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Persistence of Nasal Colonization with Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* in Pig Farmers after Holidays from Pig Exposure

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Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is frequently transmitted from pigs to farmers. This study analyzed whether an absence from direct contact with pigs during holidays had an impact on nasal MRSA colonization rates of pig farmers. Overall, 59% of the farmers did not clear MRSA colonization during their leave.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been reported to colonize livestock (pigs, cattle, and poultry) in many countries, affecting up to 70% of all pig farms in Germany (4, 9). Among the MRSA isolates from pigs, clonal complex 398 (CC398), as defined by multilocus sequence typing (MLST), is predominant and accounts for more than 90% of all European porcine strains (4). MRSA CC398 is frequently transmitted to persons who have direct contact with the animals, leading to colonization rates of up to 86% among farmers from MRSA-positive units (2). However, a Dutch study has recently shown that persons with a short-term occupational exposure to animals (up to 3 h daily) were positive directly after their visit on a pig farm but 94% were negative when a second nasal swab was collected 24 h later (13).

Currently, little is known about the dynamics of MRSA colonization of persons with direct and regular contact with livestock. Therefore, we investigated whether an absence from the pig farm during the summer holidays had an impact on MRSA carrier rates among pig farmers.

For this study, 35 farmers (21 male/14 female; 20 to 29 years [$n = 7$], 30 to 39 years [$n = 14$], 40 to 49 years [$n = 6$], and 50 to 65 years [$n = 8$]) in the Dutch-German Euregio with daily exposure to pigs (>3 h) took nasal swabs on three consecutive days before their summer leave in 2010. Three additional swabs were obtained during the first 3 days after return. All swabs were obtained by the farmers themselves in the morning before their first contact with the animals. For the detection of MRSA, swabs were enriched using a selective medium (phenol red-mannitol broth plus ceftizoxime/aztreonam; Mediaproducts, Groningen, Germany) and streaked onto a chromogenic medium (bioMérieux). MRSA was confirmed by Vitek2 and *mecA* PCR (1). Every first MRSA isolate of each participant was characterized by typing of the *S. aureus* protein A gene (*spa*) and by detection of resistance and virulence markers using a DNA microarray (StaphyType; Clondiag) (5, 11). the chi-square test and *t* test (IBM SPSS Statistics 20.0) were used for statistical analysis.

Among the 35 persons screened, MRSA was confirmed in at least one swab from 27 farmers (18 male/9 female; $P = 0.22$) (Table 1). The mean length of the holidays did not differ between those farmers who "lost" MRSA and those who remained MRSA positive (10.1 versus 12.4 days; $P = 0.51$). The distribution of *spa* types was t011 (63%), t034 (22%), t108 (7%), and t1197 and t1451 (each 4%), which are indicative for the CC398 lineage and have been found on regional farms before (9). All strains contained *mecA*, β-lactamase operon *blaZ-blaI-blaR* and tetracycline resistance gene *tet*(M), while *tet*(K) was detected in all but four isolates. Macrolide-lincosamide-streptogramin B (MLS_B) resistance genes were found in 10 of 27 isolates [*erm*(A) (19%), *erm*(B) (4%), *erm*(C) (11%), and *erm*(A)/*erm*(C) (4%)]. All MRSA isolates were positive for the markers *agr* group 1, capsule type 5, α- and δ-hemolysin, and a similar spectrum of genes encoding microbial surface components recognizing adhesive matrix molecules (MSCRAMM). All strains lacked genes encoding Panton-Valentine leukocidin, toxic shock syndrome toxin 1, and exfoliative toxin. These findings correspond to previous reports (5, 7) except for the detection of staphylococcal enterotoxin genes, which are rarely described (8), in four strains (15%; *entK/entQ* [$n = 3$] and *entB* [$n = 1$]).

In total, 27 of the farmers (77%) carried MRSA at least transiently, which confirms the results of other investigations (2, 3, 14). However, swabs were only taken from the anterior nares, which could underestimate the true colonization rates, because the farmers might have been colonized elsewhere.

Investigations among field workers with sporadic pig contact have suggested a high rate of transient "contamination" (e.g., via dust inhalation) (13). In contrast, for the majority of pig farmers in this study (59%), MRSA carriage did not clear after the holidays, which indicates that regular pig contact leads to rather persistent colonization. However, this finding is in contrast to a study showing that MRSA detection among veal farmers was strongly reduced (58%) after absence of animal contact (6). The reasons for this discrepancy are unclear, but different intensities of dust or animal exposure in veal and pig stables or differences in the "grade" of dust contamination with MRSA (e.g., due to variant antibiotic selective pressure) could contribute to this phenomenon.

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TABLE 1 MRSA colonization of German pig farmers before and after summer vacation

Test result	No. of farmers with result	Length of vacation in days (no. of farmers)	*spa* type(s) (no. of isolates)
Never colonized with MRSA	8	<7 (1), 7–14 (7)	
Colonized in all three samples before and all three samples after the vacation	16	<7 (1), 7–14 (13), >14 (2)	t011 (12), t034 (2), t1451 (1), t1197 (1)
Positive at least once before the vacation, negative on day 1 after return, but positive again at day 3 or day 4 after return	7	7–14 (7)	t011 (2), t034 (3), t108 (2)
Positive at least once before the vacation and negative in all three samples after return	3	7–14 (3)	t011 (2), t034 (1)
Negative in all three samples before but positive in all three samples after the vacation	1	7–14	t011

Of 10 farmers who tested positive before their vacation and negative on the first day after return, 7 tested MRSA positive again on day two or three after their return, which could either show a recontamination after the first animal contact or be explained by a false-negative result of the nasal swab taken on the first day. In this context, we highlight that contamination can occur within hours when working in a pig holding (13) and that we have used an enrichment technique to culture MRSA in order to increase the test sensitivity, both of which argue for a recontamination. However, it might be a limitation of this study that only the first MRSA isolate of each farmer was subjected to typing, so that we cannot differentiate between persistent colonization with a false-negative test result on day 1 after return and recontamination on day 2 by comparison of both isolates using more discriminatory typing methods than *spa* typing (12).

Furthermore, our findings indicate that, if a pig farmer tests MRSA positive in a screening, e.g., prior to an elective surgical procedure, it is not sufficient to absent him from the barn for 7 to 14 days as a single measure to passively clear the colonization before the intervention. Consequently, active MRSA decolonization (e.g., mupirocin ointment), although less effective for persons with daily pig exposure (10), should be applied in addition to reduction of exposure to decrease the risk of infection.

In conclusion, absence from pig contact during the summer leave mostly did not have an impact on MRSA colonization of pig farmers. Only 9% of the farmers lost MRSA during their leave and remained negative for 3 days after their return. Our results indicated that farmers are more likely to be persistently MRSA colonized than transiently contaminated in the nares.

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