Colonization Kinetics of Different Methicillin-Resistant *Staphylococcus aureus* Sequence Types in Pigs and Host Susceptibilities

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In this study, we investigated the kinetics of colonization, the host susceptibility and transmissibility of methicillin-resistant *Staphylococcus aureus* (MRSA) after nasal treatment of pigs with three different MRSA strains of distinctive clonal lineages (sequence type 398 [ST398], ST8, and ST9), and origin in weaning piglets. The colonization dose of \(5.0 \times 10^6\) CFU/animal was determined in preliminary animal studies. A total of 57 piglets were randomly divided into four test groups and one control group. Each of three test groups was inoculated intranasally with either MRSA ST8, MRSA ST9, or MRSA ST398. The fourth group was a mixture of animals inoculated with MRSA ST398 and noninoculated “sentinel” animals. Clinical signs, the nasal, conjunctival, and skin colonization of MRSA, fecal excretion, and organ distribution of MRSA, as well as different environmental samples were examined. After nasal inoculation with MRSA piglets of all four test groups showed no clinical signs of an MRSA infection. MRSA was present on the nasal mucosa, skin, and conjunctiva in all four test groups, including sentinel animals. Likewise, fecal excretion and internal colonization of MRSA ST8, ST9, and ST398 could be shown in each group. However, fecal excretion and the colonization rate of the nasal mucosa with MRSA ST9 were significantly lower in the first days after infection than in test groups infected with ST8 and ST398. The results of this study suggest differences in colonization potential of the different MRSA types in pigs. Furthermore, colonization of lymph nodes (e.g., the ileocecal lymph node) with MRSA of the clonal lineage ST398 was demonstrated.

*Staphylococcus aureus* colonizes mucous membranes and the nasopharynx of healthy animals and humans. Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a serious threat to public health. MRSA is an important cause of hospital-acquired (nosocomial) infections in humans, including wound infections, abscesses, ventilator-associated pneumonia, meningitis, endocarditis, and septicemia. In the past 2 decades such infections have been reported worldwide, especially from hospitals (18, 19, 30, 39).

Animal-associated MRSA may be transmitted to humans and causes various disease conditions (11, 32, 37, 40). The frequent occurrence of MRSA in food-producing animals has led to growing concern. People with direct daily contact with animals, such as farmers, veterinarians, and slaughterhouse workers, are more frequently colonized with MRSA than those without frequent contact with animals (17, 21, 23, 41).

The transmission of MRSA and its different clonal lines as well as the factors influencing transmission are largely unclear in animal husbandry (23).

There is a growing body of literature on MRSA infections in large domestic and production animals. However, these are mainly field studies dealing with the prevalence of MRSA (14, 28, 35) in naturally contaminated animals on farms and in slaughterhouses. There are no reports about the rate of recovery of MRSA from internal organs of large domestic animals after experimental inoculation with MRSA. Experimental studies with MRSA in animals were carried out mainly with respect to certain diseases. Intravenous inoculation of *S. aureus* was examined a few times in pigs with regard to hepatic function (22) and acute induced pyemia (27). Osteomyelitis was studied after intravenous inoculation in pigs (16, 20) and chickens (12). In another study rat femur was directly contaminated with *S. aureus* via surgery (5).

Effectiveness of different antibiotics was tested in various rabbit eye and rat animal models (4, 7, 8, 9).

In a recent study, piglets and a sow were colonized with MRSA under experimental conditions. Six piglets were challenged by nasal and gastrointestinal inoculation with a mixture of four strains including sequence types 398 and 9 (ST398 and ST9, respectively) after treatment with antimicrobials. One sow was inoculated vaginally with the same MRSA mixture shortly before farrowing. Internal organs were not examined (25).

In this study, we investigated the colonization kinetics and host susceptibility of three different clonal lines of MRSA (ST8, ST9, and ST398) to test three distinct hypotheses: (i) inoculation of clinically healthy weaned piglets with MRSA at a sufficiently high dose to trigger colonization does not result in clinical signs in pigs; (ii) the degree of colonization differs between different strains of different origins; and (iii) MRSA is readily transmitted from inoculated animals to noninoculated sentinel housed in the same pen.

