ORIGINAL ARTICLE

Resistance to Zinc and Cadmium in *Staphylococcus aureus* of Human and Animal Origin

Rajeshwari Nair, MPH;[1,2] Dipendra Thapaliya, MPH;[3] Yutao Su;[1] Tara C. Smith, PhD[1,3]

OBJECTIVE. Studies conducted in Europe have observed resistance to trace metals such as zinc chloride and copper sulfate in livestock-associated *Staphylococcus aureus*. This study was conducted to determine the prevalence of zinc and cadmium resistance in *S. aureus* isolated in the United States.

DESIGN. Cross-sectional study of convenience sample of *S. aureus* isolates.

PARTICIPANTS. Three hundred forty-nine *S. aureus* isolates, including methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) obtained from human, swine, and retail meat were included in the sample set.

METHODS. Polymerase chain reaction was used to test for the presence of genes for zinc and cadmium resistance (*czrC*), methicillin resistance (*mec*A), and staphylococcal complement inhibitor (*scn*). Antibiotic susceptibility of isolates was tested using the broth microdilution method. Data were analyzed using the multivariable logistic regression method.

RESULTS. Twenty-nine percent (102/349) of *S. aureus* isolates were *czrC* positive. MRSA isolates were more likely to be *czrC* positive compared to MSSA (MRSA *czrC* positive: 12/61, 19.6%; MSSA *czrC* positive: 12/183, 6.6%). After adjustment for oxacillin and clindamycin susceptibility in analysis, multidrug-resistant *S. aureus* was observed to have low odds of being *czrC* positive ($P = .03$). The odds of being *czrC* positive were observed to be significantly high in tetracycline-resistant *S. aureus* isolated from noninfection samples ($P = .009$) and swine ($P < .0001$).

CONCLUSIONS. Resistance to zinc and cadmium was observed to be associated with MRSA, a finding consistently observed in European studies. Prolonged exposure to zinc in livestock feeds and fertilizers could propagate resistance to the metal ion, thereby hindering use of zinc-based topical agents in treating *S. aureus* infections.

Infect Control Hosp Epidemiol 2014;35(S3):S32-S39

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first identified among hospitalized patients and considered to be predominantly acquired nosocomially for almost 3 decades.[1] MRSA infections originating in the community have been reported to be a common occurrence after the bacteria's appearance in the United States.[2] MRSA strains of clonal complex 5 (CC5) and CC8 are identified to be the largest and most diverse. In addition, many other MRSA clones are observed to be in circulation worldwide.[3,4] CC398 has emerged as an important colonizer mainly among food-producing animals, particularly swine, and in humans reporting close contact with these animals (designated livestock-associated MRSA [LA-MRSA]).[5,6] Studies on LA-*S. aureus* (LA-SA) have also observed infections due to both MRSA and methicillin-susceptible *S. aureus* (MSSA) CC398 in humans and animals.[7,8] Additionally, studies conducted in Europe[9] and the United States[9,10] have suggested retail meat to be a potential vehicle for MRSA CC398. However, prevalence and the type of meat contaminated with CC398 may vary by geography.

Previous studies have hypothesized that tetracycline use in the animal industry may function as a driving force in the emergence of the MRSA CC398 sequence type.[11,12] Subsequent research suggested that resistance to antimicrobial metals may be a larger driver of antibiotic resistance than use of specific antimicrobials, as animals are frequently fed supplements containing metal products.[13,14] This is particularly noted for metals such as zinc and copper that are observed to have antibacterial and antimycotic effects against diarrhea and growth depression in weanling pigs.[14,15] However, resistance to these trace elements was observed in *S. aureus* strains isolated from livestock.[14]

Extensive studies identified heavy metal-resistant determinants against compounds such as zinc, cadmium, mercury, and arsenic to be coded on plasmids and mercury on the

Affiliations: 1. Center for Emerging Infectious Diseases, College of Public Health, University of Iowa, Iowa City, Iowa; 2. Department of Epidemiology, College of Public Health, University of Iowa, Iowa City, Iowa; 3. Department of Biostatistics, Environmental Health Sciences and Epidemiology, College of Public Health, Kent State University, Kent, Ohio.

Received May 22, 2014; accepted June 3, 2014; electronically published September 15, 2014.

