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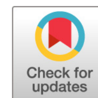

Zinc Resistance within Swine-Associated Methicillin-Resistant *Staphylococcus aureus* Isolates in the United States Is Associated with Multilocus Sequence Type Lineage

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ABSTRACT Zinc resistance in livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) sequence type 398 (ST398) is primarily mediated by the *czrC* gene colocated with the *mecA* gene, encoding methicillin resistance, within the type V staphylococcal cassette chromosome *mec* (SCC*mec*) element. Because *czrC* and *mecA* are located within the same mobile genetic element, it has been suggested that the use of zinc in feed as an antidiarrheal agent has the potential to contribute to the emergence and spread of methicillin-resistant *S. aureus* (MRSA) in swine, through increased selection pressure to maintain the SCC*mec* element in isolates obtained from pigs. In this study, we report the prevalence of the *czrC* gene and phenotypic zinc resistance in U.S. swine-associated LA-MRSA ST5 isolates, MRSA ST5 isolates from humans with no swine contact, and U.S. swine-associated LA-MRSA ST398 isolates. We demonstrated that the prevalence of zinc resistance in U.S. swine-associated LA-MRSA ST5 isolates was significantly lower than the prevalence of zinc resistance in MRSA ST5 isolates from humans with no swine contact and swine-associated LA-MRSA ST398 isolates, as well as prevalences from previous reports describing zinc resistance in other LA-MRSA ST398 isolates. Collectively, our data suggest that selection pressure associated with zinc supplementation in feed is unlikely to have played a significant role in the emergence of LA-MRSA ST5 in the U.S. swine population. Additionally, our data indicate that zinc resistance is associated with the multilocus sequence type lineage, suggesting a potential link between the genetic lineage and the carriage of resistance determinants.

IMPORTANCE Our data suggest that coselection thought to be associated with the use of zinc in feed as an antimicrobial agent is not playing a role in the emergence of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) ST5 in the U.S. swine population. Additionally, our data indicate that zinc resistance is more associated with the multilocus sequence type lineage, suggesting a potential link between the genetic lineage and the carriage of resistance markers. This information is important for public health professionals, veterinarians, producers, and consumers.

KEYWORDS *Staphylococcus aureus*, zinc resistance

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Staphylococcus aureus commonly colonizes the skin and mucosal surfaces of mammalian and avian species and is present in the anterior nares of 20 to 30% of healthy humans (1). *S. aureus* is also a major opportunistic human pathogen with diverse clinical manifestations, ranging from mild skin and soft tissue infections to severe

systemic infections and fatal sepsis. Prior to the availability of antibiotics, fatality rates for human cases of *S. aureus* bacteremia were estimated at 80% (2). Increased access to antibiotics has reduced the case fatality rate of *S. aureus* bacteremia to around 20 to 30% (2), but the capacity of *S. aureus* to acquire resistance to antibiotics has made multidrug-resistant strains a major public health concern (3).

Methicillin-resistant *S. aureus* (MRSA) was first reported in 1961 (4) and rapidly became endemic in hospitals (i.e., hospital-associated MRSA [HA-MRSA]) in many countries. During the 1990s, an increasing number of MRSA infections occurred in persons with no known risk factors for HA-MRSA infection (5). These infections developed in healthy members of the general community and were termed community-associated MRSA (CA-MRSA). *S. aureus* is considered a clonal organism, and genotypes associated with hospital infections typically differed from those associated with community infections, as well as varying geographically (6).

Although MRSA was first reported in food animals (dairy cattle) in 1972 (7), animal reservoirs were not considered to play a significant role in MRSA epidemiology until 2004, when an atypical MRSA variant was detected in three people in the Netherlands and was attributed to their residence on a swine farm (8). These initial isolates could not be typed by pulsed-field gel electrophoresis using SmaI restriction digestion, due to a variation in methylation by the type I restriction modification system. Multilocus sequence typing (MLST) revealed that these isolates belonged to a novel sequence type (ST), ST398 (9). This genotype was found to be widespread in the Dutch pig industry and to be present in other animal species, including cattle, poultry, and horses (1, 10, 11). Subsequent research revealed more complex epidemiology, and the predominant genotypes of MRSA found in swine vary geographically. In most Asian countries, ST9 variants are most common (12, 13); in the United States and Canada, both ST398 and ST5 MRSA variants appear to be relatively common, with ST9 MRSA being detected sporadically (14–17).