MRSA prevalence on skin, nasal mucosa, and conjunctiva and fecal shedding and distribution patterns of MRSA in internal organs of weaned piglets were studied. To the best of our knowledge, this is the first experimental study on the degrees of colonization, including the dissemination of MRSA in different internal organs, after nasal challenge of weaned piglets with three different MRSA strains.
TABLE 1 Characteristics of MRSA isolates used in the study

<table>
<thead>
<tr>
<th>Group</th>
<th>Strain identifier</th>
<th>Source (place of sampling)</th>
<th>spa type</th>
<th>MLST type</th>
<th>SCCmeC type</th>
<th>Resistance phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST8</td>
<td>09S01631</td>
<td>Pork (at retail)</td>
<td>t008</td>
<td>ST8</td>
<td>NT</td>
<td>OXA</td>
</tr>
<tr>
<td>ST9</td>
<td>09S02535</td>
<td>Broiler meat (at retail)</td>
<td>t1430</td>
<td>ST9</td>
<td>IVa</td>
<td>OXA-TET-ERY-CLI-CIP-SXT</td>
</tr>
<tr>
<td>ST398</td>
<td>08S00974</td>
<td>Fattening pig (at farm)</td>
<td>t011</td>
<td>ST398</td>
<td>V</td>
<td>OXA-TET-CLI</td>
</tr>
<tr>
<td>Sentinel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Typing was performed as described by Argudín et al. (1). SCCmeC, staphylococcal cassette chromosome mec; NT, not typeable according to Zhang et al. (42).

b Susceptibility tests were carried out using the broth microdilution method according to CLSI guidelines (6). OXA, oxacillin; TET, tetracycline; ERY, erythromycin; CLI, clindamycin; CIP, ciprofloxacin; SXT, sulfamethoxazole-trimethoprim.

MATERIALS AND METHODS

Animal experiments were approved by the State Office of Health and Social Affairs Berlin, Germany (Landesamt für Gesundheit und Soziales [LAGeSo] number 0031/10).

Animals. Fifty-seven weaned piglets from 10 litters (Landrace × Large White) were included in the main study. They originated from one farm with no history of MRSA colonization. Results from sixty pigs, including test animals of our study and their sows, were negative for MRSA by nasal, skin, and rectal swabs 2 weeks and directly before the study. Furthermore, results from feed, dust, and feces from this farm were tested negative for MRSA. Experimental animals were still negative for MRSA immediately prior to challenge. Experimental facilities were disinfected 3 days before challenge and tested negative for MRSA 2 days before challenge. Results from all persons with contact with animals and samples throughout the whole study were negative for MRSA before the study. Before entering a pen, personnel were subjected to disinfection, and a complete change of protective clothing for the respective sluce was required.

Animal study. Preliminary studies were carried out in 28 MRSA-free weaned piglets with MRSA ST398 to determine the inoculation dose (data not shown). Piglets of four groups of seven animals were inoculated intranasally with 1 ml of broth (0.5 ml per nasal cavity) containing ~5.0 × 10^8 CFU/ml. Colonization was considered successful if more than 50% of animals showed MRSA colonization of skin, conjunctiva, nose, and feces and if MRSA could be found in inner organs 21 days after colonization. Observation and sampling protocols for animals in the prestudies were identical to the main study.

In the main study a total of 57 weaned piglets from 10 litters (Landrace × Large White) were investigated according to the following study design. Piglets were weaned at 28 days postpartum (p.p.) and randomly divided into five groups (ST8, ST9, ST398, sentinel, and a control group). Groups were housed simultaneously in separate pens in the experimental facilities. The Federal Institute for Risk Assessment at Berlin to avoid cross contamination between groups. Piglets of the ST8 (n = 13), ST9 (n = 13), and the ST398 (n = 12) groups were challenged by nasal application, with the MRSA suspension dropped slowly into both nasal vestibules.

