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PUBLIC AND ENVIRONMENTAL HEALTH MICROBIOLOGY

Methicillin-Resistant *Staphylococcus aureus* Sequence Type (ST) 5 Isolates from Health Care and Agricultural Sources Adhere Equivalently to Human Keratinocytes

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ABSTRACT *Staphylococcus aureus* is part of the nasal microbiome of many humans and has become a significant public health burden due to infections with antibiotic-resistant strains, including methicillin-resistant *S. aureus* (MRSA) strains. Several lineages of *S. aureus*, including MRSA, are found in livestock species and can be acquired by humans through contact with animals. These livestock-associated MRSA (LA-MRSA) isolates raise public health concerns because of the potential for livestock to act as reservoirs for MRSA outside the hospital setting. In the United States, swine harbor a mixed population of LA-MRSA isolates, with the sequence type 398 (ST398), ST9, and ST5 lineages being detected. LA-MRSA ST5 isolates are particularly concerning to the public health community because, unlike the isolates in the ST398 and ST9 lineages, isolates in the ST5 lineage are a significant cause of human disease in both the hospital and community settings globally. The ability of swine-associated LA-MRSA ST5 isolates to adhere to human keratinocytes *in vitro* was investigated, and the adherence genes harbored by these isolates were evaluated and compared to those in clinical MRSA ST5 isolates from humans with no swine contact. The two subsets of isolates adhered equivalently to human keratinocytes *in vitro* and contained an indistinguishable complement of adherence genes that possessed a high degree of sequence identity. Collectively, our data indicate that, unlike LA-MRSA ST398 isolates, LA-MRSA ST5 isolates do not exhibit a reduced genotypic or phenotypic capacity to adhere to human keratinocytes.

IMPORTANCE Our data indicate that swine-associated livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) ST5 isolates are as capable of adhering to human skin and have the same genetic potential to adhere as clinical MRSA ST5 isolates from humans. This suggests that humans in contact with livestock have the potential to become colonized with LA-MRSA ST5 isolates; however, the genes that contribute to the persistence of *S. aureus* on human skin were absent in LA-MRSA ST5 isolates. The data presented here are important evidence in evaluating the potential risks that LA-MRSA ST5 isolates pose to humans who come into contact with livestock.

KEYWORDS LA-MRSA, *Staphylococcus aureus*, swine

Humans and several other mammals harbor *Staphylococcus aureus* as a component of their nasal and skin microbiome. Although this organism is a commensal in 25 to 33% of humans in developed countries (1), it can cause opportunistic infections that

Received 19 September 2017 **Accepted** 28 October 2017

Accepted manuscript posted online 3 November 2017

Citation Hau SJ, Kellner S, Eberle KC, Waack U, Brockmeier SL, Haan JS, Davies PR, Frana T, Nicholson TL. 2018. Methicillin-resistant *Staphylococcus aureus* sequence type (ST) 5 isolates from health care and agricultural sources adhere equivalently to human keratinocytes. Appl Environ Microbiol 84:e02073-17. https://doi.org/10.1128/AEM.02073-17.

Editor Edward G. Dudley, The Pennsylvania State University

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range in severity from mild skin infections to severe systemic infections (2, 3). Treatment of these infections is challenging due to the rapid acquisition of antimicrobial resistance genes, including the staphylococcal cassette chromosome *mec* element (SCC*mec*) element, which carries the *mecA* gene, conferring methicillin resistance (4, 5). These isolates, deemed methicillin-resistant *S. aureus* (MRSA), have become a significant public health burden in the United States, annually causing thousands of infections, which result in significant health care costs and losses in productivity (6, 7).

MRSA isolates are classified by the source from which they are acquired to form the following categories: hospital-acquired MRSA (HA-MRSA), community-acquired MRSA (CA-MRSA), and livestock-associated MRSA (LA-MRSA) (8). These subsets of isolates possess unique characteristics that allow them to thrive in each environment. For example, HA-MRSA isolates tend to possess a large number of antimicrobial resistance genes that enable them to survive in a hospital setting, where the use of antimicrobial agents is more common (9). Alternatively, CA-MRSA isolates more commonly possess the arginine catabolic mobile element (ACME), which improves their survival on the skin of healthy humans through the degradation of polyamines and pH modulation at the skin surface (10). Finally, LA-MRSA isolates are thought to have adapted to the colonization of livestock species through the loss of human-specific virulence factors and, in some cases, the gain of virulence factors specific to their livestock host species (11–13).

With the discovery of LA-MRSA sequence type 398 (ST398) isolates in swine (14), significant concerns arose due to the potential that livestock species can be reservoirs for MRSA and LA-MRSA may contribute to the risk for human infections in the community. This precipitated research investigating the prevalence of LA-MRSA and the associated infection risk. Studies found that while ST398 was the predominant lineage in Europe (15), outside Europe other lineages were more prevalent. In Asian swine, LA-MRSA ST9 was the most common lineage. Swine in North America were found to harbor a more diverse population of LA-MRSA isolates, with isolates of the ST398, ST9, and ST5 lineages being found (16, 17). Evidence indicates that the ST398 and ST9 lineages are animal adapted and less able to colonize and cause disease in humans (11, 12, 18). This has not been shown for the ST5 lineage, which is a globally disseminated and highly successful *S. aureus* clone in humans (19).

Nasal colonization with *S. aureus* contributes to *S. aureus* infections in the host, especially within the hospital setting (20, 21). This becomes important for humans with livestock contact, as these individuals are significantly more likely to carry MRSA than their counterparts with no contact with livestock (14, 22). Persistent colonization is a complex interaction between host tissues and the microbiota of the nasal cavity or skin. While the impact of a few genes has been experimentally verified (23–25), many genes are thought to contribute to the adherence and colonization of *S. aureus* through their interaction with host proteins, such as fibrinogen and fibronectin (26, 27). Furthermore, many genes are suspected to function in adherence, on the basis of the identification of motifs consistent with the motifs for other adherence genes (28), although their specific ligand has yet to be identified. Genetic investigation examining known and suspected adherence genes indicates variability in the presence or absence of these genes in different lineages, such as the *sdr* genes, which are not uniformly present in all lineages (29). Additional genes, such as ACME and *speG*, are found on mobile genetic elements (MGEs) and are thought to promote bacterial survival on the skin and contribute to long-term colonization with *S. aureus* (9, 10, 30).

