Colonization and Transmission of Methicillin-Resistant Staphylococcus aureus ST398 in Nursery Piglets

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A transmission experiment was performed to evaluate the spread of methicillin-resistant Staphylococcus aureus (MRSA) ST398 in nursery piglets. Reproduction ratios (R0) in three experimental groups were found to vary between 3.92 and 52.54, indicating that after introduction, MRSA ST398 will spread easily among weaned piglets, with a tendency to become established.

Methicillin-resistant Staphylococcus aureus (MRSA) ST398 has been reported worldwide to prevail in livestock, on retail meat, and in people in close contact with affected animals (2, 7, 9, 12, 18, 19, 21). To gain a better understanding, a number of recent studies have focused on the colonization and transmission dynamics of MRSA ST398 in pigs, in the field as well as under experimental conditions (1, 4, 16, 17, 24). However, so far, quantitative data on the progression of MRSA colonization in a group of susceptible animals are quite sparse. Furthermore, various methods have been applied in previous experimental studies, all bearing certain limitations. For example, in a recent study from Denmark, the MRSA status of the 2-week-old animals was investigated only once a week by nasal sampling, hampering a detailed analysis of the transmission dynamics (17). In another recent study from the Netherlands, Broens et al. (4) found that nasal inoculation of piglets with MRSA did not result in stable colonization. For that reason, the researchers had to resort to an oral inoculation model which led to the development of lethal necrotizing pneumonia in 80% of their inoculated animals.

In this paper, we report on the successful use of a combined nasal-skin inoculation model for the colonization of weaned piglets without the use of antimicrobials. Furthermore, we quantified the spread of MRSA ST398 among nursery piglets by estimating the basic reproduction ratio (R0). By additionally investigating environmental contamination and the presence of methicillin-susceptible Staphylococcus aureus (MSSA) on the piglets, we aimed at gaining more insight into the transmission and colonization dynamics of MRSA ST398.

Thirty-one 3-week-old cross-bred piglets (Landrace by Piétrain, Malèves-St. Marie, Belgium) from four different sows were obtained from a MRSA-free herd. The MRSA-negative status of all piglets was confirmed upon arrival at the experimental site and once more 4 days before inoculation, as described below. The animals were equally distributed over three experimental groups (1–3) of eight piglets and one negative control group of seven animals. After 1 week of acclimatization, at 28 days of age, two piglets (seeder piglets) from each experimental group were randomly selected for inoculation with a suspension of MRSA strain C26 (ST398, spa type t011, SCCmeC type V). To achieve this suspension, overnight brain heart infusion (BHI) broth (Bio-Rad, France) cultures were diluted 1:250, grown to exponential phase, and centrifuged (2,167 × g, room temperature, 10 min). After being washed twice with 1× phosphate-buffered saline (PBS), pH 7.4 (Invitrogen, Belgium), the bacterial pellet was resuspended in PBS, pH 7.4, and adjusted to an optical density at 600 nm (OD600) of 0.5, corresponding to a concentration of ~3 × 10⁸ CFU/ml. Inoculation was done using a sterile 1-ml syringe (Becton Dickinson, Belgium) by administering 1 ml (~3 × 10⁸ CFU) of the MRSA suspension in both nares and 0.5 ml (~1.5 × 10⁸ CFU) on two spots of the skin behind the ears, for a total of ~9 × 10⁸ CFU. To ensure that the correct amounts of CFU were applied, the concentration of the inoculum was checked afterwards by a serial dilution on 5% sheep blood agar plates (Bio-Rad, Belgium). Similar to the seeders, five piglets of the negative-control group were inoculated with sterile PBS at pH 7.4.