Six animals of the sentinel group (n = 12) were inoculated with MRSA ST398, whereas the other six animals remained untreated. A fifth group served as a negative-control group (n = 7). According to the results of the prestudies, an inoculation dose of ~5.0 × 10^8 CFU/pig was used.

Five piglets of the ST8, ST9, and ST398 groups were randomly selected and sacrificed on day 3 postinoculation (p.i.). The remaining animals were monitored for a period of 3 weeks and sacrificed on day 21 p.i. All animals of the sentinel group were monitored for a period of 4 weeks and sacrificed on day 42 p.i. At necropsy, palatine tonsils, mandibular lymph nodes, spleen, lung, and ileocecal lymph nodes were collected and examined for MRSA qualitatively and quantitatively.

During the observation period, the following clinical parameters were monitored: (i) evaluation of general condition (daily); (ii) rectal temperature (daily); (iii) prevalence of MRSA on skin, nasal mucosa, and conjunctiva and in feces (daily for the first 3 days p.i.; thereafter, twice a week); and (iv) body weight (twice a week).

Bacterial strains. For the nasal inoculation of pigs, three distinct MRSA strains of the multilocus sequence typing (MLST) sequence types ST8 (ST8 group), ST9 (ST9 group), and ST398 (ST398 and sentinel group) and with differing characteristics and antimicrobial susceptibility profiles were chosen (Table 1).

Each MRSA strain was cultured in Mueller-Hinton broth (catalogue number CM405; Oxoid, Wesel, Germany) supplemented with 6% NaCl (MHB+) at 37°C overnight. Portions (0.5 ml) of each preculture were used to inoculate 50 ml of prewarmed MHB+ and grown at 37°C to an optical density of 0.6 measured with a digital photometer (LP25; Lange, Berlin, Germany). This value corresponded to an average bacterial count of approximately 5.0 × 10^8 CFU/ml (ST8, 5.7 × 10^8 CFU/ml; ST9, 3.5 × 10^8 CFU/ml; and ST398, 6.4 × 10^8 CFU/ml), as determined per plate culture. Each pig, except sentinel pigs and controls, was inoculated with 1 ml (0.5 ml per nostril) of the respective culture for its group.

Sampling. Swab samples were taken by holding each animal individually, without any contact with the sampling areas. Samples were collected in individual sterile reservoirs and transported immediately into the labs for further investigations. Nasal swabs (Nerbe Plus, Winsen/Luthe, Germany) were dry cotton swabs (3 mm) for sampling one nose vestibule. Conjunctival and rectal samples were also collected using sterile dry cotton swabs (3 mm and 5 mm, respectively). Skin swab samples were obtained from the skin surface behind one ear using sterile moist cotton swabs (Sarstedt AG and Co., Nümbrecht, Germany) humidified by phosphate-buffered saline (PBS). Organ tissue samples were collected during necropsy immediately after animals were sacrificed.

Environmental samples were taken from the doors and exhaust air and supply air shafts by using sterile dry cotton swabs. Shafts were generally swabbed all over, whereas doors were swabbed on the upper angle.

Air samples were collected via impingement. An All-Glas-Impinger (AGI-30; Ace Glass Inc., Vineland, NJ) filled with 30 ml of phosphate-buffered saline was used. The collection time was 30 min, and the recorded airflow was approximately 11.5 liters per min.

Qualitative detection of MRSA in animal and environmental swabs. All samples were analyzed on the day of sampling. All swabs from nose, skin, conjunctiva, feces, and the environment were investigated individually with enrichment. Samples were enriched using Mueller-Hinton broth with 6% NaCl (MHB+) and tryptone soy broth containing 75 mg/liter aztreonam and 3.5 mg/liter cefoxitin (TSB+) subsequently. After 24 h of incubation at 37°C, 1 ml of MHB+ was transferred into 9 ml of TSB+. This selective broth was incubated for 17 h at 37°C. A loopful of TSB+ was streaked onto chromogenic MRSA screen agar (CHROMagarMRSA; MAST Diagnostica GmbH), followed by incubation at 37°C for 24 h.