Reports indicate that LA-MRSA ST398 isolates have a reduced ability to adhere to human keratinocytes, seem to colonize humans more transiently than isolates of other lineages, and seem to be less transmissible between humans than their HA-MRSA counterparts (18, 31–33). These LA-MRSA ST398 isolates possess genetic differences from other MRSA strains, such as the truncation of the adherence genes *clfA*, *clfB*, and *fnbB* and the absence of *sdrE* (18), which are thought to contribute to the reduced capacity of LA-MRSA ST398 to colonize and cause disease in humans. The adherence properties and genetic factors contributing to adherence have not been investigated

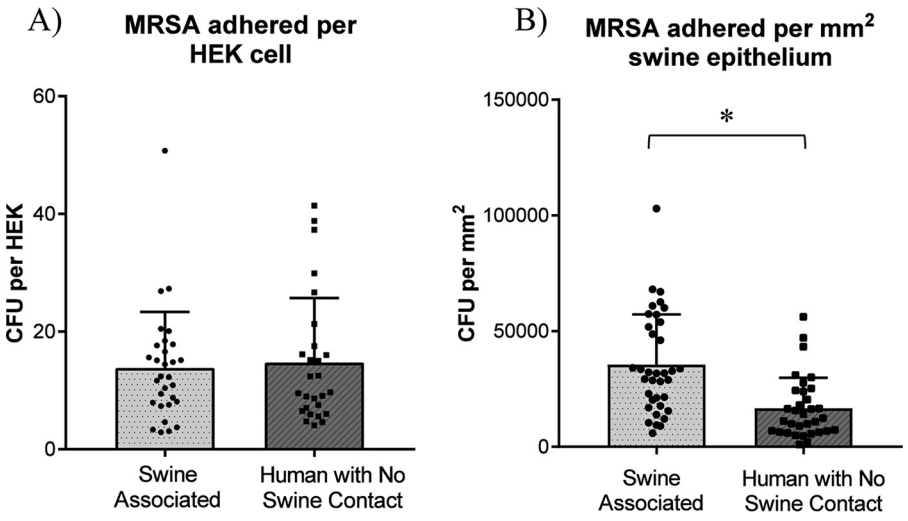

FIG 1 Capacity of swine-associated LA-MRSA ST5 isolates and clinical MRSA ST5 isolates from humans with no swine contact to adhere to human keratinocytes and swine skin biopsy specimens. (A) Number of CFU adhered per HEK cell for LA-MRSA ST5 isolates and clinical MRSA ST5 isolates, with each point representing the average for three technical replicates. (B) Number of MRSA CFU adhered per square millimeter of swine skin biopsy specimen for LA-MRSA ST5 and clinical MRSA ST5 isolates. *, $P < 0.05$.

for LA-MRSA ST5 isolates. In this study, we present a comparison of the *in vitro* adherence capability of LA-MRSA ST5 isolates and MRSA ST5 isolates from humans with no swine contact and compare the identified and suspected adherence genes in these populations of *S. aureus*.

RESULTS

Adherence to human epidermal keratinocytes. Adherence assays were used to determine the capacity of swine-associated LA-MRSA ST5 and MRSA ST5 isolates from humans with no swine contact to colonize human skin. LA-MRSA ST5 isolates and MRSA ST5 isolates from humans with no swine contact adhered to the human epidermal keratinocyte (HEK) cell line equivalently (Fig. 1). There was no significant difference in the number of CFU that adhered per HEK cell ($P = 0.92$) when the isolates were compared as subsets (Fig. 1A). There was considerable variability in the ability of individual isolates to adhere to HEK cells (Fig. 2A). For example, the average number of CFU per HEK cell that adhered for isolate UCI27 was higher than the average number of CFU per HEK cell that adhered for isolate ISU980 (Fig. 2A). In some cases, there were also wide ranges in the number of CFU per HEK cell that adhered between biological replicates of an individual isolate, as was seen for ISU978 (Fig. 2A).

Adherence to swine skin biopsy specimen. To assess the capacity of the selected isolates to colonize swine epithelial tissue, adherence assays were completed using swine skin biopsy specimens. Swine-associated LA-MRSA ST5 isolates adhered in greater numbers of CFU per square millimeter than MRSA ST5 isolates from humans with no swine contact ($P < 0.0001$) (Fig. 1B). Similar to the adherence patterns for the HEK cells, differences in the adherence capacity of individual isolates as well as variations between biological replicate were noted (Fig. 2B).

Comparison of adherence genes. To examine genomic differences that may influence how MRSA ST5 isolates interact with their host and environment, we compared the nucleotide sequences of 22 genes encoding factors associated with adherence and skin colonization (29). The percent identity of the sequence of each of the adherence-associated genes in LA-MRSA ST5 and MRSA ST5 isolates from humans with no swine contact to the sequence of *S. aureus* Mu3 was determined for each isolate (Fig. 3; see also Fig. S1 and Table S1 in the supplemental material). This analysis indicated that the adherence genes harbored by both subsets of isolates showed a high degree of nucleotide sequence identity. Specifically, a nucleotide sequence identity to the

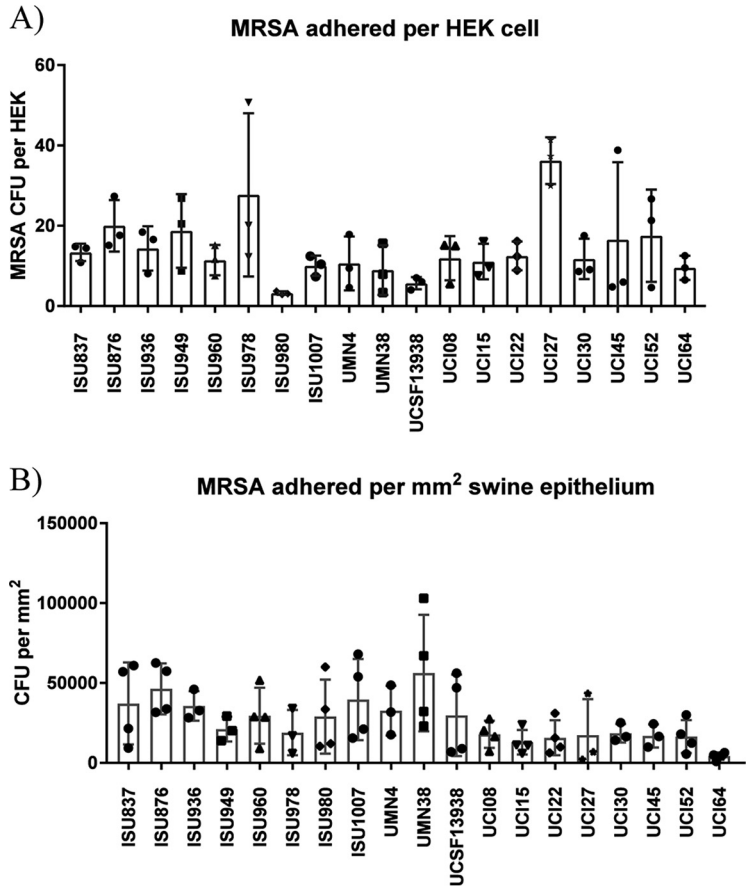

FIG 2 Patterns of adherence of individual isolates to HEKs and swine skin biopsy specimens. Differential patterns of adherence to HEKs (A) and swine skin biopsy specimens (B) were noted between individual isolates. Differences in the adherence pattern between biological replicates of an individual isolate, such as the number of CFU per HEK cell for isolate ISU978 (A) or the number of CFU per square millimeter for isolate UMN38 (B), were also noted.

