January 2013

Epidemiology And Characterization Of Methicillin-Resistant Staphylococcus Aureus From Swine Workers In Romania

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Epidemiology and Characterization of Methicillin-Resistant *Staphyloccous aureus* from Swine Workers in Romania

By

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A Thesis Presented to

The Faculty of the Department of Epidemiology and Public Health

Yale University

In Candidacy for the Degree of

Master of Public Health

2013

Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a potent human pathogen associated with high morbidity and mortality worldwide. Livestock are known reservoir for MRSA, which pose substantial health risks for livestock farmers and their community contacts. However, little is known about MRSA among swine workers in Eastern Europe. In this study, we conducted a prevalence survey of swine workers in Romania. Three livestock-associated *S. aureus* strains (*spa* type t034, t011, and t4872) and one community-associated *S. aureus* strain (*spa* type t321) were isolated from workers on seven commercial swine farms in Transylvania. The rate of MRSA carriage in workers was 6.8%. Veterinarian visit and hospitalization were associated with MRSA carriage status, suggesting control and preventive measures are needed to minimize the transmission of MRSA from pig farmers to their community members and vice versa.

Acknowledgement

I would like to thank Dr. Peter Rabinowitz for all his guidance and support, for providing me this research opportunity, and for being an outstanding mentor throughout the whole process; I would also like to thank Dr. Melinda Pettigrew for being an instrumental thesis reader; and Dr. Lynda-Martins Osadebe for her mentorship, and for taking time off from her busy schedule to help me preparing for this project.

I would like to acknowledge my mentors in Romania, Dr. Anca Gurzau and Dr. Marina Spinu, for offering me exceptional support and guidance. I would like to recognize my translator, Adriana Opincariu, for her contribution to this project; and colleagues at the Environmental Health Center and Faculty of Veterinary Medicine at University of Agricultural Sciences and Veterinary Medicine for their help and collaboration.

I appreciate Dr. Tara Smith, Blake Hanson, and Ashley Kates at The University of Iowa, College of Public Health for working on the molecular analysis portion of this project. Their skills and knowledge are invaluable.

I would like to acknowledge the farm managers who permit us to visit their facility, and big thanks to all the workers who participated in this study. We would like to thank JMI Laboratories in North Liberty, IA for providing positive control DNA for cfr PCR, and Yale School of Public Health, Yale Animal Human Medicine Project, and Environmental Health Center at Cluj-Napoca, Romania for their financial support.

Lastly, I would like to extend my sincere gratitude to my family and friends, thank you for your love and encouragement.

Table of Contents

List of Tables

Introduction

Staphylococcus aureus is a Gram-positive bacterium that colonizes approximately 30% of the population in the United States (1). Asymptomatic carriage of *S. aureus* is an important risk factor for staphylococcal infection (1), which accounts for substantial morbidity and mortality worldwide (2). The virulence of *S. aureus* is manifested by its ability to adapt to variety of environmental conditions and to obtain antibiotic resistance efficiently. Methicillin-resistant *S. aureus* (MRSA) carries the *mecA* gene, which equips the bacterium with the ability to resist beta-lactam antibiotics (3). This gene resides on a transposable genetic element known as the staphylococcal cassette chromosome *mec* (SCC*mec*) that encodes an altered penicillin binding protein (PBP2a) with a low affinity for beta-lactam antibiotics. At least five different SCC*mec* types (I-V) and several subtypes have been identified (4).

MRSA is a highly infectious pathogen that can lead to life-threatening diseases. Even though MRSA associated infections are commonly found in hospitals, individuals without any previous hospitalization can acquire MRSA from their community contacts (5). Serious invasive CA-MRSA infections such as necrotizing pneumonia are associated with up to 75% of all mortality (6).

