RESEARCH REPORT
PUBLIC HEALTH/WORKER SAFETY

Title: Investigation and Characterization of *Staphylococcus aureus* Bacteriophages that Inhabit Swine Production Environments – **NPB #16-143**

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INDUSTRY SUMMARY

Rationale: *Staphylococcus aureus* is an opportunistic pathogen of major concern in both human and animal health. While this bacterium can cause life-threatening illness, it more commonly asymptomatically colonizes the nasal passages and skin of healthy people. *S. aureus* can also be carried by livestock animals including swine, and there is evidence that *S. aureus* can be transmitted back and forth between humans and swine. This makes swine a potential reservoir for *S. aureus*, which may pose a risk to workers in swine production facilities, and potentially to the larger community. Bacteriophages (phages) are bacterial viruses and they are major predators of bacteria in natural environments. The continued emergence of antibiotic resistant bacteria, including resistant strains of *S. aureus*, has led to increased interest in novel antibacterial strategies, including the use of phages. **Objectives:** Our preliminary data suggested that swine production environments may be a rich source of phages infecting *S. aureus* that could be used as antibacterials. The goal of this project was to conduct an initial survey of swine production environments across the US to i) estimate the prevalence and diversity of phages in these environments and ii) assemble a large working collection of *S. aureus* phages that could potentially be used as antibacterials. **Methods:** Swab samples were collected from swine barns at 20 sites in 10 states and tested for the presence or absence of phage against a panel of 10 *S. aureus* strains of both human and animal origin. Phage-positive samples were further processed to isolate individual phages from the samples. **Results:** Twelve of the 20 sample sites were positive for the presence of phage against at least one of the 10 *S. aureus* host strains used. Two sites contained phage capable of infecting nine of the 10 host strains tested. Host strains of both human and swine origin were roughly equally susceptible to phages contained in the environmental samples, suggesting that the phages are not "specialized" for swine environments and that there is not a strong distinction between human and swine *S. aureus*. Eighty-four phage isolates were initially collected from the environmental samples, with 51 of these surviving subculture and storage to allow for characterization. This collection of 51 phages was first analyzed by restriction digestion of phage genomic DNA, which placed the phages into one of five groups. Transmission electron microscopy of 13 phage isolates to date showed that the phages belong to one of three distinct morphological types. Fourteen of the phages have been analyzed by genome sequencing, which also placed them into three major groups. Two of these groups are related to known *S. aureus* phages. The third group has proved to be refractory to normal DNA sequencing approaches, suggesting it may be a new type of *S. aureus* phage that contains highly modified DNA bases that interfere with DNA sequencing. Work to obtain DNA sequence for these phages is ongoing.

Industry significance: Bacteriophages of *S. aureus* are a common component of the normal microbial flora present in swine production environments. Many of the phages identified to date are related to one of two known *S. aureus* phage types, but a subset of our phage collection has been resistant to normal methods of molecular characterization and may represent a previously undescribed phage type. This project has generated a library of 51 *S. aureus* phages that may prove useful for controlling *S. aureus* populations in swine production environments.

KEYWORDS: *Staphylococcus aureus*, MRSA, bacteriophage, phage therapy, occupational health, swine production

SCIENTIFIC ABSTRACT

Staphylococcus aureus is a pathogen of significant concern in both humans and livestock. Asymptomatic carriage of *S. aureus* by swine, particularly multidrug-resistant (MDR) strains, poses a potential risk to workers involved with livestock production and to the larger community.

Bacteriophages are the most abundant form of life in the biosphere and are major predators of bacteria in natural environments. Recent interest in phages as novel antimicrobials has raised the possibility that phages infecting *S. aureus* may provide an alternative means for modulating this pathogen in the livestock environment and reducing the risk of transmission to humans. In this study, an environmental survey of 20 swine production facilities across the United States was conducted to determine the prevalence of *S. aureus* phages. Swab samples were collected and enriched against 10 *S. aureus* strains representing seven common lineages (USA300, USA800, ST5, ST9, ST80 and ST398) in two mixed-enrichment panels. Twelve of the 20 sample sites contained detectable phage. Host lineage or host source (human or animal) were not strong predictors of phage sensitivity. A collection of 51 phages was isolated from the environmental samples. These phages were placed into five molecular groups by restriction digestion of phage genomic DNA by the enzyme DraI, indicating overall phage diversity is low in these samples. Initial analysis of the collection by transmission electron microscopy and genome sequencing indicates the presence of three major phage types in the collection: temperate siphophages related to phiETA, virulent phi29-like podophages, and large myophages which are morphologically similar to *S. aureus* phage K but have been refractory to DNA sequencing. This last group may represent a novel class of *S. aureus* myophage with genomic DNA containing hypermodified bases.

