



Short communication

Suspected transmission and subsequent spread of MRSA from farmer to dairy cows



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ABSTRACT

In the present study we describe an outbreak where PVL positive MRSA belonging to *spa*-type t002 and multi-locus sequence type ST2659 persisted in a Swedish dairy herd for at least two years, despite efforts to hinder transmission between animals and between the farmer and his animals. This is the first description of persistence and spread of MRSA in a dairy herd in Sweden. Sampling of animals in the herd was initiated by the finding of MRSA in the farmer and was performed at eight occasions from November 2012 to September 2014. In total, MRSA was detected in 25 animals and in 16 of these MRSA was detected in milk samples. In addition, MRSA was also detected in bulk milk samples. Whole genome sequencing (WGS) of twelve isolates from farmer ($n = 1$), animals ($n = 9$) and bulk milk ($n = 2$) revealed high relatedness, implying a common source. MRSA may initially have been transmitted from humans to cows with further spread within the herd. WGS showed minor differences in one isolate (loss of phage Φ N315) which could indicate adaption of the strain to an animal host.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been isolated from dairy herds in several countries (Lim et al., 2013; Luini et al., 2015; Tenhagen et al., 2018; Vanderhaeghen et al., 2010), although it does not appear to be as common in dairy cattle as in pigs. Occurrence of MRSA in dairy cows in Sweden has been sporadic with ten incidents reported up to and including 2017 (Swedres-Svarm, 2017, 2018). However, methicillin-sensitive *S. aureus* is one of the most important udder pathogens in dairy cows worldwide (Barkema et al., 2006), and in Sweden, it is the most common pathogen found in milk samples from dairy cows with clinical mastitis (Ericsson Unnerstad et al., 2009). *S. aureus* in dairy cattle is generally considered as a pathogen of contagious nature with the bovine udder as one of the primary sites of infection (Keefe, 2012). The close contact between humans and dairy cows in some milking systems enables transmission of bacteria between milking personnel and udder.

In September 2012, a dairy farmer with skin lesions was diagnosed with PVL positive MRSA belonging to *spa*-type t002. The farmer owned a dairy herd that in November 2012 consisted of 68 cattle of which 25

were lactating cows. Cows and young stock were kept in tie stalls and younger calves in boxes with 1–5 animals per box. During summer, cows and young stock were kept on pasture. There was a history of several years with sporadic cases of mastitis caused by beta-lactamase susceptible *S. aureus* in lactating cows at the farm. In light of the farmer's MRSA infection and known *S. aureus* infections in the cows, the question of an association between farmer and cows was raised and an investigation started at the farm, including sampling of cattle and review of biosecurity and milking routines. Moreover, in September 2012, an isolate of PVL positive MRSA of *spa*-type t002 (IndexM) was confirmed at the National Veterinary Institute (SVA), Uppsala, Sweden. The isolate was from an anonymous monitoring of *S. aureus* from routine clinical submissions of milk samples to SVA. At that time, the herd of origin was not known, but it is now suspected that this could be the first isolate from the present herd.

The purpose of this paper is to describe the findings of the investigations in the herd including further characterization of the isolated MRSA using whole genome sequencing (WGS).

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Table 1
Number of animals of different categories sampled, and sites sampled, at Samplings a–h and number of bulk milk samples collected.

Animal category	Sample type	Samplings (year and date)											
		2012		2013			2014				2015		
		a Nov 20	b Dec 18	c Sep 16	d Oct 1	e Oct 29	f Mar 5	– Apr 14	g Apr 23	– May-Sep	h Sep 11	– Sep-Dec	– Jan Dec
Lactating cows	M	10/19			1/3		1/3			3/25			
	M, N		7/9				0/1		0/14				
	M, G	1/2											
	M, H	0/4											
	M, N, G			3/18									
	M, N, W								0/4				
	M, N, P					0/2							
	M, N, G, T			0/3									
Dry cows	M, N, G, T, W			0/1									
	N		0/1										
Young cattle, calves	N, G	1/1							0/1				
	N, G	0/39		0/3					0/10				
	N, P			1/17		5/25			0/32				
	N, G, P				1/1								
	N, G, U, W			0/2									
	N						0/4			2/8			
	N, G, W	0/1											
	N, G, E	0/1											
Total animals		12/68	7/10	4/44	2/4	5/27	1/8	1	0/61		5/33		
	N, H	0/1											
Bulk milk	BM	1/1		1/1			0/1	0/1	0/1	1/6	0/1	1/7	1/10
	SCC BM	360	212	375	163	204	147	199			126		