Epidemiology of Livestock-Associated MRSA

Specific MRSA strains associated with livestock (LA-MRSA) have been documented in many countries in Europe, Asia, and North America (7-13). Studies have shown that hospitals situated in areas with high density of MRSA-positive swine facilities have higher MRSA infection rate compare to hospitals located in areas without or limited livestock facilities (14). For instance, MRSA infections increased three-folds in a Dutch

hospital situated in a pig-dense area, and 22% of patients were colonized with MRSA in a German hospital located in a region with intense livestock farming (15).

It is well established that pigs are frequent carriers of LA-MRSA (16, 17), which place swine workers at a particular high risk for MRSA colonization. In Europe, among individuals who had contact with MRSA-positive pigs or veal calves, 23-38% were MRSA carriers, and 4% of the family members without direct exposure to agriculture animals were colonized (18). These studies indicate that regular contact with live pigs is a significant risk factor for acquiring LA-MRSA, and swine workers play a vital role in LA-MRSA and CA-MRSA transmission.

Epidemiology of S. aureus in Romania

According to the European Antimicrobial Resistance Surveillance, Romania had one of the highest European prevalence of invasive HA-MRSA in 2007 (26.2%), 2008 (33.3%), and 2010 (39.1%) (www.rivm.nl/earss/database). Studies have shown that MRSA frequently colonizes hospitals, posing substantial risks for HA-MRSA infections (19, 20). In addition, the diversity of *S. aureus* found in healthcare settings is remarkable, indicating a continuing evolution and transmission of various *S. aureus* strains in healthcare settings in Romania (19). Even though Romania is considered a "hot spot" for antimicrobial drug resistance, no study has explored the diversity and epidemiology of livestock-associated MRSA in this country.

Objective and Goals

This thesis explores the epidemiology and characteristics of LA-MRSA and methicillin-susceptible *S. aureus* (MSSA) strains isolated from swine workers in

Romania. Potential risk factors for *S. aureus* colonization are also examined. Specifically, we hypothesize that biosafety practice differs between MRSA carriers and non-carriers, given MRSA can be easily transmitted through close contacts with other MRSA carriers and contaminated surfaces (21, 22).

Materials and Methods

Sample Collection

Workers from seven commercial swine farms in northwestern Romania participated in this study. Farm A consisted of multiple age-segregated nurseries, finishing, and wean-to-finish sections. Farms B, C, D, E, F, and G consisted of several sections with finishing pigs only. At farm visits, both environmental and human sampling for S. aureus took place. Five environmental samples were collected from randomly chosen corners of pigpens on each farm. Both sides of the wall corner involving an area of 10x10 centimeters and one meter above the floor were sampled using sterile swabs.

Demographic data, biosafety practice, information on medical history and contacts with animals were obtained using questionnaire. All participants provided a written informed consent prior to the enrollment of this study. Protocols were approved by the institutional review board (IRB) of Yale University and the Environmental Health Center at Cluj-Napoca, Romania.

Isolation of Staphylococcus aureus

Swabs of nasal, oropharygneal, and environmental surfaces were cultured separately using protocols previously described in the Official Journal of the European

Union (23). In summary, swabs were stored in Stuart's medium at 4°C during transportation. Samples were inoculated in 3 mL Mueller-Hinton broth supplemented with 6.5% NaCl, and incubated at 37°C for 24 hours. Samples were then diluted (1:10) in Tryptone Soy Broth containing 3.5mg/l cefoxitin and 75 mg aztreonam, and incubated for 24 hours at 37°C. One loopful of broth was inoculated onto selective MRSA chromogenic agar plates (Brilliance ™ MRSA 2 Agar, Oxoid) and incubated for 48 hours at 37°C. Presumptive positive colonies were shipped to an experienced microbiology laboratory at Iowa State University, United States for further molecular assessments.

Characterization of MRSA Isolates

Presumptive positive colonies were streaked onto Columbia CNA with 5% sheep blood (Becton Dickinson and Company, Sparks, Maryland, USA), and incubated for 24 hours at 35°C. All plates were tested for *S. aureus* and MRSA using the catalase test, coagulase test, and Pastorex Staph-plus latex agglutination assay (Bio-Rad, Redmond, Washington, USA). All isolates testing positive for *S. aureus* were subjected to molecular testing and antimicrobial susceptibility testing (AST).