Detection of MRSA in air samples. A total of 500 μl of the original collection fluid of the impinger was streaked onto the chromogenic MRSA screen agar. Simultaneously, 10 ml of the collection fluid was filtered using a nitrocellulose filter with a pore size of 0.22 μm. This filter was placed onto a chromogenic MRSA screen agar and incubated as described above.

Detection of MRSA in internal organs. Tissue samples of 10 g (lung and spleen) or whole organs (<10 g for tonsils and mandibular and ileocecal lymph nodes) were immersed in 95% ethanol, flamed, minced aseptically, and homogenized with MHB+ (1:10) using a Stomacher 400 (Seward, London, United Kingdom) for 2 min at high speed. All samples were cultured onto the respective plates as described above.

Organ samples were removed aseptically and transferred into 10 ml of chromogenic MRSA screen agar (CHROMagarMRSA; MAST Diagnostica GmbH), followed by incubation at 37°C for 24 h.
were examined for MRSA quantitatively by direct plating and qualitatively as described above.

**Confirmation and typing of MRSA isolates.** Isolates were confirmed as MRSA using a multiplex PCR (29) and spa typed (31). Due to the high number of isolates and for practical reasons, typing was performed on a representative subset of isolates from each animal.

**Statistical analysis.** Statistical analysis was conducted using the software SPSS, version 16.0 (SPSS, Inc., Chicago, IL). Logistic regression was applied to estimate the effect of the inoculation strain and the day of sampling on the test results for all different samples from the colonization and the sentinel study. The test result for the respective sample type was the binary outcome; test day and inoculation strain were included as categorical covariates.

A χ² test was performed to calculate the differences between test groups on different days of investigation regarding the number of MRSA-positive samples. Differences were considered significant if P was <0.05.

**RESULTS**

**Clinical signs.** During the complete postinoculation observation period of 21 (MRSA ST398, ST8, and ST9) and 42 (sentinel) days, no clinical signs indicative of infection were observed in inoculated, sentinel, and control animals. The development of body weight of individual piglets in all test groups was similar to that of the control group throughout the whole study.

**MRSA on skin, nasal mucosa, and conjunctiva.** None of the seven control pigs was positive for MRSA during the whole study period. Results of logistic regression revealed that nasal swabs were more often positive in the ST398 group than in the ST8 and ST9 groups. For swabs collected from the conjunctiva and the skin, the difference between the strains was not significant. Day of sampling had a significant effect on the results of nasal, conjunctival, and skin swabs.

MRSA was found on day 1 p.i. in all except two nasal swabs in the ST398 group (10/12) and in all except one in the ST8 group (12/13) (Fig. 1a). In contrast, in the ST9 group all nasal swabs were MRSA negative on day 1 p.i. On day 2 p.i., all nasal swabs in the ST8 and ST398 groups tested positive and in 77% (10/13) of the animals; in the ST9 group MRSA was detected in nasal mucosa as well.

The proportion of MRSA-positive nasal swab samples tended to decrease in all three groups from day 2 to day 10 (Fig. 1a). After day 10 the proportion of positive swabs increased again. On day 21 p.i., 50% (4/8) of the nasal swabs in the ST8 and ST9 groups and 85% (6/7) in the ST398 group were MRSA positive.

MRSA was found on day 1 p.i. in all skin swabs in the ST9 group (13/13) and in all but one swab in the ST8 group (12/13) (Fig. 1b). However, the number of MRSA-positive skin samples was significantly lower (50%) in the ST398 group than in the other two groups. In each group the highest skin colonization rate was observed in the first week. In the second week the number of MRSA-positive skin samples decreased. However, in the third week of the observation period, the proportion of positive skin samples increased again. On day 17 p.i., all animals in the ST398 group tested positive in the skin swabs, in contrast to the ST8 (2/8) and ST9 (3/8) groups. On day 21 p.i., 37% (5/13), 75% (6/8), and 71% (5/7) of skin swabs were positive in the ST8, ST9, and ST398 groups, respectively. The χ² test revealed on day 3 p.i. significantly more MRSA-positive skin swabs in animals in the ST8 (P = 0.018) and ST398 (0.001) groups than in the ST9 group.

