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Longitudinal study on the colonisation and transmission of methicillin-resistant *Staphylococcus aureus* in pig farms

Patrick Daniel Bangerter[a], Xaver Sidler[b], Vincent Perreten[a], Gudrun Overesch[a,*]

[a] Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Länggassstrasse 122, CH-3012 Bern, Switzerland
[b] Department of Farm Animals, Division of Swine Medicine, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, CH-8057 Zurich, Switzerland

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ABSTRACT

Knowledge about the dynamics of methicillin-resistant *Staphylococcus aureus* (MRSA) in pigs lacks detail at the level of individual animal. The aim of our study was therefore to determine the colonisation status of MRSA in individual pigs from birth to slaughter in order to gain a better understanding of substantial factors involved in transmission. Two farrow-to-finish and two grow-to-finish herds were included in the study. A total of 1728 nasal swabs from 390 pigs and 592 environmental wipes were collected at 11 different time points.

Intermittent colonisation throughout the entire production cycle was conspicuous in the tracking of MRSA in individual pigs. Almost all pigs from a MRSA-positive herd changed MRSA status several times, which implies that pigs are transiently rather than permanently colonised. We highly recommend the definition of MRSA status at herd level rather that at the level of the individual pig when considering prevention measures against MRSA. Therefore, to avoid the further spread of MRSA in countries with moderate prevalence, such as in Switzerland, defining farms as MRSA positive or MRSA negative and allowing the trade of pigs only within herds of the same status seems feasible. This will also be important for combating the further dissemination of livestock-associated (LA)-MRSA into healthcare facilities and the community via humans who have close contact with animals.

1. Introduction

The rapid spread of LA-MRSA in pigs and farm animals worldwide has raised major public health concerns (Crombé et al., 2013; Verkade and Kluytmans, 2014; Voss et al., 2005). Colonised animals may act as a MRSA reservoir not only for livestock but also for humans with close contact to animals, i.e., farmers and veterinarians. As a consequence, higher colonisation rates and cases of infections have been reported in these professions at risk (Lewis et al., 2008; van Rijen et al., 2008; Wettstein Rosenkranz et al., 2014).

In 2009, official monitoring was launched for MRSA in pigs at slaughterhouses in Switzerland. The prevalence of MRSA in 2009 was very low at 2% (95% CI 0.9–3.9) but reached 20.8% (95% CI 16.7–25.45) in 2013 (Büttner et al., 2014; Overesch et al., 2012).

To date, little is known about the dynamics of MRSA in pigs because only a few longitudinal studies have been conducted. Those longitudinal studies that have been conducted examined the MRSA status in pigs mostly until slaughter age (Broens et al., 2012; Burns et al., 2014; Verhegghe et al., 2013) or even just until the age of 70 days (Weese et al., 2011). Moreover, these studies did not provide results from individual pigs. Other studies examined only one MRSA-positive farm (Burns et al., 2014; Weese et al., 2011), and the results are unlikely to be generally applicable. Other researchers (Broens et al., 2011) considered the prevalence of MRSA before and after transportation of the pigs from farm to abattoir, but they did not examine the changes in the MRSA status of individual pigs at the farm while simply analysing pooled samples, from which individual changes could not be demonstrated. The dramatic increase of MRSA in Swiss slaughter pigs during recent years necessitates the introduction of measures to combat the further spread of MRSA in the Swiss pig population. However, until now, there have been no precise studies of the individual colonisation dynamics of MRSA throughout each pig production stage; these studies are needed to gain a better understanding of the substantial factors considering the prevention of the spread of MRSA and to identify targets for possible intervention measures.

* Corresponding author. Fax: +41 31 6312634.
E-mail addresses: patrick_bangerter@bluewin.ch (P.D. Bangerter), xsidler@vetclinics.uzh.ch (X. Sidler), vincent.perreten@vetsuisse.unibe.ch (V. Perreten), gudrun.overesch@vetsuisse.unibe.ch (G. Overesch).

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For that reason, we selected MRSA-positive and negative farms with different management practices, such as all in/all out and continuous animal flow systems and determined the MRSA status in the individual pig from birth throughout each production stage, up to and including transport and slaughtering.

2. Materials und methods

2.1. Farm characteristics and animals

Pigs from four Swiss pig farms were recurrently tested for the presence of MRSA between May and December 2014 during a production cycle of approximately 150–175 days, as well as additional pigs from three other farms on transportation to slaughterhouses. We chose two farrow-to-finish farms (ff-I, ff-II) as well as two grow-to-finish farms (gf-I, gf-II) for analysis, to determine if any variances between the different management systems exists. One farrow-to-finish farm was chosen as a MRSA-negative control farm whereas the other farrow-to-finish farm was MRSA-positive. Furthermore, one of the grow-to-finish farms was purchaser of grower pigs from a farrow-to-finish study-farm while the other grow-to-finish farm was not associated to any of the farms.