Antimicrobial Susceptibility Testing

All S. *aureus* isolates were tested for antimicrobial susceptibility by minimum inhibitory concentration methodology as described by the Clinical Laboratory Standards Institute (24). Isolates were tested for susceptibility to oxacillin, tetracycline, erythromycin, clindamycin, trimethoprim-sulfamethoxazole, gentamycin, levofloxacin, vancomycin, daptomycin, quinupristin/dalfopristin, linezolid, and rifampin.

Molecular Testing

Genomic DNA was extracted using the Wizard Genomic DNA purification kit (Promega Corporation, Madison, Wisconsin, USA). Total DNA for plasmid analysis was extracted via heat lysis. The presence of the *mecA* (25), *cfr*, *fexA* (26), and *dfrK* genes (27), as well as PVL was determined by PCR (28). *Spa* typing was carried out using the primers described by Ridom Bioinformatics (ridom.de/doc/Ridom_spa_sequencing.pdf), and sequences were interpreted utilizing the Ridom StaphType software (Ridom GmbH, Würzburg, Germany). All molecular procedures employed known positive and negative controls.

Statistical Analysis

Univariate analysis was performed to determine the prevalence of MRSA colonization in swine workers. Associations between worker characteristics and *S. aureus* colonization status were assessed using Pearson chi-squared test and Fisher's exact test. Multivariate logistic regression was used to model *S. aureus* carriage status. Due to the small sample size, descriptive epidemiology was employed to analyze environmental samples. Significant association was set at $P < 0.05$. All statistical analyses were performed using SAS software version 9.3.

Results

Characteristics of Swine Farms

Table 1 shows farm characteristics including number of pigs, farm type, and number of workers in June 2012. Farm A was a farrow-to-finish operation housed

approximately 33,000 pigs. Roughly 10,000 finishing pigs were raised on farms B and C, and 1,300 finishing pigs were raised on farms D, E, F, and G. Farm A had greatest number of workers (n=69), followed by Farm B (n=19) and Farm C (n=13). Farms D through G had fewer than five workers each.

Prevalence of S. aureus Colonization in Workers

Table 2 presents the prevalence of *S. aureus* among swine workers from seven commercial farms in June 2012. A total of 103 nasal and 103 oropharygneal swabs were collected. More than eighty percent of the workers from farms A and C were sampled for *S. aureus* colonization. For Farm B, twelve of the nineteen workers agreed to be sampled. One hundred percent worker participation rates were obtained from farms D through G. The rate of human MRSA colonization was considerably lower on the farrow-to-finish farm. In detail, workers on Farm A were colonized by MSSA strains, while workers on farms B, C, D, E, F, and G were colonized by MRSA. In total, twenty-three *S. aureus* strains were isolated from twenty-one workers on six farms. Among 103 workers who had provided nasal and oropharygneal samples, six individuals (5.83%) from five farms were tested positive for MRSA. Two were colonized in both nares and throat, two were colonized in throat only, and two were colonized in nares only. MSSA carriers (n=14) were only found on Farm A. Eleven individuals from this farm were colonized in the nares, and two were colonized in the throat. The prevalence of human MRSA carriage ranged from 0% to 67% on farms, and the prevalence of MSSA carriage was 21% on the farrow-to-finish farm. Fisher's exact test suggested that farm location was a risk factor for *S. aureus* colonization (P=0.042), but it was not statistically significant after being added to the multivariate logistic regression model (P>0.05).

