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Colonization with methicillin-resistant *Staphylococcus pseudintermedius* in multi-dog households: A longitudinal study using whole genome sequencing

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ABSTRACT

Despite a worldwide increase in the presence of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) in dogs and its potential to cause serious canine health problem, the understanding of the transmission and long-term carriage of MRSP is limited.

The objective of this study was to investigate the transmission of MRSP to contact dogs living in multiple dog households where one or more of the dogs had been diagnosed with a clinically apparent infection with MRSP.

MRSP carriage was investigated over several months in 11 dogs living in four separate multiple dog households where an MRSP infection in a dog had been diagnosed. Whole-genome sequencing was used for genotypic characterization.

Contact dogs were only MRSP-positive if the index dog was positive on the same sample occasion. Three contact dogs were consistently MRSP-negative. The data from whole genome sequencing showed similarities between isolates within each family group, indicating that MRSP was transmitted within each family.

The results show that the risk of MRSP-colonization in dogs living with an MRSP-infected dog is reduced if the index dog becomes MRSP negative. All of the contact dogs will not carry MRSP continuously during the time the index dog is MRSP-positive. The information yielded from whole genome sequencing showed the methodology to be a promising additional tool in epidemiologic investigations of MRSP transmission.

1. Introduction

Staphylococcus pseudintermedius, previously typed as *Staphylococcus intermedius,* is both a skin and mucous membrane commensal in the dog and the most frequent bacterial pathogen isolated from clinical canine specimens (Bannoehr and Guardabassi, 2012). It is a well-known cause of dermatologic infections, such as pyoderma, otitis externa and wound infections, and it may also cause infections in other body tissues (Bannoehr and Guardabassi, 2012; Cox et al., 1984; Devriese et al., 2005; Lehner et al., 2014; Muller, 2001; Sasaki et al., 2007; Weese and van Duijkeren, 2010). An increase in methicillin-resistant *S. pseudintermedius* (MRSP) strains isolated from asymptomatic as well as clinically infected dogs has been reported worldwide during the last decade (Bannoehr and Guardabassi, 2012; van Duijkeren et al., 2011a). In Europe, including Sweden, the clonal lineage sequence type (ST)71 spa-type t02 carrying Staphylococcal chromosome cassette *mec* (SCC*mec*) types II–III has so far appeared to dominate (Borjesson et al., 2012; Perreten et al., 2010). Methicillin resistance is mediated by the *mec*A gene, which encodes the penicillin-binding protein (PBP) 2a, which has a low affinity for all β-lactam

Abbreviations: BLAST, Basic Local Alignment Search Tool; CLSI, Clinical and Laboratory Standards Institute; MLST, Multilocus sequence type; MRSP, Methicillin-resistant *S. pseudintermedius*; MSSP, Methicillin sensitive *S. pseudintermedius*; PBP, Penicillin-binding protein; PCR, Polymerase chain reaction; PFGE, Pulsed-field gel electrophoresis; SCCmec, Staphylococcal Chromosome Cassette *mec*; SNP, single nucleotide polymorphism; ST, sequence type; WGS, Whole genome sequencing.

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antimicrobials (Chambers, 1997). In addition to β-lactam resistance, a high occurrence of resistance to a wide range of antimicrobials has been reported for MRSP isolates (Beever et al., 2015; Borjesson et al., 2012; Gold et al., 2014; Siak et al., 2014). Because of the multidrug resistance phenotype of this important canine pathogen, infection with MRSP is recognized as a serious canine health problem. Knowledge of infection control measures is needed, such as longitudinal studies on carriage in and transmission between dogs (Bannoehr and Guardabassi, 2012; Beck et al., 2012; Laarhoven et al., 2011; van Duijkeren et al., 2011b; Windahl et al., 2012).