INTRODUCTION

There are an estimated 80,000 invasive methicillin resistant *Staphylococcus aureus* (MRSA) infections in the United States annually [1]. *S. aureus* is the leading cause of bacteremia, skin, soft tissue and device-related infections [2]. These *S. aureus* infections, particularly MRSA infections, can be extremely difficult and costly to treat. In one study, the median cost to treat a surgical site infection with MRSA was $92,363 [3]. MRSA infections are mainly divided into three main sources of infection: hospital associated (HA-MRSA), community associated (CA-MRSA) and livestock associated (LA-MRSA).

Carriage of *S. aureus* in the general public in the continental US ranges from 26% to 32% [4]. An estimated 1.3% of that *S. aureus* being MRSA [5]. However, in individuals in the US that are swine farmers, production workers or veterinarians, carriage of multi-drug resistant *S. aureus* (MDRSA) is two to six times greater than individuals in the community, or those who are not exposed to swine [6, 7]. MDRSA is defined by an *S. aureus* isolate that demonstrates resistance to three or more classes of antimicrobials [1].

While there have been few human infections from ST398 LA-MRSA recorded, there have been several cases that had severe consequences, such as a case of lethal necrotizing pneumonia and several cases of bacteremia [8, 9]. Recently, there has been growing concern for bloodstream infections caused by several ST398 isolates that have appeared in multiple hospital infections, leading the authors to investigate if ST398 has once again become a “human adapted subclone” in France [10, 11]. Therefore, LA-MRSA strains may still pose a threat to swine worker safety.

In a recent survey of *S. aureus* carriage among swine in the US, Sun et al., 2015 examined 36 different swine herds; this sample represented 11 different states and 9 different breed stock companies. Results from this

study found that only two out of 1200 *S. aureus* isolates were not categorized as MLST lineages: ST9, ST398 or ST5. Additionally, for 21 out of 35 herds the prevalence of *S. aureus* exceeded 80%. However, this prevalence is *S. aureus* in general, all isolates in this study with the exception of a known MRSA positive herd were MSSA. This study indicates that MRSA prevalence in the USA appears to be currently lower than in European countries [12]. In the Netherlands, MRSA prevalence among swine herds is approximately 69% [13].

To combat the transfer of MRSA strains from swine to humans and to the community new safety measures should be employed. While elimination of *S. aureus* from swine environments as well as swine workers does not seem likely, one study found that people carrying MSSA had an 83% decreased risk of MRSA acquisition when samplings were conducted months apart [14]. Therefore, if a product could serve to decolonize MRSA carriage in swine and workers temporarily, this may allow for an MSSA strain to fill the niche. Bacteriophages, viruses that infect bacteria, could have a part in resolving this problem.

There are two known life cycles a phage can pursue: lytic or lysogenic. In the lytic life cycle a phage adsorbs to a bacteria, it then injects its DNA into the host cell and replicates, producing virions. After replicating in the cell, the phage will lyse the cell to release its progeny. In contrast, in the lysogenic life cycle a phage will adsorb to the cell, as well as inject its DNA, however, this DNA will either be integrated into the chromosome of the bacteria or exist as its own entity such as a plasmid. Either way, the phage DNA will replicated each time the bacterial DNA is replicated; damage and stress to the bacterial cell can cause these phage to excise from the bacterial genome and begin the lytic life cycle [15].

Strictly lytic phages are considered to be the best option for therapeutic applications, as lysogenic phage present safety concerns with their ability to integrate into the host genome and possibly disseminate bacterial DNA. For the *S. aureus* bacteriophages, lytic phages of serogroups D and G are of most interest for therapeutic use; other serogroups of *S. aureus* phage were commonly used to type strains o*f S. aureus* for clinical usage, however these phage are lysogenic [16]. These novel phage could have both great therapeutic and genetic potential.