Farm ff-I consisted of 75 sows, 50 replacement gilts, two farrowing rooms for 18 sows in each room, one weaner accommodation and a finishing unit with a capacity for 200 fattening pigs. The farm had a three-week batch monitoring system. One cohort of ten pregnant sows approaching delivery was selected for starting sampling and placed after washing in a cleaned and disinfected farrowing room. Four sows were placed in pens with possible direct contact to the neighbouring pen through an open fence. The other six sows had no contact.

Farm gf-I purchased grower pigs from farm ff-I but also from other breeders. The farm had seven finishing pens with a capacity for 280 fattening pigs. One finishing pen containing 37 fattening pigs grown on farm ff-I was selected for sampling.

Farm ff-II consisted of 42 sows, one farrowing room with 12 farrowing pens, one weaner accommodation and a finishing unit with a capacity for 250 fattening pigs. Replacement gilts were purchased. The farm had no regular batch-monitoring system and routinely used post-weaning prophylactic feed supplementation with lincomycin and spectinomycin for three weeks. One cohort of three pregnant sows approaching delivery was selected for starting sampling. All three sows were placed in pens with possible direct contact to the neighbouring pen through an open fence. Five other pens were also occupied. The status of those sows was unknown.

Farm gf-II had one finishing pen with a capacity for approximately 90 fattening pigs. A total of 87 fattening pigs purchased from one breeder were selected for sampling.

More details of the farms are given in Table 1. Samples and time points are listed in Table 2.

2.2. Collection of samples

Nasal swabs from individual pigs were taken at the different time points during a production cycle up to and including slaughtering. When indicated, additional environmental wipes were also taken (Table 2). At the two farrow-to-finish farms (ff-I, ff-II), sows were sampled three times and their offspring nine times. At approximately four to five weeks of age, the piglets were merged into new groups. Each group was housed in a separate pen in one room in the weaner accommodation. At the age of nine to ten weeks at tp7, the grower pigs were merged into new groups and moved to first stage finishing. At tp8, at approximately 14 weeks of age, fattening pigs were moved to second stage finishing.

On the two grow-to-finish farms (gf-I, gf-II) grower pigs were purchased and housed in the finishing pens, where sampling began. They were sampled five times.

At the end of fattening, on all four farms (ff-I, ff-II, gf-I, gf-II), samples were taken at three different times, i.e., before (tp9i) and after (tp9ii) transportation and after stunning or bleeding (tp9iii). Slaughter pigs from the four farms were transported to three different commercial abattoirs (slaughterhouse sh-I, sh-II and sh-III), namely farm ff-I and gf-I to slaughterhouse sh-I, farm ff-II to slaughterhouse sh-II and farm gf-II to slaughterhouse sh-III. The lairages at slaughterhouse sh-I and sh-III were unused and clean, whereas the lairages at slaughterhouse sh-II had already been used earlier that day and were therefore not clean.

On transportation to the abattoir from farm ff-1, additional pigs ($n = 42$) from one other farm were picked up by the same lorry. Initial nasal samples were taken from these pigs on the farm immediately before transportation (tp9i). During transport from farm gf-II, additional pigs from two other farms (farm 1, $n = 56$; farm 2, $n = 21$) also were picked up by the same lorry. Initial nasal samples from these pigs were taken on one day and two days before transport (tp9i). Additional pigs from the other farms were located in separate lorry sections, but contact between the pigs was possible. Moreover, for the transportation of batch ff-I and batch gf-I, 10 and 5 supplemental pigs, respectively, which were not part of the study, were transported and tested as well but only at tp9i, 9ii and 9iii.

Nasal samples were collected using transport swabs (Transwab® Amies MW172, MWE Medical Wire, Corsham, England and Uni-Ter Amies CLR, Meus S.r.l., Piove Di Sacco, Italy) from both nares of the pigs. Environmental wipes (lairs, wall, watering place, manger and steel parts) were collected from the farrowing pens, the weaner accommodation, the fattening units and the lairages at the slaughterhouse, as well as from the lorries, using wipes (Triko-Tex® 18 × 32 cm, Chicopee Europe, Katwijk, The Netherlands) moistened with distilled water. Samples were taken by wiping the surface of the steel parts, each part with one wipe, and the wall at the height of the snout. In each box, one wall was selected for sampling. Each wipe was individually placed in a sterile Stomacher bag. Depending on the transfer or death of the pigs, the number of

Table 1
Characteristics of the farms.

Farm no	MRSA status[a]	Operation type	Other livestock on farm	Antimicrobial group treatment[b]
ff-I	Positive	Farrow- to-finish farm Continuous flow	No	No
gf-I	Positive	Grow-to-finish farm Continuous flow	Yes (calves)	No
ff-II	Negative	Farrow-to-finish farm Continuous flow	No	Yes
gf-II	Positive	Grow-to-finish farm All in–all out flow	Yes (dairy cows)	No

[a] According to previous screening.
[b] During the production cycle.