Characteristics of S. aureus Isolates

In total, four *spa* types including t011 (65.2%), t034 (21.7%), t0321 (4.3%), and t4872 (4.3%) were identified in twenty-three human *S. aureus* isolates. As expected, most isolates belong to *spa* types t034 and t011, which are associated with LA-MRSA strain ST-398 (29). *Spa* type t4872 is also ST398-associated both by repeats and by previously documented MLST (29). *Spa* type t321 is a CA-MRSA strain related to ST-001 (30). No isolate was found to carry Panton-Valentine Leukocidin (PVL) toxin. Resistant genes *drfK* and *fexA* were present in seven MRSA isolates with *spa* type t4872 or t011. For MSSA, five isolates with *spa* type t321, t011, or t034 carried *drfK* and *fexA* gene, and one MSSA isolate with *spa* type t011 tested positive for *cfr* resistant gene.

Three MRSA strains with *spa* type t321, t011, or t034 were detected on Farms C, D, and F, respectively. Resistant genes *fexA*, *drfK*, and *cfr* were discovered in t034 strain, but not in t321 and t011 strains. One MSSA strain with *spa* type t034 was found on Farm D. None of these strains tested positive for PVL toxin.

The antibiotic resistant pattern varied among isolates (Table 3). Resistance to tetracycline, oxacillin, erythromycin, and clindamycin were commonly found in ST-398 associated strains. One-quarter of them were also resistant to co-trimoxazole (TMP-SMX). Two isolates with *spa* type t011 and t4872 had intermediate resistant to quinupristin/dalfopristin (quino/dalfo), and two t034 typed isolates were fully resistant to quino/dalfo. In addition, two isolates with *spa* type t034 from farms D and E were linezolid-resistant. Similar to LA-MRSA associated strains, t321 typed strains exhibited resistant to tetracycline, oxacillin, erythromycin, but neither to clindamycin nor TMP-

SMX. One t321 typed strain from Farm C was also resistant to gentamycin and levofloxacin. All strains were susceptible to vancomycin, daptomycin, and rifampin.

Risk Factors for S. aureus Colonization

More than half of the *S. aureus* carriers were male workers who had spent fewer than eight years in school. The average age for carriers was 42.5 years and the average number of cigarette consumed per day on worksite was 15.3. Interestingly, *S. aureus* carriers tended to wear PPE more often on a daily basis, and had similar work hours compare to those of non-carriers. Carriers also responded that they never take work clothes home, and always shower outside of the workplace at the end of the day. None of the biosafety practices was a significant risk factor for *S. aureus* colonization ($P>0.05$).

When asked about contacts with animals outside of work, one-third of the workers who were colonized with *S. aureus* had contact with poultry and swine outside of the workplace within last year. In addition, carriers tended to have fewer pets at home compare to non-carriers. Contact with veterinarians was associated with *S. aureus* carriage status ($P=0.038$).

For general health status, most workers did not participate in sport activities regularly, but majority believed to have excellent or good health ($n=81$). Among carriers, none had skin disease within last year; three were hospitalized in last 12 months, which was a significant risk factor for *S. aureus* colonization ($P=0.026$). Both veterinarian visit and hospitalization within last year remained statistically significant in the multivariate logistic regression model for *S. aureus* colonization.

Discussion

This is the first study to evaluate the prevalence of MRSA among swine workers in Romania. Result suggests that swine workers are frequently colonized with *S. aureus* strains varied in genotype and antimicrobial resistant patterns. Prevalence of MRSA in Romanian swine workers (6.8%) is relatively low compare to that of other European countries (Germany: 24%, Netherland: 42%, Spain: 9.3%) (31-33), but substantially higher than the MRSA prevalence of the general population (<0.1%) (34), indicating swine workers may play a role in bridging MRSA transmission between pigs and other human contacts in their community.

This study is also the first to document livestock-associated MRSA in Romania. Three ST398-associated *spa* types (t011, t034, and t04872) were identified in this Romanian cohort. The genotype for MRSA isolates appears diverse, given that three *spa* types were uncovered from nine MRSA strains. In addition to livestock-associated strains t034 and t011, one community-associated strain with *spa* type t321 was detected. Ridom sequences indicate this strain is genetically distinct from livestock-associated strains (Table 4), suggesting human colonization by community-associated strains may occur on livestock farms. MSSA strains were predominantly t011 typed; one strain had *spa* type t4872, which was previously reported only in Spain (Ridom Database: http:/spa.ridom.de/). This is the first time these four *spa* types are documented in Romania.

