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Methicillin-Resistant *Staphylococcus aureus* in Commercial Swine Herds Is Associated with Disinfectant and Zinc Usage

Mackenzie Jonathan Slifierz,[a] Robert M. Friendship,[b] J. Scott Weese[a]

Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada[a]; Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada[b]

Methicillin-resistant *Staphylococcus aureus* (MRSA) originating from swine is concerning for public health, but an understanding of the emergence and persistence of MRSA in nursery herds is lacking. The aim of this study was to determine whether MRSA in nursery pigs is associated with particular herd-level parameters, including the use of antimicrobials, disinfectants, and heavy metals, which may be driving the selection and persistence of antimicrobial resistance. Nasal cultures for MRSA were completed for 390 pigs from 26 farms at the end of the suckling phase and again at 3 weeks postweaning. Herd-level information was collected, and a random subset of MRSA isolates was screened for resistance to zinc and quaternary ammonium compounds (QACs). Multivariate analysis revealed that in-feed concentrations of zinc ($P < 0.001$) and frequent disinfection of nursery pens ($P < 0.001$) are associated with MRSA shedding in nursery pigs. Furthermore, 62.5% (25/40) of MRSA isolates carried the zinc resistance gene *czrC* and demonstrated decreased susceptibility to zinc. All MRSA isolates carried at least 1 QAC resistance gene. The most common genotype was *qacG qacH smr*, which occurred in 32.5% (13/40) of isolates. Seven isolates (17.5%) demonstrated a significant tolerance to benzalkonium chloride, indicating a potential to survive commercial QAC exposure in the presence of organic matter. Overall, these findings indicate that high levels of in-feed zinc and QAC-based disinfectants are important drivers in the selection and persistence of MRSA in commercial swine herds, and these agents may be coselecting for other antimicrobial resistance genes.

Staphylococcus aureus is one of the leading causes of opportunistic infections in humans. Those strains with multiresistant phenotypes, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), are of concern due to the risk of treatment failure, increased hospitalization, and increased use of medical resources (1). Hence, it is understandable that since the first report of MRSA in pigs and the implication of pigs as a source of human infections (2), there has been escalating concern about the use of antimicrobials in livestock production systems and the potential public health risk of MRSA originating from animals.

However, previous investigations of the association between antimicrobial usage and the presence of MRSA in swine production systems have yielded conflicting results. Several studies have documented the commonness and long-term persistence of MRSA in pigs raised without exposure to antimicrobials (3–5) and in organic husbandry (6, 7). Furthermore, a Dutch study of 202 pig herds and a German study of 291 pig herds were unable to find an association between MRSA and antimicrobial usage (8, 9), and a recent meta-analysis of risk factors for MRSA in grower-finisher herds determined no difference between organic and conventional herds but did report group treatment with antimicrobials as a risk factor (10). Regional differences in MRSA carriage by pigs also are paradoxical; Denmark and the Netherlands, which have legislated restraints for antimicrobial use in livestock production, report a considerably higher prevalence of MRSA among pigs than that in the midwestern United States (9, 11, 32). In addition, further research has demonstrated no difference in the presence of MRSA in conventional and antibiotic-free pork products (12).

The lack of a clear association between antimicrobial usage and MRSA in swine production indicates that there are additional factors which may play a synergistic or independent role in the selection and perpetuation of antimicrobial resistance in staphylococci. Some recent evidence suggests that the use of disinfectants and zinc are risk factors for MRSA, as these compounds are associated with the coselection of resistance genes (13, 14). However, these factors have not been thoroughly investigated in nursery pig herds despite the relatively high prevalence of MRSA and commonness of antimicrobial use during this phase of production. Therefore, the objective of the present study was to investigate risk factors for MRSA shedding in pigs in commercial nursery herds with a particular focus on antimicrobials, heavy metals, disinfectants, biosecurity, and management practices.

MATERIALS AND METHODS

Study design. The use of animals in this study was approved by the Animal Care Committee at the University of Guelph. Twenty-two cohorts of pigs ($n = 390$) were monitored from farrowing to 3 weeks postweaning on 26 participating farms (4 farrow-to-wean, 4 wean-to-finish, and 18 farrow-to-finish farms) located throughout 10 counties in southern Ontario (November 2013 to October 2014). A variety of different farm sizes and management types were enrolled in the study from a sampling frame of southern Ontario swine farms that have associations with the University of Guelph, although the composition of this sampling frame does not represent a probability selection of all southern Ontario swine herds. At

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Address correspondence to Mackenzie Jonathan Slifierz, mslifier@uoguelph.ca.
M.J.S., R.M.F., and J.S.W. contributed equally to this work.

