 animals tested were found to carry MRSA. Two MRSA isolates were confirmed to carry *mecC* and belonged to *spa* type t843 (ST130). The third MRSA carried *mecA* and belonged to t034 (ST398).

Twenty-six *spa* types were detected, including six new *spa* types (Table 1). The *spa* types formed three BURP clusters related to CC133 (10 *spa* types, cluster 1), a mix of CC133 and CC15 (two *spa* types, cluster 2), and CC15 (two *spa* types, cluster 3), respectively (Fig. 1). Nine *spa* types were singletons and three were excluded from the BURP analysis due to low repeat number (≤ 5) (Table 1). Analysis of the distribution of the *spa* clusters showed that cluster 1 was spread through the whole territory, whereas cluster 2 was detected in two slaughterhouses in Lolland Island and cluster 3 was found at two farms in North Jutland and one farm in Lolland Island.

The 74 ovine isolates showed 24 different *spa* types, and 10 STs (including three new STs, ST2305, ST2328 and ST2423) that belonged to eight CCs. The 11 caprine isolates displayed five *spa* types and four STs (ST9, ST30, ST39 and ST133) belonging to three CCs (Table 1). Ten *spa* types found in small ruminants had previously been reported in the national databases of human MRSA and SAB cases (Table 1). CC133 was rare in human infections in Denmark as only two human isolates had *spa* types related to this CC (t1166 and t1403). *spa* type t1293 (ST59), which was found in seven sheep sampled at the same slaughterhouse, had been recorded in two cases of human infection. The other seven *spa* types previously reported in human cases were rare among small ruminant isolates (≤ 3 isolates). Of these, the most common *spa* type in the human databases was t034 (ST398, $n = 483$) followed by t843 (ST130, $n = 77$).

4. Discussion

The isolation frequency of *S. aureus* was relatively high (43.3%), especially in goats (64.7%)compared to that previously observed in dairy sheep (29%) (Vautor et al., 2005). However, it should be noted that this is not a true prevalence study since the methodology used for *S. aureus*