The inoculation day was set as day 0 postinoculation (0 DPI). Two DPI, the seeders were returned to their pens. Transmission was monitored during a period of 6 weeks, corresponding to the nursery period mostly applied under field conditions in Western Europe (15). Swab samples were collected separately from both anterior nares, the skin behind the ears, and the perineum, using a moistened sterile swab. Environmental contamination was examined once a week by streaking 10 cm² of the wall (piglet height) and the feeder (floor height) with a sterile moistened sponge. After euthanasia (43 DPI), samples were taken from the throat. This study was approved by the Ethical Committee for Animal Experiments of the Veterinary and Agrochemical Research center (VAR), Brussels, Belgium.

All samples were processed within 24 h. Swab samples were inoculated in 3 ml of Mueller-Hinton broth (MH) (Oxoid, Germany) supplemented with 6.5% NaCl, while sponges were inoculated in 50 ml of MH supplemented with 6.5% NaCl. Both swabs and sponges were incubated aerobically for 24 h at 37°C. Then 1 μl of each broth was inoculated on ChromID MRSA plates (bioMérieux, France) and incubated aerobically at 37°C for 48 h. Swabs taken ~4.2 (nasal swab samples only), 14, 22, 34, and 43 DPI were also investigated for the presence of MSSA, using ChromID S. aureus plates (bioMérieux, France). Suspected MRSA and MSSA colonies...
were purified on Columbia agar plates with 5% sheep blood (BioRad, Belgium). From purified isolates, DNA was extracted as previously described (20), and both MRSA and MSSA were identified by multiplex PCR using the method of Maes et al. (14).

To certify the isolates' characteristics, one MRSA strain originating from one randomly selected animal of each group per sampling occasion was characterized by \( \text{spa} \) typing (10). From each MSSA-positive piglet on /H11002 and 43 DPI, one strain was likewise characterized. Additionally, MRSA and/or MSSA strains isolated from the environmental samples on 4 and 42 DPI were characterized by \( \text{spa} \) typing (10). Multilocus sequence typing (MLST) was effectuated on one MSSA isolate of each \( \text{spa} \) type (8).

Quantification of transmission of MRSA was done using a stochastic infection model as described by Velthuis et al. (22). Briefly, transmission among piglets was assumed to be in accordance with the susceptible-infectious-susceptible (SIS) model. In this model, it is assumed that animals can carry MRSA intermittently. An animal was classified as infectious when MRSA was detected on at least one of the three sampling sites and as susceptible when MRSA was not detected. Given that the population (\( N \)) is composed of susceptible animals (\( S \)) and infectious animals (\( I \)), two events can occur: an infection event, \( (S, I) \rightarrow (S - 1, I + 1) \), and a recovery event without immunity, \( (S + 1, I - 1) \). The rate at which an infection event occurs depends on the density of \( S \) animals, the number of \( I \) animals, and the transmission parameter \( \beta \) (i.e., the number of cases affected by \( I \) animals during every period out of the number of \( S \) animals at the start of the period). The rate at which a recovery event occurs depends on the number of \( I \) animals and the recovery parameter \( \alpha \) (i.e., the number of recovered animals per unit of time) (22, 23). From these expression results, the average number of secondary cases caused by one infectious individual in a totally susceptible population, termed the reproduction ratio (\( R_0 \)), equals \( \beta/\alpha \) (23). Consequently, \( R_0 \) can be assessed after estimating these transmission parameters. This was done separately for the three experimental groups.

We estimated the transmission parameter \( \beta \) using a generalized linear model (GLM) with a complementary log-log link function and log \( I/N \) as the offset variable, as described by Velthuis et al. (22). The duration of the infectious period (1/\( \alpha \)) was defined as the average number of days at which each animal was classified as infectious during the experiment.