Resistance to tetracycline antibiotics has been almost universal in *S. aureus* isolates from pigs. Additionally, a prominent feature of livestock-associated MRSA (LA-MRSA) ST398 isolates from Europe and North America is the high prevalence (61 to 74%) of zinc resistance seen in swine-associated isolates (18–20), relative to isolates from veal calves (42%) or humans (48%) (19, 21). Zinc resistance in these MRSA isolates has been attributed to colocalization of the *czrC* gene (conferring zinc and cadmium resistance) on the type V staphylococcal cassette chromosome *mec* (SCC*mec*) element, which contains the *mecA* gene (conferring methicillin resistance). A strong correlation between phenotypic zinc resistance and the presence of *czrC* was reported previously, with 99% of MRSA ST398 isolates harboring *czrC* showing phenotypic zinc resistance and 96% of isolates exhibiting zinc resistance harboring the *czrC* gene (21). Dietary zinc supplementation at $>$2,400 ppm (compared with the minimum nutritional requirement of 100 to 165 ppm) for 5 to 10 days is commonly used in weaned pigs to control enteric disease. Since *czrC* and *mecA* are colocated on the SCC*mec* element, it has been suggested that the use of high concentrations of zinc in feed might have contributed to the emergence and spread of MRSA in swine, by increasing the selection pressure to maintain the SCC*mec* element in swine-associated ST398 isolates (20, 22–24).

While many reports detailing the prevalence of zinc resistance in LA-MRSA ST398 and ST9 isolates have been published, little to no information exists regarding the prevalence of zinc resistance in LA-MRSA ST5 isolates (21). Here we report the prevalence of zinc resistance in U.S. swine-associated LA-MRSA ST5 isolates and compare it with the prevalence in MRSA ST5 isolates obtained from humans with no swine contact and that in U.S. swine-associated LA-MRSA ST398 isolates, as well as that in previous studies reporting zinc resistance in LA-MRS ST398 isolates.

RESULTS

Prevalence of the *czrC* gene. The *czrC*-specific PCR demonstrated that none of the tested swine-associated MRSA ST5 isolates (0/82 isolates) harbored the *czrC* gene (Table 1). In contrast, all LA-MRSA ST398 isolates (14/14 isolates) tested harbored the *czrC*

TABLE 1 Prevalence of phenotypic zinc chloride resistance and *czrC* presence in isolates from humans with no swine contact and swine-associated isolates

Characteristic	No. of isolates/total no. of isolates (%)		
	MRSA ST5 from humans with no swine contact	LA-MRSA ST5	LA-MRSA ST398
czrC prevalence	16/73 (21.9)[a]	0/82 (0)	14/14 (100)[a]
Phenotypic zinc chloride resistance	18/73 (24.7)[a]	0/82 (0)	14/14 (100)[a]

[a]Statistical significance ($P < 0.0001$), compared to LA-MRSA ST5.

gene. The prevalence of *czrC* in LA-MRSA ST5 isolates associated with swine was significantly lower than that in swine-associated LA-MRSA ST398 isolates ($P < 0.0001$) (Table 1) and also was lower than that reported for other LA-MRSA ST398 isolates ($P < 0.0001$) (21). Over one-fifth of MRSA ST5 isolates obtained from humans with no swine contact (16/73 isolates [22%]) contained the *czrC* gene (Table 1). The prevalence of the *czrC* gene among MRSA ST5 isolates obtained from humans with no swine contact was significantly higher than that among swine-associated ST5 isolates ($P < 0.0001$) (Table 1). Information on individual isolates is provided in Table S1 in the supplemental material.

Zinc chloride susceptibility testing. Susceptibility testing revealed that no swine-associated MRSA ST5 isolates (0/82 isolates) were resistant to zinc chloride, while phenotypic resistance was seen for all LA-MRSA ST398 isolates (14/14 isolates). The prevalence of phenotypic resistance to zinc among MRSA ST5 isolates obtained from humans with no swine contact was 25% (18/73 isolates), greater than among swine-associated MRSA ST5 isolates ($P < 0.0001$) (Tables 1 and 2). Two MRSA ST5 isolates obtained from humans with no swine contact exhibited phenotypic resistance despite not harboring the *czrC* gene. Phenotypic zinc chloride resistance in the absence of *czrC* was reported previously for MRSA ST398 and non-ST398 isolates by Cavaco et al. (21), which indicates that an alternative mechanism for zinc resistance is also present in MRSA ST5 isolates.