sequence of reference isolate Mu3 of greater than 90% was observed for all genes evaluated (Table 1 and Table S1). The gene displaying the greatest sequence divergence was *clfA* (Fig. 3 and S1); however, it was intact in all the isolates, and the variation was associated with single nucleotide polymorphisms, insertions, and deletions that did not result in a premature stop codon or removal of large segments of the *clfA* gene. The greatest variation in *clfA* was present in LA-MRSA ST5 isolates and was due to an insertion of 162 bp at position 2125 and a deletion of 24 bp at position 2799, which were detected in the *clfA* genes of 19 of the isolates (Fig. 4). There were isolates that lacked the sequences associated with specific adherence genes (Fig. 3 and S1 [in black]; Table S1). This may be associated with gaps in the draft genome sequence rather than a true absence of the gene of interest.

Several adherence genes were not conducive to sequence identity analysis due to the presence of variable repeat regions (*sasG*) or the absence of the gene in the reference genome (ACME and *speG*). Therefore, isolate subsets were compared on the basis of the presence or absence of these adherence genes (Table 2). The adherence gene *sasG* was found at an equal prevalence in both subsets of isolates ($P = 0.26$). Significantly more MRSA ST5 isolates from humans with no swine contact than swine-associated isolates harbored the ACME *arc* gene cluster ($P = 0.02$) as well as *speG* ($P < 0.0001$). The adherence-associated genes *cna* and *sasX* were not found in ST5 isolates obtained from swine-associated sources or from humans with no known swine contact.

The *agr* genes encode a two-component sensor system that functions as a global regulator and a pivotal regulator of virulence factors and adherence genes (34, 35).

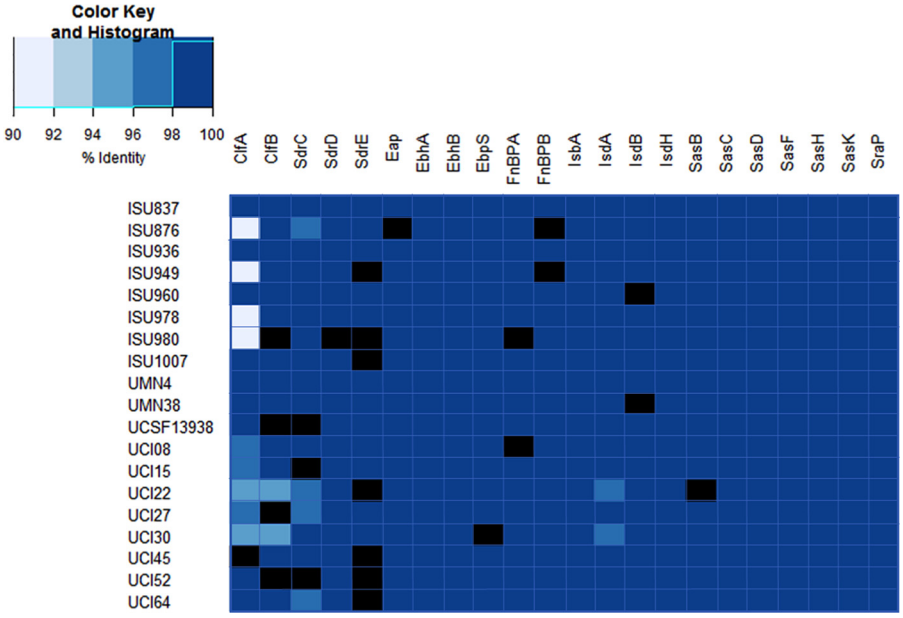

FIG 3 Percent nucleotide sequence identity of adherence-associated genes for isolates evaluated by the phenotypic assay. Each gene was compared to the gene in the reference isolate, Mu3. The percent identity of all screened genes was greater than 90%. The greatest variation was seen in the *clfA* gene. This was true for both LA-MRSA ST5 isolates and clinical MRSA ST5 isolates; however, a subset of LA-MRSA ST5 isolates (isolates ISU876, ISU949, ISU978, and ISU980) showed added variation due to nucleotide insertions and deletions. Genes not found in the draft genomes are depicted in black.

Swine-associated LA-MRSA ST5 and MRSA ST5 isolates from humans with no swine contact were evaluated for their *agr* types. All isolates with detectable *agr* genes harbored a type II *agr*, including 78/82 of the LA-MRSA ST5 isolates (95.1%) and 69/71 of clinical the MRSA ST5 isolates (97.2%) (Table S1). No difference in *agr* type was

TABLE 1 Percent nucleotide sequence identity for the sequences of the adherence factor genes in LA-MRSA ST5 isolates and MRSA ST5 isolates from humans with no swine contact compared to those of MRSA ST5 isolate Mu3

	Minimum-maximum (avg) % identity	
Adherence factor	LA-MRSA ST5 isolates[a]	MRSA ST5 isolates from humans with no swine contact[b]
clfA	90-100 (97)	93-100 (96)
clfB	93-100 (99)	91-100 (97)
sdrC	97-100 (99)	96-100 (98)
sdrD	99-100 (100)	97-100 (99)
sdrE	98-100 (100)	99-100 (100)
eap	100-100 (100)	100-100 (100)
ebhA	100-100 (100)	99-100 (100)
ebhB	100-100 (100)	100-100 (100)
ebpS	100-100 (100)	100-100 (100)
fnbpA	98-100 (100)	97-100 (100)
fnbpB	100-100 (100)	95-100 (100)
isbA	100-100 (100)	100-100 (100)
isdA	99-100 (100)	97-100 (99)
isdB	100-100 (100)	100-100 (100)
isdH	100-100 (100)	100-100 (100)
sasB	98-100 (100)	100-100 (100)
sasC	100-100 (100)	100-100 (100)
sasD	100-100 (100)	100-100 (100)
sasF	99-100 (100)	100-100 (100)
sasH	100-100 (100)	100-100 (100)
sasK	100-100 (100)	100-100 (100)
sraP	99-100 (100)	100-100 (100)

[a]Includes 82 swine-associated LA-MRSA ST5 isolates.
[b]Includes 72 human clinical MRSA ST5 isolates from humans with no swine contact.

