This item is the archived peer-reviewed author-version of:

A longitudinal study of ****Staphylococcus aureus**** colonization in pigs in Ireland

Reference:

Burns A., Shore A.C., Brennan G.I., Malhotra Surbhi, et al.- A longitudinal study of ****Staphylococcus aureus**** colonization in pigs in Ireland

Veterinary microbiology - ISSN 0378-1135 - 174:3-4(2014), p. 504-513

Full text (Publishers DOI): <http://dx.doi.org/doi:10.1016/j.vetmic.2014.10.009>

Handle: <http://hdl.handle.net/10067/1232710151162165141>

A longitudinal study of *Staphylococcus aureus* colonization in pigs in Ireland

A. Burns¹, B.K. Markey¹, A.C. Shore², M.C. Galligan³, J.S. Sabirova⁶, J.F. Gibbons¹, D.C.

Coleman², S. Malhotra-Kumar⁶, M. Gutierrez⁵, J. Egan⁵, S. Fanning⁴, F.C. Leonard^{1*}

¹ School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland

² Microbiology Research Unit, Dublin Dental University Hospital, University of Dublin

Trinity College Dublin, Ireland

³ School of Mathematical Sciences, University College Dublin, Belfield, Dublin 4, Ireland

⁴ School of Public Health, Physiotherapy and Population Science, Science Centre, University

College Dublin, Belfield, Dublin 4

⁵ National Reference Laboratory for Antimicrobial Resistance (Food, Feed and Animal

Health), Department of Agriculture, Food and the Marine, Backweston Laboratories,

Celbridge, Co. Kildare, Ireland

⁶ University of Antwerp, Antwerp, Belgium

***Corresponding author:** Finola Leonard, Veterinary Science Centre, University College

Dublin, Belfield, Dublin 4. Tel: 353 1 7166179; Fax: 353 1 7166185

email: nola.leonard@ucd.ie

26

27 **Abstract**

28 The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in livestock has
29 refocused attention on *S. aureus* colonization and transmission in pigs. This study aimed to
30 determine if the colonization status of a sow has an effect on the colonization status of her
31 piglets, whether pigs carry the same strain of *S. aureus* throughout production and to fully
32 characterise *S. aureus* strains circulating in an Irish pig herd. Six healthy sows and their
33 piglets were serially sampled. Nasal swabs were collected from piglets two days after birth
34 and then two days before and two days after they were moved into each subsequent
35 production stage, i.e. days 2, 17, 21, 45, 49, 96 and 100. The overall average prevalence of *S.*
36 *aureus* colonization on those days was 73 %, 28 %, 36 %, 39 %, 26 % 69 % and 69 %
37 respectively. The odds of being *S. aureus* positive were estimated to be almost 12 times
38 higher for piglets born to nasal-positive sows than those born to negative sows. Furthermore,
39 the odds of being *S. aureus* positive were estimated to be three times higher than this again
40 for piglets born to sows which were both nasal and vaginal positive. Strains isolated from
41 piglets immediately after birth were indistinguishable from those of the dam as shown by
42 phenotypic and molecular typing, including microarray analysis and optical mapping. The
43 effect of the dam decreased as the piglets aged. Mixing of pigs from different sows at
44 weaning was associated with changes in colonization with *S. aureus*. Strains of *S. aureus*
45 isolated from pigs post weaning were more resistant to antibiotics, coinciding with the
46 introduction of in-feed antibiotics. All isolates were CC9 and the majority exhibited a new
47 *spa* type t10449, which has not been identified previously. In conclusion, the findings show
48 that the status of the sow influences the colonization status of her piglets in the early
49 production stages but strains carried by pigs change over time. Multiresistant *S. aureus* was
50 detected, in particular post weaning. The results suggest that sow status and management

practices, including mixing of pigs and antimicrobial usage at weaning, should be considered if implementing control measures for *S. aureus* on a farm.

Keywords

Staphylococcus aureus, pigs, colonization, longitudinal study, typing.

Introduction

Staphylococcus aureus is a common commensal organism of the skin and mucosal membranes of both humans and animals (Werckenthin et al., 2001). Antimicrobial resistance in *S. aureus*, in particular methicillin-resistance, is a concern in both human and veterinary medicine. Livestock-associated methicillin-resistant *S. aureus* (LA-MRSA) ST398 was first identified in Europe in the early 2000s and was found chiefly in pigs and pig handlers (Voss et al., 2005; Meemken et al., 2009). This event refocused attention on staphylococci of animal origin as a potential public health problem, in particular staphylococci of intensively farmed animals such as pigs, which are frequently exposed to high levels of antimicrobials.

Staphylococcus aureus, including MRSA, is a commensal organism in pigs and seldom results in clinical signs in this species although there are some reports of disease in pigs caused by methicillin-susceptible *S. aureus* (MSSA) or MRSA (Armand-Lefevre et al. 2005; Park et al., 2013; Schwarz et al., 2008; van Duijkeren et al., 2007). The transmission dynamics of *S. aureus* in individual pigs are of interest when examining possible control measures for MRSA and other multidrug-resistant *S. aureus* which may emerge in the future. This is especially true in light of recent findings in which colonization with MSSA was found to be protective against MRSA colonization in pig farmers (van Cleef et al., 2014). A small number of studies have investigated the host-organism relationship with *S. aureus* in pigs,

focusing on LA-MRSA; however there are no previous studies on other strains of *S. aureus*. A longitudinal study by Weese et al. (2010) indicated that there was a significant association between sow and piglet colonization. In addition, their findings suggested that colonization was age related (Weese et al., 2010). Studies by Broens et al. (2011) and Verhegghe et al. (2011) also found an association between colonization status of sows and their piglets. However, these studies examined only MRSA and did not evaluate the isolates further, either phenotypically or genotypically (Broens et al., 2011; Verhegghe et al., 2011). No study to date has investigated and characterized strains of methicillin-susceptible *S. aureus* (MSSA) or MRSA colonizing pigs throughout each production stage. Such studies are essential to provide a better understanding of transmission patterns of this pathogen as a basis for the design of future control programmes. A primary objective of this study was to evaluate *S. aureus* colonization in individual pigs over time, including whether pigs carry the same strain of the bacterium throughout production. A second objective was to determine the effect of a sow's colonization status on her piglets. The final objective was to fully characterize *S. aureus* isolated during the different production stages, including antimicrobial resistance patterns. At the time of the study (2011), Irish pigs were considered free of MRSA (EFSA, 2009; Horgan et al., 2011)

Materials and Methods

Animals and sampling

Pigs in a large, 2000-sow farrow-to-finish commercial unit were sampled and found to be positive for *S. aureus*. The unit was selected because it was typical of large units in Ireland and the farmer was willing to cooperate. It was a closed farm whereby all the piglets were born on the farm and no pigs were purchased. There were 30 members of staff who were each assigned to work with pigs in a particular production stage only. First stage weaned pigs received in-feed antimicrobials for the duration of their time in the first stage weaner accommodation (weeks three to seven), receiving tilmicosin (at an inclusion rate of 1kg/tonne feed) from weeks three to five, followed by trimethoprim/sulphadiazine (inclusion rate 2kg/tonne feed) from weeks five to seven. Second stage weaned pigs (weeks seven to 14) received in-feed antimicrobials for the first four days (trimethoprim/sulphadiazine at the same inclusion rate).

