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Short communication: Methicillin-resistant *Staphylococcus aureus* in conventional and organic dairy herds in Germany

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) have been described repeatedly in dairy herds. In this study, we compared the prevalence and antimicrobial resistance of MRSA in bulk tank milk from conventional and organic dairy herds in Germany. Samples were collected from 372 conventional and 303 organic dairy herds throughout Germany. Bulk tank milk (25 mL) was tested for MRSA using an established double selective enrichment method. The MRSA isolates were typed using *spa* typing and tested for resistance to 19 antimicrobials using the broth microdilution method. Methicillin-resistant *Staph. aureus* was detected more frequently in bulk tank milk from conventional (9.7%) than from organic (1.7%) dairy herds. Herd size and region were associated with differences in prevalence. Most isolates (38/41) were from *spa* types associated with the livestock-associated clonal complex CC398. Isolates from conventional herds tended to be more resistant to antimicrobials; however, because of the limited number of isolates from organic herds, no statistical tests were performed. In conclusion, prevalence of MRSA in dairy herds in Germany seems to be increasing and is more prevalent in regions with high livestock density. Organic herds are also affected although at a lower level. Therefore, MRSA should be specifically included in biosecurity protocols for dairy herds, and effective control measures need to be investigated.

Key words: methicillin-resistant *Staphylococcus aureus* (MRSA), organic, conventional, bulk tank milk

Short Communication

Staphylococcus aureus is an important mastitis pathogen for dairy cows (Tenhagen et al., 2009). In recent years, methicillin-resistant *Staph. aureus* (MRSA) has

been repeatedly described in dairy herds, with herd-level prevalence up to 9.9% (Nemeghaire et al., 2014; Tenhagen et al., 2014; Parisi et al., 2016). Within-herd prevalence was up to 60% (Spohr et al., 2011; Locatelli et al., 2017). About 10% of the dairy herds in Belgium with cases of dairy cow mastitis caused by *Staph. aureus* had a problem with MRSA (Vanderhaeghen et al., 2010).

In Germany, previous investigations estimated MRSA prevalence in bulk tank milk (BTM) from dairy herds to be 4.1 and 4.7% in 2009 and 2010, respectively (BVL, 2012; Tenhagen et al., 2014). However, in contrast to the current investigation, those studies did not differentiate between conventional and organic herds. Methicillin-resistant *Staph. aureus* has been shown to be associated with both clinical and subclinical mastitis (Fessler et al., 2010; Spohr et al., 2011; Silva et al., 2014). Moreover, people working on MRSA-positive dairy farms are at increased risk of carrying MRSA and could therefore introduce MRSA into the healthcare system when receiving medical treatment (Spohr et al., 2011; Antoci et al., 2013).

The risk of acquiring MRSA through the consumption of milk has been estimated to be low due to mandatory heat treatment of milk before marketing (Spohr et al., 2011). However, this estimation might differ for the consumption of raw milk and raw milk products, because this exposes consumers to viable bacteria. So far, no reports indicating the spread of livestock-associated MRSA to humans via milk have been published.

Resistant bacteria such as MRSA are fostered by the routine use of antimicrobials. European Council Regulation (EC) No. 834/2007 limits the number of antimicrobial treatments permitted in organic dairy cows. Therefore, organic farms in Germany and elsewhere in Europe are considered to use lesser amounts of antimicrobials than conventional farms, and organic herds might be less prone to harboring MRSA than conventional herds. This has already been shown for pigs, where MRSA prevalence was lower at both the herd and intraherd level in organic farms (Heine, 2011).