 0899-823X/2014/35S3-0006$15.00. DOI: 10.1086/677834

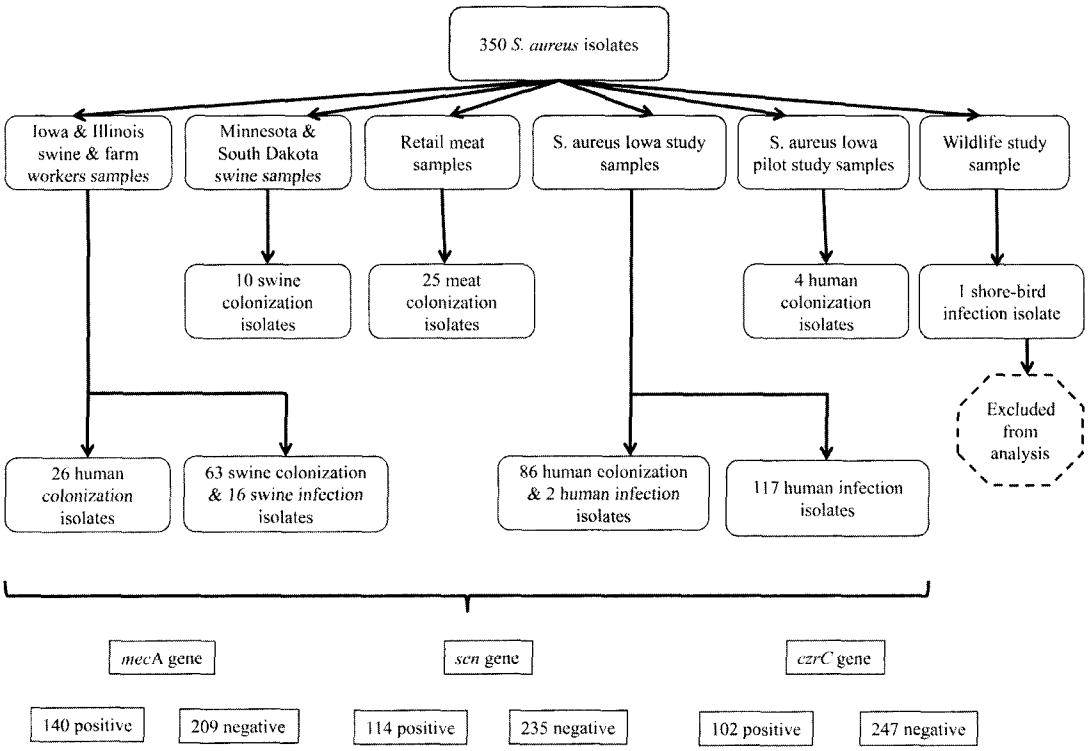

FIGURE 1. *Staphylococcus aureus* study isolate source and characteristics. *mec*A, *scn*, and *czrC* genes were tested by polymerase chain reaction. *czrC*, gene encoding zinc and cadmium resistance; *mec*A, gene encoding methicillin resistance; *scn*, gene encoding staphylococcal complement inhibitor.

chromosome.[16-18] Hence, it was speculated that resistance determinants to other metals such as zinc and cadmium could also be located on a chromosomal mobile element capable of horizontal transfer among *S. aureus* strains.[16] This hypothesis was supported by identifying a gene responsible for resistance to zinc and cadmium (*czrC*) linked to the staphylococcal chromosome cassette (SCC)*mec* element in MRSA CC398 isolates.[17] A similar finding showed clustering of metal resistance determinants on the SCC*mec* element, closely linking it to the *mec*A gene.[18] The loss of resistance determinants for zinc and tetracycline, both frequently used in farming, resulted in complete or partial loss of the SCC*mec* element.[19,20]

This relationship between phenotypic and genotypic presence of zinc and cadmium resistance and methicillin resistance was further strengthened when all tested MSSA isolates were found to be negative for the *czrC* gene.[11,21] *Staphylococcus aureus* isolated from livestock, environmental, retail meat, and humans from both colonization and infection samples was tested, but antibiotic susceptibility data, potential genetic predictors for livestock association (*scn*), and their interactions with zinc and cadmium resistance were not tested. It is crucial to include such data when modeling transmission or spread of *S. aureus* in humans and livestock.

Our study objective was to identify prevalence of the *czrC* gene in *S. aureus* colonizing and infecting isolates from humans, swine, and retail meat contamination samples mostly obtained in Iowa. Associations between potential risk factors and presence of the *czrC* gene as determinants for zinc and cadmium resistance will also be analyzed for the tested isolates. In addition, Iowa contributes about 30% to the production of hogs in the United States, and MRSA CC398 has been identified among Iowa swine and swine workers.[22,25] Individuals with farm and swine exposure in Iowa may also be at an increased risk for colonization with multidrug-resistant *S. aureus* (MDRSA) and tetracycline-resistant strains (Wardyn et al, unpublished observations). The presence of LA-SA strains and the dependence on agriculture and livestock warrant testing for zinc and cadmium resistance and its impact on resistance to other antibiotics.

MATERIAL AND METHODS

An observational study was conducted to assess the prevalence of *czrC*, and a descriptive analysis was conducted to identify predictors for genotypic resistance to zinc and cadmium in *S. aureus*. A convenience sample of 349 *S. aureus* isolates obtained from 2008 to 2013 was tested (Figure 1).[10,23-27] Most isolates were obtained from human nasal and throat colonization and infection samples collected via a longitudinal cohort study of individuals with agricultural

https://doi.org/10.1086/677834 Published online by Cambridge University Press

exposures (via the Iowa Agricultural Health Study) and those living in rural areas but without farming occupations (via the Iowa voter registration database). Infection surveillance was carried out using a subset of Iowa's clinical diagnostic laboratories. Characteristics for farm workers (such as work grade) and patient information for infection surveillance isolates were not available for comparison. *Staphylococcus aureus* isolated from retail meat contamination swabs (raw pork) from Iowa, Minnesota, and New Jersey was also tested for the *czrC* gene. Information on antibiotic and metal ion exposure in sampled asymptomatic swine and retail meat was not available for analyses.