A total of 50 sows were screened for *S. aureus* colonization and six sows were selected for inclusion in this study based on their nasal and vaginal colonization status. In line with the study objectives, two sows testing nasal and vaginal negative, two sows testing nasal positive but vaginal negative and two sows testing both nasal and vaginal positive, were selected. All six sows and their litters were studied in parallel and farrowed within one to two days of each other. Each farrowing room housed approximately 14 sows; all six study sows were not housed within the same room. Both nasal and vaginal swabs (Cotton swab, VWR, Ireland) were collected seven days prior to farrowing. Sows and piglets were sampled two days after farrowing. Thereafter, each pig was individually tagged and samples (nasal swabs) were collected from piglets only. A total of 73 pigs was followed from farrow to finish. Piglets were weaned at approximately three weeks of age. Sampling was conducted on days 17 and 21, two days before and two days after moving to first stage weaner accommodation; on days

45 and 49, two days before and after moving to second stage weaner accommodation; and on days 96 and 100, before and after moving to the finishing houses. Following the farrowing stage piglets were merged, at random, into two groups of 30-40 pigs and they stayed in these groups in the first stage and second stage weaner houses. Each group was housed in a separate pen within one room in the first stage weaner house; the room contained four pens and formed one air space. For commercial reasons, there were a few pigs in each of the pens housing the two groups which did not originate from the six litters included in the study. In the finisher stage groups were sub divided into smaller groups but were not mixed with unfamiliar pen-mates. Pens housing finisher pigs were of Trobridge design, i.e. rows of pens side-by-side and opening onto an open-air passage.

Microbiological analysis

Swabs were placed in 10 ml Mueller-Hinton broth (MHB) (Oxoid, UK) supplemented with 6.5 % NaCl (Sigma-Aldrich, Ireland), vortexed and incubated for 18-24 h at 37°C. After incubation samples were plated onto Baird-Parker agar plates (Oxoid, UK) and statically incubated for 24 h at 37°C. Five suspect *S. aureus* colonies from each plate were then sub-cultured onto Columbia Blood Agar Base (Oxoid, UK) supplemented with 5% defibrinated sheep blood (Oxoid, UK) and incubated for 24 h at 37°C. A single colony from the five suspect colonies displaying the typical characteristics of *Staphylococcus aureus* was then selected and tested using a commercial latex agglutination test for clumping factor and protein A (Pastorex Staph-Plus, Bio-Rad, France). Identification of suspect isolates was confirmed using a polymerase chain reaction (PCR) assay, as described previously (Maes et al., 2002; Poulsen et al. 2003).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the disk diffusion method of the Clinical and Laboratory Standards Institute (CLSI, 2009). One hundred and seventy-one of a total of 281 *S. aureus* isolates collected were tested on Mueller-Hinton agar (Oxoid, UK). Isolates were selected in order to ensure pigs from all litters were represented at each sampling point. Isolates from a number of pigs which were positive on multiple occasions were also selected for testing in order to determine changes in isolates from individual pigs over time. All *S. aureus* isolates were tested against gentamicin (10 µg), amikacin (30 µg), kanamycin (30 µg), neomycin (30 µg), streptomycin (25 µg), tobramycin (10 µg), ampicillin (10 µg), erythromycin (15 µg), lincomycin (2 µg), fusidic acid (10 µg), tetracycline (30 µg), vancomycin (30 µg), rifampicin (5 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), mupirocin (5 µg), mupirocin (200 µg), sulphonamide (300 µg), trimethoprim (5 µg), linezolid (30 µg), and ceftiofur (30 µg) (Oxoid, UK). Spectinomycin (500 µg), ethidium bromide (60 µg), mercuric chloride, phenyl mercuric acetate and cadmium acetate (Abtek Biologicals Ltd, UK) were also employed for testing. Zones of inhibition were measured and resistance was determined using the interpretative criteria of the CLSI (2009) and as described by Rossney et al. (2007). The *S. aureus* reference strain NCTC 8325 was used as a control standard. Ceftiofur resistance was used as a marker for MRSA.

Molecular Typing

A total of 60 *S. aureus* isolates was typed using pulsed-field gel electrophoresis (PFGE) following digestion of high molecular weight chromosomal DNA in agarose plugs with the restriction endonuclease *Sma*I as described by O'Mahony et al, (2005). All isolates from sows which were nasal-positive two days after farrowing were typed. A selection of isolates recovered from the piglets from each sow were chosen for typing based on their colony morphology and antimicrobial susceptibility patterns. At least one isolate representing each

antimicrobial resistance pattern was typed. All isolates from a single pig, which was positive on all sampling days (animal no. 230), were typed. The *S. aureus* reference strain NCTC 8325 was used as a control strain.

All isolates that were analysed using PFGE were also *spa* typed. *Spa* typing was performed as described by Shopsisin et al. (1999). The *spa* repeat region was amplified using primers *spa*-1113F (5'-AAAGACGATCCTTCGGTGAGC-3') and *spa* 1514R (5' CAGCAGTAGTGCCGTTTGCTT-3'), described previously by Hasman et al. (2009). The *spa* types were determined using Ridom StaphType 1.5.0 software and clustered in *spa*-derived clonal complexes (*spa*-CC) by Based Upon Repeat Pattern (BURP) as described by Mellmann et al. (2007).

A subgroup of 23 isolates from the 60 isolates was subjected to DNA microarray analysis using the StaphyType Kit (Alere Technologies GmbH, Germany) as described by Monecke et al (2008). These isolates were chosen in order to ensure at least one of each antimicrobial susceptibility pattern, PFGE pattern and *spa* type was analyzed. In addition, isolates obtained from pigs that were repeatedly positive for *S. aureus* during production were analyzed.

Two isolates, one from pig no. 230 (230 D2) and one from its dam (no. 4069N), both obtained on day 2 after farrowing, were analysed using whole genome mapping. The isolates were gently lysed using Argus® HMW DNA isolation kit (Opgen, Inc) to generate high molecular weight DNA (HMW-DNA). HMW-DNA was loaded on a MapCard and digested in-situ with the restriction endonuclease *Nco*I, stained with a fluorescent DNA dye, JoJo-1 (Invitrogen), and processed using the Argus Optical Mapping System (OpGen, Inc). The *Nco*I-generated restriction fragments detected by the Argus system were re-assembled into overlapping contigs to eventually create a circular restriction map. Generated restriction maps were compared with *in silico* maps of all sequenced *S. aureus* strains available in the NCBI database and edited using an *in silico* generated optical map of *S. aureus* ECT-R2 (NCBI

accession no: NC_017343) as a reference. Edited maps were then compared with each other using MapSolver (OpGen, Inc).