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each farm, 1 to 3 pigs were selected from each available litter (maximum of 20 pigs enrolled per farm) and assigned a unique identifier. There were no selection criteria for either the commercial farms or the pigs.

A survey of production parameters, biosecurity, and herd health was completed for each farm. The investigator completed an additional observational survey of the biosecurity and management practices on the farm. Documentation of antimicrobial usage and diet also were collected for each nursery herd. Lastly, a nasal swab of both nares was collected from each pig just prior to weaning and once again at 3 weeks postweaning. The samples were transported and stored at 4°C before being processed on the same day.

Detection of methicillin-resistant *Staphylococcus aureus*. Methicillin-resistant *S. aureus* was detected using a previously described enrichment protocol (15). Briefly, nasal swabs were inoculated into an enrichment broth (10 g/liter tryptone, 75 g/liter NaCl, 10 g/liter mannitol, 2.5 g/liter yeast extract) and incubated at 35°C overnight. The enrichment then was inoculated onto MRSA chromogenic agar (BBL CHROMagar MRSA; Becton, Dickinson and Company, Sparks, MD, USA) and incubated at 35°C for 48 h. Suspect colonies were confirmed to be MRSA by coagulase test, *S. aureus* latex agglutination assay (Pastorex Staph-plus; Bio-Rad, Marnes-la-Coquette, France), and a penicillin binding protein 2a latex agglutination assay (MRSA latex agglutination test; Oxoid Ltd., Hants, United Kingdom). One isolate from each MRSA-positive pig was stored for further analysis.

Molecular characterization of isolates. Five isolates of MRSA were randomly chosen from each MRSA-positive nursery herd for further characterization. Extraction of DNA was completed with InstaGene Matrix (Bio-Rad Laboratories) in accordance with the instructions of the manufacturer. Multiplex PCR was used for detection of *mecA* and *mecC* (16), and PCR detection of Panton-Valentine Leukocidin (PVL) genes and *scn* was completed as previously described (17, 18). The staphylococcal protein A gene (*spa*) also was amplified and sequenced (19), and isolates were assigned a *spa* type from the Ridom SpaServer database (http://www.spaserver.ridom.de).

Susceptibility to zinc was determined by agar dilution, with inoculation of a 0.5 McFarland suspension of each isolate on Muller-Hinton II agar plates containing $ZnCl_2$ (0.25 to 16 mM) (20). Plates were read after 24 h of incubation at 35°C, and the MIC of $ZnCl_2$ was recorded. Isolates with a MIC of >2 mM were considered zinc resistant (14). Identification of the zinc resistance gene *czrC* was completed by PCR amplification (21).

A selection of genes responsible for tolerance to quaternary ammonium compounds (QACs) (*qacAB*, *qacG*, *qacH*, *qacJ*, and *smr*) was evaluated using a real-time PCR protocol (22). The amplification and melting curves were assessed for agreement with controls, and the products were confirmed by sequencing. Phenotypic susceptibility to QACs was completed using the protocol developed by Sundheim et al. (23). Bacteria were suspended (final concentration of 10^5 cells/ml) in microdilutions of Muller-Hinton broth containing benzalkonium chloride (0.5 to 12.0 μg/ml). The microtiter plates were incubated for 48 h and read on a spectrophotometer using a 630-nm filter. The MIC was recorded as the concentration needed to completely prevent regrowth. Two technical replicates were performed for this protocol.

Statistical analysis. The group-level nursery data initially were analyzed using univariate methods. Fisher's exact test was used for dichotomous predictors of MRSA status, and the Wilcoxon rank-sum test was used for continuous predictors of MRSA status. Further individual-based analysis was performed to investigate the associations observed in the group-level data. A multivariate random-effect logistic regression model was constructed using individual data from 311 pigs that were not shedding MRSA prior to weaning. The model was built manually, and *a priori* decisions were made to assess confounding as a >30% alteration in the coefficient and to assess two-way interactions between variables demonstrating a tendency toward significance ($P < 0.10$). Additional descriptive univariate statistics were used to validate the presence or lack of confounding variables. The colinearity of variables also was assessed. Variables were retained in the final model only if they were confounders, were part of an interaction term, or demonstrated statistical significance. Standardized residuals and the best linear unbiased predictions of the random effect were used to assess the fit of the model, and the final model was accepted only if the assumptions of normality and homoscedasticity were met. Statistical analysis was completed using STATA 10.0 I/C, and the null hypothesis was rejected at $P < 0.05$ for all statistical tests used in this study.