SCC*mec* typing. The swine-associated LA-MRSA ST5 isolates carried SCC*mec* type III (17/82 isolates [21%]) or type IV (42/82 isolates [51%]) or could not be typed using the primer sets published previously (23/82 isolates [28%]) (Table 3). Of the 23 untypeable isolates, 20 (24.4% of LA-MRSA ST5 isolates) carried a class D *mec* gene complex, which has not been assigned to a *mec* type, and 3 carried a class A *mec* gene complex without the traditional *ccrA-ccrB* gene combination. All LA-MRSA ST398 isolates tested harbored *SCCmec* type V (14/14 isolates). The MRSA ST5 isolates from humans with no known swine contact mostly carried SCC*mec* type II (69/73 isolates [95%]); the others were type IV (4/73 isolates [5.5%]).

***czrC* localization.** To determine the location of the *czrC* gene within the genomes of the 16 MRSA ST5 isolates obtained from humans with no swine contact that harbored the *czrC* gene, draft genome sequences were obtained, along with the complete genome sequences for two of the strains (UCI28 and UCI62) (25, 26). The gene content and organization of the *SCCmec* region and the surrounding mobile genetic elements for strains UCI28 and UCI62, along with strains Mu3 and S0385 for reference,

TABLE 2 Results from phenotypic zinc chloride resistance screen

Isolate type	No. of isolates with susceptibility of[a]:						
	0.25 mM	0.5 mM	1 mM	2 mM	4 mM	8 mM	16 mM
MRSA ST5 from humans with no swine contact ($n = 73$)	6	0	20	29	14	4	0
LA-MRSA ST5 ($n = 82$)	0	20	53	9	0	0	0
LA-MRSA ST398 ($n = 14$)	0	0	0	0	14	0	0

[a]Values of >2 mM indicate resistance.

TABLE 3 SCC*mec* type and *czrC* gene prevalence among LA-MRSA ST5 and ST398 isolates and MRSA ST5 isolates from humans with no swine contact

Isolate type	SCC*mec* type	*czrC* prevalence (no. of isolates/total no. of isolates)
LA-MRSA ST5	III	0/17
	IV	0/42
	Untypeable[a]	0/23
MRSA ST5 from humans with no swine contact	II	16/69
	IV	0/4
LA-MRSA ST398	V	14/14

[a]Isolates that are unable to be classified into an SCC*mec* type due to the presence of a *ccr* gene or *mec* complex unable to be determined using available primer sets or the presence of a *ccr* and *mec* complex combination not currently assigned an SCC*mec* type.

are shown in Fig. 1. The *SCCmec* region for strains Mu3 and UCI28 contained pUB110 within the J3 region of the SCC*mec* element (Fig. 1A and B). Strain UCI28 and 12 other isolates (UCI3, UCI9, UCI19, UCI21, UCI28, UCI43, UCI45, UCI46, UCI48, UCI52, UCI56, and UCI64) harbored *czrC* downstream of *speG* and upstream of pUB110 and the SCC*mec* element (Fig. 1B) (25, 26). The nucleotide sequences containing *speG* and *czrC* located between the 23S methyltransferase and the second transposase were observed to be 100% identical between strains UCI28 and UCI62. The SCC*mec* elements of Mu3, UCI28, and UCI62 were observed to be 95.8% identical, with nucleotide differences being found in the J2 region. Isolate UCI62, as well as UCI11and UCI27, harbored *czrC* downstream of *speG* and upstream of the arginine catabolic mobile element (ACME) genes and the SCC*mec* element (Fig. 1C) (25, 26). The *czrC* gene for all 16 clinical isolates, even those lacking ACME, was located downstream of *speG*, a spermidine acetyltransferase that functions in the detoxification of spermidine and is often found within the ACME composite island. The location of the *czrC* gene within the SCC*mec* region of these isolates is different than the location of *czrC* in the LA-MRSA ST398 reference strain S0385, in which *czrC* is located downstream of the *mecA* gene within the type V SCC*mec* (Fig. 1D) (27).