Staphylococcus aureus strain types identified to be sequence types (ST) 398 and ST9 in previous studies were included in this study. Isolates were also included if they belonged to *spa* types within 6 steps of t034 (ST398) and t193 (ST9) using the Based Upon Repeat Pattern (BURP) algorithm[28] or belonged to these sequence types on multilocus sequence type. Additionally, we included isolates identified as *spa* type t002 and its BURP cluster. Previous studies have identified *S. aureus* t002 in livestock; hence, we included these *spa* types regardless of origin.[29] Isolates were also included if they demonstrated complete or intermediate resistance to tetracycline, given the association of resistance with livestock strains.[30,31]

This study was approved by the University of Iowa Human Subjects Office as an exempt study. Isolates were grown and confirmed to be *S. aureus* as described previously.[10]

Staphylococcus aureus Genetic Analysis

Amplification of the *spa* fragment was performed using methods and primers as described previously.[32] Identification of *spa* type for each isolate and *spa* cluster complexes using BURP was performed using Ridom StaphType (ver 2.2.1; Ridom).[28,33] All isolates were tested for methicillin resistance (*mec*A gene),[34] staphylococcal complement inhibitor (*scn* gene),[31] and zinc-cadmium resistance (*czrC* gene),[17,21] as per previously published standard polymerase chain reaction (PCR) protocols. Positive and negative controls were used in all molecular assays.

Antimicrobial Susceptibility Testing (AST)

The antimicrobial susceptibility of isolates was tested by the broth microdilution method for susceptibility to oxacillin, gentamicin, erythromycin, clindamycin, tetracycline, trimethoprim/sulfamethaxozole, imipenam, levofloxacin, linezolid, vancomycin, and daptomycin, in accordance with Clinical Laboratory Standards Institute standards.[35] High-level resistance to mupirocin (MIC $\geq$ 512 mg/L) and inducible clindamycin resistance were also examined. All AST-confirmed MRSA isolates were considered to be MDR. MSSA isolates nonsusceptible to $\geq$1 antimicrobial agent in $\geq$3 discrete antimicrobial classes were also classified as MDR, as per a recently published report on standardization of bacterial antimicrobial resistance profiles.[36] Of the 349 *S. aureus* isolates, 244 (70%) were tested for antibiotic susceptibility. Results on antimicrobial susceptibility and risk predictors for being *czrC* positive are presented for these 244 observations to maintain completeness of data.

Statistical Analysis

Presence or absence of *czrC* was analyzed as the outcome variable. Prevalence of genetic and antibiotic susceptibility predictors was compared between *S. aureus czrC*-positive and *czrC*-negative groups using the χ^2 (or Fisher's exact) test for categorical variables. Due to limitations of sample size, exposures such as the origin of sample (swine, human, retail meat), *S. aureus* strain type (LA, non-LA, nontypeable) and results on antibiotic susceptibility (susceptible, resistant/intermediate) were analyzed individually and as groups in broad categories (sample origin: swine and other, strain type: LA and non-LA, antibiotics: susceptible and resistant).

Effect modifications between covariates were investigated using the Cochran-Mantel-Haenszel method. Multivariable analyses were performed using PROC LOGISTIC to observe for crude and adjusted associations. Variables with *P* values $\leq$.2 in the bivariate analysis were included in model selection. Predictors for zinc and cadmium resistance were identified using a manual backward selection process. The Hosmer-Lemeshow test, Akaike's Information Criterion, and the *c* statistic were used to assess fit for the final parsimonious model. Data analysis was performed using SAS statistical software (ver 9.3; SAS Institute).

RESULTS

Of the 350 *S. aureus* isolates tested and analyzed, 235 were isolated from humans, 89 from swine, 25 from retail meat, and 1 from a wildlife study. The wildlife isolate was excluded from final analysis. Roughly 61% of *S. aureus* isolates were from noninfection samples (214/349) and MSSA (209/349, 59.9%). Presence of *czrC* was observed in 102/349 (29%) of *S. aureus* isolates (Table 1).

There was good concordance between methicillin resistance tested by broth microdilution and PCR. Eight isolates were observed to have discordant oxacillin resistance phenotype-genotype.

Antibiotic Drug Resistance Phenotypes

Complete or intermediate resistance was observed with all tested antibiotics except clarithromycin, rifampin, imipenem, linezolid, daptomycin, tigecycline, and chloramphenicol in the tested isolates. Multidrug resistance was observed in 105/244 (43%) of tested *S. aureus* isolates and 44/183 (24%) of MSSA isolates. Prevalence of MDR was significantly greater in *czrC*-negative isolates (89/105, 84.8%) compared to *czrC*-positive isolates. Resistance to oxacillin, ciprofloxacin, and clindamycin was significantly different among *czrC*-negative and *czrC*-positive *S. aureus* (Table 2).

