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Short communication: *Staphylococcus aureus* isolated from colostrum of dairy heifers represent a closely related group exhibiting highly homogeneous genomic and antimicrobial resistance features

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ABSTRACT

In heifers, intramammary infections caused by *Staphylococcus aureus* affect milk production and udder health in the first and subsequent lactations, and can lead to premature culling. Not much is known about *Staph. aureus* isolated from heifers and it is also unclear whether or not these strains are readily transmitted between heifers and lactating herd mates. In this study, we compared phenotypic characteristics, *spa* types, and DNA microarray virulence and resistance gene profiles of *Staph. aureus* isolates obtained from colostrum samples of dairy heifers with isolates obtained from lactating cows. Our objective was to (1) characterize *Staph. aureus* strains associated with mastitis in heifers and (2) determine relatedness of *Staph. aureus* strains from heifers and lactating cows to provide data on transmission. We analyzed colostrum samples of 501 heifers and milk samples of 68 lactating cows within the same herd, isolating 48 and 9 *Staph. aureus* isolates, respectively. *Staphylococcus aureus* strains from heifers, lactating herd mates, and an unrelated collection of 78 strains from bovine mastitis milk of mature cows were compared. With 1 exception each, characterization of all strains from heifers and lactating cows in the same herd yielded highly similar phenotypic and genotypic results. The strains were Staphaurex latex agglutination test negative (Oxoid AG, Basel, Switzerland) and belonged to *agr* type II, CC705, and *spa* types t529 and t12926. They were susceptible to all antimicrobial agents tested. In contrast, the strains from mature cows in other herds were spread across different clonal complexes, *spa* types, and SplitsTree clusters (<http://www.splitstree.org/>), thus displaying a far higher degree of heterogeneity. We conclude that strains isolated from colostrum of heifers and mastitis milk of lactating cows

in the same herd feature highly similar phenotypic and genomic characteristics, suggesting persistence of the organism during the first and potentially subsequent lactations or transmission between heifers and mature herd mates.

Key words: *Staphylococcus aureus*, dairy heifer, bovine mastitis, genotyping

Short Communication

Heifer mastitis leads to considerable financial losses in the dairy industry, as it affects not only future milk performance and udder health, but also leads to premature culling (Compton et al., 2007; Piepers et al., 2010). To date, it is still poorly understood, which sources of infection and routes of transmission play a role in *Staphylococcus aureus* mastitis in heifers. Heifers have never been milked and have, thus, not been exposed to the milking process and vacuum, which represent major risk factors for contagious mastitis in lactating cows. However, *Staph. aureus* can be detected in milk samples of up to 15% of heifers prepartum and in up to 8% of heifers at first parturition (Fox, 2009). Whereas heifers were suggested to represent a reservoir of *Staph. aureus* to uninfected herd mates (Roberson et al., 1994), another study suggested that the udder of lactating cows might be an infection source of contagious major pathogens for heifers (Piepers et al., 2011). In a recent review, De Vlieghe et al. (2012) summarized the controversial discussion on transmission of *Staph. aureus* between heifers and mature cows and declared the need for further studies using strain typing.

In this study, we compare DNA microarray profiles and *spa* types, as well as phenotypic characteristics of *Staph. aureus* isolates obtained from colostrum samples of dairy heifers to isolates obtained from mastitis milk of lactating cows. Our objective was to (1) characterize *Staph. aureus* strains associated with mastitis in heifers and (2) determine the relatedness of *Staph. aureus* strains from heifers and lactating herd mates to provide data on transmission.

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Colostrum samples of 501 heifers from a randomized selection of 72 farms in Switzerland were collected. Sampling was performed within 24 h postpartum. In addition, a total of 68 samples from lactating cows within the same herd, including first-lactation animals, were taken on 7 farms, which harbored heifers that had been tested positive for *Staph. aureus*. Only milk from lactating cows with clinical signs of mastitis or milk that had been tested positive in the California mastitis test was collected. For all tested cows, quarter milk samples were collected by the farmers. We compared strains from heifers (**H**) and lactating cows (**LC**) within the same herd to strains from an unrelated collection (**C**) of 78 *Staph. aureus* strains that were used in a comprehensive study investigating bovine mastitis isolates from mature cows in Switzerland (Moser et al., 2013).