Statistical analysis

A logistic mixed effects model was fitted to the data using the lmer package (Bates et al., 2012) in R (R Development Core Team, 2012) to investigate the probability of a piglet having *S. aureus*, depending on the status of its dam. Random effects were included for pig and sow. Covariates/cofactors included age, sow status and production stage. The *S. aureus* status of the sow seven days pre-farrowing was included as two indicator variables; N_pos for nasal positive pre-farrowing, and NV_pos for vaginal and nasal positive. When both indicators are set to zero, the model predicts the *S. aureus* status of piglets from sows that were *S. aureus*-negative in both the nose and vagina.

Linear, quadratic and cubic terms for age and interaction effects between age and indicators of sow status were included. The quadratic and cubic terms included were age² and age³, divided by 100 and 1000 respectively to ensure numerical stability of the model-fitting algorithm.

To investigate the effect of moving between production stages on the probability of a piglet testing positive for *S. aureus*, three indicator variables were used. Production stage 1 (birth to weaning, farrowing house) was set to be the baseline level and the effect of each production stage was estimated relative to stage 1. The p-values were calculated from likelihood ratio tests (LRT) obtained by comparing reduced models to the full model, fitted with all terms of interest. Terms of interest included age (linear, quadratic and cubic), production stage (3 indicator variables), sow status and all two-way interactions between sow status with age. The model was then re-fitted with only statistically significant terms included.

Results

Sow status

Nasal swabs from a total of 16 of the 50 sows sampled seven days prior to farrowing were positive and of these, four sows were positive in both the vagina and nares. Six of the sows were chosen for inclusion in this study as follows; two nasal positive sows, two nasal and vaginal positive sows and two negative sows. Of the six sows that were chosen and sampled two days after farrowing both of the nasal positive sows remained colonised, one of the nasal and vaginal positive sows was positive at both sites and only one of the negative sows was negative at the time of this sampling. Individual piglets from each sow were followed from farrowing to the finishing stage.

Piglet carriage of S. aureus

Figure 1 shows the average prevalence of *S. aureus* carriage in piglets from litters grouped according to the status of the sows seven days prior to farrowing. Additional information on how many individual piglets changed or maintained their carriage status between sampling points is given in Tables S1a to S1d in supplemental materials. One pig tested positive on all seven sampling occasions (pig 230) and all samples from one pig tested negative. Most piglets (60 of 73) changed status at least twice during the course of the study. The highest prevalence of *S. aureus* carriage was observed on day two following farrowing (in piglets of positive sows), but decreased prior to leaving this production stage. The lowest prevalence of *S. aureus* was observed in piglets from the negative sows. The prevalence of *Staphylococcus aureus* increased in pigs from all sows on day 21, two days after weaning, with a further increase on day 45, just prior to leaving first stage weaner accommodation. The prevalence of *S. aureus* decreased in pigs on day 49 but another increase was observed at day 96 prior to moving to finishing houses.

Statistical analysis

The significance of the effect of the nasal status of the sow on the *S. aureus* status of the piglets was assessed from the fitted model by examining together the main effect and the two interaction effects (with the linear and quadratic terms for age) (Table 1).

The main effects alone are not statistically significant at the 0.05 level (although the main effect for nasal status of the sow is statistically significant at the 0.1 level). However, the interaction effects between age (linear and quadratic term) and nasal status of the sow, as well as the interaction between age (linear term) and vaginal status of the sow are highly significant ($p < 0.01$) (Table 1).

The odds of a piglet being *S. aureus* positive were predicted to be almost 12 times higher (Odds Ratio (OR) = 11.822) for pigs born to sows that were nasal positive at the farrowing stage, than for those born to sows that were nasal negative (Table 1). The odds of a piglet being *S. aureus* positive were estimated to be a further 3 times higher for a piglet born to a sow who was also vaginal positive, compared to piglets from sows that were nasal positive alone (OR = 3.149). The effect of the sow on the bacterial status of piglets (for sows that are nasal positive) reduced as piglets got older (Figure 2). By the time the pigs were approximately 60 days old, the predicted odds ratio was greatly reduced (to approximately 0.5), indicating that piglets from sows that were nasal positive pre-farrowing are predicted to be only half as likely to be *S. aureus* positive than piglets from sows that were *S. aureus* negative. Finally, the predicted difference in the probability of testing *S. aureus* positive between piglets from nasal positive and negative sows reduced towards zero as the pigs reached the final stage of production (i.e. the odds ratio moved towards one) (Figure 2). Figure S1 shows that piglets from sows that were vaginal positive pre-farrowing have a higher predicted odds of testing *S. aureus* positive than those piglets from sows that were not, but after they are around 60 days old, their odds of being *S. aureus* positive are predicted to

become lower than for piglets from sows that were either *S. aureus* negative, or only nasal positive.

In comparison to piglets in the farrowing house, the probability of pigs testing positive for *S. aureus* was estimated to be lowest in second stage weaned pigs. The indicator variable for second stage weaner house was the only significant indicator variable of the three included for production stage, and hence is the only one of the three included in the final model (Table 1). The estimated odds ratio for pigs being nasal positive on entering the second stage weaner production stage is approximately 0.25 ($p=0.03$), i.e. the odds of piglets being *S. aureus* positive during production stage weaner 2 was estimated to be about four times less than at other stages of production.

Antimicrobial Resistance

In total, 175? isolates identified as *Staphylococcus aureus* by PCR assay were subjected to antimicrobial susceptibility testing and results are shown in Table 2 with more details given in Table S2. MRSA was not detected, based on ceftiofur resistance. All isolates from sows were resistant to tetracycline and 65 % of isolates from piglets on day 2 had the same resistance pattern as that of the sows. The resistance patterns post weaning were different from those pre-weaning. Resistance to ampicillin, erythromycin and lincomycin was commonly observed in isolates from first and second stage weaners. Tetracycline resistance was not observed in the *S. aureus* isolates from first and second stage weaners but was observed in a small percentage (0.06 %) of isolates from pigs in the finisher stage. A variety of resistance patterns was detected in isolates from pigs in the finishing stage (Tables 2 and S2).

Molecular Typing

A total of 60 isolates were typed using pulsed field electrophoresis (PFGE) and two *Sma*I-PFGE patterns, A and B, were identified (Figure S2). All isolates except one were assigned to pattern A (n=59).

The same 60 isolates which were typed using PFGE analysis were subjected to *spa* typing. Two different *spa* types were identified with t10449 (n=55) being the dominant type over t1334 (n=5).

