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Experimental nasal colonization of piglets with methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* sequence type (ST)398 is widely spread among livestock. People in contact with livestock have a higher risk of testing positive for MRSA. Several experimental settings have been described to study *in vivo* colonization of MRSA in pigs, each having its own limitations. The aim of this study was to develop a nose-colonization model in pigs to quantitatively study the colonization of MRSA and the co-colonization of MSSA and MRSA.

Two experiments were performed: in the first experiment piglets received an intranasal inoculation with MRSA ST398, *spa*-type t011, and in the second experiment piglets received an intranasal inoculation with two MSSA strains (ST398, *spa*-type t011 and t034) and two MRSA strains (also ST398, *spa*-type t011 and t034) to investigate co-colonization. Colonization was quantitatively monitored for 2 weeks in both experiments.

Nasal colonization was successfully established in all piglets with stable numbers of *S. aureus* between 10^4 and 10^6 CFU. MSSA and MRSA were able to co-colonize.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is widely spread among livestock in many parts of Europe, US, Canada, and Asia (Anonymous, 2009; Graveland et al., 2011). This Livestock Associated MRSA (LA-MRSA) can particularly colonize pigs and calves and belongs mainly, depending on the geographical region, to Clonal Complex (CC)398 (Western countries) or CC9 (Asia).

It has been suggested that LA-MRSA of CC398 has adapted to its host after its transmission from humans and subsequently acquired resistance against methicillin and tetracycline (Price et al., 2012). It is believed that at the same time it lost the human virulence genes and the capacity for colonization in humans was reduced (Price et al., 2012). However, in an experimental setting it has been shown that MRSA is still able to colonize healthy humans (Slingerland et al., 2012).

People in contact with livestock are at increased risk of acquiring MRSA (Van den Broek et al., 2009; Van Cleef et al., 2010). Furthermore, human *S. aureus* colonization is a recognized risk factor for infection (Wertheim et al., 2004). Any reduction of MRSA in pigs (in prevalence or load) is

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expected to reduce incidence in humans. Currently, there are no effective intervention methods available to reduce colonization in pigs.

Co-occurrence of multiple genotypes of *S. aureus* in humans has been reported, but a co-occurrence of MSSA and MRSA strains is not mentioned (Bloemendaal et al., 2009; Mongkolrattanothai et al., 2011). Conversely, it is suggested that colonization with MSSA protects against co-colonization with MRSA (Dall'Antonia et al., 2005; Huang et al., 2011). However, it is not yet known if co-colonization can be maintained in pigs. Knowledge of the underlying mechanism of colonization of *S. aureus* in pigs and the dynamics of colonization in an individual animal under controlled conditions is facilitated by the availability of an *in vivo* model. This may also help to understand the successful spread of specific clones and to evaluate intervention methods.

Already some *in vivo* models for studying colonization in pigs have been described (Moodley et al., 2011; Broens et al., 2012; Szabo et al., 2012; Crombe et al., 2012). Although these studies have merits for specific aspects, these models do not allow the challenge with multiple strains in the same quantity or did not collect quantitative data.

The aim of this study was to develop a nose-colonization model in pigs in order to quantitatively study MRSA colonization and possible co-colonization with MSSA.

2. Materials and methods

2.1. Experiment 1: colonization with MRSA

Eight Caesarean Derived, Colostrum Deprived (CD/CD) piglets – from a single sow – were obtained, housed and fed as described before (Dekker et al., 2013). Four piglets were housed per isolator. Nasal samples were taken to assess the presence of staphylococci using a Rayon swab (Copan, Brescia, Italy). Samples were plated on Columbia agar with sheep blood (BA) (Oxoid, Badhoevedorp, The Netherlands) and the swab was enriched in Mueller Hinton Broth with 6.5% NaCl (MH+; Tritium Microbiology, Eindhoven, The Netherlands). After overnight (18–24 h; O/N) incubation at 37 °C BA was visually assessed for the presence of staphylococci and MH+ was plated on Mannitol Salt Agar (MSA, Oxoid, Badhoevedorp, The Netherlands), which was assessed for the presence of *S. aureus*.