TABLE 1 Observed concentration of zinc in nursery rations and methicillin-resistant *Staphylococcus aureus* carriage in 22 swine cohorts

In-feed zinc concn (mg zinc[a]/kg feed)	No. (%) of:	
	Cohorts	MRSA-positive cohorts
<500	1 (4.5)	0
500–999	5 (22.7)	0
1,000–1,499	0	0
1,500–1,999	0	0
2,000–2,499	2 (9.1)	1 (4.5)
2,500–3,000	13 (59.1)	7 (31.8)
>3,000	1 (4.5)	0

[a] All zinc was supplemented in the ration as zinc oxide.

RESULTS

Descriptive statistics of nursery herds. The swine herds participating in the study had an average of 524 sows (standard deviation [SD], 440; range, 25 to 1,500) and weaned an average of 951 pigs per month (SD, 758; range, 30 to 2,600). Ten (45.4%) of the 22 nursery herds identified as operating as continuous-flow operations, while the remaining 12 nursery herds identified as being all-in/all-out operations. Two of the farrow-to-finish herds raise pigs without exposure to antimicrobials and receive a premium at slaughter.

The prevalence of MRSA among suckling pigs was 24.1% (94/390), and MRSA was detected in 27.3% (6/22) of the cohorts at the end of the suckling phase. At 3 weeks postweaning, 23.3% (90/387) of pigs carried MRSA and 36.4% (8/22) of the cohorts were MRSA positive (Table 1). All cohorts testing positive prior to weaning also were positive at 3 weeks postweaning. The swine operations testing positive for MRSA (2 farrow-to-wean, 2 wean-to-finish, and 6 farrow-to-finish sites) were located in 7 of the 10 counties visited in southern Ontario.

Risk factors for MRSA in nursery herds. Univariate analysis of risk factors associated with MRSA carriage in nursery herds is presented in Table 2. Nursery herds testing positive for MRSA reported more frequent use of zinc therapy and disinfectants, as well as having a higher stocking density. Interestingly, the presence of companion animals (dogs and cats) on the farm was negatively associated with MRSA status. The presence of MRSA was not associated with any particular antimicrobial therapy, the number of antimicrobials used, or the route of administration in the nursery herds.

After analyzing the data with a multivariate random-effect logistic regression model, only two variables remained significantly associated with MRSA in nursery herds: in-feed zinc concentration (mg zinc per kg feed; odds ratio [OR], 1.000915; 95% confidence intervals [CI], 1.000405 to 1.001425; $P < 0.001$) and disinfection of the nursery for each new group of incoming pigs (OR, 14.12; 95% CI, 4.36 to 45.77; $P < 0.001$). Based on the predictions of this model, the odds of MRSA carriage in pigs consuming a

TABLE 2 Factors associated with methicillin-resistant *Staphylococcus aureus* in nursery herds

Parameter	Mean or proportion of parameter for:		*P* value[a]
	MRSA-positive cohorts ($n = 8$)	MRSA-negative cohorts ($n = 14$)	
Herd size (no. of sows, SD)	698 (433)	425 (427)	0.076
Nursery was off site (%)	25	14.3	0.602
Continuous nursery flow (%)	25	57.1	0.204
Avg weaning age (days, SD)	22.4 (2.6)	24.5 (4.8)	0.351
No. of pigs weaned per month (SD)	1,224 (696)	795 (711)	0.088
Nursery stocking density (no. of pigs/m^2, SD)	3.22 (1.23)	2.47 (1.17)	0.048
Direct pig-to-pig contact between pens (%)	50	42.9	0.999
Exposure to wooden surfaces (%)	25	21.4	0.999
Temp upon entrance to nursery (°C, SD)	27.6 (1.23)	26.7 (2.79)	0.273
No outside breeding stock replacements (%)	62.5	71.4	0.999
Danish entry (%)	37.5	42.9	0.999
Bootbath (%)	0	28.6	0.254
Shower-in/shower-out required (%)	62.5	28.6	0.187
Danish entry, bootbath, or shower (%)	75	71.4	0.999
Older pigs sometimes mixed with new pigs coming into the nursery (%)	25	35.7	0.999
Nursery pens disinfected for incoming pigs every time (%)	100	50	0.022
Corridors are disinfected on a weekly to monthly basis (%)	87.5	42.9	0.074
Cat(s) and/or dog(s) on the property (%)	37.5	92.9	0.011
Pets allowed into the barn (%)	0	71.4	0.115
Live rodents observed in barn at sampling (%)	12.5	7.1	0.999
Wild birds observed in barn in past year (%)	12.5	42.9	0.193
Antibiotic administration (%)			
Feed	87.5	78.6	0.999
Water	37.5	42.9	0.999
Injection	87.5	71.4	0.613
Antimicrobial treatment (%)[b]			
Tetracycline (in-feed)	62.5	85.7	0.309
Tiamulin (in-feed)	25	42.9	0.649
Penicillin (in-feed/injection)	50	50	0.999
Sulfamethazine (in-feed/in-water)	25	21.4	0.999
Zinc therapy (≥2,000 ppm in-feed)	100	50	0.022
No. of different antibiotics administered at the group-level (SD)	1.88 (1.13)	2.43 (1.09)	0.345

