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Characterization of methicillin-resistant *Staphylococcus aureus* CC398 obtained from humans and animals on dairy farms

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ABSTRACT

In this study MRSA isolates from dairy farms were investigated for their genetic relationships and antimicrobial susceptibility. In total, 125 MRSA isolates from 26 dairy farms were studied, including isolates from milk samples ($n = 46$), dairy cattle ($n = 24$), calves ($n = 6$), dust samples from pig ($n = 16$) and veal calf sheds ($n = 1$), dogs ($n = 2$), a horse, a sheep and humans ($n = 28$). CC398-specific PCRs, *spa* typing, SCC*mec* typing and ApaI macrorestriction analysis were conducted. Susceptibility testing was performed by broth microdilution. All 125 isolates belonged to CC398. Eight *spa* types (t011, t108, t034, t567, t1184, t1451, t2287 and t3934) were detected. SCC*mec* elements of types IV ($n = 48$) and V ($n = 67$) were identified with 10 isolates being non-typeable. Six main macrorestriction patterns – with up to 23 sub-patterns – and twelve resistance patterns were identified. Sixty-eight isolates showed a multiresistance phenotype. Farm-by-farm analysis revealed different scenarios: in some farms, the MRSA CC398 isolates from dairy cattle, humans, pig sheds and/or sheep were indistinguishable suggesting an interspecies exchange of the same MRSA CC398 subtype. In other farms, several MRSA CC398 subtypes were detected in different host species/sources with occasionally even more than one MRSA CC398 subtype from the same host species/source. These latter results may suggest that either different MRSA subtypes associated with humans or animals have been imported into the respective farm or that one MRSA CC398 strain has undergone diversification, reflected by more or less expanded changes in PFGE patterns, *spa* type or resistance pattern, during colonization of different hosts on the same farm.

1. Introduction

Although there are many studies on methicillin-resistant *Staphylococcus aureus* (MRSA) of the clonal complex (CC) 398 available, there is still a lack of information on their reservoirs and transmission routes. During the last years several studies have been performed to gain information on the presence/prevalence of MRSA CC398 in different geographical regions and animal populations (Voss et al., 2005; Nemati et al., 2008; Kadlec et al., 2009; Feßler et al., 2010b; Graveland et al., 2010; Spohr et al., 2010; Vanderhaeghen et al., 2010; Argudín et al., 2011; Graveland et al., 2011; Pletinckx et al., 2011). Since MRSA CC398 has been found in different animal species, a transmission of these MRSA isolates between different animal hosts and humans is very likely. In contrast to the wealth of data on MRSA CC398 in swine

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farms and farm personnel, comparatively little is known about MRSA CC398 among cattle. Vanderhaeghen et al. (2010) investigated the MRSA prevalence on dairy farms in Belgium by analyzing 118 *S. aureus* isolates from unrelated cases of bovine mastitis with 11 (9.3%) isolates being *mecA*-positive. The in-herd prevalence for MRSA of four randomly chosen farms, in which MRSA has been detected previously, was 0–7.4% (Vanderhaeghen et al., 2010). Possible risk factors for the transmission of MRSA CC398 between veal calves and people living on the same farms were investigated in The Netherlands and a positive correlation was seen for the intensity of the contact and the number of MRSA-positive animals on the farms (Graveland et al., 2010). Small-scale investigations in Germany revealed indistinguishable or closely related characteristics of the MRSA CC398 isolates from cases of mastitis and from asymptomatic nasal carriage of workers on the respective dairy farms (Feßler et al., 2010b; Spohr et al., 2010). These studies strongly suggest that an exchange of MRSA occurs between different animal species and humans in the farm environment. The present study was performed to gain information on the presence and the characteristics of MRSA among different animal species and humans on dairy farms.

2. Materials and methods

2.1. MRSA isolates, sampling and identification

During this study, a total of 2306 samples from different animal species, animal-related sources and humans collected on 26 Dutch dairy farms, designated A–Z, were investigated for the presence of MRSA. In each of these 26 farms, MRSA had been isolated previously from milk samples during routine surveillance conducted by GD Animal Health Service (Deventer, The Netherlands). The numbers of samples taken from animals (milk samples, nasal or skin swabs from dairy cattle, calves, sheep, horses, cats and dogs), from the environment of animals (dust samples from pig, veal calf and poultry sheds) and from humans (nasal swabs) working on these farms, but also the numbers of MRSA-positive samples as well as the numbers of MRSA isolates further investigated in this study are shown in Table 1. The nasal swabs from humans were provided on a voluntary basis. All animal isolates were obtained from samples taken by trained personnel of GD Animal Health Service during the period from 2007 to 2009. Only one isolate per sample/animal was further characterized in this study. The differences between the numbers of MRSA-positive samples from milk and skin swabs of dairy cattle and the corresponding numbers of MRSA isolates included in further analyses are based on the fact that occasionally more than one sample from the same animal was taken. In total, 125 MRSA isolates were included in this study.

2.2. PCR-based typing methods and macrorestriction analysis

Two CC398-specific PCR assays, designated A07 and C01, were performed (van Wamel et al., 2010). All isolates were subjected to *spa* typing (http://spaserver.ridom.de/). SCC*mec* typing was performed using the multiplex PCR assays according to Kondo et al. (2007). All isolates were subjected to macrorestriction analysis with subsequent pulsed-field gel electrophoresis (PFGE). Since MRSA ST398 are non-typeable with the enzyme SmaI, the enzyme ApaI was used according to a previously described protocol (Kadlec et al., 2009). For the comparison of the PFGE patterns, the criteria published by Tenover et al. (1995) were applied. The unrelated main patterns ($\geq$7 fragments difference) were numbered 1–6, while patterns that were closely related (2–3 fragments difference) or possibly related (4–6 fragments difference) to one of the main patterns were indicated by additional lower-case letters (Tables 2a and 2b).

2.3. Susceptibility testing

Susceptibility testing was performed by broth microdilution according to the recommendations given in the CLSI document M31-A3 (CLSI, 2008). For this, custom-made microtitre plates (MCS Diagnostics, Swalmen, The

Table 1
Overview of the dairy farms sampled, the numbers of samples taken, the numbers of MRSA-positive samples and the numbers of MRSA isolates from different sources.

Sample source	No. of farms sampled	No. of MRSA-positive farms	No. of samples	No. of positive MRSA-samples	No. of MRSA isolates included in this study
Cows – milk	26	26	1839	62	46
Cows – nasal swab	26	4	32	4	4
Cows – skin swab	26	12	153	27	20
Calves – nasal swab	24[a]	6	33	6	6
Veal calves – dust sample	1[a]	1	3	1	1
Pigs – dust sample	14[a]	7	72	16	16
Poultry – dust sample	5[a]	0	5	0	0
Cats – throat swab	12[a]	0	13	0	0
Dogs – nasal swab	17[a]	2	25	2	2
Sheep – nasal swab	3[a]	1	4	1	1
Goats – nasal swab	2[a]	0	2	0	0
Horses – nasal swab	5[a]	1	8	1	1
Humans – nasal swab	26	18	117	28	28
$\sum$			2306	148	125

[a] The numbers indicate the numbers of farms in which the respective other animals were available for sampling.