MRSA-strain V0608892/1, isolated from the nose of a healthy pig, was used for colonization. This strain was of MLST ST398 (Enright et al., 2000; Van Wamel et al., 2010; Van Meurs et al., 2013), and staphylococcal protein A (*spa*)-type t011 (Harmsen et al., 2003); see Table 1. At the age of 6 days the piglets were administered 500 μL inoculum within 1 h after preparation. The bacterial suspension for colonization was prepared by making a 1:15 dilution of an O/N liquid culture in BHI. Cultures were re-incubated while shaking until an OD_{600} of 0.5 was reached. Sixty milliliter of the log-phase culture was centrifuged at $2000 \times g$ for 10 min and cells were washed with phosphate buffered saline (PBS; Lonza, Belgium); the pellet was suspended in 12 mL PBS. Quantitative culture showed the inoculum contained 3.5×10^8 colony forming units (CFU) per administration (500 μL).

To monitor colonization, nasal samples were obtained at 13 time points (see Fig. 1). Samples were processed as follows within 2 h after sampling: swab was suspended in 1 mL PBS and a serial dilution to 10^{-3} of the original suspension was prepared. Fifty microliter of the undiluted and the 10^{-3} suspension were plated on Brilliance MRSA agar 2 (Oxoid, Badhoevedorp, The Netherlands) using an Eddy-Jet spiral plater (IUL Instruments, Barcelona, Spain) in E-mode. Remainder of the undiluted suspension was cultured in MH+ for enrichment, after O/N incubation MH+ was plated on Brilliance MRSA 2 agar. Plates were enumerated according to the manufacturer's instructions.

Six days after inoculation the piglets received an intranasal administration of 1 mL glycerol per day for 5 days to assess the effect of a carrier that can be used in pharmaceutical preparations.

All bacterial concentrations were transformed to $\log_{10}$ values. The two-tailed Wilcoxon Rank Sum test was used to compare the concentrations at $t = 12$ (before first glycerol administration) and $t = 18$ (after last administration).

2.2. Experiment 2: co-colonization with MRSA and MSSA

Eleven CD/CD piglets were obtained, housed and fed as described above and kept in groups of four, four and three piglets. Animals originated from two sows.

Table 1
Characteristics of the *Staphylococcus aureus* strains that were used for colonization.

	V0608892/1	10KE0026	10KE0027	10KE0028	10KE0029
Origin	Pig, diagnostic screening	Calf, (Graveland et al., 2010)	Calf, (Graveland et al., 2010)	Calf, (Graveland et al., 2010)	Calf, (Graveland et al., 2010)
*mec*A	+	+	–	+	–
*mec*C	–	–	–	–	–
PFGE	N/A	F′	F	A′	A
MLST	ST398	ST398	ST398	ST398	ST398
Spa	t011	t011	t011	t034	t034
RAPD	N/A	A	A	A	A
MLVA	N/A	6-6––	6-6––	6-6––	6-6–4

N/A, data not available.

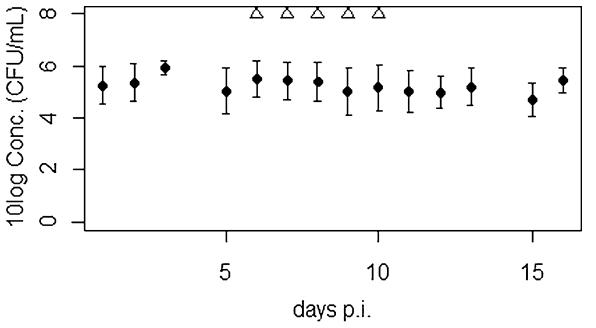

Fig. 1. Experiment 1: mean concentration (CFU/swab) of MRSA ± standard deviation in nasal swabs of pigs in isolators (A) and (B). Each isolator housed four piglets. (Δ) indicate the nasal administration of glycerol. Individual data per pig are shown in Fig. S1.

Nasal samples were obtained before inoculation and analyzed as described above to check for the presence of staphylococci before the start of the experiment.