[a] Univariate statistics (Fisher's exact or Wilcoxon rank sum).
[b] Group-level exposure to antimicrobial between birth and 3 weeks postweaning.

ration containing 3,000 mg zinc/kg feed was 12.4 times greater than the odds of MRSA carriage in pigs consuming a ration containing 250 mg zinc/kg feed (95% CI, 3.04 to 50.25; $P < 0.001$), which was the lowest in-feed zinc concentration observed in this study. The average concentration of zinc used in nursery feed was 2,284 mg zinc/kg feed (range, 250 to 7,000 mg zinc/kg feed), and 15 nursery herds (68.2%) used a therapeutic concentration (2,000 to 3,000 mg zinc/kg feed) (Table 1). There were no significant two-way interaction terms or confounding variables detected in the final multivariable model.

Molecular characterization of MRSA isolates. Twenty-five (62.5%) of the 40 isolates of MRSA, originating from 6 (75%) of the 8 MRSA-positive nursery herds, carried the *czrC* gene, but 90% (36/40) of isolates were phenotypically resistant to zinc chloride (MIC of >2 mM). Two nursery herds carried both *czrC*-positive and *czrC*-negative genotypes of MRSA. The MICs toward $ZnCl_2$ are presented in Table 3 for *czrC* genotypes of MRSA.

Each MRSA isolate harbored at least 1 resistance gene for QACs, and 30 (75%) of the 40 isolates harbored 2 or more QAC resistance genes. The most common genotype was *qacG qacH smr*, which was detected in 32.5% (13/40) of the tested isolates which originated from 6 (75%) of the 8 MRSA-positive nursery herds. One MRSA isolate (*spa* type t571) carried *qacAB* along with the *qacG*, *qacH*, and *smr* genes. The *qacJ* gene was not detected in any

TABLE 3 MIC of zinc chloride ($ZnCl_2$) among methicillin-resistant *Staphylococcus aureus* isolates from nursery pigs

MRSA genotype	No. of isolates	No. of farms	No. of isolates with $ZnCl_2$ MIC (mM) of:								
			0.25	0.5	1.0	2.0	4.0	6.0	8.0	12.0	16.0
czrC positive	25	6						6	13	3	3
czrC negative	15	4				4	11				

TABLE 4 MIC of BKC[a] among methicillin-resistant *Staphylococcus aureus* isolates from nursery pigs

MRSA genotype	No. of isolates	No. of farms	No. of isolates with indicated *spa* type				No. of isolates with BKC MIC (μg/ml) of:									
			t034	t571	t3075	t002	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5
qacG	6	3	6						2			1	2	1		
qacH	3	1		3					3							
smr	1	1		1							1					
qacG qacH	6	4	4	1		1			2				1	1	2	
qacG smr	10	5	6	3	1				5			1	3		1	
qacG qacH smr	13	6	7	6					3		1	3	4	1	1	
qacG qacH smr qacAB	1	1		1									1			

[a] BKC, benzalkonium chloride.

of the tested isolates. In addition, there were 7 isolates (17.5%) from 4 farms with a MIC of ≥4.5 μg/ml, indicating a potential to survive exposure to commercial QAC preparations in the presence of organic matter. A summary of the QAC susceptibility profiles is presented in detail in Table 4.