TABLE 1. Characteristics of *Staphylococcus aureus* Isolates by Origin of Sample

	Noninfection/ infection		Methicillin resistance		Livestock association		Zinc and cadmium resistance		
	NI	I	*mec*A+	*mec*A−	*scn*−	*scn*+	*czrC*+	*czrC*−	Total
Human	116	119	60	175	126	109	23	212	235
Swine	73	16	64	25	86	3	70	19	89
Retail meat	25	0	16	9	23	2	9	16	25
Total	214	135	140	209	235	114	102	247	349

NOTE. Data on the wildlife isolate were excluded. A plus sign indicates that the gene was gene present; a minus sign indicates that it was absent. *czrC*, gene encoding zinc and cadmium resistance; I, infection isolates; *mec*A, gene encoding methicillin resistance; NI, noninfection (includes colonization and retail meat swab samples); *scn*, gene encoding staphylococcal complement inhibitor.

Predictors of Zinc and Cadmium Resistance in *S. aureus*

Bivariate analysis of the association between exposures and resistance to zinc and cadmium is shown in Table 3. The proportion of zinc- and cadmium-resistant isolates was significantly different by *mec*A, *scn*, MDR, oxacillin, erythromycin, and clindamycin resistance; origin of sample (swine vs other), and source of sample (noninfection vs infection). Isolates positive for *mec*A (odds ratio [OR], 3.5; 95% confidence interval [CI], 1.48–8.25), negative for *scn* (OR, 2.7; 95% CI, 1.05–7.16), and MDR (OR, 2.94; 95% CI, 1.21–7.17) were observed to have significantly greater likelihood of being *czrC* positive compared to *mec*A-negative, *scn*-positive, or non-MDR *S. aureus*, respectively. Additionally, *S. aureus* noninfection isolates (OR, 4.09; 95% CI, 1.47–11.3) and isolates of swine origin (OR, 29.78; 95% CI, 7.06–125.7) had significantly greater odds of being *czrC* positive compared to *S. aureus* isolated from infections and nonswine samples (ie, human and meat).

Staphylococcus aureus isolates negative for *scn* were more likely (OR, 2.54; 95% CI, 0.96–6.7; $P = .05$) to be *czrC* positive after controlling for MDR. We also observed a significant association between MDR and *czrC* adjusted for *scn* (OR, 2.78; 95% CI, 1.13–6.84; $P = .02$). Additionally, we observed an increased odds (OR, 4.7) of *czrC*-positive *S. aureus* in noninfection isolates adjusted for tetracycline susceptibility (95% CI, 1.38–16.2; $P = .009$). *Staphylococcus aureus* isolates were also observed to differ in presence of the *czrC* gene by origin (swine vs other) after controlling for tetracycline susceptibility ($P < .0001$).

Several multivariable analyses were performed including variables from bivariate analysis in the logistic regression model. We included the variable tetracycline susceptibility in models since at least 1 study has observed *czrC* and *tet*(K) to be encoded in the same region in the SCC*mec* complex of porcine *S. aureus* isolates, suggesting the potential for selective antibiotic resistance.[20] Table 4 shows the crude and adjusted multivariable models. Methicillin resistance variables by PCR and AST were both included in multivariable models. MRSA was observed to have increased odds of being *czrC* positive compared to MSSA on both crude ($P = .76$) and adjusted ($P = .0005$) analysis. The odds of zinc and cadmium resistance in *scn*-negative *S. aureus* significantly decreased when adjusted for oxacillin and clindamycin susceptibility (OR, 0.21; $P = .11$). MDRSA was also observed to have significantly lower odds of being *czrC* positive compared to non-MDRSA on adjusted analysis (OR, 0.06; $P = .03$). The *c* statistic for the final model is 0.86.

Subgroup Analysis

We conducted a subgroup analysis to establish observed associations for zinc and cadmium resistance in human colonization and infection samples. This analysis included 209 *S. aureus* isolates, of which only 8 (3.8%) were *czrC* positive. Roughly 22% were MRSA isolates, 101 (48.3%) *scn* negative, and 82 (39.2%) were MDR. Forty-three percent of 209 *S. aureus* human isolates were from colonization samples, and 53% were identified as study-defined LA-SA strains.

Human MRSA isolates were observed to have greater odds of being *czrC* positive compared to human MSSA isolates, although the association was not statistically significant (OR, 1.26; 95% CI, 0.25–6.5). Additionally, we observed lower odds of LA-SA strains isolated from humans being *czrC* positive (OR, 0.52; 95% CI, 0.12–2.22) compared to non-LA strains. Factors such as *scn*-negative *S. aureus*, MDR, and noninfection strains were observed to have a low odds of being *czrC* positive in humans, albeit not significant.