A wide range of antimicrobial resistant patterns was also observed among *S. aureus* isolates from the workers. Similar to strains found in Romanian hospitals, livestock-associated *S. aureus* strains exhibited a high frequency in tetracycline-

resistance and vancomycin-susceptibility. However, these livestock-associated strains were largely resistant to clindamycin and erythromycin, and susceptible to gentamicin and rifampicin (19), suggesting that the driving force for antibiotic-resistant in MRSA may differ between these two settings. We were told by farm managers that antibiotics were not employed on swine farms, and we were unable to collect information on livestock feed. However, survey conducted by European Commission in 2010 suggests that antibiotics are widely used in Romania: more than half of the human population use antibiotics, which can be obtained from pharmacies without medical prescription (35). Given that resistance can develop under selective antibiotic pressures in many settings (36, 37), extensive practice of antibiotics in the community may contribute to the diverse antimicrobial resistant patterns observed in this study. Acquisition of antibiotic resistant genes pose a major challenge to the treatment of MRSA associated infections. Even though workers claimed that no antibiotics were used on farms, usage of antibiotics in the community should be restricted as a potential strategy to mitigate selective antibiotic pressures and hamper the emergence of novel antibiotics resistant genes.

Previous studies have shown that weaning piglets are more frequently colonized with MRSA compare to pigs in other age groups (7, 38, 39). Thus, workers who have contacts with young piglets may have higher risk for acquiring MRSA, resulting in a higher colonization rate. In contrast, our findings indicate that workers on finishing farms have higher rate of MRSA colonization than that of workers on the farrow-to-finish farm. According to the information provided by the farm manager, Farm A is an operation that does not import pigs from other swine facilities, while farms B, C, D, E, F, and G do purchase pigs from other swine operations in Romania and neighboring

countries. Importation of pigs could have introduced MRSA to these swine facilities. Due to the sheer complexity of meat production and transportation system in Europe, and widely reported MRSA presence in pig populations in many European countries (7, 33, 40-47), we speculate that contacts with imported colonized pigs as a risk factor for acquiring MRSA (48). However, further studies are needed to understand the route of transmission between pigs and swine workers in Romania.

The unusual rate of *S. aureus* carriage on Farm B (0%) may be explained by a lack of evidence. Only 63.1% (12/19) of the workers on this farm agreed to provide their nasal and oropharygneal swabs for our study. We were unable to collect information from other seven workers. In addition, given that MSSA isolates from workers on Farm A grew on selective MRSA chromogenic agar plates, we may have underestimated the MSSA prevalence of workers on other farms. Previous evaluation indicates that Brilliance MRSA agar has high sensitivity but relatively low specificity compare to other chromogenic media (49), which may explain the growth of some MSSA isolates on this MRSA-selective agar. Thus, our data on MSSA colonization rate may not be entirely accurate. Nevertheless, this study provides important evidence that swine workers in Romania are frequent carriers of livestock-associated *S. aureus*. Furthermore, the discovery of CA-MRSA strain (t321) on a worker from Farm C implies that swine workers can import CA-MRSA strains to the farm and have the potential to transmit them to livestock. This pathway can give rise to novel *S. aureus* strains, as suggested by a previous study on the evolution of LA-MRSA strains (50).

Our results also show that contacts with veterinarians are associated with *S. aureus* carriage. The odds for acquiring *S. aureus* increases by three-fold if the worker

had visited a veterinarian in last 12 months. This could have a number of possible explanations. Veterinarians have been reported to have high rates of for MRSA colonization (51), so there is some possibility of person-to-person transmission. It is also possible that handling sick animals or administering antibiotics to animals could have predisposed to MRSA carriage. We were unable to directly test veterinarians, thus it is difficult to know which of these possibilities is more likely. Since swine workers who had contact with veterinarian were colonized by livestock-associated *S. aureus* strains, and livestock veterinarians tend to have a higher rate of MRSA colonization than other veterinary professions (51-53), further investigations are needed to identify the source of MRSA strains uncovered from these swine workers.