A total of 23 isolates were characterised using DNA microarray profiling for assigning isolates to MLST clonal complexes (CCs) and sequence types (STs) and for the detection of genes encoding antibiotic resistance and virulence genes (Table 3). All isolates were negative for (i) the Panton-Valentine leukocidin (PVL) toxin genes, (ii) the exfoliative and enterotoxin genes, and (iii) the immune evasion complex genes. A number of isolates contained genes encoding resistance to β -lactam [*bla*Z], tetracycline [*tet*(K)], pleuromutilin-streptogramin A [*vga*(A)] and macrolide-lincosamide-streptogramin (MLS) antimicrobials [*erm*(C)] antimicrobials. One isolate did not harbour any resistance genes. A total of eight isolates harboured *bla*Z, *erm*(C) and *vga*(A). Other isolates only harboured one resistance gene such as *tet*(K) or *vga*(A).

Table 3 summarizes the genotypic characteristics of 23 isolates. All isolates belonged to clonal complex 9 (CC9). Isolates from both sows which were nasal-positive harboured the resistance gene *tet*(K) and were phenotypically resistant to tetracycline. Pig 230 was the only pig that was positive for *S. aureus* on all seven sampling occasions. However, isolates from this pig harboured different resistance genes and exhibited different resistance phenotypes at different production stages. Isolates obtained post weaning had a greater number of resistance genes.

326

327 Pig 155, whose dam was both nasal and vaginal positive, was positive for *S. aureus* on four
328 of the seven sampling occasions. Similar to pig 230, isolates from pig 155 had a greater
329 number of resistance genes post weaning. One isolate from one pig (205 D2), whose dam was
330 negative for *S. aureus*, did not harbour any detectable resistance genes.

331 Whole genome mapping (WGM) was employed to further corroborate the clonal relatedness
332 of *S. aureus* isolates recovered from piglet no. 230 and its dam, sow 4069, immediately after
333 birth (230 D2 and 4069N, respectively). Matrix similarity cluster using unweighted-pair
334 group method using arithmetic averages (UPGMA) showed 100% map similarity between the
335 two strains (Figure 3a). Moreover, comparison of the two maps with those of *in silico*
336 generated optical maps of *S. aureus* reference strains identified their close homology to *S.*
337 *aureus* ECT-R2 (97.5% of map similarity) (Figure 3b), which recently caused a clonal
338 outbreak in Sweden (Lindqvist et al., 2012). *S. aureus* ECT-R2 was originally isolated from
339 a human wound and is a multiresistant methicillin-susceptible *S. aureus* (Lindqvist et al.,
340 2012). Comparison of 230 D2 and 4069N optical maps with the *in silico* map of ECT-R2
341 identified a genomic deletion in 230 D2 and 4069N; both isolates lack a 38265 bp part of a
342 genomic island which is present in ECT-R2. The island contains part of bacteriophage ϕ 11,
343 encoding phage-related functions responsible for lysogeny, DNA replication and
344 transcriptional regulation (ECTR2_1841 to ECTR2_1874) and also some phage-unrelated
345 genes encoding haemolysin, cytolysin, chaperonin GroL, accessory gene regulator B family
346 protein, staphylococcal AgrD family protein and accessory gene regulator C (ECTR2_1876
347 to ECTR2_1891) (Figure 3c). The partial loss of ϕ 11 was also detected in the genomes of
348 some isolates of livestock-associated MRSA, namely RF122, LGA251, ED133, and ST398,
349 as well as in *S. aureus* COL, whereas its intact copy was found in genomes of *S. aureus*

NCTC8325, Newman, USA300 (FPR3757 and TCH1516), JKD6008, MSSA476, MRSA252, and in 71193.

Discussion

This study investigated the transmission and persistence of *S. aureus* in individual pigs throughout the production cycle. Significant findings include the identification and detailed characterization for the first time of *S. aureus* isolates from Irish pigs and demonstration of the influence of the *S. aureus* colonization status of the sow on the status of her piglets. In addition, this study provided further information on the possible influence of management practices at weaning on *S. aureus* colonization patterns. It documented changes in the antimicrobial resistance profile of *S. aureus* over time and provided data that suggest that antimicrobial use may be an important factor promoting these changes. Antimicrobial use is also thought to be a factor in the emergence and transmission of LA-MRSA in pigs (Crombe et al., 2013) and deserves further investigation.

With regard to the impact of the colonization status of the sow on the colonization status of her piglets, piglets born to sows which carried *S. aureus* prior to farrowing were more likely to carry *S. aureus* at two days after birth than piglets born to negative sows. This finding confirms the results of a small number of other studies that examined MRSA nasal colonization of sows and piglets (Verheghe et al., 2011; Weese et al., 2010). However this study also examined the effect of a sow being both nasal and vaginal positive on her piglet's status, which has not been reported previously. For piglets from nasal- and vaginal-positive sows, the odds of being *S. aureus* positive were predicted to be three times higher than for piglets born to nasal positive sows, which is consistent with the suggestion by Moodley et al. (2011) that the likely source of MRSA transmission from sows to piglets was through direct

contact with the snout, skin and vagina of the sows. Previous reports on transmission of *Staphylococcus hyicus* from sow to piglet (Wegener and Skov-Jensen, 1992) also provide evidence that transmission of staphylococci from the vagina to the piglet at time of farrowing is significant. In addition, the antimicrobial resistance patterns (Table 2), microarray and whole genome mapping (Table 3, Figure 5) data from this study strongly support the view that both the sows and the piglets on day 2 carry the same strain of *S. aureus*. In addition, results indicated that the effect of dam status decreased over time. The predicted odds ratio of a piglet from a nasal-positive sow being positive compared to a piglet from a nasal-negative sow fell below zero after approximately day 40 (Figure 2). This suggests that being born to a positive sow may be in some way protective against *S. aureus* carriage at later production stages. This finding is worthy of further study as a recent publication reported that prior colonization with MSSA appeared to be protective against acquisition of MRSA in pig farmers (van Cleef et al., 2014).

The prevalence of *S. aureus* varied in each production stage. This study found the average carriage rate of *S. aureus* was at its highest on day 2 after farrowing, followed by a decrease prior to weaning. Similar findings were reported by Weese et al. (2010) and Verheghe et al. (2011) for MRSA. These results, together with the finding that the great majority of pigs changed carriage status at least twice during the 100-day study suggests that piglets are normally transiently rather than permanently colonized from birth. The prevalence of carriage in all pigs increased post weaning on day 21 and showed a further increase when pigs were sampled on day 45. Weese et al, (2010) and Dewaele et al, (2011) suggested that the increase in MRSA-positive pigs recorded at weaning was due to the commingling of positive and negative pigs, stress during weaning, age related susceptibility and contamination of other sites on the farms. Weaning may represent a point at which controls could be implemented in

order to reduce transmission of *S. aureus* of public health significance such as LA-MRSA.