DISCUSSION

The recent emergence of MRSA in livestock throughout the world has become a focal point in discussions regarding the role of antibiotic use in food animal production

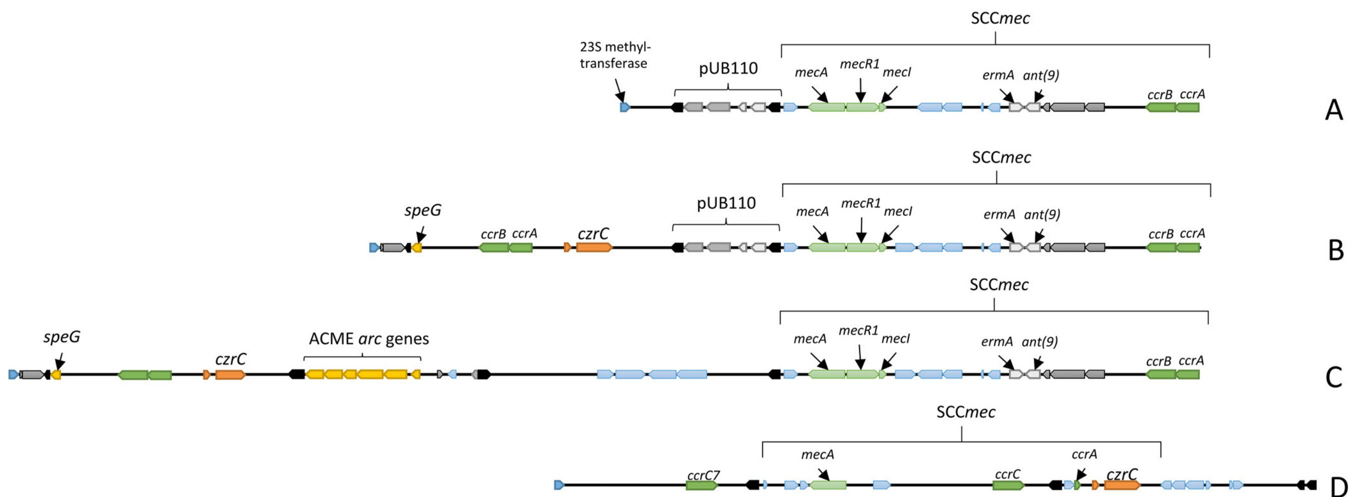

FIG 1 SCC*mec* region and surrounding mobile genetic elements for Mu3 (A), UCI28 (B), UCI62 (C), and S0385 (D). All regions start at the 23S methyltransferase (blue pentagons). Insertion sequences are depicted as solid black pentagons. The locations of the *SCCmec* elements, *czrC*, *speG*, and pUB110 are indicated, along with other previously annotated genes of interest. The SCC*mec* element of S0385 (D) is given as a reference for the location of *czrC* within the type V SCC*mec* element in LA-MRSA ST398.

and the development of antibiotic-resistant clinical infections in humans. However, the mechanisms and factors responsible for this emergence, as well as the factors contributing to the geographical variations in genotypes of swine-associated MRSA found globally, are poorly understood. Although some causal role of antibiotic use in the emergence of LA-MRSA is hypothesized and may seem obvious, epidemiological evidence of such relationships has not been readily demonstrated (28). It is clear that other factors, including disinfectants and metals, may play selective roles in the emergence of particular MRSA clones in humans and animals (20, 29, 30).

In this study, zinc resistance mediated by the *czrC* gene was examined as a potential contributor to the prevalence of LA-MRSA ST5 on swine farms in the United States. A documented association exists between the presence of *czrC* and the *mecA* gene in LA-MRSA ST398 isolates obtained from swine farms, with swine-associated isolates having a higher prevalence of *czrC* than LA-MRSA ST398 isolates obtained from veal calves or humans (19, 21). The strong correlation (99%) reported between isolates harboring the *czrC* gene and phenotypic zinc resistance in LA-MRSA ST398 indicates that this gene orchestrates the predominant mechanism mediating zinc resistance in this lineage (21). The specific importance of the *czrC* gene and the physical link between *mecA* and *czrC* within the SCC*mec* element provide a mechanism by which dietary supplementation of zinc in swine rations could contribute to the persistence of methicillin resistance through coselection (19, 20, 22, 23). Evidence of the practical relevance of this mechanism comes from Denmark, where widespread use of zinc in weaned pig diets as an alternative to antibiotic therapy for controlling enteric disease followed the banning of antibiotics for growth promotion in 2000, approximately a decade before LA-MRSA ST398 became highly prevalent in the Danish swine industry (22).