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Table 1. Prevalence of methicillin-resistant *Staphylococcus aureus* in bulk tank milk of conventional and organic dairy herds in Germany in 2014 by region and herd size

Variable	Conventional		Organic	
	Samples assigned ¹	No./total (%)	Samples assigned ¹	No./total (%)
Region				
Northwest	147	21/141 (14.9)	59	0/51 (0)
Southwest	163	6/156 (3.8)	279	5/207 (2.4)
East	74	9/75 (11.9)	46	0/45 (0)
Herd size ²				
<50		6/95 (6.3)		3/114 (2.6)
50–79		6/70 (8.6)		2/99 (2.0)
80–199		9/96 (8.6)		0/56 (0)
200–499		6/41 (14.6)		0/9 (0)
≥500		7/20 (35.0)		0/0
Total		36/372 (9.7)		5/303 (1.7)

¹Samples assigned according to the sampling plan.

²Only 612 herds included because of missing data.

However, published data on dairy cattle comparing organic and conventional farming are limited to a US study that found only one MRSA in BTM from 288 tested herds (Cicconi-Hogan et al., 2014); therefore, potential differences could not be analyzed.

The purpose of this study was to estimate the herd-level prevalence of MRSA in organic and conventional dairy herds in Germany. The study was part of a national monitoring program for zoonotic agents and resistant bacteria in the food chain carried out by the regional veterinary services in cooperation with national reference laboratories (Kaesbohrer et al., 2012; BVL, 2016).

Selection of herds was based on a national sampling plan designed to cover the different German federal states (*Länder*) according to their share of the national conventional and organic dairy cow population. On the national level, the number of herds to be sampled was calculated for each region based on the distribution of cows rather than herds to account for substantially different distributions of herd sizes in different regions in Germany (i.e., smaller herds in the south and large herds in the east). However, farm size was not considered when choosing herds within a state. Data on the distribution of dairy cows were provided by the national bureau of statistics (www.destatis.de). Minimum herd size was 20 lactating cows. Separate sampling plans were designed for the 2 categories of herds as the proportion of organic dairy herds is still low. Selection of the individual herds was the responsibility of the veterinary authorities of the *Länder*.

For data analysis, Germany was divided into 3 major regions: (1) the northwest, characterized by an overall high density of livestock of all species in medium-sized herds, included Schleswig-Holstein, Lower Saxony, and North Rhine-Westphalia; (2) the southwest, character-

ized by heterogeneous animal densities and smaller herds (Rhineland-Palatinate, Hesse, Bavaria, Baden-Württemberg, Saarland); and (3) the east, characterized by an overall low animal density but large herds (Mecklenburg-Western Pomerania, Brandenburg, Saxony-Anhalt, Thuringia, Saxony). Regional distribution of cattle differed between conventional and organic cows, with relatively more organic cows in the southwest and fewer in the east and northwest, leading to differing sample numbers per region (Table 1).

To sample and detect MRSA, 25 mL of bulk tank milk was collected on farm, transported to the regional state laboratory at 4°C, and examined using a harmonized double selective enrichment protocol, as described previously (Tenhagen et al., 2014), within 48 h of arrival at the laboratory. Presumptive MRSA (one randomly chosen isolate from the selective agar or sample) were submitted to the National Reference Laboratory (NRL; Berlin, Germany) for coagulase-positive staphylococci, including *Staph. aureus*, where isolates were confirmed as MRSA by an in-house multiplex PCR simultaneously targeting the 23S rDNA gene specific for *Staphylococcus* species (Straub et al., 1999), the nuclease gene *nuc*, which is specific for *Staph. aureus*, and the resistance gene *mecA* (Poulsen et al., 2003). Isolates of *Staph. aureus* that were resistant to ceftiofur but negative for *mecA* would have additionally been tested for *mecC* but no isolate fulfilled these criteria. Furthermore, MRSA isolates were typed according to the repeat pattern of their *spa* gene (Shopsin et al., 1999). Assignment of *spa* types to multilocus sequence types (MLST) was done based on previously confirmed associations in the NRL. Multilocus sequence typing (Enright et al., 2000) was done on isolates that either could not be assigned to a *spa* type (1 isolate) or showed *spa* types not previously seen in the NRL. The antimicrobial susceptibility

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