Possible control measures could include minimizing the mixing of litters at weaning and reducing antimicrobial use.

The findings of this study are similar to those of previous studies with the major difference that the highest prevalence of *S. aureus* in pigs was observed on days 2 and 96 rather than at weaning. One possible explanation is that this study sampled sows which were positive and negative for *S. aureus* prior to and two days after farrowing whereas the study by Weese et al. (2010) sampled sows which were negative at the time of farrowing. The findings of the study reported here and those of Weese et al. (2010) suggest that the colonization status of the sow has a significant influence on a piglet's colonization status in the farrowing stage.

A previous longitudinal study by Broens et al. (2011b) suggested that pigs which received antimicrobial agents such as β -lactam antibiotics and tetracyclines were at a higher risk of MRSA ST398 colonization (Broens et al., 2011). Pigs in this study received macrolide in-feed antibiotics (tilmicosin) upon entering first stage weaner pens and this coincided with the detection of a multidrug resistant (MDR) strain of *S. aureus* resistant to the macrolides (erythromycin). The feed used was changed shortly before pigs entered 2nd stage weaner accommodation to a different feed medicated with potentiated sulphonamides. The reduction in prevalence of *S. aureus* observed at this time suggests that the use of antibiotics containing sulphonamides and trimethoprim to which the *S. aureus* was susceptible, may have contributed to the reduction in prevalence. However, once the use of in-feed antimicrobials ceased after day 51, increased carriage of *S. aureus* was detected on day 96. Coinciding with cessation of in-feed antimicrobials, the percentage of multi-resistant isolates decreased on

days 96 and 100 and a much greater variety of antimicrobial resistance patterns was detected at this time (Table 3).

All isolates investigated in this study were identified as belonging to CC9-MSSA, a lineage not previously reported in Ireland. Previous studies have reported *S. aureus* ST9 colonization in pigs in China and Malaysia (Neela et al., 2009; Wagenaar et al., 2009), including methicillin-resistant strains. This lineage appears to predominate in Asia whereas CC398 is dominant in North America and Europe (De Neeling et al., 2007; Khanna et al., 2008). However, *S. aureus* CC9 has been reported in France and Italy in both pigs and humans working with pigs (Armand-Lefevre et al., 2005; Battisti et al., 2009). Other studies have also reported this lineage in pigs, cattle and poultry in Europe and occasionally in healthy humans (Grundmann et al., 2002; Hasman et al., 2009, Richter et al., 2012; Monecke et al., 2013).

The majority of the 60 isolates which were *spa* typed belonged to a new *spa* type, t10449. This *spa* type has not been reported previously in animals or humans and belongs to CC9. A small number of isolates in this study belonged to another *spa* type, t1334 (4 repeat differences from t10449). This *spa* type has been previously found in the USA and clusters with t337, a dominant *spa* type associated with CC9 (Dressler et al., 2012).

The microarray data in relation to the resistance genes supports most of the phenotypic findings. However, resistance was detected against some antimicrobial agents the genes for which may not be located on the microarray and this is considered a limitation of the method rather than a questionable result. For example, the gene for trimethoprim resistance located on the array is *dfrS1*. This gene is common in staphylococci however other trimethoprim resistance genes such as *dfr(G)* have been observed in staphylococci in healthy pigs

(Stegmann and Perreten, 2010). A novel trimethoprim resistance gene, designated *dfpK* has been found in porcine MRSA ST398 isolates (Kadlec et al., 2012). These genes are not located on the microarray.

Utilizing high resolution WGM, we found the two strains isolated from piglet 230 and its dam to be completely identical and closely related to the human outbreak strain ECT-R2, which harbors only remnants of an *SCCmec* element and is a multiresistant methicillin-susceptible *S. aureus* (Lindqvist et al., 2012). In addition, in comparison to ECT-R 2, both porcine strains showed a genomic deletion comprising bacteriophage ϕ 11 genes as well as genes encoding a number of virulence factors, such as haemolysin, cytolysin and accessory gene regulators. This genomic downscaling has been suggested to be an adaptive mechanism to alleviate fitness costs (Lynch, 2006). It thus seems plausible to suggest that the success of this clone as livestock-associated *S. aureus* might be related to the simplification of its genome. Prolonged treatment with high levels of non-beta-lactam antibiotics as has occurred in Ireland for many years (Beechinor, 2011) would require resistance mechanisms other than those against beta-lactam antimicrobial agents to develop and would mean high fitness costs associated with the simultaneous presence of genes for methicillin resistance. This could thus be one of the reasons for the partial deletion of *SCCmec* element in *S. aureus* resulting in methicillin susceptibility (Deplano et al., 2000; Grubb and Annear, 1972; Noto et al., 2008).

In conclusion, this study found that the colonization status of the sow has an important influence on the colonization status of her piglets but that this effect decreases as the piglets get older and move through the production stages. The findings suggest that consideration of sow status and management practices such as mixing of pigs at weaning and antimicrobial usage, would be useful in the formulation of strategies to control LA-MRSA or other strains of MSSA of public health significance which may emerge in pigs in the future.

Acknowledgements

This research was funded by the Department of Agriculture, Food and the Marine under the Food Institutional Research Measure, project 08 RD D 680, National Development Programme. The authors wish to express their thanks to the farmer and staff who facilitated the study on their unit and to Dr G. Sayers who assisted with the statistical analysis.

References

- Armand-Lefevre, L., Ruimy, R., Andremont, A., 2005, Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. *Emerging infectious diseases* 11, 711 - 714.
- Bates, D., Maechler, M. and Bolker, B. 2012, lme4: Linear mixed-effects models using Eigen and EigenClasses (2012). R package version 0.999999-0 <http://CRAN.R-project.org/package=lme4>
- Battisti, A., Franco, A., Merialdi, G., Hasman, H., Iurescia, M., Lorenzetti, R., Feltrin, F., Zini, M., Aarestrup, F.M., 2009, Heterogeneity among methicillin-resistant *Staphylococcus aureus* from Italian pig finishing holdings. *Veterinary Microbiology* 142, 361-366.
- Beechinor, J.G. (2011). Report on consumption of veterinary antimicrobials in Ireland during 2010. Available at: http://www.imb.ie/images/uploaded/documents/Report_consumption_vet_antimicrobial_2010_Nov_2011_final_clean.pdf
- Broens, E.M., Espinosa-Gongora, C., Graat, E.A.M., Vendrig, N., Van Der Wolf, P.J., Guardabassi, L., Butaye, P., Nielsen, J.P., De Jong, M.C.M., Van De Giessen, A.W.,

497 2011, Longitudinal study on transmission of MRSA CC398 within pig herds. BMC
 498 veterinary research 8, 58.