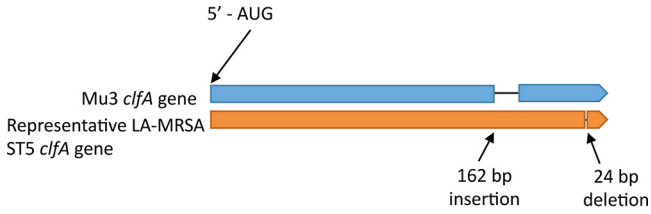

FIG 4 Representative image of the alignment of *clfA* from 19 LA-MRSA ST5 isolates and *clfA* from Mu3. In these 19 LA-MRSA ST5 isolates, the *clfA* gene showed a reduced percent nucleotide sequence identity to the *clfA* gene of Mu3. The *clfA* genes in the LA-MRSA ST5 isolates with a reduced identity possessed an insertion of 162 bp at position 2125 and a deletion of 24 bp at position 2799. The insertion and deletion did not result in a frameshift mutation, and the *clfA* genes in these isolates remained intact.

observed between the isolate subsets. The isolates in which an *agr* system was not identified are likely missing the *agr* gene due to gaps in the draft genome sequences.

DISCUSSION

Reports of the colonization capacity of LA-MRSA ST398 indicate that these isolates possess a reduced capacity for adherence to human epithelium (18), which results in transient colonization with ST398 isolates and the reduced transmissibility of these isolates between humans in both the hospital and the community settings (31, 33, 36–39). The reduction in adherence to human keratinocytes is suspected to be due to the absence of genes known to be involved in adherence, such as *sdrE*, and mutations and truncations in adherence genes, such as those noted in *clfA* and *clfB* (18). These changes are hypothesized to contribute to the adaptation of LA-MRSA ST398 isolates to livestock species (18). Although adherence capacity and transmissibility have been reported for LA-MRSA ST398 isolates, there are no reports to date addressing these concerns in LA-MRSA ST5 isolates.

In this study, we investigated the ability of LA-MRSA ST5 isolates and human clinical MRSA ST5 isolates to adhere to human keratinocytes and swine skin biopsy specimens to better understand the potential for these isolates to colonize humans and pigs. We found no difference between the human keratinocyte adherence capacity of LA-MRSA ST5 isolates and MRSA ST5 isolates from humans with no swine contact. We further determined that the keratinocyte adherence patterns exhibited by both subsets of isolates were consistent with the adherence-related genes that the isolates harbored. Notably, adherence genes reported to be absent or truncated in LA-MRSA ST398 isolates were present and intact in LA-MRSA ST5 isolates, including *clfA*, *clfB*, *sdrC*, *sdrE*, and *fnbB* (18). In the case of LA-MRSA ST5 isolates, there was a high proportion of nucleotide sequence identity with the sequence of the specific gene in the reference genome (Mu3), which was also seen for the human clinical MRSA ST5 isolates (Table 1; Fig. 3; see also Fig. S1 in the supplemental material). This indicates a genetic capacity for adherence that is equivalent between the two subsets of isolates and that was

TABLE 2 Prevalence of adherence genes in LA-MRSA ST5 isolates and MRSA ST5 isolates from humans with no swine contact

Adherence factor	No. of isolates with the gene/total no. of isolates tested (%)	
	LA-MRSA ST5	MRSA ST5 from humans with no swine contact
sasG	67/82 (81.7)	63/71 (88.7)
ACME		
arc gene cluster	0/82 (0)	5/71 (7.0)[a]
speG	0/82 (0)	14/71 (19.7)[a]
can	0/82 (0)	0/71 (0)
sasX	0/82 (0)	0/71 (0)

[a]Significant difference ($P < 0.05$) compared to LA-MRSA ST5.

confirmed with *in vitro* testing. Although adherence to human keratinocytes was equivalent between the two subsets of isolates, the LA-MRSA ST5 isolates did show significantly greater adherence to swine epithelium *in vitro* than human clinical MRSA ST5 isolates (Fig. 1B). This difference may reflect the adaptation of swine-associated LA-MRSA ST5 isolates to colonization of the swine epithelium through unidentified mechanisms.

Evaluation of multiple isolates of LA-MRSA ST5 and MRSA ST5 from humans with no swine contact indicated that there is a large amount of individual isolate variation as well as variation between test replicates for an individual isolate (Fig. 2). Variability between different isolates was anticipated; however, the large variability between test replicates for the same isolate was not expected. The variation in replicates may be associated with differences in adherence gene expression during an individual test and is controlled for by screening each isolate for *in vivo* adherence multiple times.

Overall, we conclude that, unlike LA-MRSA ST398 isolates, LA-MRSA ST5 isolates do not have a reduced capacity to adhere to human keratinocytes. This was seen both *in vitro* and through *in silico* analysis of adherence genes, which showed greater than 90% identity in all isolates. While the adherence capacity of the LA-MRSA ST5 isolates was not different from that of the MRSA ST5 isolates from humans with no swine contact, this does not directly indicate their ability to colonize and cause disease. Colonization is a complex interaction between the host, the bacterium, and the microbiota, and this interaction is difficult to replicate in an *in vitro* setting. It is also important to note that adhesion alone is not sufficient to cause disease and many virulence factors in *S. aureus* that contribute to colonization and mediate disease were not found in the LA-MRSA ST5 isolates (40). This includes ACME, which was found only in clinical MRSA ST5 isolates in this study and contributes to the persistence of *S. aureus* on the skin of hosts. We have also previously shown that immune evasion genes, such as those found in the β-hemolysin-converting bacteriophage, are absent in the LA-MRSA ST5 isolates evaluated in this study (40). Collectively, this study indicates that LA-MRSA ST5 isolates are able to adhere to human keratinocytes equivalently to clinical MRSA ST5 isolates. This may result in humans that come into contact with livestock harboring LA-MRSA ST5 isolates becoming colonized with those isolates; however, LA-MRSA ST5 isolates are suspected to be less virulent than clinical MRSA ST5 isolates and less capable of causing disease in humans.

MATERIALS AND METHODS

Isolate acquisition and selection. MRSA isolates were acquired from Iowa State University ($n = 73$), the University of Minnesota ($n = 9$), the University of California, Irvine ($n = 64$), and the University of California, San Francisco ($n = 7$) (17, 41). These isolates included 82 LA-MRSA ST5 isolates from swine-associated sources, including pigs ($n = 38$); the environment within swine buildings ($n = 26$); veterinary students with short-term swine contact sampled after a one-time visit to a swine farm ($n = 9$); and swine veterinarians with long-term, occupational swine exposure ($n = 9$). Clinical MRSA ST5 isolates from humans residing in urban areas with no known swine contact were included ($n = 72$). All isolates were confirmed to be ST5 by multilocus sequence typing and *spa* typed prior to acquisition (see Table S1 in the supplemental material). *Staphylococcus aureus* Mu3 (ATCC 700698; ATCC, Manassas, VA) was used for comparison during analysis of the results of the adherence assays and as a reference genome for adherence factor gene analysis.