The MRSA isolates tested in this study primarily belonged to the clonal complex (CC) 398-associated *spa* types t034 (57.5%) and t571 (37.5%), along with single isolates of t3075 (CC398 associated) and t002 (CC5 associated). The *spa* types t034 and t571 were from 5 (22.7%) and 3 (13.6%) nursery herds, respectively. The *spa* types t002 and t3075 were from two different farms, each coexisting with *spa* type t034. All of the tested MRSA isolates were negative for *mecC*, *scn*, and PVL. The molecular characteristics of each *spa* type are summarized in Table 5. Lastly, it was observed that isolates belonging to *spa* type t034 were more likely to carry *czrC* ($P < 0.001$) and demonstrated an increased tolerance to zinc ($P < 0.001$) compared to isolates of *spa* type t571.

DISCUSSION

The finding that MRSA in nursery pigs is associated with the concentration of zinc in the nursery ration and the frequent use of disinfectants to clean the nursery, in addition to the commonness of resistance to zinc and QACs among MRSA isolates, indicates that these compounds are important drivers in the selection and persistence of MRSA in swine production systems. Resistance genes for zinc and QACs have been found to colocate with antibiotic resistance genes in mobile genetic elements in the chromosomal genome and on plasmids (14, 24), forming a biological basis for the observed statistical association in this investigation. This is concerning, as exposure to these compounds may cause coselection or coretention of antibiotic resistance genes in environments with low or no antibiotic exposure, as has been demonstrated in laboratory experiments (13, 15, 25, 26).

The association between frequent disinfection of the nursery and MRSA carriage in nursery pigs is consistent with the findings of a meta-analysis which also determined that regular disinfection of fattening pig holdings was associated with MRSA carriage (10). The use of therapeutic levels of in-feed zinc also has been shown to affect the shedding of MRSA in nursery pigs in previous controlled experiments (15, 26). The evidence indicates that therapeutic levels of zinc and routine use of QAC-based disinfectants is exerting a selective pressure on MRSA in commercial nursery herds. The means by which such a selective advantage leads to within-herd changes in the persistence of MRSA still is unclear, but it may be due to a combination of increased survival in the environment and increased host susceptibility to colonization.

Supplementation of the diets of pigs in the postweaning period with high levels of zinc is a common and an increasingly used approach to prevent postweaning diarrhea (most often associated with *E. coli*). Increased public concern about the use of antimicrobials in pork production and the premiums paid for antibiotic-free pork presumably are important driving factors. This study demonstrates that the use of rations containing therapeutic levels of zinc is commonplace in Ontario nursery herds and highlights the complexity of antimicrobial resistance. These data cannot be taken as indicating that movement toward antibiotic-free pork production is harmful; however, they indicate that replacing traditional antimicrobials with compounds that are not considered to be classical anti-infective drugs (yet are being used for their antimicrobial ability) is not an effective means of reducing antimicrobial resistance pressure.

TABLE 5 Distribution of zinc and QAC resistance by methicillin-resistant *Staphylococcus aureus spa* type

Resistance gene/phenotype	No. (%) of isolates with indicated *spa* type[a]				
	t034 ($n = 23$)	t571 ($n = 15$)	t002 ($n = 1$)	t3075 ($n = 1$)	Total ($n = 40$)
qacAB	0 (0)	1 (6.7)	0	0	1 (2.5)
qacG	23 (100)	11 (73.3)	1 (100)	1 (100)	36 (90)
qacH	11 (47.8)	11 (73.3)	1 (100)	0	23 (57.5)
qacJ	0	0	0	0	0
smr	13 (56.5)	11 (73.3)	0	1 (100)	25 (62.5)
≥2 QAC resistance genes	17 (73.9)	11 (73.3)	1 (100)	1 (100)	30 (75)
Mean MIC of BKC (μg/ml)	3.6	3.0	2	2	3.3
czrC	23* (100)	1* (6.7)	0	1 (100)	25 (62.5)
Mean MIC of $ZnCl_2$ (mM)	8.78*	3.73*	2	16	6.9

[a] An asterisk indicates significant differences between *spa* types ($P < 0.05$).

Interestingly, all qualitative questions relating to the reason for using therapeutic levels of zinc were left unanswered by the producers (data not shown). This suggests that producers have a minimal role in setting the in-feed zinc dosage, and further studies should investigate the role of the herd veterinarian and nutritionist with respect to setting the level of in-feed zinc, as it is essential that these findings be communicated to the appropriate target group. Furthermore, it was observed that a single production system was using a nursery ration containing 7,000 mg zinc/kg feed, which is surprising and indicates that there is an opportunity to better educate the industry on the appropriate use of zinc in swine feed.