499 CLSI (2009). Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved
 500 Standard—Tenth Edition (M02-A10). Clinical and Laboratory Standards Institute,
 501 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

502 De Neeling, A.J., van den Broek, M.J.M., Spalburg, E.C., van Santen-Verheuvél, M.G.,
 503 Dam-Deisz, W.D.C., Boshuizen, H.C., van de Giessen, A.W., van Duijkeren, E.,
 504 Huijsdens, X.W., 2007, High prevalence of methicillin resistant *Staphylococcus*
 505 *aureus* in pigs. Veterinary Microbiology 122, 366-372.

506 Deplano A, Tassios PT, Glupczynski Y, Godfroid E, Struelens MJ (2000) In vivo deletion of
 507 the methicillin resistance mec region from the chromosome of *Staphylococcus aureus*
 508 strains. Journal of Antimicrobial Chemotherapy 46: 617–620.

509 Dewaele, I., Messens, W., De Man, I., Delputte, P., Herman, L., Butaye, P., Heyndrickx, M.,
 510 Rasschaert, G., 2011, Sampling, prevalence and characterization of methicillin-
 511 resistant *Staphylococcus aureus* on two Belgian pig farms. Veterinary Science
 512 Development 1, e1.

513 Dressler, A., Scheibel, R., Wardyn, S., Harper, A., Hanson, B., Kroeger, J., Diekema, D.,
 514 Bender, J., Gray, G., Smith, T., 2012, Prevalence, antibiotic resistance and molecular
 515 characterisation of *Staphylococcus aureus* in pigs at agricultural fairs in the USA.
 516 Veterinary Record 170, 495-495.

517 EFSA, 2009, Analysis of the baseline survey on the prevalence of methicillin-resistant
 518 *Staphylococcus aureus* (MRSA) in holdings with breeding pigs, in the EU 2008 [1] -
 519 part a: MRSA prevalence estimates. EFSA Journal 17, 1376-1458.

520 Grubb WB, Annear DI (1972) Spontaneous loss of methicillin resistance in *Staphylococcus*
 521 *aureus* at room-temperature. Lancet 2,1257.

522 Grundmann, H., Hori, S., Enright, M.C., Webster, C., Tami, A., Feil, E.J., Pitt, T., 2002,
523 Determining the genetic structure of the natural population of *Staphylococcus aureus*:
524 a comparison of multilocus sequence typing with pulsed-field gel electrophoresis,
525 randomly amplified polymorphic DNA analysis, and phage typing. Journal of Clinical
526 Microbiology 40, 4544-4546.

527 Hasman, H., Moodley, A., Guardabassi, L., Stegger, M., Skov, R., Aarestrup, F.M., 2009,
528 *Spa* type distribution in *Staphylococcus aureus* originating from pigs, cattle and
529 poultry Veterinary Microbiology 141, 326-331.

530 Ho, J., O'Donoghue, M., Guardabassi, L., Moodley, A., Boost, M., 2012, Characterization of
531 Methicillin Resistant *Staphylococcus aureus* Isolates from Pig Carcasses in Hong
532 Kong. Zoonoses and Public Health 59, 416-423.

533 Horgan, M., Abbott, Y., Lawlor, P.G., Rossney, A., Coffey, A., Fitzgerald, G.F., McAuliffe,
534 O., Paul Ross, R., 2011, A study of the prevalence of methicillin-resistant
535 *Staphylococcus aureus* in pigs and in personnel involved in the pig industry in
536 Ireland. The Veterinary Journal. 190, 255-259

537 Kadlec, K., Fessler, A., Hauschild, T., Schwarz, S., 2012, Novel and uncommon
538 antimicrobial resistance genes in livestock-associated methicillin-resistant
539 *Staphylococcus aureus*. Clinical Microbiology and Infection 18, 745-755.

540 Khanna, T., Friendship, R., Dewey, C., Weese, J.S., 2008, Methicillin resistant
541 *Staphylococcus aureus* colonization in pigs and pig farmers. Veterinary Microbiology
542 128, 298-303.

543 Lindqvist M, Isaksson B, Grub C, Jonassen TO, and Hallgren A (2012) Detection and
544 characterisation of SCCmec remnants in multiresistant methicillin-susceptible
545 *Staphylococcus aureus* causing a clonal outbreak in a Swedish country. European
546 Journal of Clinical Microbiology and Infectious Disease 31, 141-147.

547 Lynch M (2006) Streamlining and simplification of microbial genome architecture. Annu
548 Reviews in Microbiology 60, 327–349.

549 Maes, N., Magdalena, J. Rottiers, S., De Gheldre Y. and M. J. Struelens. 2002.
550 Evaluation of a triplex PCR to Discriminate *Staphylococcus aureus* from Coagulase-Negative
551 Staphylococci and to Determine Methicillin Resistance from Blood Cultures. Journal
552 of Clinical Microbiology, 40, 1514 -1517.

553 Meemken D, Blaha T, Tegeler R, Tenhagen BA, Guerra B, Hammerl JA, Hertwig S,
554 Käsbohrer A, Appel B, Fetsch A. (2010). Livestock associated methicillin-resistant
555 *Staphylococcus aureus* (LaMRSA) isolated from lesions of pigs at necropsy in
556 northwest Germany between 2004 and 2007. Zoonoses Public Health. 2010
557 Dec;57(7-8):e143-8

558 Mellmann A, Weniger T, Berssenbrügge C, Rothgänger J, Sammeth M, Stoye J, Harmsen D.
559 2007. Based Upon Repeat Pattern (BURP): an algorithm to characterize the long-term
560 evolution of *Staphylococcus aureus* populations based on spa polymorphisms.
561 BMC Microbiology, 29;7:98.

562 Moodley, A., Latronico, F., Guardabassi, L., 2011, Experimental colonization of pigs with
563 methicillin-resistant *Staphylococcus aureus* (MRSA): insights into the colonization
564 and transmission of livestock-associated MRSA. Epidemiology and infection 139,
565 1594-1600.

566 Monecke S, Ruppelt A, Wendlandt S, Schwarz S, Slickers P, Ehricht R, Jäckel SC. (2013)
567 Genotyping of *Staphylococcus aureus* isolates from diseased poultry. Vet
568 Microbiol;162(2-4):806-12.

569

570 Neela, V., Zafrul, A.M., Mariana, N.S., Van Belkum, A., Liew, Y.K., Rad, E.G., 2009,
571 Prevalence of ST9 methicillin-resistant *Staphylococcus aureus* among pigs and pig
572 handlers in Malaysia. *Journal of Clinical Microbiology* 47, 4138-4140.

573 Noto MJ, Fox PM, Archer GL (2008) Spontaneous deletion of the methicillin resistance
574 determinant, *mecA*, partially compensates for the fitness cost associated with high-
575 level vancomycin resistance in *Staphylococcus aureus*. *Antimicrobial Agents and*
576 *Chemotherapy* 52: 1221–1229.