For inoculation two matching pairs of highly related MRSA and MSSA strains were used. Strains 10KE0026 (MRSA) and 10KE0027 (MSSA) were of ST398, *spa*-type t011. These four strains were isolated from veal calves in a different study (Graveland et al., 2010). None of the isolates contained the *mec*C-gene (Garcia-Alvarez et al., 2011). Additionally, Random Amplification of Polymorphic DNA (RAPD) (Damiani et al., 1996) and Pulsed Field Gel Electrophoresis (PFGE)-typing (Ichiyama et al., 1991; Cookson et al., 2007) was performed. The MLVA-patterns of strains 10KE0026, 10KE0027, and 10KE0028 were identical, but the pattern of strain 10KE0029 had an additional fragment (6-6—4, see Table 1 for strain characteristics).

For the inoculum all strains were prepared as described above and pooled in equal volumes (the ratio between CFU and OD was found to be equal for all isolates) and 2.4×10^7 CFU was delivered to each piglet. After plating the inoculum, 50 colonies were picked and characterized by mecA-PCR and spa-typing to confirm all strains were present in the inoculum.

Nasal swabs were obtained at nine time points in experiment 2 (see Fig. 2) and analyzed as described for experiment 1. Additionally, samples were plated on MSA for the enumeration of all *S. aureus*. The number of MSSA per sample was calculated by subtracting the number of MRSA from the total number of *S. aureus*. Fifteen colonies per sample were picked from MSA to differentiate between MSSA and MRSA at 1 ($n = 165$), 8 ($n = 165$) and 15 ($n = 165$) days after inoculation. Isolates were screened on nutrient agar supplemented with 8 μg/mL oxacillin, which was proven to discriminate between the MSSA and MRSA strains that were used in this study.

Both experiments were performed in accordance with the Dutch law on Animal Welfare and were approved by the ethical committee of Utrecht University under registrations 2010.II.05.098 and 2011.II.11.180.

3. Results

3.1. Experiment 1

All piglets were screened negative for the presence of staphylococci in nasal samples at the age of 5 days, 1 day before inoculation. All samples were quantitatively cultured and mean culture results for MRSA per isolator (four piglets) are shown in Fig. 1; individual data is displayed in Fig. S1. Numbers of MRSA were found of approximately 10^4–10^6 CFU/swab and fluctuated slightly during the experiment. However, the mean concentrations per sampling moment remained stable. Following the Wilcoxon Rank Sum Test no statistically significant change in MRSA concentration after the last administration of glycerol was found when compared to the concentration prior to the first treatment ($p > 0.05$).

Supplementary Fig. S1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vetmic.2014.09.019.

3.2. Experiment 2

All piglets were screened negative for the presence of staphylococci at the age of 5 days, 1 day before inoculation. After plating and quantifying the inoculum fifty colonies were picked randomly from the agar plates with 50–500 colonies and characterized. It confirmed that all strains could be recovered: 14/50 colonies originated of strain 10KE0026 (1.34×10^7 CFU/mL), 3/50 colonies of 10KE0027 (2.88×10^6 CFU/mL), 6/50 colonies of 10KE0028 (5.76×10^6 CFU/mL) and 27/50 colonies of 10KE0029 (2.59×10^7 CFU/mL).

Nasal swabs were quantitatively cultured. There was more MRSA found than the total number of *S. aureus* in five samples – so the concentration of MSSA would be negative – (pig 6 $t = 11$; pig 7, $t = 3$ and $t = 4$; pig 8, $t = 6$; and pig 9,

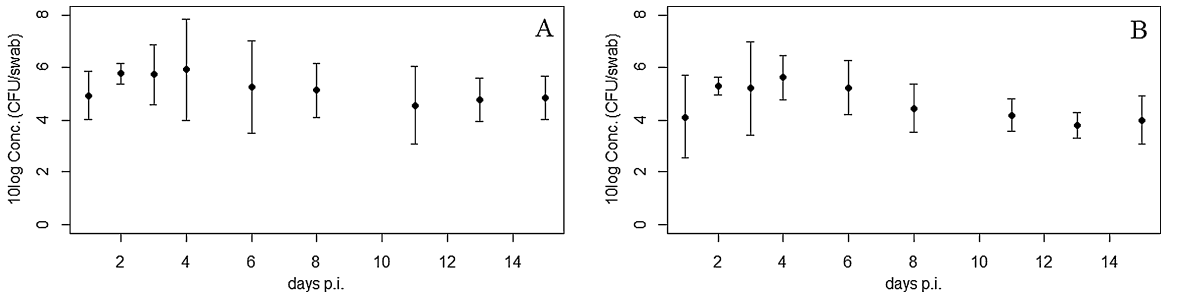

Fig. 2. Experiment 2: mean ± standard deviation of the concentrations of (A) MSSA and (B) MRSA in nasal swabs. Individual data per pig are shown in Fig. S2.