Interestingly, the odds for being a *S. aureus* carrier decreases by four-fold if the worker were hospitalized in last 12 months (Table 6). Given that no HA-MRSA strain was detected in this cohort, it is unclear if the protective effect is directly related to hospitalization. A study with larger sample size is needed to confirm this finding.

We did not find any association between biosafety practice and the status of MRSA carriage as we had originally predicted. We did, however, discovered a cluster of LA-MSSA strains with *spa* type t011 on Farm A, suggesting clonal spread and potential zoonotic cross-transmission among workers on this farm. This observation specifies a need for worker education as well as implementation of control and prevention measures for MRSA and other zoonotic pathogens on the farms.

Our study has several limitations. The prevalence of *S. aureus* colonization in pigs remains unknown. Even though we did not obtain any pig samples for MRSA testing, given the high frequency of LA-MRSA colonization in workers, as well as

evidence from previous studies that pigs can harbor MRSA (54), we speculate that some pigs on there farms are *S. aureus* carriers as well. These animals can also play a vital role in inter- and intra-species transmission of *S. aureus*. Due to limited data, we were unable to assess whether swine workers acquired MRSA from pigs or from their community contacts. Further studies are needed to address the transmission route of LA-MRSA in farm settings. In addition, no information on antimicrobial usage was available to fully understand the origin and development of antibiotic resistance genes observed in these *S. aureus* strains. Since this is a cross-sectional study, we are unable to obtain longitudinal data on potential MRSA infections as a result of *S. aureus* colonization.

Conclusion

In conclusion, our study shows that swine workers in Romania are frequently colonized with LA-MRSA, suggesting a need for education as well as implementation of control and prevention measures on the farms to minimize potential LA-MRSA related infections. Routine surveillance of MRSA colonization in pigs, swine workers, and their community contacts are needed to assist public health professionals to better understand the epidemiology and transmission of livestock associated *S. aureus* in Romania.

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Appendix I: Tables

Table 1. Characteristics of swine farms

	Farms						
Variables	A	B	C	D	E	F	G
No. of pigs[b]	33,000	11,000	9000	1200	1300	1350	1300
Farm type	Farrow-to-Finish	Finish	Finish	Finish	Finish	Finish	Finish
No. of workers	69	19	13	4	4	3	2

[b] Estimated number of pigs on the farm in June 2012

Table 2. Prevalence of *S. aureus* among swine workers in Romania, June 2012

	Farms (Fisher's exact test: P=0.262; Logistic regression: P=0.042) Percentage of positive samples (no. of positive/no. of tested)						
Workers	A	B	C	D	E	F	G
MRSA[a]	0(0/67)	0(0/12)	9.1(1/11)	50(2/4)	25(1/4)	66.7(2/3)	50(1/2)
MSSA[b]	20.9(14/67)	0(0/12)	0(0/11)	0(0/4)	0(0/4)	0(0/3)	0(0/2)

[a] *Staphylococcus aureus* with *mecA* element

[b] *Staphylococcus aureus* without *mecA* element

Table 3. Characteristics of *S. aureus* strains isolated from swine workers and farms in Romania