Sequencing studies have demonstrated that the *czrC* gene is located within the type V and type VIII SCC*mec* elements of ST398 MRSA (19, 31). The majority of European LA-MRSA ST398 isolates investigated carried the type V SCC*mec* element containing the *czrC* gene downstream of the *mecA* gene (27), but none of the LA-MRSA ST5 isolates we examined carried the SCC*mec* type V element. All of the MRSA ST5 isolates from humans with no swine contact that carried the *czrC* gene contained the SCC*mec* type II element (Table 3), which has not been previously associated with the *czrC* gene. In those isolates, the *czrC* gene was located upstream of the SCC*mec* element and possibly transferred with the *speG* gene, which confers resistance to spermidine and a potential selective advantage for isolates colonizing and infecting humans (32). Importantly, the fact that none of the swine-associated LA-MRSA ST5 isolates harbored the SCC*mec* types seen in ST5 MRSA isolates from clinical infections provides further evidence that the animal and human reservoirs of ST5 MRSA appear to be phylogenetically distinct (33).

Previous reports examining *czrC* in LA-MRSA isolates indicated a higher prevalence of this gene in MRSA clonal complex 398 (CC398) isolates (72.5%), compared to all non-CC398 isolates evaluated (25.5%) (21). An absence of *czrC* in European and Asian LA-MRSA isolates of the CC5 and CC9 lineages has been reported, which is consistent with our results evaluating LA-MRSA ST5 isolates from the United States. Collectively, our results and previously published data indicate that the *czrC* gene has a lineage association and is prevalent in the ST398 lineage but is absent or rare among livestock-associated ST5 and ST9 lineages (21). An alternate explanation for the elevated prevalence of the *czrC* gene in the ST398 lineage is the selection pressure incurred with the use of elevated levels of zinc in feed. However, the prevalence of the *czrC* gene in non-ST398 LA-MRSA isolates from European swine was reported to be 30% in the tested isolates, while the phenotypic zinc resistance was reported to be 60% in the same isolates (21), arguing against selection pressure incurred with the use of elevated levels of zinc in feed being the sole factor controlling MRSA prevalence in swine, because the majority of these isolates lacked an SCC*mec* element carrying *czrC*. Although no national data concerning the use of zinc in swine rations exist, the practice is thought to be widespread in the United States (M. Tokach, personal communication).

This appears not to have played the same role in propagating methicillin resistance in livestock isolates of *S. aureus* in the United States, as the majority of herds tested in recent studies were MRSA negative (14, 34).

Our results reported here, combined with previously reported results (21), open new avenues of research to be explored. First, the *czrC* gene has been identified in two methicillin-susceptible *S. aureus* ST398 isolates (18). The presence of this gene without the SCC*mec* element should be evaluated to determine whether *czrC* is a remnant from a previously methicillin-resistant isolate or whether the *czrC* gene has been integrated through a different mechanism. Both LA-MRSA isolates (21) and swine-associated methicillin-susceptible *S. aureus* isolates (J. Sun, R. S. Singer, S. J. Hau, T. L. Nicholson, and P. R. Davies, unpublished data) that show phenotypic zinc resistance without carrying the *czrC* gene have been identified. Such isolates should be screened for other mechanisms of zinc resistance, to determine the impact of other genes in conferring a resistant phenotype. Evaluation of the impact of *czrC* in non-ST398 LA-MRSA, specifically the ability of LA-MRSA ST5 isolates to acquire and to harbor *czrC*, and the impact of zinc in feed on the capability of LA-MRSA ST398 isolates to outcompete other lineages in swine also bears further investigation. Ultimately, zinc resistance in LA-MRSA is more complex than the presence or absence of *czrC* or the use of zinc in feed as an antimicrobial agent to combat disease in livestock. Further investigation is needed to determine the mechanisms leading to zinc resistance and to illuminate the impact of selective pressure on the emergence of particular MRSA clones in humans and animals.

Overall, the data reported here indicate that coselection associated with zinc supplementation in feed has not contributed to the persistence or prevalence of LA-MRSA ST5 in the U.S. swine population. This conclusion is contrary to theories surrounding the dissemination of LA-MRSA ST398 in Europe and, considering the presence of *czrC* in LA-MRSA ST398 isolates in the United States, indicates a potential link between the genetic lineage and the carriage of specific resistance markers, such as that seen for *qacA* resistance in CC22 in the hospital setting (30). Furthermore, the data reported here indicate that multiple mechanisms contribute to fitness and the ability of LA-MRSA ST5 and other lineages to compete and to persist in the nasal microbiota of pigs.