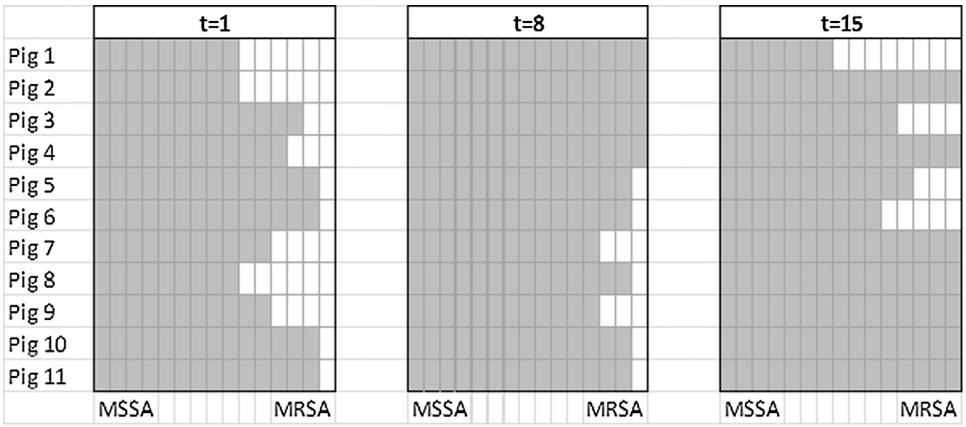

Fig. 3. Presence of MSSA in the nasal samples of the co-colonization in experiment 2. Shaded cells represent MSSA, non-shaded cells represent MRSA. Each cell represents one of the 15 tested colonies for each sample.

$t = 1$). The mean results for the enumeration of MSSA and MRSA are shown in Fig. 2. Individual data per piglet is provided as supplementary data in Figs. S2 and S3. The concentration of MSSA varied mainly between 10^5 and 10^7 CFU/swab and was present in a larger proportion than MRSA, of which the concentrations varied between 10^4 and 10^6 CFU/swab (Wilcoxon Rank Sum test; $p < 0.01$). Fifteen colonies per sample were picked and characterized to distinguish between MRSA and MSSA to confirm the findings of the numbers of MSSA. As can be observed in Fig. 3 colonies of MSSA could be found in these cultures in larger quantities than MRSA, and in some cases MRSA could not be retrieved by this method. Nevertheless, the quantitative culture on Brilliance agar showed that MRSA is clearly present in these samples.

Supplementary Figs. S2 and S3 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vetmic.2014.09.019.

4. Discussion

Colonization of MSSA and MRSA in the nose of piglets was successfully established. In humans there is abundant information about the host factors that play a role in colonization with *S. aureus* (Peacock et al., 2001; Ruimy et al., 2010; Mulcahy et al., 2012). For animals, however, there is no information available about individual susceptibility for *S. aureus* colonization.

In this study the challenge with bacteria was standardized and the inoculum was directly administered into the nose. Therefore, this model offers the possibility to perform quantitative experiments to assess the interaction between strains during colonization and to assess differences in colonization properties of individual strains. This is an asset above a published model, which promoted colonization by secondary inoculation (intra-vaginal infection of the sow and exposure of the piglets during delivery). The latter model does, however, reflect a more natural route of colonization and also practices an early exposure of the piglets that do not yet have a natural flora established in their nose.

The use of CD/CD piglets and housing of the animals in isolators was necessary to successfully establish colonization with the selected strains. Naturally born piglets develop nasal microbiota upon birth, which would limit the ability of the administered strains to colonize the host at a later stage (Broens et al., 2012). The influence of a microbiota – among many other factors – plays an important role in the success of colonization as bacteria need a niche to be able to colonize (Van Belkum et al., 2009; Edwards et al., 2012). Housing the animals in isolators prevents the uptake of other staphylococci from the environment.

Administration of glycerol did not lower the concentrations of MRSA, which showed the robustness of nasal colonization in these animals and the lack of interference with the detection of *S. aureus*. This indicates that this model can be used for testing intervention studies on *S. aureus* colonization, also when glycerol is used as carrier in a pharmaceutical preparation.