Isolates were purposefully selected from swine-associated sources to represent the potential diversity among the isolates during adherence assays. Ten swine-associated LA-MRSA isolates were selected from the 82 total isolates. They were selected such that each farm was represented in the adherence assay by an environmental isolate ($n = 3$) or a swine isolate ($n = 4$) or was represented by an isolate from a human after that person had visited the farm ($n = 1$). Two isolates from humans with long-term swine contact were also selected for phenotypic screening. Nine isolates from humans with no known swine contact were randomly selected for inclusion in the adherence assay. The name and source information for isolates included in adherence assays can be found in Table 3.

Genome sequencing. Draft genome sequences were generated using a previously described protocol (42–47). In short, isolates were grown in Trypticase soy broth (TSB; BD Biosciences, Sparks, MD), and genomic DNA was extracted using a High Pure PCR template preparation kit (Roche Applied Science, Indianapolis, IN). A Nextera XT DNA sample preparation and index kit (Illumina, San Diego, CA) was used to generate libraries of DNA that were sequenced using a MiSeq (v2) 500-cycle reagent kit (Illumina, San Diego, CA) on an Illumina MiSeq instrument. Sequence reads were assembled using the MIRA (v4.0.2) program (http://mira-assembler.sourceforge.net/docs/DefinitiveGuideToMIRA.html). The whole-genome

TABLE 3 Isolates selected for adherence assays

Isolate name	Isolate source
ISU837	Environment (farm 10)
ISU876	Pig (farm 24)
ISU936	Pig (farm 39)
ISU949	Pig (farm 42)
ISU960	Environment (farm 38)
ISU978	Pig (farm 39)
ISU980	Environment (farm 41)
ISU1007	Human, short-term contact
UMN4	Human, long-term contact
UMN38	Human, long-term contact
UCI08	Human clinical isolate
UCI15	Human clinical isolate
UCI22	Human clinical isolate
UCI27	Human clinical isolate
UCI30	Human clinical isolate
UCI45	Human clinical isolate
UCI52	Human clinical isolate
UCI64	Human clinical isolate
UCSF13938	Human clinical isolate

sequence for Mu3 was obtained from NCBI (GenBank accession number AP009324.1) for use for adherence gene comparisons.

Gene comparisons. The adherence genes analyzed can be found in Tables 1 and 2. The percent identity of the nucleotide sequence of each gene with the sequence of the gene in the Mu3 reference genome was determined using multiple-sequence alignments in the Geneious (v.9.0.5) program (Biomatters Ltd., Auckland, New Zealand). Percent identities relative to the sequence of Mu3 were then used to generate heatmaps with the R program (48). For *sasG*, the gene was designated present or absent due to the gene structure, including B repeats consisting of 384-nucleotide repeats, which prevented adequate alignment of the entire gene. The genes composing ACME, *cna*, and *sasX* were also designated present or absent, as these genes are not found in Mu3, as determined by screening of the draft genome sequences *in silico* using the Geneious (v.9.0.5) program.

Accessory gene regulator (*agr*) typing. The *agr* type of the isolates was determined by screening the draft genome sequences *in silico* with the Geneious (v.9.0.5) program for type-specific regions using the following primer sets: for *agrI*, 5′-GTC ACA AGT ACT ATA AGC TGC GAT-3′; for *agrII*, 5′-TAT TAC TAA TTG AAA AGT GGC CAT AGC-3′; for *agrIII*, 5′-GTA ATG TAA TAG CTT GTA TAA TAA TAC CCA G-3′; and for *agrIV*, 5′-CGA TAA TGC CGT AAT ACC CG-3′ (as described previously [49]).

Human epidermal keratinocyte adherence assay. Human epidermal keratinocytes (HEKs; ATCC PCS-200-010; ATCC, Manassas, VA) were obtained to screen isolates for *in vitro* adherence to human keratinocytes. These cells were grown in dermal cell basal medium (DCBM) (ATCC, Manassas, VA) supplemented with the reagents from the keratinocyte growth kit (ATCC, Manassas, VA). For the assay, HEKs of passage 5 or lower were plated into 24-well plates (Becton, Dickinson and Company, Franklin Lakes, NJ) at 5,000 cells/cm^2 and allowed to grow to confluence.

An overnight culture of MRSA grown in TSB was used to inoculate 5 ml of TSB and was incubated at 37°C until logarithmic growth phase. The bacterial culture was diluted to an optical density at 600 nm (OD_{600}) ranging from 0.50 to 0.59, and 60 μl was inoculated into 10 ml of supplemented DCBM to reach an average inoculum concentration of 3.96×10^6 CFU/ml. Wells of HEKs were inoculated with 750 μl of diluted culture from each isolate ($n = 3$), resulting in an average multiplicity of infection (MOI) of 45, and an uninoculated DCBM control was included on each plate ($n = 3$). The plate was centrifuged at 400 $\times$ *g* for 5 min, followed by a 1-h incubation at 37°C with 5% CO_2. After incubation, each well was washed gently eight times with phosphate-buffered saline (PBS). Each well was then treated with 200 μl of 0.1% trypsin to dislodge the cells and adherent bacteria. The trypsin was collected and combined with 800 μl of PBS.

Serial dilutions of the inoculum and the recovered contents from each well were generated, plated onto Trypticase soy agar (BD Biosciences, Sparks, MD) plates, and incubated for 24 h at 37°C. The contents of three nontreated control wells were collected from each plate in the same manner used for the test wells, and a Scepter (v.2.0) cell counter (Millipore, Billerica, MA) was used to calculate the average number of keratinocytes per well in the plate. Each isolate was screened with three biological replicates generated from the average of three technical replicates. For each biological replicate, the number of CFU per HEK cell was calculated for comparison.