DISCUSSION

Findings from the study document that zinc and cadmium resistance exists in human and livestock *S. aureus* isolates in the United States. Of the 349 tested isolates, roughly 79% of *S. aureus* isolated from swine was positive for the *czr*C gene, compared to 10% of human and 36% of retail meat contamination *S. aureus* isolates. Our study observed 19.6% of MRSA isolates to be *czrC* positive compared to other studies from Europe that observed prevalence of 74% (MRSA from swine farms),[11] 58% (human and swine MRSA),[17] 70% (swine MRSA), and 41% (veal MRSA).[21] The relatively low prevalence could be due to exclusion of *S. aureus* isolates that were MRSA by presence of the *mec*A gene but lacked antibiotic susceptibility

https://doi.org/10.1086/677834 Published online by Cambridge University Press

TABLE 2. Antibiotic Susceptibility Pattern in Zinc- and Cadmium-Resistant and Zinc- and Cadmium-Susceptible *Staphylococcus aureus* Isolates

	Metal resistance testing by PCR					
	czrC positive		*czrC* negative			
Antibiotics	% resistant	% susceptible	% resistant	% susceptible	No. of isolates tested for antibiotic	*P*
Penicillin	13.6	86.4	26.4	73.6	128	.28
Ampicillin	0	100	9.3	90.7	106	.35
Oxacillin	50	50	22.3	77.7	244	.003
Augmentin	0	100	10.5	89.5	106	.2
Unasyn	0	100	10.5	89.5	106	.2
Cephalosporins	0	100	14.6	85.4	116	.12
Ciprofloxacin	0	100	18.4	81.6	130	.04
Levofloxacin	8.3	91.7	17.3	82.7	244	.39
Norfloxacin	0	100	1.3	98.7	95	1.00
Moxifloxacin	0	100	2.1	97.9	115	1.00
Erythromycin	37.5	62.5	35.3	64.7	242	.83
Clindamycin	54.2	45.8	33.3	66.7	237	.04
Azithromycin	0	100	1.3	98.7	97	1.00
Clarithromycin	0	100	0	100	94	...
Gentamicin	0	100	5.2	94.9	217	.6
Rifampin	0	100	0	100	216	...
Imipenem	0	100	6.3	93.7	100	.58
Meropenem	0	100	6.3	93.7	100	.58
Nitrofurantoin	0	100	1.02	98.98	119	1.00
TMP/SMX	0	100	8.2	91.8	244	.23
Linezolid	0	100	0	100	211	...
Daptomcyin	0	100	0	100	202	...
Tigecycline	0	100	0	100	109	...
Chloramphenicol	0	100	0	100	100	...
Synercid	4.4	95.6	2.8	97.2	199	.53
Tetracycline	83.3	16.7	72.7	27.3	244	.33
Vancomycin	0	100	0.5	99.5	244	1.00
MDR	16/105 (15.2%)		89/105 (84.8%)		...	.01

NOTES. Antimicrobial susceptibility was tested by broth microdilution method. Multidrug-resistant (MDR) isolates are resistant to >3 antibiotics. $P \leq .05$ is significant. *czrC*, gene encoding zinc and cadmium resistance; PCR, polymerase chain reaction; TMP/SMX, trimethoprim/sulfamethaxozole.

results for analysis. Previous studies observed all tested MSSA isolates of human and swine origin to be susceptible to zinc and cadmium.[11,21] However, our study observed genotypic zinc and cadmium resistance in 6.6% of MSSA isolates, suggesting a potential reservoir for this resistance in MSSA. The odds of zinc and cadmium resistance were observed to be 48% lower in study-defined LA-SA strains isolated from human samples. These results in conjunction with our finding of significantly increased odds of *czrC* positivity in swine isolates potentially suggest a relatively strong link of zinc and cadmium resistance in livestock *S. aureus*, particularly swine. This finding is supported by data on the use of zinc in fertilizers and livestock feeds, particularly in weanling and nursery pigs (initially about 2,975 ppm, later reduced to 83 ppm before sending to market) to increase market production.[15,37,38] Breeding (gestation and lactation) sows are also exposed to 150 g/t of zinc in their feed.[38] These practices pose a concern for the emergence of resistance to metals such as zinc and cadmium in organisms of livestock origin. There is also potential for the spread of such organisms to humans, resulting in coresistance to different antimicrobials, adding to the burden of existing antimicrobial resistance.

We observed a significant association of zinc and cadmium resistance with methicillin resistance on bivariate and adjusted analysis. This finding is consistent with results observed in European studies, further strengthening the evidence of the *czrC* gene on the SCC*mec* element.[17,21] A recent study on antimicrobial resistance classified MRSA as MDR due to coresistance to other β-lactams and cephalosporins.[36] This explains the association of *S. aureus* zinc and cadmium resistance with MDR observed in our study. However, the association between MDR and *czrC* was reversed on adjusting for oxacillin susceptibility. This finding suggests that oxacillin/methicillin resistance could be a strong predictor for zinc and

https://doi.org/10.1086/677834 Published online by Cambridge University Press

TABLE 3. Phenotypic and Genotypic Characteristics of *Staphylococcus aureus* by Zinc and Cadmium Susceptibility