Previous studies have shown that the presence of MSSA protects against methicillin-resistant strains (Dall'Antonia et al., 2005; Huang et al., 2011). However, in these studies it is expected that study subjects are challenged with MSSA and MRSA separately (in a natural environment), while in the present study we challenged with both variants simultaneously. As described above, this resulted in co-colonization of MSSA and MRSA. This suggests that when MSSA colonizes first, MRSA can no longer colonize because the attachment-site is already occupied. However, when MSSA and MRSA are administered simultaneously (as described here) they can both colonize because the attachment sites are still available. In a previous study it has been shown that resistance genes on large mobile genetic elements are not a burden for healthcare-associated MRSA (Knight et al., 2013). However, in the present study it was shown that MSSA colonization was statistically higher than MRSA colonization. After characterizing the colonies that were selected at $t = 1$ all strains but MSSA 10KE0027 (ST398, *spa*-type t011) could be retrieved (data not shown). This indicates that strain characteristics other than presence of *mec*A seem to contribute to colonization differences.

Although the strains that were used in the second experiment originated from calves, they were able to colonize pigs in our model. This is in agreement with the observation that MRSA strains of spa type t011 and t034 and MLST ST398 were found in pigs before (Hasman et al., 2010). For inoculation two genetically different MSSA and MRSA strain-pairs were used to increase the chance of colonization in case the hosts showed lower affinity to certain *S. aureus* genotypes. During the course of the experiment the two different MSSA and MRSA strains were not quantitatively differentiated as this exceeded the scope of the experiment.

In conclusion, the described nasal colonization model in piglets is suitable for quantitative analysis of colonization of *S. aureus* in pigs and that MSSA and MRSA can colonize simultaneously in piglets.

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References

Anon., 2009. Analysis of the Baseline Survey on the Prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) in Holdings with Breeding Pigs, in the EU, 2008, Part A: MRSA Prevalence Estimates; on request from the European Commission.

Bloemendaal, A.L., Fluit, A.C., Jansen, W.T., Vriens, M.R., Ferry, T., Amorim, J.M., Pascual, A., Stefani, S., Papaparaskevas, J., Borel Rinkes, I.H., Verhoef, J., 2009. Colonization with multiple *Staphylococcus aureus* strains among patients in European intensive care units. Infect. Control Hosp. Epidemiol. 30, 918–920.

Broens, E.M., Graat, E.A., van de Giessen, A.W., Broekhuizen-Stins, M.J., de Jong, M.C., 2012. Quantification of transmission of livestock-associated methicillin resistant *Staphylococcus aureus* in pigs. Vet. Microbiol. 155, 381–388.

Cookson, B.D., Robinson, D.A., Monk, A.B., Murchan, S., Deplano, A., de Ryck, R., Struelens, M.J., Scheel, C., Fussing, V., Salmenlinna, S., Vuopio-Varkila, J., Cuny, C., Witte, W., Tassios, P.T., Legakis, N.J., van Leeuwen, W., van Belkum, A., Vindel, A., Garaizar, J., Haeggman, S., Olsson-Liljequist, B., Ransjo, U., Muller-Premru, M., Hryniewicz, W., Rossney, A., O'Connell, B., Short, B.D., Thomas, J., O'Hanlon, S., Enright, M.C., 2007. Evaluation of molecular typing methods in characterizing a European collection of epidemic methicillin-resistant *Staphylococcus aureus* strains: the HARMONY collection. J. Clin. Microbiol. 45, 1830–1837.

Crombe, F., Vanderhaeghen, W., Dewulf, J., Hermans, K., Haesebrouck, F., Butaye, P., 2012. Colonization and transmission of methicillin-resistant *Staphylococcus aureus* ST398 in nursery piglets. Appl. Environ. Microbiol. 78, 1631–1634.

Dall'Antonia, M., Coen, P.G., Wilks, M., Whiley, A., Millar, M., 2005. Competition between methicillin-sensitive and -resistant *Staphylococcus aureus* in the anterior nares. J. Hosp. Infect. 61, 62–67.