Swine skin biopsy specimen adherence assay. The external epidermis of the pinna and the base of the ear was cleaned of visible surface debris, scrubbed with gauze soaked in 7.5% povidone-iodine surgical scrub (Purdue Pharma L.P., Stamford, CT), and rinsed three times with gauze soaked in 95% ethanol. A section of scrubbed skin was excised and washed in Gibco EpiLife cell culture medium (Thermo Fisher Scientific Inc., Oakwood Village, OH) supplemented with Gibco human keratinocyte growth supplement (Thermo Fisher Scientific Inc., Oakwood Village, OH) containing 100 IU/ml penicillin G, 100 μg/ml streptomycin, 50 μg/ml gentamicin sulfate, and 1.0 μg/ml amphotericin B. The skin

segment was moved to supplemented EpiLife medium containing 100 IU/ml penicillin G and incubated for 2 h at 37°C. The skin was then transferred to supplemented EpiLife medium without antibiotic and incubated for 30 min at 37°C. The hypodermis was removed, and punch biopsy specimens were generated using a Miltex sterile disposable 8-mm punch biopsy tool (Thermo Fisher Scientific Inc., Oakwood Village, OH). The biopsy specimens were placed into 48-well plates (Corning Inc., Corning, NY) with the epidermis exposed. One biopsy specimen from each skin section was used for each isolate screened, and one biopsy specimen treated with uninoculated EpiLife medium was used as a control to detect contamination. Pigs were housed in biosafety level 2 (BSL2) containment facilities and cared for in compliance with the Institutional Animal Care and Use Committee of the National Animal Disease Center.

A culture of each isolate grown overnight in TSB was used to inoculate 5 ml of TSB and grown to logarithmic growth phase. The cultures were diluted to an OD_{600} ranging from 0.50 to 0.59, and 500 μl was inoculated into 4.5 ml of supplemented EpiLife medium, generating an average MRSA inoculum concentration of 4.18×10^6 CFU/ml. The cultures were mixed well, and 25 μl was inoculated onto each skin biopsy specimen, resulting in an average inoculum distribution of 8.32×10^4 MRSA/mm^2. After each skin biopsy specimen in the plate was inoculated, the plate was incubated for 1 h at 37°C with 5% CO_2. The skin biopsy specimens were then washed four times with PBS to remove nonadherent bacteria, 50 μl of 0.1% trypsin was added to each skin biopsy specimen, and the skin biopsy specimens were incubated for 15 min at 37°C to dislodge the adhered bacteria. After trypsinization, the skin biopsy specimens were moved to centrifuge tubes with 950 μl of supplemented EpiLife medium and vortexed to suspend the bacteria.

Serial dilutions of the inoculum and the bacteria retrieved from the skin biopsy specimens were made after trypsinization. Dilutions were plated onto Trypticase soy agar plates containing 1 μg/ml oxacillin. The plates were incubated for 48 h at 37°C, and the colonies were counted and used to determine the number of CFU per milliliter for the inoculum and skin biopsy specimens. Each isolate was tested with at least three biological replicates consisting of the average of three technical replicates. For each biological replicate, the number of CFU per square millimeter of surface area of the skin biopsy specimen was calculated.

Statistical analysis. All statistics were completed using GraphPad Prism (v.7.01) software (GraphPad Software, Inc., La Jolla, CA). Mann-Whitney tests were used to compare the number of CFU per HEK cell and the number of CFU per square millimeter between LA-MRSA ST5 isolates and clinical MRSA ST5 isolates from humans with no swine contact. Fisher's exact tests were used to compare the prevalence of individual genes between the subsets of isolates.

Accession number(s). The draft genome sequences for all isolates were deposited in DDBJ/ENA/GenBank with the following accession numbers: LKVI00000000 to LKWJ00000000, LKWY00000000 to LKYQ00000000, LKYS00000000 to LLBD00000000, LLBG00000000 to LLBI00000000, and LLBK00000000 to LLBW00000000 (the letters in each range are sequential, though the numbers for each entry are 00000000). Individual isolate accession numbers are listed in Table S1 in the supplemental material (42–47).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AEM.02073-17.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.1 MB.

ACKNOWLEDGMENTS

We thank Susan Huang from the University of California, Irvine, School of Medicine and Binh Diep from the Department of Medicine, University of California, San Francisco, for providing the clinical strains used in this study. Additional assistance with completion of the adherence assays was provided by our student employees, Maya Weese and Isabella Smith.

Financial support was provided by the National Pork Board, the U.S. Department of Agriculture (USDA), and the Oak Ridge Institute for Science and Education (ORISE).

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.

REFERENCES

1. Sivaraman K, Venkataraman N, Cole AM. 2009. Staphylococcus aureus nasal carriage and its contributing factors. Future Microbiol 4:999–1008. https://doi.org/10.2217/fmb.09.79.
2. Foster T. 1996. Staphylococcus. *In* Baron S (ed), Medical microbiology, 4th ed. The University of Texas Medical Branch at Galveston, Galveston, TX.
3. Fridkin SK, Hageman JC, Morrison M, Sanza LT, Como-Sabetti K,

