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Evidence of possible methicillin-resistant *Staphylococcus aureus* ST398 spread between pigs and other animals and people residing on the same farm

Larissa J. Pletinckx[a,b,*], Marijke Verhegghe[c,d], Florence Crombé[d,f], Jeroen Dewulf[e], Yves De Bleecker[a], Geertrui Rasschaert[c], Patrick Butaye[d,f], Bruno M. Goddeeris[b], Ingrid De Man[a]

[a] *Catholic University College South-West-Flanders (KATHO), Department HIVB, Wilgenstraat 32, 8800 Roeselare, Belgium*
[b] *Catholic University Leuven, Department of Biosystems, Division of Gene Technology, Kasteelpark Arenberg 30 - bus 2456, 3001 Heverlee, Belgium*
[c] *Institute for Agricultural and Fisheries Research (ILVO), Technology and Food Science Unit, Food Safety, Brusselsesteenweg 370, 9090 Melle, Belgium*
[d] *Ghent University, Faculty of Veterinary Medicine, Department of Pathology, Bacteriology and Poultry Diseases, Salisburylaan 133, 9820 Merelbeke, Belgium*
[e] *Ghent University, Faculty of Veterinary Medicine, Veterinary Epidemiology Unit, Department of Reproduction, Obstetrics and Herd Health, Salisburylaan 133, 9820 Merelbeke, Belgium*
[f] *Department of Bacteriology and Immunology, Veterinary and Agrochemical Research Centre (VAR), Groeselenberg 99, 1180 Brussels, Belgium*

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged in a wide variety of animal species. However, little is known about the transmission routes of MRSA ST398 between different animal species, the barn environment and people residing on the same farm. In this study, two pig farms, two poultry-pig and two dairy-pig farms were investigated with respect to the presence of MRSA. On each farm, samples were collected from all animal species present, the barn environment, the farmer, household members and the herd veterinarians. Besides the MRSA prevalence, the obtained *spa*-, SCC*mec*-type and antimicrobial susceptibility profiles were also compared. Multilocus sequence typing (MLST) showed that MRSA ST398 was found in all animal species, in humans present on the farms and also in the pig barn environment. The presence of MRSA with the same *spa*-, SCC*mec*-type and antibiotic profile in the different animal species in direct or indirect contact with pigs suggests MRSA transfer. Furthermore, different pig age categories were investigated, with weaned piglets having the highest MRSA prevalence (86.3%). The herd-level prevalence was highly correlated ($r = 0.86$, $p = 0.03$) between sows and pre-weaned piglets. The results also indicate that companion animals, rats, mice and farmers could play an important role in the dissemination of MRSA, emphasizing the importance of internal biosecurity. However, external biosecurity is equally important because other *spa*-, SCC*mec*-types or antimicrobial resistances can be introduced through purchase of gilts. In this study we demonstrated that MRSA likely spreads between animal species, humans and the pig barn environment, which is why it is important to accurately implement control practices, in which not only pigs should be targeted, but also all other animal species present on farms.

1. Introduction

Since 2005, the presence of a distinct clone of methicillin-resistant *Staphylococcus aureus* (MRSA) has been reported in a wide variety of animal species and

* Corresponding author at: Catholic University College South-West-Flanders (KATHO), Department HIVB, Wilgenstraat, 32, 8800 Roeselare, Belgium. Tel.: +32 51232330; fax: +32 51228258.
E-mail address: larissa.pletinckx@katho.be (L.J. Pletinckx).

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has been referred to as livestock-associated MRSA (LA-MRSA). LA-MRSA with sequence type (ST) 398 has been described mainly in pigs, but also in cattle, horses and poultry (Catry et al., 2010; Graveland et al., 2010; De Neeling et al., 2007; van den Eede et al., 2009; Vanderhaeghen et al., 2010; Pletinckx et al., 2011). Previous studies have established that pigs are a reservoir for LA-MRSA ST398 from which humans can be infected and ST398 is currently considered the most prevalent sequence type (Voss et al., 2005; Wulf et al., 2008). However, the interaction between animal species and humans in contact with livestock and their influence on the spread of MRSA are not yet fully understood. Therefore, the main objective of this study was to improve the understanding of the potential transmission routes between animal species, humans and the barn environment on the same farm by (i) determining the MRSA prevalence, (ii) comparing the obtained *spa*-, SCC*mec*-types and antibiotic profiles in the different animal species, animal workers and the barn environment, (iii) determining if MRSA carriage in pigs was age-related, (iv) evaluating whether a correlation existed between MRSA-positive sows and suckling piglets. Accordingly, a better understanding of the different sources of MRSA can be achieved for developing adequate control measures.

