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Prevalence of methicillin-resistant *Staphylococcus aureus* carrying *mecA* or *mecC* in dairy cattle

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ABSTRACT

In the Netherlands, livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) has been found in pigs, veal calves, horses and poultry. However, little is known about its prevalence in healthy dairy cattle. Recently, a new *mec* gene, called *mecC*, has been found in methicillin-resistant *S. aureus* (MRSA) isolates from humans and animals in several countries. The objective of our pilot study was to investigate the prevalence of MRSA (*mecA* and *mecC*) in dairy cows at a large slaughterhouse. Samples from the skin between the udder and hind leg were taken from 411 cows. The samples were incubated in Mueller–Hinton enrichment broth with 6.5% NaCl, followed by selective enrichment and plated onto Columbia agar with 5% sheep blood, Brilliance MRSA 2 agar and Baird–Parker agar. Suspected colonies were tested by PCR for a *S. aureus* specific DNA fragment, the *mecA* and *mecC* genes and the Panton-Valentine leucotoxin (PVL) genes. All MRSA isolates and methicillin-susceptible *S. aureus* (MSSA) isolates were typed by *spa* typing and MLVA typing. Sixteen of 411 (3.9%) cows, all originating from different farms, were found to be MRSA positive and this prevalence is lower than in Dutch pigs, veal calves and broilers. All MRSA isolates belonging to livestock-associated MLVA complex 398, were PVL-negative and *spa* type t011 predominated. MSSA isolates ($n = 39$) were of many different MLVA types and *spa* type t543 was found most often. Four MSSA isolates belonging to MLVA clonal complex 398 and *spa* types t011 ($n = 2$), t108 and t034 were isolated from different MRSA-negative animals. In conclusion, the prevalence of MRSA in dairy cows was low and isolates carrying the *mecC* gene were not found, indicating that it is absent or has a low prevalence ($<0.73\%$) in Dutch dairy cows.

1. Introduction

In Europe, livestock-associated methicillin-resistant *S. aureus* (LA-MRSA) are mainly comprised of isolates belonging to multilocus sequence type 398. In The Netherlands, the prevalence of LA-MRSA has been investigated in healthy pigs, veal calves, horses and broilers (Busscher et al., 2006; Broens et al., 2011; Graveland et al., 2010; Geenen et al., 2013). In Dutch dairy cattle, LA-MRSA have been identified in cows with (sub)-clinical mastitis and in-contact animals (Tavakol et al., 2012; Feßler et al., 2012). However, data on its prevalence in healthy cows are lacking. In 2011, a new *mecA* homologue *mecC* (formely called mec_{LGA251}) was identified in milk samples from 15 dairy cows in the United Kingdom (UK) and from human clinical samples in the UK and Denmark (Shore et al., 2011; García-Álvarez et al., 2011). These isolates were from three different multilocus sequence type lineages (CC130,

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CC705, and ST425). In humans and in cows, *spa* type t843 (associated with CC130) carrying SCC*mec* type XI predominated and it was suggested the bovines might be the source of this clone. Since this first report, MRSA carrying *mecC* have been found in many animal species e.g., sheep, dogs, cats, rats, hedgehogs, a rabbit, lynx, seal, bird, otter, and brown hare in different countries (Eriksson et al., 2013; Medhus et al., 2013; Paterson et al., 2012; Loncaric et al., 2013). Recently, MRSA carrying *mecC* have been detected in milk samples from cows in Sweden (Unnerstad et al., 2013), Belgium (Vandendriessche et al., 2013), France (Laurent et al., 2012) and Finland (Gindonis et al., 2013). In Denmark, two human cases of *mecC* MRSA infection have been linked to livestock as the isolates from the humans and ruminants (cow and sheep respectively) on the farms the patients lived on appeared to be nearly identical as determined by whole genome sequencing (Harrison et al., 2013; Petersen et al., 2013). This is a public health concern, as severe human infections with *mecC*-carrying MRSA have been reported (Barraud et al., 2013; Garcia-Garrote et al., 2013). The objective of the present study was to investigate the prevalence of MRSA carrying *mecA* or *mecC* in Dutch dairy cows.