Isolates	Farms	Sites[a]	*mecA*	*spa* type	PVL[b]	*fexA*[c]	*cfr*[d]	dfrK[e]	Antimicrobial resistance[g]
R10	A	N	-	t011	-	-	-	Yes	T, ER, CL, QD
R13	A	N	-	t011	-	-	-	-	T, ER, CL, QD[φ]
R6	A	N	-	t011	-	Yes	-	Yes	T, ER, CL, TS, QD
R22	A	N	-	t011	-	-	-	-	T, ER, CL
R29	A	N	-	t011	-	Yes	-	Yes	T, ER, CL, TS, QD
R18	A	N	-	t011	-	Yes	-	Yes	O, T, ER, CL, TS, QD
R3	A	N	-	t011	-	-	-	-	T, ER, CL, QD
R8	A	N	-	t011	-	-	-	-	T, ER, CL, QD
R4	A	N	-	t011	-	-	-	-	T, ER, CL, QD
R2	A	O	-	t011	-	-	-	-	O, T, ER, CL, QD
R16	A	N	-	t4872	-	Yes	-	Yes	T, ER, CL, QD[φ]
R21	A	O	-	t011	-	Yes	Yes	Yes	T, ER, CL, TS, QD
R5	A	O	-	NT	-	-	-	-	ER
R27	A	N	-	t011	-	Yes	-	Yes	T, ER, CL, QD
R14	C	O	Yes	t321	-	Yes	-	Yes	O, T, ER,
R11	C	E	Yes	t321	-	-	-	-	O, T, ER, G, LE
R7[γ]	D	O	Yes	t034	-	-	-	Yes	O, T, CL, QD[φ]
R31[γ]	D	N	Yes	t034	-	Yes	-	Yes	O, T, CL, QD[φ]
R15	D	N	Yes	t011	-	Yes	-	Yes	O, T, ER, CL, QD[φ]
R9	D	E	Yes	t011	-	-	-	-	O, T, CL, QD[φ]
R30	D	E	No	t034	-	Yes	Yes	Yes	O, T, ER, CL, TS, QD, LI
R17	E	N	Yes	t034	-	Yes	-	Yes	O, T, CL, QD[φ]
R24[ρ]	F	N	Yes	t011	-	Yes	-	Yes	O, T
R26[ρ]	F	O	Yes	t011	-	Yes	-	Yes	O, T
R12	F	N	Yes	t034	-	-	-	Yes	O, T, ER, CL, LE, QD
R19	F	E	Yes	t034	-	Yes	Yes	Yes	O, T, CL, TS, QD, LI
R23	G	O	Yes	t034	-	Yes	-	Yes	O, T, ER, CL, QD

[a] N, nares; O, oropharynx; E, environmental sample
[b] PVL, Panton-Valentine Leukocidin; -, not detected
[c] *fexA* gene; -, not detected
[d] *cfr* gene; -, not detected
[e] *dfrK* gene; -, not detected
[f] *ermT* gene; -, not detected
[g] O, oxacillin; T, tetracycline; ER, erythromycin; CL, clindamycin; TS, TMP/SMX; G, gentamycin; LE, levofloxacin; V, vancomycin; D, daptomycin; QD, Quin/Dalfo; LI, linezolid; R, rifampin
[φ] antibiotic resistant level: intermediate
[γ] Isolates R7 and R31 came from the same worker
[ρ] Isolates R24 and R26 came from the same worker

Table 4. *Spa* type identified in *S. aureus* isolates from swine workers

Spa Type	Ridom Profile	Worker Prevalence
t011	08-16-02-25-34-24-25	14/103 (13.6%)
t034	08-16-02-25-02-25-34-24-25	4/103 (3.9%)
t321	07-23-16-34-33-13	1/103 (0.97%)
t4872	08-16-02-25-34-24-25-34-24-25	1/103 (0.97%)

Table 5. Characteristics of swine workers and *S. aureus* carriage[a]