Detection rates were highest on the conjunctiva (Fig. 2a) on day 1 p.i. for all groups. MRSA was found in 77% (10/13), 69% (9/13), and 75% (9/12) of conjunctival swabs in the ST8, ST9, and ST398 groups, respectively. On day 2 p.i., significantly more MRSA-positive conjunctival swabs were found in the ST398 group than in the ST9 group. On day 21 p.i., one animal in the ST8 (1/8) and ST398 (1/7) groups and two animals (2/8) in the ST9 group were MRSA positive on the conjunctiva.

**Fecal shedding of MRSA.** MRSA was isolated at 1 day p.i. from feces of animals in the ST8 (5/13) and ST398 (5/12) groups (Fig. 2b) but not in the ST9 group. The proportion of MRSA-positive fecal samples was significantly lower in the ST9 group than in the other two groups on days 1 and 3 p.i. After day 2 p.i., 20 to 40% of

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**Fig 1** Percentages of MRSA-positive nasal and skin swabs from piglets of the ST8, ST9, and ST398 groups over the entire study period of 21 days.
animals of all three test groups excreted MRSA in feces in the first week. Thereafter, 10 to 25% of animals in all groups showed fecal excretion of MRSA until the end of the study. On day 21 p.i., the number of MRSA-positive rectal samples increased and became significantly higher in the ST398 group than in the other two groups. Logistic regression revealed no significant association of day of sampling or inoculation strain with the test results for the rectal swabs.

**Dissemination of MRSA in internal organs.** MRSA bacteria of all three inoculation strains were recovered from all investigated tissue types in all three groups. No significant association of inoculation strain and test results for the internal organs was observed (Table 2). In all three groups the detection rate was highest in tonsils and mandibular and ileocecal lymph nodes and tended to be lower in spleen and lung.

Quantification of MRSA in tissue samples revealed that both the ST8 (up to log 5.01 CFU/g) and ST398 (up to log 4.76 CFU/g) groups had the highest MRSA concentration in the tonsils on day 21 p.i. In the ST9 group, MRSA counts were below the detection limit of the quantitative method in all tissue samples. Quantification of MRSA in the mandibular lymph nodes was possible in the ST8 group only on day 21 p.i. (up to log 2.48 CFU/g). MRSA counts were below the detection limit (10 CFU/g) in spleen, lung, and ileocecal lymph nodes in all test groups.

**Transmission trial.** MRSA was detected in nasal swabs of all inoculated (6/6) and sentinel (6/6) animals in the sentinel group on day 1 p.i. (Fig. 3a). Thereafter, detection rates in nasal swabs developed similarly (30 to 50% MRSA-positive nasal swabs) in inoculated and sentinel animals until the end of the observation period.

On day 1 p.i., MRSA was detected in skin swabs of 67% (4/6) of inoculated and 83% (5/6) of sentinel animals (Fig. 3b). On days 7 and 10 p.i., numerically more MRSA-positive skin swabs were found in the inoculated animals. At other times, skin colonization of inoculated and sentinel animals was largely identical, with three to four out of six of the animals testing positive for MRSA.