577 O'Mahony, R., Abbott, Y., Leonard, F. C., Markey, B. K., Quinn, P. J., Pollock, P. J.,
578 Fanning, S. & Rossney, A. S. (2005) Methicillin-resistant *Staphylococcus aureus*
579 (MRSA) isolated from animals and veterinary personnel in Ireland. *Veterinary*
580 *Microbiology* 109, 285-296

581 Park J, Friendship RM, Poljak Z, Weese JS, Dewey CE. (2013). An investigation of
582 exudative epidermitis (greasy pig disease) and antimicrobial resistance patterns of
583 *Staphylococcus hyicus* and *Staphylococcus aureus* isolated from clinical cases.
584 *Can Vet J*.;54(2):139-44.

585 Poulsen, A.B., Skov, R., Pallesen, L.V., 2003. Detection of methicillin resistance in
586 coagulase-negative staphylococci and in staphylococci directly from simulated blood
587 cultures using the evigene MRSA detection kit. *Journal of Antimicrobial.*
588 *Chemotherapy*. 51, 419-421.

589 R Development Core Team, 2012. R: A Language and Environment for Statistical
590 Computing. R Development Core Team. R Foundation for Statistical Computing,
591 Vienna, Austria. <http://www.R-project.org/>

592 Richter, A.; Sting, R.; Popp, C.; Rau, J.; Tenhagen, B. A.; Guerra, B.; Hafez, H. M.; Fetsch,
593 A. (2012). Prevalence of types of methicillin-resistant *Staphylococcus aureus* in

turkey flocks and personnel attending the animals. *Epidemiology and Infection*, 140, 12, 2223-2232,

Rossney AS, Shore AC, Morgan PM, Fitzgibbon MM, O'Connell B, Coleman DC. 2007. The emergence and importation of diverse genotypes of methicillin-resistant *Staphylococcus aureus* (MRSA) harboring the Panton-Valentine leukocidin gene (pvl) reveal that pvl is a poor marker for community-acquired MRSA strains in Ireland. *Journal of Clinical Microbiology*, 45, 2554-2563.

Schwarz, S., Kadlec, K., and Strommenger, B. (2008). Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* detected in the Bft-GermVet monitoring programme 2004–2006 in Germany. *J. Antimicrob. Chemother.* 61, 282–285.

Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, Bost DA, Riehman M, Naidich S, Kreiswirth BN. 1999. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *Journal of Clinical Microbiology*, 37, 3556-3563.

Stegmann, R., Perreten, V., 2010, Antibiotic resistance profile of *Staphylococcus rostri*, a new species isolated from healthy pigs. *Veterinary Microbiology* 145, 165-171.

van Cleef BA, van Benthem BH, Verkade EJ, van Rijen M, Kluytmans-van den Bergh MF, Schouls LM, Duim B, Wagenaar JA, Graveland H, Bos ME, Heederik D, Kluytmans JA. (2014). Dynamics of MRSA and MSSA carriage in pig farmers: a prospective cohort study. *Clin Microbiol Infect.* 2014 Feb 4. doi: 10.1111/1469-0691.12582. [Epub ahead of print

van Duijkeren E, Jansen MD, Flemming SC, de Neeling H, Wagenaar JA, Schoormans AH, van Nes A, Fluit AC. (2007). Methicillin-resistant *Staphylococcus aureus* in pigs with exudative epidermitis. *Emerg Infect Dis.*;13(9):1408-10

618 Verhegghe, M., Pletinckx, L.J., Crombe, F., Van Weyenberg, S., Haesebrouck, F., Butaye, P.,
 619 Heyndrickx, M., Rasschaert, G., 2011, Cohort study for the presence of livestock-
 620 associated MRSA in piglets: effect of sow status at farrowing and determination of
 621 the piglet colonization age. *Veterinary Microbiology* 162, 679-686.

622 Voss, A., Loeffen, F., Bakker, J., Klaassen, C., Wulf, M., 2005, Methicillin-resistant
 623 *Staphylococcus aureus* in Pig Farming. *Emerging Infectious Diseases* 11, 1965-1966.

624 Wagenaar, J.A., Yue, H., Pritchard, J., Broekhuizen-Stins, M., Huijsdens, X., Mevius, D.J.,
 625 Bosch, T., Van Duijkeren, E., 2009, Unexpected sequence types in livestock
 626 associated methicillin-resistant *Staphylococcus aureus* (MRSA): MRSA ST9 and a
 627 single locus variant of ST9 in pig farming in China. *Veterinary Microbiology* 139,
 628 405-409.

629 Weese, J.S., Zwambag, A., Rosendal, T., Reid-Smith, R., Friendship, R., 2010, Longitudinal
 630 Investigation of Methicillin-Resistant *Staphylococcus aureus* in Piglets. *Zoonoses and*
 631 *Public Health* 58, 238-243.

632 Wegener HC, Skov-Jensen EW. 1992. A longitudinal study of *Staphylococcus hyicus*
 633 colonization of vagina of gilts and transmission to piglets. *Epidemiology and*
 634 *Infection*, 109(3):433-44.

635 Werckenthin, C., Cardoso, M., Martel, J.-L., Schwarz, S., 2001, Antimicrobial resistance in
 636 staphylococci from animals with particular reference to bovine *Staphylococcus*
 637 *aureus*, porcine *Staphylococcus hyicus*, and canine *Staphylococcus intermedius*.
 638 *Veterinary Research* 32, 341-362.

639

Table 1. Parameter estimates, odds ratios with 95% confidence intervals and p-values from likelihood ratio tests for the fitted model assessing the significance of nasal status of the sow on the *S. aureus* status of the piglets.

	Estimate		95% confidence interval		Likelihood ratio test
Parameter		Odds ratio	lower	upper	p-value
age	-0.202	0.847	0.75	0.891	<0.001
age ² /100*	0.675	1.965	1.543	2.501	<0.001
age ³ /1000*	-0.046	0.955	0.94	0.971	<0.001
2 nd stage weaner house	-1.403	0.246	0.135	0.448	0.033
N_pos	2.47	11.822	3.554	39.329	0.096
VN_positive	1.147	3.149	1.263	7.852	0.465
age:N_pos	-0.089	0.915	0.87	0.963	0.006
age:VN_pos	-0.019	0.981	0.967	0.996	0.009
(age ² /100):N_pos	0.064	1.066	1.018	1.115	0.006

*The quadratic and cubic terms age² and age³ were divided by 100 and 1000 respectively to ensure numerical stability of the model-fitting algorithm
N_pos = nasal positive, VN_pos = nasal and vaginal positive

Table 2. Days sampled, the two most common antimicrobial resistance patterns and each production stage and the number of isolates showing each pattern from a total of 171 isolates tested. Isolates obtained from 4 positive and 2 negative sows sampled on day 2 and from their piglets on all 7 sampling occasions

Days	Group medication	No. of antimicrobial patterns	The two most common antimicrobial resistance patterns detected	Number of isolates showing pattern
<u>Sows</u>	None			
Positive Sows		1	Te*	6/6
<u>Piglets</u>	None	3	Te	17/26
Day 2			SuTe	6/26
Day 17		3	Te	7/13
			SuTe	3/13
<u>1st Stage Weaners</u>	Tilmicosin			
Day 21	(days 21 to 35)	4	ApErLn	10/19
	Trimethoprim/ Sulphadiazine		ApErLnSt	5/19
Day 45	(days 36-47)	4?	ApErLn	20/28
			ApErLnSu	5/28
<u>2nd Stage Weaners</u>	Trimethoprim/ Sulphadiazine			
Day 49	(days 48 to 52)	2	Susceptible ?	3/16
			ApErLn	13/16
Day 96		3?	ApErLn	17/33
			ApErLnSt	7/33
<u>Finishers</u>	None	13	Er Ln	8/30
Day 100			ApErLn	7/30

*Ap, ampicillin; Er, erythromycin; Ln, lincomycin; PMA, Su, sulphonamide; St, streptomycin; Te, tetracycline.