2. Material and methods

Samples were taken from cows at a large slaughterhouse slaughtering 600 cattle per day from all over The Netherlands and from abroad (mainly Belgium and France). The samples were taken immediately after stunning and bleeding using Sodibox wipes (Sterile cloth with Ringer's solution; SodiBox, France). Samples were taken by gently rubbing the wipe 3 times over the skin between the left hind leg and the udder on a surface of approximately 10 cm^2 after the animal was hanging on the slaughter line. All samples were taken by the same person. Exclusion criteria were cows with visible dirt at the sample site and bulls/steers. In addition, animals originating from countries outside The Netherlands were also excluded. The samples were taken on 4 sampling days between October 2011 and January 2012 and were cultured within 24 h. The wipes were incubated in 100 ml of Mueller–Hinton enrichment broth (BBL, France) with 6.5% NaCl and incubated for 18 h at 37 °C. For selective enrichment, 1 ml of broth was transferred to 9 ml phenolred mannitol broth with 5 mg/L ceftizoxim and 75 mg/L aztreonam (bioMérieux, France), incubated for 18 h at 37 °C and subsequently plated onto Columbia agar with 5% sheep blood (Oxoid, The Netherlands), Brilliance MRSA 2 agar (Oxoid, The Netherlands) and Baird–Parker agar (Oxoid, The Netherlands) containing R.P.F Supplement (Oxoid, The Netherlands) and incubated for 18 h at 37 °C. In order to assure that MRSA isolates carrying the *mecC* gene can grow on the media used, 6 different *mecC* positive isolates identified during routine surveillance of the National Institute for Public Health and the Environment were used as positive controls. Suspected colonies were tested by PCR for a *S. aureus* specific DNA fragment described by Martineau et al. (Martineau et al., 1998), the *mecA* and the Panton-Valentine leucotoxin (PVL) genes as described (De Neeling et al., 1998; Lina et al., 1999). An additional PCR for *mecC* was performed (García-Álvarez et al., 2011). All MRSA isolates and methicillin-susceptible *S. aureus* (MSSA) isolates were typed by *spa* typing and multiple–locus variable number tandem repeat analysis (MLVA) typing as described (Harmsen et al., 2003; Schouls et al., 2009). MRSA isolates were tested for their susceptibility to oxacillin by a microbroth dilution method (Trek Diagnostic systems, East Grinstead, UK) and using the EUCAST breakpoint (www.EUCAST.org).

3. Results and discussion

In total 16 of 411 cows sampled were found to be MRSA positive yielding a prevalence estimate of 3.9% (95% CI 2.03–5.77%). All animals originated from different farms located in 11 provinces in The Netherlands. All MRSA isolates were *mecA* positive, *mecC* negative, belonged to MLVA clonal complex 398 and were PVL negative. In The Netherlands 56% of pig farms, 88% of veal calve farms and 8% of broiler farms were found to be LA-MRSA positive (Geenen et al., 2013; Broens et al., 2011; Graveland et al., 2010). Although our study design differed from the aforementioned studies, it seems that the prevalence in healthy cows is lower than in other livestock. Tavakol et al. (2012) investigated approximately 38,000 milk samples and found only 14 MRSA which supports our data. In the present study the predominant *spa* type found among the MRSA isolates was t011 ($n = 11$) (Table 1). This is in agreement with data from veal calves in The Netherlands (Graveland et al., 2010) and isolates from cows with mastitis and in-contact animals (Feßler et al., 2012; Tavakol et al., 2012). Other *spa* types found were t108 ($n = 1$), t567 ($n = 1$), t588 ($n = 2$) and t1457 ($n = 1$). Although our study was not designed to study the prevalence of MSSA, *S. aureus* isolates negative for the *mecA* and *mecC* gene ($n = 39$) were also typed. All MSSA isolates originated from different animals. The diversity in *spa* types ($n = 15$)

Table 1
spa types and MLVA types of the MRSA and MSSA isolates.

spa type	MSSA (*n*)	MRSA (*n*)	MLVA type (MT)-MLVA complex (MC)
t011	2	11	MT0398-MC0398
t021	1	0	MT0390-MC0030
t034	1	0	MT0569-MC0398
t108	1	1	MT0572-MC0398
t224	1	0	MT3359-MC none
t267	3	0	MT3366-MC none
t289	1	0	MT0007-MC0007
t524	2	0	MT3357-MC none
t529	4	0	MT3371-MC none
t543	14	0	MT3358/3360/3361/3362/3364/3365-MC none
t567	0	1	MT0567-MC0398
t588	0	2	MT0567-MC0398
t843	1	0	MT0429-MC0429
t1403	5	0	MT3362/3369-MC none
t1457	0	1	MT0564-MC0398
t1508	1	0	MT3018-MC none
t2678	1	0	MT3368-MC none
t9855	1	0	MT3358-MC none
Total	39	16	