As depicted in Fig. 1, highly efficient transmission of MRSA strain C26 from seeders to contact animals was observed. In all three experimental groups, all contact animals were MRSA positive on the first sampling occasion (4 DPI), 2 days after the introduction of the seeders with the contact animals. In contrast, all animals of the negative control group remained MRSA negative during the whole experiment. The corresponding \( R_0 \) values were all significantly higher than 1 (Table 1). In general, an \( R_0 \) score above 1 means that a primary case generates more than one secondary case and that the agent spreads in the population (6). Consequently, when our results are extrapolated to a field situation, a single introduction of a MRSA ST398-positive piglet in a susceptible population of weaned piglets is likely to cause an infection of multiple animals. In other words, our results prove that MRSA ST398, or at least the strain we used (\( \text{spa} \) type t011, SCC\( \text{mec} \) type

![FIG 1](http://aem.asm.org/) MRSA detection status of the piglets during the time of the experiment (expressed in days postinoculation [DPI]). (A) Distribution of MRSA-positive (MRSA+) seeder animals (\( n = 2 \)/group). (B) Distribution of MRSA-positive contact animals (\( n = 6 \)/group) and control animals (\( n = 7 \)). * day on which seeders and contacts were placed together.

### TABLE 1: Estimation of transmission parameters by the generalized linear model method

<table>
<thead>
<tr>
<th>Group</th>
<th>( \beta ) (95% CI)</th>
<th>1/( \alpha ) (SD; median; min-max; ( n ))</th>
<th>( R_0 ) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.89 (0.51–1.54)</td>
<td>13.00 (10.53; 15; 1–25; 15)</td>
<td>11.57 (6.63–20.02)</td>
</tr>
<tr>
<td>2</td>
<td>1.14 (0.78–1.66)</td>
<td>3.44 (2.99; 2; 1–13; 32)</td>
<td>3.92 (2.68–5.71)</td>
</tr>
<tr>
<td>3</td>
<td>2.69 (1.30–5.57)</td>
<td>19.53 (11.45; 13; 5–39; 15)</td>
<td>52.54 (25.39–108.78)</td>
</tr>
</tbody>
</table>

* \( \beta \), transmission parameter; CI, confidence interval; SD, standard deviation; 1/\( \alpha \), infectious period in days; \( n \), number of observations; \( R_0 \), reproduction ratio.
Transmission of MRSA ST398 in Piglets

V), has high spreading potential in weaned piglets. Recently, two other studies have investigated the transmission potential of MRSA ST398 in piglets (4, 17). Even though different methodologies were used in these studies, efficient transmission and persistence of MRSA ST398 among the animals were also described, illustrating the robustness of this observation.

An important issue to consider is the dose required for successful transmission of MRSA ST398 to susceptible animals. In this study, the dose used to inoculate the seeders was relatively high (~9 × 10^8 CFU), which possibly resulted in a higher level of "infectiousness" of the seeders, i.e., a higher number of bacteria transmitted than the number of bacteria transmitted under field conditions (23). Still, preliminary data showed that such a high dose was necessary to achieve colonization (unpublished data). In this study, the amount of bacteria present on the animals was not quantified, rendering it unclear whether the contact animals were carrying doses similar to those of the seeders or whether the animals were only transiently contaminated. However, it must be noted that semiquantitative approaches have already shown a high variability in MRSA counts in naturally colonized animals (17). In addition, one must consider that quantification techniques will always be hampered by the sampling procedure as well as by the sensitivity of the isolation method. At this time, well-defined criteria to describe true colonization with S. aureus in animals are absent. Numerous studies have reported MRSA ST398 in pigs or other animals and in their caretakers, but without indicating whether all the positive individuals were truly colonized.

It appears that a MRSA-positive animal is not the sole source for contamination of susceptible pen mates (11). Our study suggests that the environment may also play a role in the spread of MRSA ST398. Indeed, after introduction of the seeders, the environmental samples from all three experimental groups were shown to harbor the inoculated strain (Table 2). This means that besides the seeders (and their secondary cases) the environment can start to function as a source of MRSA supply. Hence, our study suggests that a pen housing MRSA-negative piglets will, upon introduction of a MRSA-positive animal, become a "dynamic system" of interacting reservoirs, regardless of whether the entities of this system are truly colonized or not. Even if certain entities in this system, such as an individual animal, "lose" the MRSA ST398 strain for a while, other sources will be present to resupply it. In this respect, it is not hard to imagine that the farmer or other contact animals, such as dogs or rats, might act as vectors to spread MRSA ST398 through different parts of the farm, expanding the dynamic system from a single pen to the entire farm.