658

659

661 **Table 3. Characteristics of *S. aureus* isolates from nasal positive, nasal and vagina positive and negative sows and their pigs, including clonal**
662 **complex, *spa* types, PFGE patterns, antimicrobial resistance patterns and resistance genes**

Strain No:	Sow status	Sample origin	Typing		Antimicrobial resistance pattern		Resistance genes
			Clonal complex	<i>spa</i> type	PFGE pattern		
Pig identifier including day of sampling*	Nasal positive, nasal and vagina positive and negative sow						
4069N*	Nasal positive sow	Sow	CC9	t10449	A	Te ‡	vga(A), tet(K)
230 D2**	Nasal positive sow	Piglet	CC9	t10449	A	Te	tet(K)
230 D17	Nasal positive sow	Piglet	CC9	t10449	A	Ln	vga(A)
230 D21	Nasal positive sow	Weaner	CC9	t10449	A	ApErLnSt	blaZ, erm(C), vga(A)
230 D45	Nasal positive sow	Weaner	CC9	t10449	A	ApErLnSt	blaZ, erm(C), vga(A)
230 D49	Nasal positive sow	Weaner	CC9	t10449	A	ApErLn	blaZ, erm(C), vga(A)
230 D96	Nasal positive sow	Weaner	CC9	t10449	A	SuTe	tet(K)
230 D100	Nasal positive sow	Finisher	CC9	t10449	A	ErLnSu	erm(C), vga(A)
237 D17	Nasal positive sow	Piglet	CC9	t10449	A	PmaSuTe	blaZ, erm(C), vga(A)
4072N	Nasal positive sow	Sow	CC9	t10449	A	Te	tet(K)
155 D17	Nasal and vagina positive sow	Piglet	CC9	t10449	A	Te	tet(K)
155 D21	Nasal and vagina positive sow	Weaner	CC9	t10449	A	ApErLnSt	blaZ, erm(C), vga(A)
155 D49	Nasal and vagina positive sow	Weaner	CC9	t10449	A	ApErLn	blaZ, erm(C), vga(A)
155 D96	Nasal and vagina positive sow	Weaner	CC9	t10449	A	ErLn	erm(C), vga(A)

157 D100	Nasal and vagina positive sow	Finisher	CC9	t1334	A	ApErLnSuTeTp	blaZ, erm(C), vga(A)
159 D100	Nasal and vagina positive sow	Finisher	CC9	t10449	A	ApLn	blaZ, vga(A)
173 D21	Nasal and vagina positive sow	Weaner	CC9	t10449	A	ApErLnSu	tet(K)
3631N	Negative sow ***	Sow	CC9	t10449	A	Te	tet(K)
205 D2	Negative sow	Piglet	CC9	t10449	A	Susceptible	No resistance genes
205 D100	Negative sow	Finisher	CC9	t1334	A	ApErLnTeTp	blaZ, erm(C), vga(A)
221 D96	Negative sow	Weaner	CC9	t1334	A	ApLnTeTp	blaZ, vga(A)
212 D100	Negative sow	Finisher	CC9	t10449	A	ErLnPma	erm(C), vga(A)
219 D17	Negative sow	Piglet	CC9	t10449	A	LnTe	vga(A), tet(K)

663

664 * 4069N signifies sow no. 4069 sampled from the nares on day 2

665 ** 230 D2 signifies pig no.230, sampled on day 2 etc

666 *** Sow 3631 was negative before farrowing but positive on day 2. The results of analysis of the day 2 isolate are shown

667 ‡ see footnote on table 2

Figure captions

Figure 1. Mean percentage prevalence of *S. aureus*-positive piglets from negative sows, nasal-positive sows and nasal- and vaginal-positive sows from day 2 to day 100

Figure 2. The odds ratio of a piglet from a nasal positive sow being *S. aureus* positive to that of a piglet from a negative sow plotted against age (x-axis), illustrating the estimated main effect of sows being nasal positive as well as the interactions of this variable with age.

Figure 3. The odds ratio of a piglet from a nasal and vaginal positive sow being *S. aureus* positive to that of a piglet from a sow that was only nasal positive is plotted against age (x-axis), illustrating the combined main effect of being vaginal positive, as well as its interaction with age.

Figure 4. PFGE analysis of *S. aureus* isolates following macro-restriction of genomic DNA with *Sma*I showing PFGE patterns A and B. Lanes 1, 8,15 DNA size marker; lanes 2-7, 9-13, pattern A; lane 14, pattern B.

Figure 5. **A:** Comparison of whole-genome maps of *S. aureus* from piglet no.230 (230 D2) and its dam (4069N); **B:** Comparison of WGMs of 230 D2 and 4069N to an *in silico* map of ECT-R 2; **C:** Enlarged view of the region encoding phage 11 in ECT-R 2 (marked in red) and the corresponding regions in 230 D2 and 4069N demonstrating the partial loss of the phage ϕ 11-encoding genomic island.

Figure 2. Mean percentage prevalence of *S. aureus*-positive piglets from 2 negative sows (n = 25 piglets), 2 nasal-positive sows (n = 24 piglets) and 2 nasal- and vaginal-positive sows (n = 24 piglets) from day 2 to day 100

Figure 2. The odds ratio of a piglet from a nasal positive sow being *S. aureus* positive to that of a piglet from a negative sow plotted against age (x-axis), illustrating the estimated main effect of sows being nasal positive as well as the interactions of this variable with age.

Figure 3. A: Comparison of whole-genome maps of *S. aureus* from piglet no.230 (230 D2) and its dam (4069N); **B:** Comparison of WGMs of 230 D2 and 4069N to an *in silico* map of ECT-R 2; **C:** Enlarged view of the region encoding phage 11 in ECT-R 2 (marked in red) and the corresponding regions in 230 D2 and 4069N demonstrating the partial loss of the phage ϕ 11-encoding genomic island.

731

732