Quaternary ammonium compounds are routinely used on swine farms and in food-processing facilities as disinfectants, primarily due to the bactericidal activity of these compounds in the presence of organic burdens and the low cost (27). It has been demonstrated that MRSA isolates with a benzalkonium chloride MIC of ≥4.5 μg/ml, as exhibited by 17.5% of isolates in the current study, are able to survive exposure to commercial QAC-based disinfectants in the presence of organic matter (23). Hence, the finding that QAC resistance genes are common among MRSA of porcine origin is concerning and may provide explanations for the observed association between frequent disinfection and MRSA in the present study and the reported ineffectiveness of QAC-based disinfectants for elimination of MRSA from swine holdings (28). Additionally, genes conferring resistance to QACs, particularly *qacG* and *smr*, also have been identified in MRSA ST9, originating from pig carcasses in Hong Kong (29).

Herd size is a documented risk factor in several studies (8–10), but in this study it was not significantly associated with MRSA status; however, it did approach significance. This may be due to limited sample size and the decision not to categorize herd size during analysis, contrary to the approach used in other studies (8–10). Stocking density was associated with MRSA carriage in nursery pigs according to univariate statistics, and although not statistically significant in the final model, it should be investigated further as the rate of contact between pigs can play an important role in MRSA transmission (30). Additionally, the inverse association between cohort-level MRSA status and the presence of companion animals is similar to reports of multispecies farms acting as a protective factor (10), although the reason for this relationship still is unclear.

The lack of an association between MRSA and various measures of antibiotic usage is consistent with previous reports (8, 9). One possible explanation for this lack of association is the use of therapeutic concentrations of in-feed zinc as a replacement for antibiotic therapy. As observed in this study, MRSA-positive cohorts appeared to use fewer antibiotics and higher levels of in-feed zinc than the MRSA-negative cohorts. However, although no confounding effect was detected in the present data set, future studies should consider the possibility for zinc to act as a confounder that can influence the association between antibiotic usage and MRSA status of swine herds. It is also worth mentioning that high fitness and superior host adaptation may contribute to long-term persistence of MRSA in the absence of antimicrobials (31).

One of the limitations of the present study is the limited number of cohorts that were enrolled. This sample size did reduce statistical power when detecting differences at the cohort level, and future studies should investigate these relationships using a larger number of swine herds. Another limitation was a potential for bias to be introduced into the survey results due to producers providing answers that would please the surveyor; however, most of the data were validated by documentation and investigator-completed surveys. Lastly, comparing the measurements of the current study to more comprehensive regional data indicates that the cohorts enrolled in this study were representative of the Ontario swine industry.

Overall, resistance determinants for zinc and QACs are widespread among MRSA of porcine origin and, when paired with the epidemiological evidence, it supports the hypothesis that these compounds are playing an important role in the selection and persistence of MRSA in commercial swine herds. These findings illustrate the multifactorial nature of antimicrobial resistance among staphylococci, and these results may be useful for devising control measures to reduce antimicrobial resistance in staphylococci on swine farms. However, this issue may be region specific, as resistance to zinc can vary substantially by *spa* type, and the dominant *spa* type of pig holdings also differs by region. Therefore, determination of resistance patterns to zinc and QACs in additional MRSA *spa* types from different regions is warranted.