Because swine environments can be a reservoir for *S. aureus* in this study we hypothesized that these environments would also serve as a reservoir for *S. aureus* phage. Therefore, the objectives of this study were to determine the prevalence of *S. aureus* bacteriophages from swine production environments across the US using a panel of *S. aureus* strains that are representative of the swine production environment and human clinical isolates. Additionally, isolated phage would be characterized for their host range, virulence, morphology and genomic characteristics.

OBJECTIVES

The objectives of this project are twofold. First, using a representative panel of *S. aureus* strains isolated from humans and swine production facilities, a broad, multi-state survey of commercial swine production facilities will be undertaken, in order to collect a diverse library of novel phages infecting *S. aureus*. A sub-aim of this survey will be to determine the ecological diversity of phages inhabiting a smaller number of single sites, by conducting deeper sampling of the environment and repeating samples over time to determine shift in phage populations. The second objective of this project is to then characterize this phage collection for their host range and virulence among *S. aureus* strains, and to conduct whole-genome sequencing to determine their biological character and their relationships to other known phages. We propose that this phage collection can serve as the basis for future work to develop phages as an antibacterial intervention to control *S. aureus* and MRSA in swine production environments, and reduce or eliminate the transmission of these pathogens from livestock to humans.

MATERIALS AND METHODS

Culture and maintenance of bacteria and phages. *Staphylococcus aureus* was routinely cultured on trypticase soy broth (Bacto TSB, Difco) or trypticase soy agar (TSA, TSB + 1.5% w/v Bacto agar, Difco) aerobically at 30 ºC. Phages were cultured using the double-layer overlay method [17] with 4 mL of top agar (10 g/L Bacto Tryptone (Difco)), 10 g/L NaCl, 0.5% w/v Bacto agar) supplemented with 5 mM each calcium chloride and magnesium sulfate over TSA bottom plates. Lawns were inoculated with 0.1 ml of a mid-log *S.*

aureus bacterial culture grown to an OD_{550} of ~0.5. Bacterial strains used for phage isolation and propagation are shown in Table 1.

Collection and transportation of samples. Samples for this study were collected from volunteer participant sites distributed across the continental United States from July 2016 through May 2017. Participants received a styrofoam cooler, freezer packs, gloves, swabs and an instructional packet on how to collect samples. The instructions detailed that participants should identify areas with visible residue within the barn they were sampling, such as in between slatted floors and on top of water lines. Once a site was identified the participant broke the seal on a sterile BD BBL™ swab containing liquid stuart's media (BD BBL™, VWR Scientific), wetted the swab with the sponge in the tube and then collected the sample by swabbing approximately a 2x2 inch area. Each swab collected was labeled with the type of site the participant collected it from. These swabs were then shipped overnight in the same provided cooler on ice to Texas A&M University.

Processing of environmental samples. The five environmental swabs from each site were pooled by clipping the swab heads into a 50 mL tube containing 30 ml sterile TSB. The sample was eluted by shaking at room temperature for 2 hrs, followed by centrifugation at 8,000 x g at 4 °C, 10 min and sterilization of the supernatant by passage through a 0.2 µm syringe filter (Millipore). This sterilized sample was divided and enriched with two different panels of *S. aureus* strains in a mixed-strain enrichment approach [18]. One enrichment panel consisted of a mixture of four human-associated *S. aureus* isolates and the other contained six swine-associated isolates as shown in Table 1. Enrichment inocula were prepared by mixing equal volumes of *S. aureus* cultures that had been adjusted to OD_{550} of ~0.5 as described above. Enrichment cultures were composed of 10 mL of swab eluate and 10 mL of TSB in a 250 mL flask, inoculated 1:100 with the appropriate *S. aureus* inoculum. Enrichments were incubated overnight at 30 °C with aeration. After incubation, a 10 mL aliquot of each enrichment was centrifuged and filter-sterilized as described above. Enriched samples were screened for the presence of phage by spotting 10 µl aliquots onto soft agar lawns lawns inoculated with each individual *S. aureus* strain used for enrichment; samples were scored as positive for phage if they produced clearing zones or visible plaques on a lawn of any *S. aureus* host. Phages were isolated from phage-positive samples by dilution and plating to lawns of *S. aureus* followed by picking of well-isolated plaques. Each phage was subcultured three times to ensure clonality.