Damiani, G., Telecco, S., Comincini, S., Sironi, M., Carretto, E., Marone, P., 1996. Comparison of an improved RAPD fingerprinting with different typing methods for discriminating clinical isolates of *Staphylococcus* spp. Eur. J. Epidemiol. 12, 163–169.

Dekker, N., Bouma, A., Daemen, I., Klinkenberg, D., van Leengoed, L., Wagenaar, J.A., Stegeman, A., 2013. Effect of spatial separation of pigs on spread of *Streptococcus suis* serotype 9. PLoS One 8, e61339.

Edwards, A.M., Massey, R.C., Clarke, S.R., 2012. Molecular mechanisms of *Staphylococcus aureus* nasopharyngeal colonization. Mol. Oral Microbiol. 27, 1–10.

Enright, M.C., Day, N.P., Davies, C.E., Peacock, S.J., Spratt, B.G., 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. 38, 1008–1015.

Garcia-Alvarez, L., Holden, M.T., Lindsay, H., Webb, C.R., Brown, D.F., Curran, M.D., Walpole, E., Brooks, K., Pickard, D.J., Teale, C., Parkhill, J., Bentley, S.D., Edwards, G.F., Girvan, E.K., Kearns, A.M., Pichon, B., Hill, R.L., Larsen, A.R., Skov, R.L., Peacock, S.J., Maskell, D.J., Holmes, M.A., 2011. Meticillin-resistant *Staphylococcus aureus* with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect. Dis. 11, 595–603.

Graveland, H., Duim, B., van Duijkeren, E., Heederik, D., Wagenaar, J.A., 2011. Livestock-associated methicillin-resistant *Staphylococcus aureus* in animals and humans. Int. J. Med. Microbiol. 301, 630–634.

Graveland, H., Wagenaar, J.A., Heesterbeek, H., Mevius, D., van Duijkeren, E., Heederik, D., 2010. Methicillin resistant *Staphylococcus aureus* ST398 in veal calf farming: human MRSA carriage related with animal antimicrobial usage and farm hygiene. PLoS One 5, e10990.

Harmsen, D., Claus, H., Witte, W., Rothganger, J., Claus, H., Turnwald, D., Vogel, U., 2003. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. J. Clin. Microbiol. 41, 5442–5448.

Hasman, H., Moodley, A., Guardabassi, L., Stegger, M., Skov, R.L., Aarestrup, F.M., 2010. Spa type distribution in *Staphylococcus aureus* originating from pigs, cattle and poultry. Vet. Microbiol. 141, 326–331.

Huang, S.S., Datta, R., Rifas-Shiman, S., Kleinman, K., Placzek, H., Lankiewicz, J.D., Platt, R., 2011. Colonization with antibiotic-susceptible strains protects against methicillin-resistant *Staphylococcus aureus* but not vancomycin-resistant enterococci acquisition: a nested case-control study. Crit. Care 15, R210.

Ichiyama, S., Ohta, M., Shimokata, K., Kato, N., Takeuchi, J., 1991. Genomic DNA fingerprinting by pulsed-field gel electrophoresis as an epidemiological marker for study of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. 29, 2690–2695.

Knight, G.M., Budd, E.L., Lindsay, J.A., 2013. Large mobile genetic elements carrying resistance genes that do not confer a fitness burden in healthcare-associated meticillin-resistant *Staphylococcus aureus*. Microbiology 159, 1661–1672.

Mongkolrattanothai, K., Gray, B.M., Mankin, P., Stanfill, A.B., Pearl, R.H., Wallace, L.J., Vegunta, R.K., 2011. Simultaneous carriage of multiple genotypes of *Staphylococcus aureus* in children. J. Med. Microbiol. 60, 317–322.

Moodley, A., Latronico, F., Guardabassi, L., 2011. Experimental colonization of pigs with methicillin-resistant *Staphylococcus aureus* (MRSA): insights into the colonization and transmission of livestock-associated MRSA. Epidemiol. Infect. 139, 1594–1600.

Mulcahy, M.E., Geoghegan, J.A., Monk, I.R., O'Keeffe, K.M., Walsh, E.J., Foster, T.J., McLoughlin, R.M., 2012. Nasal colonisation by *Staphylococcus aureus* depends upon clumping factor B binding to the squamous epithelial cell envelope protein loricrin. PLoS Pathog. 8, e1003092.