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REFERENCES

1. **Engemann JJ, Carmeli Y, Cosgrove SE, Fowler VG, Bronstein MZ, Trivette SL, Briggs JP, Sexton DJ, Kaye KS.** 2003. Adverse clinical and economic outcomes attributable to methicillin resistance among patients with *Staphylococcus aureus* surgical site infection. Clin Infect Dis **36:**592–598. http://dx.doi.org/10.1086/367653.
2. **Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M.** 2005. Methicillin-resistant *Staphylococcus aureus* in pig farming. Emerging Infect Dis **11:** 1965–1966. http://dx.doi.org/10.3201/eid1112.050428.
3. **Weese J, Zwambag A, Rosendal T, Reid-Smith R, Friendship R.** 2011. Longitudinal investigation of methicillin-resistant *Staphylococcus aureus* in piglets. Zoonoses Public Health **58:**238–243. http://dx.doi.org/10.1111/j.1863-2378.2010.01340.x.
4. **Osadebe L, Hanson B, Smith T, Heimer R.** 2013. Prevalence and characteristics of *Staphylococcus aureus* in Connecticut swine and swine farmers. Zoonoses Public Health **60:**234–243. http://dx.doi.org/10.1111/j.1863-2378.2012.01527.x.
5. **Park J, Friendship RM, Poljak Z, Weese JS, Dewey CE.** 2013. An investigation of exudative epidermitis (greasy pig disease) and antimicrobial resistance patterns of *Staphylococcus hyicus* and *Staphylococcus aureus* isolated from clinical cases. Can Vet J **54:**139.
6. **Buntenkoetter V, Blaha T, Tegeler R, Fetsch A, Hartmann M, Kreienbrock L, Meemken D.** 2014. Comparison of the phenotypic antimicrobial resistances and *spa*-types of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates derived from pigs in conventional and in organic husbandry systems. Berl Munch Tierarztl Wochenschr **127:**135–143.
7. **Vijver L, Tulinski P, Bondt N, Mevius D, Verwer C.** 2014. Prevalence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) in organic pig herds in the Netherlands. Zoonoses Public Health **61:**338–345. http://dx.doi.org/10.1111/zph.12076.
8. **Alt K, Fetsch A, Schroeter A, Guerra B, Hammerl JA, Hertwig S, Senkov N, Geinets A, Mueller-Graf C, Braeunig J, Kaesbohrer A, Appel B, Hensel A, Tenhagen BA.** 2011. Factors associated with the occurrence of MRSA CC398 in herds of fattening pigs in Germany. BMC Vet Res **7:**69. http://dx.doi.org/10.1186/1746-6148-7-69.
9. **Broens E, Graat E, Van Der Wolf P, Van De Giessen A, De Jong M.** 2011. Prevalence and risk factor analysis of livestock associated MRSA-