Animals positive for MRSA presented as number of animals positive in at least one sampling site / number of animals sampled. Number of bulk milk samples positive for MRSA presented as number of positive samples / number of samples analysed. Sample types: M = milk; N = nostril; G = groin; P = perineum; W = wound; T = throat; U = umbilicus; E = eye; H = hock skin; BM = bulk milk. SCC = somatic cell counts in bulk milk (1000 cells/mL milk).

2. Experimental procedures

2.1. Animal sampling

From November 2012 to September 2014 the MRSA-situation in the herd was documented by samplings of animals. In total, 105 different animals were sampled at least once during this period. Samples from animals were taken at eight occasions, Samplings a–h. Numbers and categories of animals sampled in different sites are shown in Table 1. All samples were taken by the herd veterinarian. Gloves were always worn. All samples were sent to SVA for analysis.

In November 2012 all animals in the herd (n = 68) were sampled (Sampling a). Composite milk samples were taken from all 25 lactating cows. Additionally, groin samples were taken from two of the lactating cows and hock skin samples from four. Furthermore, nasal swabs (both nostrils were sampled with the same cotton tipped swab) and groin samples were taken from one dry cow and 41 young stock, nasal swab and hock skin sample were taken from one young stock, and two young stock were sampled from an infected eye and a wound, respectively. All body sites were sampled with cotton tipped swabs. Cows that were MRSA positive in Sampling a and remained in the herd were sampled again (quarter milk samples and nasal swabs) in December 2012 (Sampling b).

In September 2013 all animals housed at the time were sampled (Sampling c). Composite milk samples were taken from all 22 lactating cows. Additionally, nasal swabs and groin samples were taken from all the lactating cows, throat samples from four of them and wound sample from one. Furthermore, nasal swabs were taken from 22 young stock, groin samples from five, perineal swabs from 17 and umbilical swabs and wound swabs from two. MRSA positive animals remaining in October 2013 were sampled again (quarter milk samples from three cows and nasal, groin and perineal swabs from one bull) (Sampling d).

Later in October 2013 animals that were on pasture in September 2013, and not available for sampling, were sampled (Sampling e). Composite milk samples were taken from two lactating cows and nasal

and perineal swabs from two lactating cows and 25 young stock. MRSA positive animals remaining in March 2014 were sampled again together with one cow that had not been positive (composite milk samples from three cows, milk sample and nasal swab from one cow and nasal swabs from four young stock) (Sampling f).

In April 2014 all animals on the farm were sampled (Sampling g). Composite milk samples were taken from all 18 lactating cows. Additionally, nasal swabs were taken from all 18 cows and wound samples from four of them. Furthermore, nasal swabs and groin samples were taken from one dry cow and from ten young stock, and nasal swabs and perineal swabs from 32 young stock. In September 2014 all lactating cows and calves born after April 2014 were sampled (Sampling h). Composite milk samples were taken from all 25 lactating cows and nasal swabs from all eight calves.

2.2. Bulk milk sampling

Bulk milk samples were collected at five animal samplings (Table 1). In addition, bulk milk samples were collected by the farmer every second week from April to December 2014 and every fourth week from December 2014 to December 2015 (Table 1). Gloves were always worn.

2.3. Somatic cell counts

Somatic cell counts in composite milk from lactating cows were collected from the Swedish Official Milk Recording Scheme (SOMRS) from the recording closest in time to milk sampling of each cow.

2.4. Bacteriological analyses of animal samples

All samples were selectively enriched for MRSA overnight in 37 °C in Tryptone Soy Broth with mannitol and phenol red, 4% NaCl, 3.5 mg/l cefoxitin and 50 mg/l aztreonam. All milk in the composite milk samples and 10 ml of the bulk milk samples were centrifuged (Hettich, EBA 20, 865 RCF for 10 min), and the pellet resuspended in enrichment

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