	czrC positive, *n* = 24	*czrC* negative, *n* = 220	OR (95% CI)	*P*
Methicillin resistance (*mec*A)				
MRSA	12 (50)	49 (22.3)	3.5 (1.48–8.25)	.0029
scn				
Negative	18 (75)	115 (52.3)	2.74 (1.05–7.16)	.03
MDRSA				
Yes	16 (66.7)	89 (40.5)	2.94 (1.21–7.17)	.01
Oxacillin-resistant *S. aureus*				
Yes	12 (50)	49 (22.3)	3.49 (1.48–8.25)	.0029
Tetracycline-resistant *S. aureus*				
Yes	20 (83.3)	160 (72.7)	1.88 (0.62–5.71)	.33
S. aureus strain				
LA	16 (66.7)	118 (53.9)	1.71 (0.7–4.17)	.23
Levofloxacin-resistant *S. aureus*				
Yes	2 (8.3)	38 (17.3)	0.44 (0.09–1.93)	.39
Erythromycin-resistant *S. aureus*				
Yes	8 (38.1)	15 (17.1)	2.9 (1.06–8.48)	.03
Clindamycin-resistant *S. aureus*				
Yes	12 (57.4)	12 (13.6)	8.44 (2.93–24.31)	<.0001
Ciprofloxacin-resistant *S. aureus*				
Yes	0 (0)	7 (7.9)	...	.34
Synercid-resistant *S. aureus*				
Yes	1 (4.8)	0 (0)	...	.19
Noninfection/infection				
Noninfection	19 (79.2)	106 (48.2)	4.09 (1.47–11.3)	.005
Origin of *S. aureus*				
Swine	7 (29.2)	3 (1.4)	29.78 (7.06–125.7)	3.649E−06[a]

NOTE. Data are no. (%) unless otherwise indicated. The *Staphylococcus aureus* strain was categorized as livestock-associated (LA) and non-LA based on Based Upon Repeat Pattern (BURP) clusters. ST398/ST9 strains and clusters were included in the LA (t034, t571, t1451, t2876, t3075, t3275, t5462, t5883, t7880, t011, t1250, t4247, t2971, t9418, t3446, t337, t193, t1456, t4571) and the non-LA (t179, t548, t4032, t002, t306, t688, t2049, t359, t267, t631, t021, t127, t304, t1149, t524, t731) groups. Four isolates were excluded from BURP due to nontypeability; nontypeable isolates were included in non-LA. Only isolates tested by AST were included in analysis. CI, confidence interval; *czrC*, gene encoding zinc and cadmium resistance; MRSA, methicillin-resistant *S. aureus*; MDRSA, multidrug-resistant *S. aureus*; OR, odds ratio; *scn*, gene encoding for staphylococcal complement inhibitor.

[a] *P* value for Fisher's test reported.

cadmium resistance in *S. aureus* compared to other antimicrobials. This change in direction of association was also applicable to *scn*-negative isolates due to a strong interaction between MDR and *scn*-negative *S. aureus*. We did not observe a significant difference in tetracycline resistance between *czrC*-positive and *czrC*-negative isolates. However, we did observe a significant interaction between tetracycline susceptibility and origin of *S. aureus*, causing us to hypothesize that tetracycline susceptibility could be an important effect modifier in the causal pathway between origin of *S. aureus* (swine vs other) and zinc and cadmium resistance.

Studies with larger sample size and extensive testing on antimicrobial susceptibility are warranted to validate these results. Nevertheless, these findings could be important when considering antimicrobial treatment options in patients with direct (occupation) or indirect (residential proximity) livestock exposure.

Our study has several limitations. One of the major drawbacks was the absence of antibiotic susceptibility results on all included isolates, resulting in potentially spurious findings such as the misclassification of isolates as MDR (or non-MDR) and bias due to loss of information. Additionally, exclusion of isolates may have potentially reduced the power to detect a significant association between methicillin resistance and zinc and cadmium resistance in *S. aureus* isolated from humans. We also did not conduct phenotypic testing for zinc and cadmium resistance. Preceding landmark studies have observed excellent agreement (Kappa coefficient 0.91[21] and 100% concordance[17]) between susceptibility results and presence of *czrC* on PCR, suggesting accuracy of our study

TABLE 4. Crude and Multivariable Model for Predictors of Zinc and Cadmium Resistance in *Staphylococcus aureus*

	Crude		Adjusted	
Variable	OR (95% CI)	*P*	OR (95% CI)	*P*
Origin (swine vs other)	<0.001 (<0.001 to >999.99)	.71	84.74 (11.35–632.62)	<.0001
Noninfection vs infection	0.23 (0.04–1.46)	.12	2.95 (0.64–13.6)	.16
Strain (LA vs non-LA)	1.03 (0.18–5.81)	.97	1.27 (0.28–5.71)	.75
*mec*A (MRSA vs MSSA)	0.64 (0.04–11.01)	.76	23.57 (3.99–139.39)	.0005
scn (negative vs positive)	1.23 (0.19–7.72)	.82	0.21	.11
MDR (yes vs no)	>999.99 (<0.001 to >999.99)	.81	0.06	.03
Oxacillin (R vs S)	11,900.5 (0 to 2.109E−30)	.76	...	...
Clindamycin (R vs S)	0.002 (0 to 2.391E−61)	.94	...	...
Tetracycline (R vs S)	0.49 (0.09–2.53)	.40	0.85 (0.17–4.29)	.84
Erythromycin (R vs S)	0.08 (0 to 3.97E−131)	.98	...	...
scn * MDR	...	...	14.09	.04
scn−, MDR+ vs MDR−	...	...	0.87 (0.16–4.8)	...
scn+, MDR+ vs MDR−	...	...	0.06 (0.005–0.74)	...
MDR−, *scn*− vs *scn*+	...	...	0.21 (0.03–1.39)	...
MDR+, *scn*− vs *scn*+	...	...	3.01 (0.36–24.96)	...