One might question what causes the animals to be MRSA negative on certain occasions. First of all, the MRSA population on a specific animal might have simply diminished and ultimately vanished because the animal was not truly colonized. However, investigation of the postmortem samples showed that MRSA was present in the throats of two contact animals of group 1 and all contact animals of group 3, as well as in five out of the six seeders (data not shown), suggesting that at least these animals were stably colonized. Hence, other possibilities also need to be considered. For example, the MRSA strain might still have been present but in a quantity below the detection threshold of the sampling and/or isolation method. Another factor that might have played a role is the presence of other bacteria in the collected samples. This will have differed for different animals and on different occasions. It has been reported that some bacteria might inhibit in vitro growth of MRSA, resulting in false-negative results (3). Of particular interest in this respect is the indigenous microflora of the individual animals, which could exert an antagonistic effect on stable colonization by MRSA (4, 5, 13, 16, 17). Dall’Antonia et al. (5) illustrated that in humans, MSSA competes with MRSA for colonization of the anterior nares. Interestingly, in our study MSSA strains (spa type t3446 and occasionally spa type t337, MLST ST9) were also isolated, and more frequently, from the animals belonging to groups 1 and 3, as well as in five out of the six seeders (data not shown), suggesting that at least these animals were stably colonized. Hence, other possibilities also need to be considered. For example, the MRSA strain might still have been present but in a quantity below the detection threshold of the sampling and/or isolation method. Another factor that might have played a role is the presence of other bacteria in the collected samples. This will have differed for different animals and on different occasions. It has been reported that some bacteria might inhibit in vitro growth of MRSA, resulting in false-negative results (3). Of particular interest in this respect is the indigenous microflora of the individual animals, which could exert an antagonistic effect on stable colonization by MRSA (4, 5, 13, 16, 17). Dall’Antonia et al. (5) illustrated that in humans, MSSA competes with MRSA for colonization of the anterior nares. Interestingly, in our study MSSA strains (spa type t3446 and occasionally spa type t337, MLST ST9) were also isolated, and more frequently, from the animals belonging to the groups where the MRSA status fluctuation was more pronounced (groups 1 and 2) (data not shown). The competitive effect between MSSA (ST9 or other STs) and MRSA is an interesting issue to consider in further studies in order to determine the true impact of interference on colonization status and its usefulness in MRSA eradication programs.

In conclusion, this study demonstrates that a combined nasal-skin inoculation model results in colonization of weaned piglets with MRSA ST398. In all three groups, the R_0 values were shown to be significantly above 1, indicating that MRSA ST398 tends to become established. In this respect, groups of pigs or whole farms might function as dynamic systems with various interacting entities that maintain a MRSA-positive status. Still, further research is needed to elucidate the mechanism involved in these processes.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sampling site</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>28</th>
<th>36</th>
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<td>1 Wall</td>
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<td>Feeder</td>
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<td>+/–/+c</td>
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<td>Feeder</td>
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<td>3 Wall</td>
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<td>Feeder</td>
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<td>Control Wall</td>
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</tr>
</tbody>
</table>

a MSSA strain spa type t3446.
b MSSA strain spa type t337.
c MRSA strain spa type t011.
+/–, MRSA-positive sample; +/–, MSSA-positive sample; +/+c, MSSA- and MRSA-positive sample; –, neither MRSA nor MSSA detected; ND, not sampled.
needed to gain more insight into the different carrier states of pigs and to investigate the usefulness of interference strains as a tool for eradicating MRSA carriage. We believe that, using our model, additional transmission studies should be performed in order to evaluate the efficacy of various treatments (e.g., medication) and to determine the influence of control strategies on the spread of MRSA ST398.

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REFERENCES