MATERIALS AND METHODS

Isolate acquisition. Swine-associated LA-MRSA ST5 cultures were isolated from swine ($n = 38$), the environment within swine facilities ($n = 26$), and persons with short-term ($n = 9$) and long-term ($n = 9$) swine contact. These isolates were provided by Iowa State University and the University of Minnesota (14). Clinical isolates from humans with no swine contact were obtained from the University of California, Irvine ($n = 64$) (35), and the University of California, San Francisco ($n = 9$), hospitals servicing urban populations in Orange County (southern California) and the San Francisco area (northern California), respectively. Swine-associated LA-MRSA ST398 cultures obtained from Iowa State University were isolated from swine ($n = 8$) or the environment within swine facilities ($n = 6$) (14). Isolates were subjected to MLST and *spa* typing prior to acquisition (14, 35). Isolate sources and *spa* types are provided in Table S1 in the supplemental material.

Zinc susceptibility testing. Zinc chloride MICs were determined by agar dilution, as described by Aarestrup and Hasman (36). Briefly, plates of Mueller-Hinton agar with an adjusted pH of 5.5 were supplemented with zinc chloride in 2-fold dilutions, with concentrations ranging from 0.25 to 16 mM. The isolate *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028 was used as a positive control, and *S. aureus* ATCC 29213 and ATCC 43300 were used as negative controls. A MIC value of >2 mM was used as the cutoff value to designate resistance, in accordance with previously published reports and the result for the positive control in this study (19, 21).

***czrC* PCR testing.** The presence of the *czrC* gene was determined by PCR using previously reported primers and protocols (19). Briefly, PCR was carried out in an MJ Research PCT-200 DNA Engine thermocycler (GMI, Ramsey, MN) using 50 ng of purified genomic DNA from the appropriate strains, the forward primer 5′-TAGCCACGATCATAGTCATG-3′, and the reverse primer 5′-ATCCTTGTTTTCCTTAGTGA CTT-3′. Reaction mixtures included 0.4 μM primers, 1 U of AmpliTaq polymerase (Applied Biosystems, Foster City, CA), 2.5 μl of 10$\times$ buffer II (100 mM Tris-HCl [pH 8.3], 500 mM KCl), 2.5 mM $MgCl_2$, and 200 μM deoxynucleoside triphosphates (dNTPs), in a final volume of 50 μl. Cycling conditions were 95°C for 2 min, 30 cycles of 95°C for 15 s, 52°C for 30 s, and 72°C for 1.5 min, and a final extension step of 72°C for 7 min. PCR products were run on a 1% agarose gel, stained with ethidium bromide, and visualized using UV light.

SCC*mec* typing. SCC*mec* typing was completed using previously designed primer sets (Table 4) (37–42). Briefly, PCR was carried out in a MJ Research PCT-200 DNA Engine thermocycler (GMI) using 50

TABLE 4 Primer sets used for SCC*mec* typing of isolates

Primer name	Nucleotide sequence	Expected product (bp) (forward primer used)	Source
ccrB-F	ATTGCCTTGATAATAGCCITCT		Ito et al. (37)
ccrA1-R	AACCTATATCATCAATCAGTACGT	694 (ccrB-F)	Ito et al. (37)
ccrA2-R	TAAAGGCATCAATGCACAAACACT	937 (ccrB-F)	Ito et al. (37)
ccrA3-R	AGCTCAAAAGCAAGCAATAGAAT	1,791 (ccrB-F)	Ito et al. (37)
ccrA4-R	GTATCAATGCACCAGAACTT	1,287 (ccrB-F)	Kondo et al. (38)
ccrC-F	CGTCTATTACAAGATGTTAAGGATAAT		Kondo et al. (38)
ccrC-R	CCTTTATAGACTGGATTATTCAAAATAT	518 (ccrC-F)	Kondo et al. (38)
mecI-F	CAAGTGAATTGAAACCGCCT		Okuma et al. (39)
mecI-R	CAAAAGGACTGGACTGGAGTCCAAA	187 (mecI-F)	Okuma et al. (39)
mecR1-R	GTCTCCACGTTAATTCCATT	1,920 (mecI-F)	Kobayashi et al. (40)
Class B-F	TATACCAAACCCGACAAC		Katayama et al. (41)
IS1272-R	AACGCCACTCATAACATATGGAA	1,996 (class B-F)	Okuma et al. (39)
Class C-F	AACGTTGTAACCACCCCAAGA		Hiramatsu et al. (42)
IS431-R	TGAGGTTATTCAGATATTTCGATGT	2,072 (class C-F)	Katayama et al. (41)