Peacock, S.J., de Silva, I., Lowy, F.D., 2001. What determines nasal carriage of *Staphylococcus aureus*? Trends Microbiol. 9, 605–610.

Price, L.B., Stegger, M., Hasman, H., Aziz, M., Larsen, J., Andersen, P.S., Pearson, T., Waters, A.E., Foster, J.T., Schupp, J., Gillece, J., Driebe, E., Liu, C.M., Springer, B., Zdovc, I., Battisti, A., Franco, A., Zmudzki, J., Schwarz, S., Butaye, P., Jouy, E., Pomba, C., Porrero, M.C., Ruimy, R., Smith, T.C., Robinson, D.A., Weese, J.S., Arriola, C.S., Yu, F., Laurent, F., Keim, P., Skov, R., Aarestrup, F.M., 2012. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. mBio 3,. http://dx.doi.org/10.1128/mBio.00305-11 (Print 2012).

Ruimy, R., Angebault, C., Djossou, F., Dupont, C., Epelboin, L., Jarraud, S., Lefevre, L.A., Bes, M., Lixandru, B.E., Bertine, M., El Miniai, A., Renard, M., Bettinger, R.M., Lescat, M., Clermont, O., Peroz, G., Lina, G., Tavakol, M., Vandenesch, F., van Belkum, A., Rousset, F., Andremont, A., 2010. Are host genetics the predominant determinant of persistent nasal *Staphylococcus aureus* carriage in humans? J. Infect. Dis. 202, 924–934.

Slingerland, B.C., Tavakol, M., McCarthy, A.J., Lindsay, J.A., Snijders, S.V., Wagenaar, J.A., van Belkum, A., Vos, M.C., Verbrugh, H.A., van Wamel, W.J., 2012. Survival of *Staphylococcus aureus* ST398 in the human nose after artificial inoculation. PLoS One 7, e48896.

Szabo, I., Beck, B., Friese, A., Fetsch, A., Tenhagen, B.A., Roesler, U., 2012. Colonization kinetics of different methicillin-resistant *Staphylococcus aureus* sequence types in pigs and host susceptibilities. Appl. Environ. Microbiol. 78, 541–548.

Van Belkum, A., Melles, D.C., Nouwen, J., van Leeuwen, W.B., van Wamel, W., Vos, M.C., Wertheim, H.F., Verbrugh, H.A., 2009. Co-evolutionary aspects of human colonisation and infection by *Staphylococcus aureus*. Infect. Genet. Evol. 9, 32–47.

Van Cleef, B.A., Broens, E.M., Voss, A., Huijsdens, X.W., Zuchner, L., Van Benthem, B.H., Kluytmans, J.A., Mulders, M.N., Van De Giessen, A.W., 2010. High prevalence of nasal MRSA carriage in slaughterhouse

workers in contact with live pigs in The Netherlands. Epidemiol. Infect. 138, 756–763.

Van den Broek, I.V., van Cleef, B.A., Haenen, A., Broens, E.M., van der Wolf, P.J., van den Broek, M.J., Huijsdens, X.W., Kluytmans, J.A., van de Giessen, A.W., Tiemersma, E.W., 2009. Methicillin-resistant *Staphylococcus aureus* in people living and working in pig farms. Epidemiol. Infect. 137, 700–708.

Van Meurs, M.L., Schellekens, J.J., de Neeling, A.J., Duim, B., Schneeberger, P.M., Hermans, M.H., 2013. Real-time PCR to distinguish livestock-associated (ST398) from non-livestock-associated (methicillin-resistant) *Staphylococcus aureus*. Infection 41, 339–346.

Van Wamel, W.J., Hansenova Manaskova, S., Fluit, A.C., Verbrugh, H., de Neeling, A.J., van Duijkeren, E., van Belkum, A., 2010. Short term micro-evolution and PCR-detection of methicillin-resistant and -susceptible *Staphylococcus aureus* sequence type 398. Eur. J. Clin. Microbiol. Infect. Dis. 29, 119–122.

Wertheim, H.F., Vos, M.C., Ott, A., van Belkum, A., Voss, A., Kluytmans, J.A., van Keulen, P.H., Vandenbroucke-Grauls, C.M., Meester, M.H., Verbrugh, H.A., 2004. Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. Lancet 364, 703–705.