TABLE 2 Prevalence of MRSA-positive organ samples postmortem

<table>
<thead>
<tr>
<th>Day p.i. and group</th>
<th>No. of animals examined (%)</th>
<th>Tonsil</th>
<th>Mandibular lymph node</th>
<th>Lung</th>
<th>Spleen</th>
<th>Ileocecal lymph node</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST398</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>ST8</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ST9</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>15 (100)</td>
<td>6 (40)</td>
<td>7 (47)</td>
<td>2 (13)</td>
<td>4 (27)</td>
<td>9 (60)</td>
</tr>
<tr>
<td><strong>Day 21</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST398</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>ST8</td>
<td>8</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>ST9</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>23 (100)</td>
<td>12 (52)</td>
<td>9 (39)</td>
<td>4 (17)</td>
<td>4 (17)</td>
<td>10 (43)</td>
</tr>
</tbody>
</table>
Colonization of the conjunctiva was infrequent in inoculated and sentinel animals (Fig. 4a). On day 1 p.i., MRSA was detected in four of six conjunctival swabs of sentinel animals, whereas the conjunctiva of only two of six inoculated animals was MRSA positive. On days 2, 3, and 14, MRSA was found only in the conjunctival swabs of inoculated animals. From day 17 until day 42 p.i., MRSA was found on the conjunctiva of two sentinel animals only. However, on day 30, MRSA was detected in conjunctival swabs of both groups of animals.

MRSA was detected in feces of 50% of the inoculated animals on day 1 p.i. In contrast, all fecal samples from sentinels were MRSA negative on day 1 (Fig. 4b). On day 2 p.i., the number of MRSA-shedding animals tended to be higher in the group of inoculated animals (4/6) than in the sentinel group (2/6). On days 3 and 7 p.i., all sentinel animals were MRSA negative in feces. After day 10 p.i., fecal shedding varied in inoculated and sentinel animals. However, no significant difference was observed between the two groups.

On day 42 p.i., MRSA was recovered from all investigated tissue samples in three of the six inoculated animals. One animal was positive for all tissue samples except the mandibular lymph nodes. Another animal was positive in tonsils, and a third one was positive in the tonsils and mandibular lymph nodes. In the sentinel animals, MRSA was found in tonsils and mandibular lymph nodes.

FIG 3 Percentages of MRSA-positive nasal and skin swabs from piglets of the sentinel group (inoculated animals, n = 6; noninoculated animals, n = 6).

FIG 4 Percentages of MRSA-positive conjunctival and rectal swabs from piglets of the sentinel group (inoculated animals, n = 6; noninoculated animals, n = 6) over the entire study period of 42 days.
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FIG 5 Percentages of MRSA-positive organ samples from piglets of the sentinel group, sacrificed at 42 days p.i. (inoculated animals, n = 6; noninoculated animals, n = 6) over the entire study period of 42 days.

In this study, we examined the MRSA prevalence of three different clonal lineages of MRSA in the nose, on skin and conjunctiva, and in feces. The proportion of positive nasal swabs was highest in pigs inoculated with the ST398 strain, followed by ST8 isolated from pork at a retail chain and ST9 that originated from the poultry food chain. This may indicate a better host adaption of the ST398 lineage. However, the mechanism behind it remains unknown, and further studies are needed to confirm the difference between the different clonal lineages.

It is not clear why the ST9 strain could not be isolated from the nasal swabs on day 1 p.i. It can be speculated that its ability to attach to the mucosal surface in pigs was less than that of the other strains used. Host specificity might have influenced these findings as the ST9 strain originated from poultry and not from pigs. This strain might have required time to adapt the new host in the present study. However, apparently it did survive in the animals and reoccurred on the mucosa on day 2.

In all three test groups, the percentage of colonized animals varied significantly over time, irrespective of the sampling material. While the percentage dropped after the second day, it increased again after 2 weeks. This pattern was most obvious in the nasal swabs. However, the variation was also observed for skin and conjunctival swabs. The reduction in the number of positive swabs after the first days was expected; the subsequent increase occurring in all groups was difficult to interpret. One potential explanation could be an increasing contamination of the pen with MRSA, leading to repeated colonization of the animals. In line with that, MRSA was detected in all environmental swab samples in all groups.

Routine diagnostic tests in the field have infrequently found MRSA present in the internal organs of pigs postmortem (24). Our study shows that this finding is most likely not an artifact caused by inadequate handling of carcasses before routine postmortem but, rather, is a consequence of dissemination of MRSA within the body in the absence of clinical signs.

All three MRSA strains disseminated in all investigated organ types after nasal inoculation. Tonsils and mandibular and ileocecal lymph nodes were the most frequently colonized organs, followed by spleen and lung.