Various DNA-based techniques have been used in the strain typing of MRSP in surveillance and the investigation of outbreaks. Pulsed-field gel electrophoresis (PFGE) is a highly discriminatory method for the bacterial typing of genetic relatedness and has so far been the method primarily used when comparing MRSP and methicillin-susceptible *S. pseudintermedius* (MSSP) isolates within dog groups, households and veterinary clinics (Bannoehr and Guardabassi, 2012; van Duijkeren et al., 2011a). Other typing methods, such has *spa*-typing, *dru*-typing and MLST have also been applied to investigations of *S. pseudintermedius*, but due to the high clonality of for example ST71-t02-SCCmecII-III they might lack the necessary discriminatory power (Borjesson et al., 2012; Perreten et al., 2010). Furthermore, *spa*-typing has been shown to be unreliable for non ST71 strains as these are generally untypeable using published *spa*-typing methods (Borjesson et al., 2012). Similar or indistinguishable PFGE patterns are often interpreted as persistent carriage or transmission of the same clone. However, PFGE may also lack the resolution needed to discriminate within a single clone in an outbreak or transmission within a household (Goering, 2010). For example, the clone ST71-t02-SCCmecII-III has shown a similarity of 80% or more on both national and European level when compared using PFGE (Borjesson et al., 2012; Perreten et al., 2010; Ruscher et al., 2010). PFGE might also not show true phylogenetic relatedness, and some strains are not possible to type (Goering, 2010). Whole-genome sequencing (WGS) has been shown to be a highly effective and reproducible method for studying outbreaks and transmission of bacteria, for example, of methicillin-resistant *Staphylococcus auerus* (MRSA) (Harris et al., 2013; Sherry et al., 2013). In addition, the method offers an extensive characterization of isolates, including the detection of toxin, virulence and resistance genes (Wyres et al., 2014). Whole-genome sequencing could therefore be a useful tool for investigations of the epidemiology of MRSP (Moodley et al., 2013).

The objective of this study was to investigate transmission of MRSP to contact dogs living in multiple dog households where at least one dog had been diagnosed with a clinically apparent MRSP-infection. Furthermore, whole-genome sequencing was applied for genetic characterisations of MRSP- isolates of known origin.

2. Materials and methods

2.1. Dogs

Four unrelated dog-owning households including a total of eleven dogs were enrolled in the study (Table 1). The inclusion criterion was an MRSP-positive sample from a clinically evident infection in at least one dog living in a multiple dog household in which MRSP according to medical charts, compulsory national registers as well as respective dog owner had not previously been detected. Each of the inclusion samples had been submitted from one of four different small animal clinics to the National Veterinary Institute, Uppsala, Sweden for culture and susceptibility testing.

The four households were labelled family group A, B, C and D. The dogs with a clinical MRSP infection at the time of inclusion were referred to as “index dogs”, their first sample “inclusion sample”, and the other dogs living in the household “contact dogs” (Table 1).

Table 1
Overview of the sample sites for MRSP positive samples from the four dog groups, A, B, C and D.

	Sampling 1	Sampling 2	Sampling 3	Sampling 4
Family group A				
Ai	mouth	mouth	neg[c]	neg[c]
Aii	neg	neg	neg[c]	neg[c]
Ac	neg	neg	neg[c]	neg[c]
Acc	mouth	neg	neg	neg
Time (months) after inclusion sample	1	4	11	12
Family group B				
Bi	mouth, perineum & wound[a]	wound[a,b]	wound[a]	wound[a]
Bc	neg	neg	neg	
Bcc	mouth & perineum	perineum	neg	perineum
Time (months) after inclusion sample	1	4	10	13
Family group C				
Ci	perineum	perineum§	neg§	neg§
Cc	wound[a,b]	neg§	neg§	neg§
Time (months) after inclusion sample	1	4	10	15
Family group D				
Di	perineum[a,b]	perineum[b]	neg[b]	Not sampled
Dc	perineum	mouth	neg	Not sampled
Time (months) after inclusion sample	1	2	7	
Total number of positive samples	11	6	1	2

Dogs are identified by family group (A–D) and if they are index dogs (i for the first index dog in a group and ii for the second) or contact dogs (c or cc). Months since the inclusion sample are given for each sampling. For MRSP-positive dogs, the positive sampling site is shown: mouth (positive in pharynx or the corner of mouth), perineum or wound.

[a] Clinical signs of skin lesions or infection. Bi had skin lesions. Cc was bitten between the Ci:s inclusion sample and the first sample occasion. Di had a clinically evident infection in a wound from which MRSP first was cultured.

[b] Treatments including visits to the veterinary clinic. Bi was prescribed a corticosteroid treatment due to an atopic dermatitis. Di was treated for an infected wound.

[c] Contact with dogs outside of the household. Family group A received dog visits prior to sample occasion three and four. Ci and Cc had contact with other dogs during occasional dog walks.