ng of purified genomic DNA from the appropriate strains; reaction mixtures included 0.4 μM primers, 1 U of AmpliTaq polymerase (Applied Biosystems), 2.5 μl of 10× buffer II (100 mM Tris-HCl [pH 8.3], 500 mM KCl), 2.5 mM $MgCl_2$, and 200 μM dNTPs, in a final volume of 50 μl. PCR for the *ccrA* and *ccrB* genes was a multiplex reaction with cycling conditions of 95°C for 2 min, 10 cycles of 95°C for 15 s, 65°C for 30 s, and 72°C for 1.5 min, 25 cycles of 95°C for 15 s, 55°C for 30 s, and 72°C for 1.5 min, and a final extension step of 72°C for 7 min. PCR of the *ccrC* gene used cycling conditions of 95°C for 2 min, 30 cycles of 95°C for 15 s, 58°C for 30 s, and 72°C for 2 min, and a final extension step of 72°C for 7 min. PCR of the *mec* element genes was completed with cycling conditions of 95°C for 2 min, 30 cycles of 95°C for 15 s, 50°C for 30 s, and 72°C for 2 min, and a final extension step of 72°C for 7 min. PCR products were run on a 1% agarose gel, stained with ethidium bromide, and visualized using UV light.

Whole-genome sequencing and analysis. Draft genome sequence data for 14 *czrC*-carrying isolates (UCI3, UCI9, UCI11, UCI19, UCI21, UCI24, UCI27, UCI43, UCI45, UCI46, UCI48, UCI52, UCI56, and UCI64) were generated using the Illumina MiSeq platform (Illumina, San Diego, CA) (25). Indexed libraries were generated and run on the MiSeq platform using the 500-cycle MiSeq v2 reagent kit. The data were assembled using MIRA 4.0.2 (http://mira-assembler.sourceforge.net/docs/DefinitiveGuideToMIRA.html) (43). Closed genomes were obtained for UCI28 and UCI62 as described previously (26). Briefly, genomic DNA was sequenced with a PacBio RSII instrument using a 10-kb insert library and one SMRT cell for each isolate. The data were assembled using PacBio SMRT Analysis 2.3.0 and CANU 1.3 software. The genomes were then polished and error corrected using Illumina MiSeq data and Broad Institute Pilon 1.18 software. Whole-genome sequence data were analyzed using Geneious 9.0.5 (Biomatters Ltd., Auckland, New Zealand). The SCC*mec* region was extracted from the closed genome sequences of Mu3 (GenBank accession number AP009324.1), UCI28 (GenBank accession number CP018768), UCI62 (GenBank accession number CP018766), and S0385 (GenBank accession number AM990992.1); these were compared visually in Geneious, using multiple sequence alignments to determine similarity. For the 14 draft genomes, the contig harboring the *czrC* gene was extracted and used for comparison. These regions were analyzed for similarity to UCI28 and UCI62 using multiple sequence alignments.

Statistical analysis. Comparisons between isolates from humans with no swine contact and swine-associated isolates were completed using Fisher's exact test using GraphPad Prism (GraphPad Software, La Jolla, CA).

Accession number(s). The whole-genome sequences for isolates UCI28 and UCI62 were deposited in DDBJ/ENA/GenBank with the following accession numbers: UCI28, CP018768 and CP018769; UCI62, CP018766 and CP018767 (26). The draft genome sequences obtained for 14 *S. aureus* ST5 isolates were deposited in DDBJ/ENA/GenBank with the following accession numbers: UCI3, LKYU00000000; UCI9, LKZA00000000; UCI11, LKZC00000000; UCI19, LKZK00000000; UCI21, LKZM00000000; UCI24, LKZP00000000; UCI27, LKZS00000000; UCI43, LLAI00000000; UCI45, LLAK00000000; UCI46, LLAL00000000; UCI48, LLAN00000000; UCI52, LLAR00000000; UCI56, LLAV00000000; UCI64, LLBD00000000 (25).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AEM.00756-17.

SUPPLEMENTAL FILE 1, XLSX file, 0.1 MB.

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Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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