Characteristic	*S. aureus* Carriage Yes (N = 21)[b]	No (N = 82)[b]	*p*[c]
Age (years)	42.5±11.0	42.1±9.9	0.869
Sex			1.000
Male	18 (85.7)	67 (81.7)	
Female	3 (14.3)	15 (18.3)	
Education			0.351
<8 years	11 (61.1)	30 (46.9)	
8-12 years	6 (33.3)	21 (32.8)	
>12 years	1 (5.6)	13 (20.3)	
Number of cigarettes smoked per day	15.3±4.1	17.2±11.4	0.701
Alcohol consumption			0.763
None	7 (33.3)	30 (36.6)	
Daily	6 (28.6)	15 (18.3)	
Weekly	5 (23.8)	21 (25.6)	
Monthly	3 (14.3)	16 (19.5)	
Work			
Farm site			0.262
Time spent on farm (hours/week)	42.1±5.9	42.5±5.0	0.801
Handle pigs (hours/week)	12.5±8.5	11.6±6.0	0.756
Remove pig wastes (hours/week)	6.1±5.4	7.5±4.2	0.562
Clean pig pens (hours/week)	9.6±5.9	9.9±6.9	0.884
Biosafety practice			
Daily PPE usage while working with pigs (%)			
Gloves	40.1±41.5	37.5±28.0	0.873
Rubber Boots	93.4±22.5	88.7±24.5	0.441
Overalls	100±0.0	95.7±16.5	0.246
Take work clothes home			1.000
Always	0 (0)	1 (1.22)	
Never	21 (100)	81 (98.8)	
Shower at the facility at the beginning of the day			1.000
Always	17 (80.9)	65 (79.3)	
Sometimes	1 (4.8)	3 (3.7)	
Rarely	0 (0)	2 (2.4)	
Never	3 (14.3)	12 (14.6)	
Shower outside of the facility at the end of the day			0.788
Always	21 (100)	75 (91.5)	
Sometimes	0 (0)	2 (2.44)	
Rarely	0 (0)	4 (4.88)	
Never	0 (0)	1 (1.22)	

Contact with animals			
Direct contact with animals in last 12 months			0.869
Chicken	5 (23.8)	24 (29.6)	
Horses	0 (0)	1 (1.2)	
Goats	1 (4.8)	2 (2.5)	
Other	6 (28.6)	23 (28.4)	
Pigs at home			0.469
Yes	7 (33.3)	35 (42.7)	
No	14 (66.7)	47 (57.3)	
Number of pigs at home	1.4±1.8	1.7±2.0	0.596
Pets at home			0.149
None	4 (19.1)	12 (14.3)	
Dog	7 (33.3)	34 (40.5)	
Cat	4 (19.1)	7 (8.3)	
Other	6 (28.6)	31 (36.9)	
Number of pets at home	1.9±1.1	2.7±2.5	0.222
Visit to veterinarian in last 12 months			0.038
Yes	4 (20.0)	39 (49.4)	
No	16 (80)	40 (50.7)	
General Health			
Participation in sport activities			0.222
None	17 (80.9)	64 (80.0)	
Once a week	4 (10.1)	6 (7.5)	
2-4 times a week	0 (0)	3 (3.8)	
>4 times a week	0 (0)	3 (3.7)	
Health status			0.306
Excellent	10 (47.6)	24(29.3)	
Good	11 (52.4)	57 (69.5)	
Poor	0 (0)	1 (1.2)	
Skin disease in last 12 months			1.000
Yes	0 (0)	3 (3.7)	
No	21 (100)	79 (96.3)	
Hospitalization in last 12 months			0.031
Yes	3 (14.3)	1 (1.2)	
No	18 (85.7)	81 (98.8)	

Only variables with significant *P* values (see boldface) were used in the logistic regression model. Boldfaced *P* values came from the multivariable logistic regression.

[a] Table values are mean ± SD for continuous variables and n (column %) for categorical variables.

[b] Numbers may not sum to total due to missing data, and percentages may not sum to 100% due to rounding.

[c] P-value is for student t-test (continuous variables) or Fisher's Exact test (categorical variables).

Table 6. Risk Factors for *S. aureus* colonization in swine workers, multivariate logistic regression analysis

Variables	Estimate	Point estimate	SE	*P* value	95% CI
Vet visit	1.303	3.68	0.6269	0.0377	1.077-12.57
Hospitalization	-1.330	0.26	0.6189	0.0317	0.079-0.89
Farm Location	0.189	1.21	0.1689	0.2621	0.868-1.683