On day 21 p.i. the number of MRSA-positive tonsils and mandibular lymph nodes was higher in the ST398 and ST8 groups than in the ST9 group. MRSA was detected without enrichment in tonsils and mandibular lymph nodes at the end of the observation period (day 42) in only the ST8 and ST398 groups. One explanation for the high levels of MRSA in these organs and the higher number of MRSA-positive tonsils and mandibular lymph nodes could be the continuous contact with other colonized pigs, their feces, or their environment, i.e., continuous oral and/or nasal recolonization from nose, skin, and feces of colonized animals. The higher colonization level of tonsils in the ST8 and ST398 groups corresponds with the high overall rate of positive nasal samples in these groups compared to the ST9 group.

This is the first experimental demonstration of a higher susceptibility of pigs to MRSA ST398 than to another MRSA type (ST9). A further hint could be a spa type alteration observed in three lymph nodes of three different pigs in the ST398 group, where the original spa type t011 turned into t1451. This alteration can be explained by the deletion of one spa repeat (t011, 8-16-2-25-34-24-25, versus t1451, 8-16-2-25-34-25), which could be interpreted as molecular mimicry. Up to the present, only a few studies have explained the deletion of one spa repeat (t011, 8-16-2-25-34-24-25, versus t1451, 8-16-2-25-34-25), which could be interpreted as molecular mimicry. Up to the present, only a few studies have...
addressed the question of how often and how fast spa types change in vivo (2). So far, no experimental studies have been published showing comparable events in pigs. These results call for further investigations.

All investigated environmental swab samples collected from the doors and exhaust air and supply air shafts were MRSA positive. This corresponds with observations from other studies that detected MRSA ST398 in dust samples from pig breeding facilities, swab samples from the animals’ environment, and environmental wipes (3, 10, 26, 34, 36).

MRSA has been previously detected in air samples (15, 33, 38). In the present study, air samples were MRSA positive on only day 1 p.i. in the ST398 and sentinel groups. This might be due to a special yet undefined characteristic of the MRSA ST398 strain since both groups were colonized with MRSA ST398.

In our study we investigated the transmission of MRSA between inoculated and noninoculated animals. Results of our study suggest that a high inoculation dose of MRSA results in a substantial MRSA prevalence in feces and internal organs. Colonization of sentinel animals must have occurred exclusively through transmission between colonized and noncolonized animals and via the environment.

It is likely that during the inoculation procedure, part of the inoculum was swallowed by the piglets. This could be the reason for a higher detection rate in feces of inoculated animals than in the sentinels in the first half of the observation period. Another reason for the increased MRSA shedding in feces could be the progeny of MRSA bacteria in the ileocecal lymph nodes of inoculated pigs. Detection of MRSA in ileocecal lymph nodes could be an important diagnostic factor to discriminate between infected and merely colonized pigs. This calls for further investigations into the presence of MRSA in enteric lymph nodes of pigs in the field.

Tonsils and mandibular lymph nodes serve as the first lymphoid filter after nasal or oral challenge with bacteria. The continuous oral and/or nasal exposure to bacteria from colonized pigs, feces, and the environment led to the presence of MRSA in tonsils and mandibular lymph nodes of sentinel animals 42 days after the beginning of the trial. Moreover, the MRSA load associated with the indirect exposure of the sentinels was probably lower, resulting in the absence of MRSA in spleen, lung, and ileocecal lymph nodes of the sentinels.

In conclusion, MRSA strains of the MLST sequence types ST8, ST9, and ST398 were able to colonize all pigs, to spread within the body of the inoculated animals, and to contaminate the environment throughout the whole study period. Colonization was less efficient with the MRSA ST9 strain (originating from the poultry food chain), as indicated by a low proportion of positive nasal swabs, a numerically reduced colonization of internal organs and skin, and reduced fecal shedding in comparison to the groups inoculated with ST8 and ST398. The results of our study suggest strain-specific colonization mechanisms of the different MRSA types that might be associated with a certain degree of host specificity.

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