2. Materials and methods

2.1. Farm characteristics

During the present study, six Belgian livestock farms (Farms A, B, C, D, E and F) were sampled between July 2009 and October 2010. These six farms were selected based on the presence of MRSA ST398 positive pigs, as determined in a previous screening of 30 livestock farms chosen randomly by Animal Health Care Flanders (DGZ) from their database. The 30 livestock farms included ten pig farms, ten poultry-pig farms and ten cattle-pig farms sampled from March to May 2009 (Verhegghe et al., 2012). Farms A and B were closed pig farms, without any other commercially bred farm animals present. Farms C and D were mixed broiler-pig farms and farms E and F were mixed dairy-pig farms. Farms A, B, E and F were located in West Flanders (minimum 15 km and maximum 32 km apart), the Belgian province with the highest pig farm density, while farm C was located in the Belgian province of Antwerp and farm D in East Flanders. All farms had a farrow-to-finish pig production with a size varying from 90 to 240 sows. Farm specific characteristics are shown in Table 1. Each farmer also completed a questionnaire on farm size, different animal species, internal and external biosecurity measures and antimicrobial drug use over the last 6 months (group and individual treatments).

2.2. Sample collection

On the different farms, convenience samples were taken from pigs, purchased gilts, the pig barn environment (floor, wall, air between animals, ventilation air, dust, drinking water, animal feed and manure), farm rats and mice, farmers, family members, herd veterinarians and companion animals. Samples were also taken from other farm animals such as broiler chickens, dairy cows and calves, when present and also from the barn environment of these animals.

Every three months a new farm was sampled, starting on farm A in July 2009. All the samples (pigs, barn environment, humans, companion animals, etc.) were collected on two separate days with one week interval on all farms. On the mixed farms (C, D, E and F) an additional sampling day was used to sample the broilers or dairy cows, their calves and the barn environment one week later.

Sample size of pigs and poultry was calculated with win-episcope (WIN EPISCOPE 2.0; http://www.clive.ed.ac.uk/cliveCatalogueItem.asp?id=B6BC9009-C10F-4393-A22D-48F436516AC4). For pigs an expected prevalence of 70% was chosen while for poultry an expected prevalence of 10% was chosen (accepted error of 10% and confidence interval of 95%) based on previous results (Crombé et al., 2012; Persoons et al., 2009; Pletinckx et al., 2011; Verhegghe et al., 2012).

Individual nasal samples combining both nares were taken from randomly selected healthy pigs in proportion to the number in each age group, together with their barn environment. The number of sampled pigs per age category per farm is given in Table 1.

On the mixed poultry-pig farms C and D, a sample of 25 and 50 broiler chickens (5–6 weeks old), respectively, was also obtained on four different anatomical sampling sites (nose shell, pharynx, skin beneath the wing and cloaca) and their barn environment. In the poultry house, an equal number of broiler chickens were sampled randomly in each corner and in the middle of the poultry house (systematic sample selection by spatial location).

In addition, on the mixed dairy cow-pig farms E and F, all the dairy cows that were milked and all their calves were sampled from three different anatomical sampling sites (nose, udder skin and perineum) as well as their barn environment. After milking but before cleaning, the unit liner of the milking machines was also swabbed. In total, 14 and 30 dairy cows, were sampled on farms E and F, respectively, after milking in the stable where they were fed. Eight and 15 calves were sampled on farm E and F, respectively. Calves were housed separately from their dams and were between 14 days and 3 months old.

Companion animals (dogs $n = 6$ and cats $n = 14$) were sampled in the nose and pharynx on the six different farms. Dogs were present on each farm except on farm E, while tame farm cats were present on farms A, B, E and F. All these companion animals had direct contact with pigs, with exception of companion animals on farm B and one dog on farm F. On farm A, nasal samples from 14 goats and 6 laying hens were also collected.

On each farm, twelve rat and mice snap-traps were placed on rat trails in the pig barn. Three consecutive traps were placed in order to maximize the probability of trapping rodents on their trails in four different compartments per farm. When after one week no rats were caught, traps were placed elsewhere in the barn. In total 17 rats and mice were captured and sampled in the nasal cavity after lateral incision and in the pharynx.