Jernigan JA, Harriman K, Harrison LH, Lynfield R, Farley MM, Active Bacterial Core Surveillance Program of the Emerging Infections Program Network. 2005. Methicillin-resistant Staphylococcus aureus disease in three communities. N Engl J Med 352:1436–1444. https://doi.org/10.1056/NEJMoa043252.
4. Chambers HF, Deleo FR. 2009. Waves of resistance: Staphylococcus aureus in the antibiotic era. Nat Rev Microbiol 7:629–641. https://doi.org/10.1038/nrmicro2200.
5. Katayama Y, Ito T, Hiramatsu K. 2000. A new class of genetic element, Staphylococcus cassette chromosome mec, encodes methicillin resistance in Staphylococcus aureus. Antimicrob Agents Chemother 44:1549–1555. https://doi.org/10.1128/AAC.44.6.1549-1555.2000.
6. Klein EY, Sun L, Smith DL, Laxminarayan R. 2013. The changing epidemiology of methicillin-resistant Staphylococcus aureus in the United States: a national observational study. Am J Epidemiol 177:666–674. https://doi.org/10.1093/aje/kws273.
7. Malani PN. 2014. National burden of invasive methicillin-resistant Staphylococcus aureus infection. JAMA 311:1438–1439. https://doi.org/10.1001/jama.2014.1666.
8. Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, MacKenzie FM. 2012. Meticillin-resistant Staphylococcus aureus (MRSA): global epidemiology and harmonisation of typing methods. Int J Antimicrob Agents 39:273–282. https://doi.org/10.1016/j.ijantimicag.2011.09.030.
9. Diep BA, Carleton HA, Chang RF, Sensabaugh GF, Perdreau-Remington F. 2006. Roles of 34 virulence genes in the evolution of hospital- and community-associated strains of methicillin-resistant Staphylococcus aureus. J Infect Dis 193:1495–1503. https://doi.org/10.1086/503777.
10. Thurlow LR, Joshi GS, Clark JR, Spontak JS, Neely CJ, Maile R, Richardson AR. 2013. Functional modularity of the arginine catabolic mobile element contributes to the success of USA300 methicillin-resistant Staphylococcus aureus. Cell Host Microbe 13:100–107. https://doi.org/10.1016/j.chom.2012.11.012.
11. Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, Pearson T, Waters AE, Foster JT, Schupp J, Gillece J, Driebe E, Liu CM, Springer B, Zdovc I, Battisti A, Franco A, Zmudzki J, Schwarz S, Butaye P, Jouy E, Pomba C, Porrero MC, Ruimy R, Smith TC, Robinson DA, Weese JS, Arriola CS, Yu F, Laurent F, Keim P, Skov R, Aarestrup FM. 2012. Staphylococcus aureus CC398: host adaptation and emergence of methicillin resistance in livestock. mBio 3:e00305-11. https://doi.org/10.1128/mBio.00305-11.
12. Aires-de-Sousa M. 2016. Methicillin-resistant Staphylococcus aureus among animals: current overview. Clin Microbiol Infect 23:373–380. https://doi.org/10.1016/j.cmi.2016.11.002.
13. Lowder BV, Guinane CM, Ben Zakour NL, Weinert LA, Conway-Morris A, Cartwright RA, Simpson AJ, Rambaut A, Nubel U, Fitzgerald JR. 2009. Recent human-to-poultry host jump, adaptation, and pandemic spread of Staphylococcus aureus. Proc Natl Acad Sci U S A 106:19545–19550. https://doi.org/10.1073/pnas.0909285106.
14. Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. 2005. Methicillin-resistant Staphylococcus aureus in pig farming. Emerg Infect Dis 11:1965–1966. https://doi.org/10.3201/eid1112.050428.
15. Andreoletti O, Budka H, Buncic S, Colin P, Collins JD, De Koeijer A, Griffin J, Halvelaar A, Hope J, Klein G, Kruse H, Magnino S, Martinez Lopez A, McLauchlin J, Nguyen-The C, Noeckler K, Noerrung B, Prieto Maradona M, Roberts T, Vagsholm I, Vanopdenbosch E. 2009. Scientific opinion of the Panel on Biological Hazards on a request from the European Commission on Assessment of the Public Health significance of meticillin resistant Staphylococcus aureus (MRSA) in animals and foods. EFSA J 993:1–73.
16. Wagenaar JA, Yue H, Pritchard J, Broekhuizen-Stins M, Huijsdens X, Mevius DJ, Bosch T, Van Duijkeren E. 2009. Unexpected sequence types in livestock associated methicillin-resistant Staphylococcus aureus (MRSA): MRSA ST9 and a single locus variant of ST9 in pig farming in China. Vet Microbiol 139:405–409. https://doi.org/10.1016/j.vetmic.2009.06.014.
17. Frana TS, Beahm AR, Hanson BM, Kinyon JM, Layman LL, Karriker LA, Ramirez A, Smith TC. 2013. Isolation and characterization of methicillin-resistant Staphylococcus aureus from pork farms and visiting veterinary students. PLoS One 8:e53738. https://doi.org/10.1371/journal.pone.0053738.
18. Uhlemann AC, Porcella SF, Trivedi S, Sullivan SB, Hafer C, Kennedy AD, Barbian KD, McCarthy AJ, Street C, Hirschberg DL, Lipkin WI, Lindsay JA, DeLeo FR, Lowy FD. 2012. Identification of a highly transmissible animal-independent Staphylococcus aureus ST398 clone with distinct genomic and cell adhesion properties. mBio 3:e00027-12. https://doi.org/10.1128/mBio.00027-12.
19. Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, Borg M, Chow H, Ip M, Jatzwauk L, Jonas D, Kadlec K, Kearns A, Laurent F, O'Brien FG, Pearson J, Ruppelt A, Schwarz S, Scicluna E, Slickers P, Tan HL, Weber S, Ehricht R. 2011. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant Staphylococcus aureus. PLoS One 6:e17936. https://doi.org/10.1371/journal.pone.0017936.
20. Lindsay JA. 2010. Genomic variation and evolution of Staphylococcus aureus. Int J Med Microbiol 300:98–103. https://doi.org/10.1016/j.ijmm.2009.08.013.
21. Davis KA, Stewart JJ, Crouch HK, Florez CE, Hospenthal DR. 2004. Methicillin-resistant Staphylococcus aureus (MRSA) nares colonization at hospital admission and its effect on subsequent MRSA infection. Clin Infect Dis 39:776–782. https://doi.org/10.1086/422997.
22. Khanna T, Friendship R, Dewey C, Weese JS. 2008. Methicillin resistant Staphylococcus aureus colonization in pigs and pig farmers. Vet Microbiol 128:298–303. https://doi.org/10.1016/j.vetmic.2007.10.006.
23. Mulcahy ME, Geoghegan JA, Monk IR, O'Keeffe KM, Walsh EJ, Foster TJ, McLoughlin RM. 2012. Nasal colonisation by Staphylococcus aureus depends upon clumping factor B binding to the squamous epithelial cell envelope protein loricrin. PLoS Pathog 8:e1003092. https://doi.org/10.1371/journal.ppat.1003092.
24. Clarke SR, Brummell KJ, Horsburgh MJ, McDowell PW, Mohamad SA, Stapleton MR, Acevedo J, Read RC, Day NP, Peacock SJ, Mond JJ, Kokai-Kun JF, Foster SJ. 2006. Identification of in vivo-expressed antigens of Staphylococcus aureus and their use in vaccinations for protection against nasal carriage. J Infect Dis 193:1098–1108. https://doi.org/10.1086/501471.
25. Corrigan RM, Miajlovic H, Foster TJ. 2009. Surface proteins that promote adherence of Staphylococcus aureus to human desquamated nasal epithelial cells. BMC Microbiol 9:22. https://doi.org/10.1186/1471-2180-9-22.
26. Ni Eidhin D, Perkins S, Francois P, Vaudaux P, Hook M, Foster TJ. 1998. Clumping factor B (ClfB), a new surface-located fibrinogen-binding adhesin of Staphylococcus aureus. Mol Microbiol 30:245–257. https://doi.org/10.1046/j.1365-2958.1998.01050.x.
27. Signas C, Raucci G, Jonsson K, Lindgren PE, Anantharamaiah GM, Hook M, Lindberg M. 1989. Nucleotide sequence of the gene for a fibronectin-binding protein from Staphylococcus aureus: use of this peptide sequence in the synthesis of biologically active peptides. Proc Natl Acad Sci U S A 86:699–703. https://doi.org/10.1073/pnas.86.2.699.
28. Roche FM, Massey R, Peacock SJ, Day NP, Visai L, Speziale P, Lam A, Pallen M, Foster TJ. 2003. Characterization of novel LPXTG-containing proteins of Staphylococcus aureus identified from genome sequences. Microbiology 149:643–654. https://doi.org/10.1099/mic.0.25996-0.
29. McCarthy AJ, Lindsay JA. 2010. Genetic variation in Staphylococcus aureus surface and immune evasion genes is lineage associated: implications for vaccine design and host-pathogen interactions. BMC Microbiol 10:173. https://doi.org/10.1186/1471-2180-10-173.
30. Joshi GS, Spontak JS, Klapper DG, Richardson AR. 2011. Arginine catabolic mobile element encoded speG abrogates the unique hypersensitivity of Staphylococcus aureus to exogenous polyamines. Mol Microbiol 82:9–20. https://doi.org/10.1111/j.1365-2958.2011.07809.x.
31. Graveland H, Duim B, van Duijkeren E, Heederik D, Wagenaar JA. 2011. Livestock-associated methicillin-resistant Staphylococcus aureus in animals and humans. Int J Med Microbiol 301:630–634. https://doi.org/10.1016/j.ijmm.2011.09.004.
32. Lewis HC, Molbak K, Reese C, Aarestrup FM, Selchau M, Sorum M, Skov RL. 2008. Pigs as source of methicillin-resistant Staphylococcus aureus CC398 infections in humans, Denmark. Emerg Infect Dis 14:1383–1389. https://doi.org/10.3201/eid1409.071576.
33. Cuny C, Nathaus R, Layer F, Strommenger B, Altmann D, Witte W. 2009. Nasal colonization of humans with methicillin-resistant Staphylococcus aureus (MRSA) CC398 with and without exposure to pigs. PLoS One 4:e6800. https://doi.org/10.1371/journal.pone.0006800.
34. Cheung GY, Wang R, Khan BA, Sturdevant DE, Otto M. 2011. Role of the accessory gene regulator agr in community-associated methicillin-resistant Staphylococcus aureus pathogenesis. Infect Immun 79:1927–1935. https://doi.org/10.1128/IAI.00046-11.
35. Novick RP. 2003. Autoinduction and signal transduction in the regulation of staphylococcal virulence. Mol Microbiol 48:1429–1449. https://doi.org/10.1046/j.1365-2958.2003.03526.x.
36. van Cleef BA, Graveland H, Haenen AP, van de Giessen AW, Heederik D,