NOTE. Variables were significant at $P \leq .2$. The model was adjusted for susceptibility to oxacillin and clindamycin. 95% CIs are not available for *scn* and MDR due to inclusion of interaction terms. An asterisk denotes interaction between the variables. A plus sign indicates that the gene was present; a minus sign indicates that it was absent. CI, confidence interval; LA, livestock-associated; MDR, multidrug resistant; MDR+ = MDR *S. aureus*; MDR− = non-MDR *S. aureus*; *mec*A, gene encoding methicillin resistance; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; OR, odds ratio; R, resistant; S, susceptible; *scn*, gene encoding for staphylococcal complement inhibitor.

results. Our study included a convenience sample of *S. aureus* isolates, both MRSA and MSSA, limiting generalizability of results to other *S. aureus* strain types or geographical variations. Nevertheless, we observed consistent associations between presence of *czrC* and methicillin resistance.

To summarize, zinc and cadmium resistance was observed in both MRSA and MSSA isolates in sampled regions in the United States. A statistically significant association was observed between methicillin resistance and zinc and cadmium resistance, although roughly 7% of MSSA isolates exhibited this resistance. A greater proportion of *S. aureus* isolated from swine was observed to be *czrC* positive, possibly resulting from consumption of zinc in feeds. *Staphylococcus aureus* isolated from humans also demonstrated zinc and cadmium resistance, potentially due to contact with fertilizers via skin or inhalation. However, these results could not be substantiated due to unavailability of fertilizer or feed exposure data in both humans and animals. Further research with sufficiently powered, prospective studies is warranted to establish a causal association of zinc and methicillin coresistance in *S. aureus* and unfavorable outcomes of exposure to zinc compounds.

ACKNOWLEDGMENTS

Financial support. Sample collection of isolates used in this study was funded in part by R18 HS019966-01 from AHRQ (TCS), K01 OH-009793 from CDC/NIOSH (TCS), a pilot grant (TCS) from U50 OH 007548 from CDC/NIOSH, and National Pork Board contract 14917 (TCS).

Potential conflicts of interest. All authors report no conflicts of interest. All authors submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and the conflicts that the editors consider relevant to this article are disclosed here.

Address correspondence to Tara C. Smith, PhD, 750 Hilltop Drive, Lowry Hall, Kent, OH 44242 (tsmit176@kent.edu).

REFERENCES

1. Dukic VM, Lauderdale DS, Wilder J, Daum RS, David MZ. Epidemics of community-associated methicillin-resistant *Staphylococcus aureus* in the United States: a meta-analysis. *PLoS ONE* 2013;8:e52722.
2. Eells SJ, McKinnell JA, Wang AA, et al. A comparison of clinical outcomes between healthcare-associated infections due to community-associated methicillin-resistant *Staphylococcus aureus* strains and healthcare-associated methicillin-resistant *S. aureus* strains. *Epidemiol Infect* 2012:1–9.
3. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000;38:1008–1015.
4. Robinson DA, Enright MC. Multilocus sequence typing and the evolution of methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect* 2004;10:92–97.
5. Smith TC, Pearson N. The emergence of *Staphylococcus aureus* ST398. *Vector Borne Zoonotic Dis* 2011;11:327–339.
6. Stegger M, Lindsay JA, Sorum M, Gould KA, Skov R. Genetic diversity in CC398 methicillin-resistant *Staphylococcus aureus* isolates of different geographical origin. *Clin Microbiol Infect* 2010;16:1017–1019.