We characterized the strains using the latex agglutination test, antimicrobial susceptibility testing, *spa* typing, and a DNA microarray. A Staphaurex latex agglutination test (**SLAT**) was performed using the Staphaurex Plus kit (Oxoid AG, Basel, Switzerland) according to the manufacturer's instructions. For susceptibility testing, the following agents were tested: ampicillin (10 µg), amoxicillin (20 µg) with clavulanic acid (10 µg), cephalothin (30 µg), ceftiofur (30 µg), erythromycin (15 µg), cefoxitin (30 µg), gentamicin (10 µg), kanamycin (30 µg), cefalexin-kanamycin (15–30 µg), penicillin (10 IU), and penicillin-novobiocin (10 IU–30 µg). Disks containing cefalexin-kanamycin (Ubrolexin) were provided by Boehringer Ingelheim GmbH (Basel, Switzerland); disks containing ceftiofur, penicillin, and penicillin-novobiocin were obtained from Oxoid AG; and all other disks were provided by Becton Dickinson AG (Basel, Switzerland). Disk diffusion was performed on Mueller-Hinton agar and *Staph. aureus* ATCC 25923 was used as a quality control. Strains were classified according to Clinical and Laboratory Standards Institute standard protocols (CLSI, 2008), except for cefalexin-kanamycin, for which preliminary interpretive breakpoints recommended by Pillar et al. (2009) were used. The *spa* types were determined as previously described (Wattlinger et al., 2012). For the DNA microarray assay, we used the *Staph. aureus* genotyping kit (Alere Technologies GmbH, Jena, Germany) to detect the absence or presence of 333 genes and their allelic variants and to assign strains to clonal complexes. Microarray data from a comprehensive study by Moser et al. (2013) on characteristic features of *Staph. aureus* isolated from mastitis milk of lactating cows (**C**) in Switzerland served as a control to assess unique features among the virulence and resistance genes of *Staph. aureus* strains isolated from **H** and **LC** in the same herd. For statistical analysis, the distribution of genes was compared based on DNA microarray results

using SPSS Statistics 21 software (SPSS Inc., Chicago, IL) to run the Pearson χ^2 test to identify significant associations between the source of the strains and the presence of the genes. The Fisher exact test was used in case the expected count was less than 5. Results were considered statistically significant for $P < 0.05$.

We isolated *Staph. aureus* from 10% of **H** colostrum samples and 13% of **LC** mastitis milk samples. Isolates exhibiting identical microarray patterns, as well as identical **CC** and *spa* type assignments were considered to represent the same strain (Supplemental Table S1; <http://dx.doi.org/10.3168/jds.2013-7721>). Six **H** strains were isolated in duplicate (**H**_5, **H**_6, **H**_7, **H**_8, **H**_15, and **H**_22), 1 **LC** strain in triplicate (**LC**_7), and 2 strains could be found among both **H** and **LC** herd mates (**H**_15 = **LC**_2 and **H**_22 = **LC**_1). Five of the 6 strains that were isolated in duplicate among **H** were isolated at farm A as well as from at least 1 **H** or **LC** in another farm (farms B, C, D, and E). Interestingly, an additional 7 **H** strains (**H**_16 to **H**_21 and **H**_29) were isolated on farm A.

With 1 exception each, characterization of all strains from **H** and **LC** in the same herd yielded highly similar phenotypic and genotypic results. The strains were **SLAT**(–) and belonged to *agr* type II, **CC**705, and *spa* types t529 ($n = 38$) and t12926 ($n = 1$). They were also susceptible to all antimicrobial agents tested in the disk diffusion assay. In addition, this group of strains formed a highly homogeneous subcluster within the **CC**705 cluster of the SplitsTree software (<http://www.splitstree.org/>; Supplemental Figure S1; <http://dx.doi.org/10.3168/jds.2013-7721>), exclusively comprising **H** and **LC** strains. In contrast, the **C** strains were spread across different clonal complexes and SplitsTree clusters, thus displaying a far higher degree of heterogeneity. Only **H**_14 and **LC**_7 were not integrated into the highly homogeneous group and exhibited a **SLAT**(+) phenotype, as well as differing clonal complexes and *spa* types (**H**_14: **CC**97 and t267; **LC**_7: **CC**45 and t7061). Whereas **H**_14 was susceptible to all tested antibiotic agents, **LC**_7 exhibited resistance to penicillin and ampicillin.

A selection of DNA microarray results is listed in Table 1 and full microarray results are provided as supplemental data (Supplemental Table S2; <http://dx.doi.org/10.3168/jds.2013-7721>). Overall, strains from **H** and **LC** herd mates exhibited highly similar prevalence rates of most resistance and virulence genes, whereas various statistically significant differences were detected when comparing these rates to the respective prevalences in the control strains. Among others, **H** and **LC** strains differed from **C** strains regarding the prevalence of various allelic variants of genes encoding adhesins, proteins involved in immunevasion, enterotoxins, and

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