Wagenaar JA, Kluytmans JA. 2011. Persistence of livestock-associated methicillin-resistant Staphylococcus aureus in field workers after short-term occupational exposure to pigs and veal calves. J Clin Microbiol 49:1030–1033. https://doi.org/10.1128/JCM.00493-10.
37. Wassenberg MW, Bootsma MC, Troelstra A, Kluytmans JA, Bonten MJ. 2011. Transmissibility of livestock-associated methicillin-resistant Staphylococcus aureus (ST398) in Dutch hospitals. Clin Microbiol Infect 17:316–319. https://doi.org/10.1111/j.1469-0691.2010.03260.x.
38. van de Sande-Bruinsma N, Leverstein van Hall MA, Janssen M, Nagtzaam N, Leenders S, de Greeff SC, Schneeberger PM. 2015. Impact of livestock-associated MRSA in a hospital setting. Antimicrob Resist Infect Control 4:11. https://doi.org/10.1186/s13756-015-0053-8.
39. Hetem DJ, Bootsma MC, Troelstra A, Bonten MJ. 2013. Transmissibility of livestock-associated methicillin-resistant Staphylococcus aureus. Emerg Infect Dis 19:1797–1802. https://doi.org/10.3201/eid1911.121085.
40. Hau SJ, Sun J, Davies PR, Frana TS, Nicholson TL. 2015. Comparative prevalence of immune evasion complex genes associated with beta-hemolysin converting bacteriophages in MRSA ST5 isolates from swine, swine facilities, humans with swine contact, and humans with no swine contact. PLoS One 10:e0142832. https://doi.org/10.1371/journal.pone.0142832.
41. Hudson LO, Murphy CR, Spratt BG, Enright MC, Elkins K, Nguyen C, Terpstra L, Gombosev A, Kim D, Hannah P, Mikhail L, Alexander R, Moore DF, Huang SS. 2013. Diversity of methicillin-resistant Staphylococcus aureus (MRSA) strains isolated from inpatients of 30 hospitals in Orange County, California. PLoS One 8:e62117. https://doi.org/10.1371/journal.pone.0062117.
42. Hau SJ, Bayles DO, Alt DP, Nicholson TL. 2017. Draft genome sequences of 14 Staphylococcus aureus sequence type 5 isolates from California, USA. Genome Announc 5:e00098-17. https://doi.org/10.1128/genomeA.00098-17.
43. Hau SJ, Bayles DO, Alt DP, Davies P, Haan JS, Nicholson TL. 2017. Draft genome sequences of nine livestock-associated methicillin-resistant *Staphylococcus aureus* sequence type 5 isolates from humans with long-term swine contact. Genome Announc 5:e01079-17. https://doi.org/10.1128/genomeA.01079-17.
44. Hau SJ, Bayles DO, Alt DP, Frana TS, Nicholson TL. 2017. Draft genome sequences of nine livestock-associated methicillin-resistant *Staphylococcus aureus* sequence type 5 isolates obtained from humans after short-term swine contact. Genome Announc 5:e01080-17. https://doi.org/10.1128/genomeA.01080-17.
45. Hau SJ, Bayles DO, Alt DP, Frana TS, Nicholson TL. 2017. Draft genome sequences of 63 swine-associated methicillin-resistant *Staphylococcus aureus* sequence type 5 isolates from the United States. Genome Announc 5:e01081-17. https://doi.org/10.1128/genomeA.01081-17.
46. Hau SJ, Bayles DO, Alt DP, Nicholson TL. 2017. Draft genome sequences of one methicillin-sensitive and seven methicillin-resistant Staphylococcus aureus sequence type 5 isolates obtained in California. Genome Announc 5:e01084-17. https://doi.org/10.1128/genomeA.01084-17.
47. Hau SJ, Bayles DO, Alt DP, Nicholson TL. 2017. Draft genome sequences of 50 methicillin-resistant *Staphylococcus aureus* sequence type 5 isolates obtained from a U.S. hospital. Genome Announc 5:e01083-17. https://doi.org/10.1128/genomeA.01083-17.
48. R Development Core Team. 2013. R: a language and environment for statistical computing. The R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.
49. Gilot P, Lina G, Cochard T, Poutrel B. 2002. Analysis of the genetic variability of genes encoding the RNA III-activating components Agr and TRAP in a population of Staphylococcus aureus strains isolated from cows with mastitis. J Clin Microbiol 40:4060–4067. https://doi.org/10.1128/JCM.40.11.4060-4067.2002.