7. Verkade E, Kluytmans J. Livestock-associated *Staphylococcus aureus* CC398: animal reservoirs and human infections. *Infect Genet Evol* 2014;21:523–530.
8. Graveland H, Duim B, van Duijkeren E, Heederik D, Wagenaar JA. Livestock-associated methicillin-resistant *Staphylococcus aureus* in animals and humans. *Int J Med Microbiol* 2011;301:630–634.
9. Agerso Y, Hasman H, Cavaco LM, Pedersen K, Aarestrup FM. 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* 2012;157:246–250.
10. O'Brien AM, Hanson BM, Farina SA, et al. MRSA in conventional and alternative retail pork products. *PLoS ONE* 2012;7:e30092.
11. Aarestrup FM, Cavaco L, Hasman H. Decreased susceptibility to zinc chloride is associated with methicillin resistant *Staphylococcus aureus* CC398 in Danish swine. *Vet Microbiol* 2010;142:455–457.
12. de Neeling AJ, van den Broek MJ, Spalburg EC, et al. High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. *Vet Microbiol* 2007;122:366–372.
13. Amachawadi RG, Shelton NW, Shi X, et al. Selection of fecal enterococci exhibiting tcrB-mediated copper resistance in pigs fed diets supplemented with copper. *Appl Environ Microbiol* 2011;77:5597–5603.
14. Aarestrup FM, Hasman H. Susceptibility of different bacterial species isolated from food animals to copper sulphate, zinc chloride and antimicrobial substances used for disinfection. *Vet Microbiol* 2004;100:83–89.
15. Hill GM, Cromwell GL, Crenshaw TD, et al. Growth promotion effects and plasma changes from feeding high dietary concentrations of zinc and copper to weanling pigs (regional study). *J Anim Sci* 2000;78:1010–1016.
16. Kuroda M, Hayashi H, Ohta T. Chromosome-determined zinc-responsible operon czr in *Staphylococcus aureus* strain 912. *Microbiol Immunol* 1999;43:115–125.
17. Cavaco LM, Hasman H, Stegger M, et al. Cloning and occurrence of czrC, a gene conferring cadmium and zinc resistance in methicillin-resistant *Staphylococcus aureus* CC398 isolates. *Antimicrob Agents Chemother* 2010;54:3605–3608.
18. Ito T, Katayama Y, Asada K, et al. Structural comparison of three types of staphylococcal cassette chromosome mec integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2001;45:1323–1336.
19. Poston SM, Li Saw Hee FL. Genetic characterisation of resistance to metal ions in methicillin-resistant *Staphylococcus aureus*: elimination of resistance to cadmium, mercury and tetracycline with loss of methicillin resistance. *J Med Microbiol* 1991;34:193–201.
20. Vandendriessche S, Vanderhaeghen W, Larsen J, et al. High genetic diversity of methicillin-susceptible *Staphylococcus aureus* (MSSA) from humans and animals on livestock farms and presence of SCCmec remnant DNA in MSSA CC398. *J Antimicrob Chemother* 2014;69:355–362.
21. Cavaco LM, Hasman H, Aarestrup FM. Zinc resistance of *Staphylococcus aureus* of animal origin is strongly associated with methicillin resistance. *Vet Microbiol* 2011;150:344–348.
22. US Department of Agriculture. *Quarterly Hogs and Pigs*. Washington, DC: National Agricultural Statistics Service, Agricultural Statistics Board; 2013.
23. Smith TC, Gebreyes WA, Abley MJ, et al. Methicillin-resistant *Staphylococcus aureus* in pigs and farm workers on conventional and antibiotic-free swine farms in the USA. *PLoS ONE* 2013;8:e63704.
24. Dressler AE, Scheibel RP, Wardyn S, et al. Prevalence, antibiotic resistance and molecular characterisation of *Staphylococcus aureus* in pigs at agricultural fairs in the USA. *Vet Rec* 2012;170:495.
25. Wardyn SE, Kauffman LK, Smith TC. Methicillin-resistant *Staphylococcus aureus* in central Iowa wildlife. *J Wildl Dis* 2012;48:1069–1073.
26. Hanson BM, Dressler AE, Harper AL, et al. Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) on retail meat in Iowa. *J Infect Public Health* 2011;4:169–174.
27. Wardyn SE, Forshey BM, Smith TC. High prevalence of Panton-Valentine leukocidin among methicillin-sensitive *Staphylococcus aureus* colonization isolates in rural Iowa. *Microb Drug Resist* 2012;18:427–433.
28. Mellmann A, Weniger T, Berssenbrugge C, et al. Characterization of clonal relatedness among the natural population of *Staphylococcus aureus* strains by using spa sequence typing and the BURP (based upon repeat patterns) algorithm. *J Clin Microbiol* 2008;46:2805–2808.
29. Hasman H, Moodley A, Guardabassi L, Stegger M, Skov RL, Aarestrup FM. Spa type distribution in *Staphylococcus aureus* originating from pigs, cattle and poultry. *Vet Microbiol* 2010;141:326–331.
30. Lozano C, Gomez-Sanz E, Benito D, Aspiroz C, Zarazaga M, Torres C. *Staphylococcus aureus* nasal carriage, virulence traits, antibiotic resistance mechanisms, and genetic lineages in healthy humans in Spain, with detection of CC398 and CC97 strains. *Int J Med Microbiol* 2011;301:500–505.
31. Price LB, Stegger M, Hasman H, et al. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. *mBio* 2012, e00305-11.
32. Shopsin B, Gomez M, Montgomery SO, et al. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *J Clin Microbiol* 1999;37:3556–3563.
33. Mellmann A, Weniger T, Berssenbrugge C, et al. Based Upon Repeat Pattern (BURP): an algorithm to characterize the long-term evolution of *Staphylococcus aureus* populations based on spa polymorphisms. *BMC Microbiol* 2007;7:98.
34. Bosgelmez-Tinaz G, Ulusoy S, Aridogan B, Coskun-Ari F. Evaluation of different methods to detect oxacillin resistance in *Staphylococcus aureus* and their clinical laboratory utility. *Eur J Clin Microbiol Infect Dis* 2006;25:410–412.
35. Clinical Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing—Twenty-Second Informational Supplement*. Wayne, PA: CLSI; 2012. CLSI document M100-S22.
36. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012;18:268–281.
37. Katouli M, Melin L, Jensen-Waern M, Wallgren P, Mollby R. The effect of zinc oxide supplementation on the stability of the intestinal flora with special reference to composition of coliforms in weaned pigs. *J Appl Microbiol* 1999;87:564–573.
38. Kansas State University College of Agriculture. Swine nutrition guide. http://www.asi.k-state.edu/species/swine/research-and-extension/swine-nutrition-guide.html.