positive pig herds in The Netherlands. Prev Vet Med **102:**41–49. http://dx.doi.org/10.1016/j.prevetmed.2011.06.005.
10. **Fromm S, Beißwanger E, Käsbohrer A, Tenhagen B.** 2014. Risk factors for MRSA in fattening pig herds—a meta-analysis using pooled data. Prev Vet Med **117:**180–188. http://dx.doi.org/10.1016/j.prevetmed.2014.08.014.
11. **Smith TC, Gebreyes WA, Abley MJ, Harper AL, Forshey BM, Male MJ, Martin HW, Molla BZ, Sreevatsan S, Thakur S.** 2013. Methicillin-resistant *Staphylococcus aureus* in pigs and farm workers on conventional and antibiotic-free swine farms in the USA. PLoS One **8:**e63704. http://dx.doi.org/10.1371/journal.pone.0063704.
12. **O'Brien AM, Hanson BM, Farina SA, Wu JY, Simmering JE, Wardyn SE, Forshey BM, Kulick ME, Wallinga DB, Smith TC.** 2012. MRSA in conventional and alternative retail pork products. PLoS One **7:**e30092. http://dx.doi.org/10.1371/journal.pone.0030092.
13. **Whitehead RN, Overton TW, Kemp CL, Webber MA.** 2011. Exposure of *Salmonella enterica* serovar Typhimurium to high level biocide challenge can select multidrug resistant mutants in a single step. PLoS One **6:**e22833. http://dx.doi.org/10.1371/journal.pone.0022833.
14. **Cavaco LM, Hasman H, Aarestrup FM.** 2011. Zinc resistance of *Staphylococcus aureus* of animal origin is strongly associated with methicillin resistance. Vet Microbiol **150:**344–348. http://dx.doi.org/10.1016/j.vetmic.2011.02.014.
15. **Slifierz M, Friendship R, Weese J.** 2014. Zinc oxide therapy increases prevalence and persistence of methicillin-resistant *Staphylococcus aureus* in pigs: a randomized controlled trial. Zoonoses Public Health http://dx.doi.org/10.1111/zph.12150.
16. **Stegger M, Andersen P, Kearns A, Pichon B, Holmes M, Edwards G, Laurent F, Teale C, Skov R, Larsen A.** 2012. Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either *mecA* or the new *mecA* homologue $mecA_{LGA251}$. Clin Microbiol Infect **18:**395–400. http://dx.doi.org/10.1111/j.1469-0691.2011.03715.x.
17. **Roberts S, O'Shea K, Morris D, Robb A, Morrison D, Rankin S.** 2005. A real-time PCR assay to detect the Panton Valentine Leukocidin toxin in staphylococci: screening *Staphylococcus schleiferi* subspecies *coagulans* strains from companion animals. Vet Microbiol **107:**139–144. http://dx.doi.org/10.1016/j.vetmic.2005.01.002.
18. **van Wamel WJ, Rooijakkers SH, Ruyken M, van Kessel KP, van Strijp JA.** 2006. The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on beta-hemolysin-converting bacteriophages. J Bacteriol **188:**1310–1315. http://dx.doi.org/10.1128/JB.188.4.1310-1315.2006.
19. **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. J Clin Microbiol **37:**3556–3563.
20. **Aarestrup FM, Hasman H.** 2004. Susceptibility of different bacterial species isolated from food animals to copper sulphate, zinc chloride and antimicrobial substances used for disinfection. Vet Microbiol **100:**83–89. http://dx.doi.org/10.1016/j.vetmic.2004.01.013.
21. **Cavaco LM, Hasman H, Stegger M, Andersen PS, Skov R, Fluit AC, Ito T, Aarestrup FM.** 2010. Cloning and occurrence of *czrC*, a gene conferring cadmium and zinc resistance in methicillin-resistant *Staphylococcus aureus* CC398 isolates. Antimicrob Agents Chemother **54:**3605–3608. http://dx.doi.org/10.1128/AAC.00058-10.
22. **Prag G, Falk-Brynhildsen K, Jacobsson S, Hellmark B, Unemo M, Söderquist B.** 2014. Decreased susceptibility to chlorhexidine and prevalence of disinfectant resistance genes among clinical isolates of *Staphylococcus epidermidis*. APMIS **122:**961–967. http://dx.doi.org/10.1111/apm.12239.
23. **Sundheim G, Hagtvedt T, Dainty R.** 1992. Resistance of meat associated staphylococci to a quarternary ammonium compound. Food Microbiol **9:**161–167. http://dx.doi.org/10.1016/0740-0020(92)80023-W.
24. **Sidhu MS, Heir E, Sørum H, Holck A.** 2001. Genetic linkage between resistance to quaternary ammonium compounds and β-lactam antibiotics in food-related *Staphylococcus* spp. Microb Drug Resist **7:**363–371. http://dx.doi.org/10.1089/10766290152773374.
25. **Braoudaki M, Hilton AC.** 2004. Adaptive resistance to biocides in *Salmonella enterica* and *Escherichia coli* O157 and cross-resistance to antimicrobial agents. J Clin Microbiol **42:**73–78. http://dx.doi.org/10.1128/JCM.42.1.73-78.2004.
26. **Moodley A, Nielsen SS, Guardabassi L.** 2011. Effects of tetracycline and zinc on selection of methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type 398 in pigs. Vet Microbiol **152:**420–423. http://dx.doi.org/10.1016/j.vetmic.2011.05.025.
27. **Amass SF, Vyverberg B, Ragland D, Dowell CA, Anderson CD, Stover JH, Beaudry DJ.** 2000. Evaluating the efficacy of boot baths in biosecurity protocols. J Swine Health Prod **8:**169–173.
28. **Merialdi G, Galletti E, Guazzetti S, Rosignoli C, Alborali G, Battisti A, Franco A, Bonilauri P, Rugna G, Martelli P.** 2013. Environmental methicillin-resistant *Staphylococcus aureus* contamination in pig herds in relation to the productive phase and application of cleaning and disinfection. Res Vet Sci **94:**425–427. http://dx.doi.org/10.1016/j.rvsc.2012.10.020.
29. **Wong T, Zhang M, O'Donoghue M, Boost M.** 2013. Presence of antiseptic resistance genes in porcine methicillin-resistant *Staphylococcus aureus*. Vet Microbiol **162:**977–979. http://dx.doi.org/10.1016/j.vetmic.2012.10.017.
30. **Broens EM, Espinosa-Gongora C, Graat EA, Vendrig N, Van Der Wolf PJ, Guardabassi L, Butaye P, Nielsen JP, De Jong MC, Van De Giessen AW.** 2012. Longitudinal study on transmission of MRSA CC398 within pig herds. BMC Vet Res **8:**58. http://dx.doi.org/10.1186/1746-6148-8-58.
31. **Crombé 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. http://dx.doi.org/10.1128/AEM.07356-11.
32. **Agersø Y, Hasman H, Cavaco LM, Pedersen K, Aarestrup FM.** 2012. Study of methicillin resistant *Staphylococcus aureus* (MRSA) in Danish pigs at slaughter and in imported retail meat reveals a novel MRSA type in slaughter pigs. Vet Microbiol **157:**246–250. http://dx.doi.org/10.1016/j.vetmic.2011.12.023.