In-depth sampling. Thirty swabs were collected from one site located in using the same methodology as described above, except that swab samples were not pooled. Swabs were individually clipped into 15 mL tubes and eluted with 5 ml TSB, filter-sterilized and enriched against the swine-derived *S. aureus* host panel in a total volume of 4 ml, and then screened for the presence of phage as described above.

Creating Phage Stocks. After subculturing the phage, parent stocks of each phage were made by the confluent plate lysate method [19]. Each phage was propagated on its original isolation host and harvested from the agar overlay with 6 mL of phage buffer (100 mM NaCl, 25 mM Tris-HCl pH 7.4, 8 mM $MgSO_4$, 0.01% w/v gelatin). Phage lysates were filter-sterilized (0.2 µm) and stored in the dark at 4 ºC.

Phage Genomic DNA Extraction. High titer (>10^8 PFU/mL) stocks of each phage were made and gDNA was then extracted from 10-20 mL of phage stock. To extract gDNA, 0.5 µL of nuclease solution per ml of lysate (10 µg/mL DNase & RNase final) was added and lysates were incubated at 37 ºC for 30 minutes. Next, precipitant solution (10% PEG-8000, 1 M NaCl final) was added to each lysate at a rate of 1:2 precipitant:lysate and incubated at 4 ºC overnight. The next day the lysate was centrifuged at 10,000 x g, 4 ºC for ten minutes and the supernatant was discarded. The remaining pellet was then resuspended in 500 µL of resuspension buffer (5 mM MgSO4) and transferred to a new 1.5 ml microcentrifuge tube. To remove any insoluble particles the sample was centrifuged for 5-10 seconds and the supernatant transferred again to a new 2 mL microcentrifuge tube. To eliminate the heat stable nuclease produced by *S. aureus* to each 500 µL aliquot of resuspended phage, 10 µL of 0.5 M EDTA pH 8 and proteinase K to a final concentration of 100 µg/mL was added and incubated at

50 ºC for 30 minutes. After allowing the sample to return to room temperature 1 mL of resin from the Promega Wizard Kit ® was added and the tube was inverted approximately five times. The resin was then run through a 3 mL syringe that had a minicolumn attached to it and then rinsed twice with 2 mL of 80% isopropanol. The minicolumn was then transferred to a 1.5 mL microcentrifuge tube and centrifuged at 13,000g for 2 minutes to dry the resin. After drying the minicolumn was transferred to a new 1.5mL microcentrifuge tube and 100 µL of preheated 80 ºC water was added to the column and centrifuged at 13,000 g for 1 minute to elute the DNA. Extracted gDNA from the phage lysate was then stored at 4 ºC.

Restriction Digest of gDNA. To distinguish different phage types approximately 300 ng of extracted gDNA was digested with both DraI (5' TTTAAA 3') (New England BioLabs Inc. https://www.neb.com). 300 ng of gDNA from each phage was incubated with enzyme and CutSmart ® Buffer individually at 37 °C overnight. After overnight incubation, 4 µL of loading dye was then added and the total 24 µL for each sample was run on a 1% agarose gel at 90 V for two and half hours.

Phage genome sequencing and annotation. DNA was sequenced in an Illumina MiSeq 250-bp paired-end run with a 550-bp insert library through the generosity of Dr. H. Morgan Scott's lab at Texas A&M College of Veterinary Medicine & Biomedical Sciences (College Station, TX, USA). FastQC (bioinformatics.babraham.ac.uk), FastX Toolkit (hannonlab.cshl.edu) and SPAdes 3.5.0 [20] were then used for read quality control, read trimming, and read assembly, respectively. Preliminary phage relationships were determined by BLASTn [21] against the nr database at NCBI. Analyses were performed via CPT Galaxy (https://cpt.tamu/edu/galaxy-pub).

RESULTS

A total of 20 swine farms were sampled across the US (Figure 1). Of the sites sampled, 45% were considered finishing barns and 30% were gestation barns. One farm (Farm 16) was sampled in-depth in November of 2016 and then again six months later; this farm was not included in phage prevalence estimates. Of the 19 farms included in this sampling, 12 (63%) were positive for phage capable of infecting at least one of the *S. aureus* host strains (Table 2). Environmental samples were enriched against two separate *S. aureus* host panels: one containing four human clinical isolates (including three representatives of major MRSA clades USA300, USA800 and ST80), and one containing six recently-collected swine-associated strains {Sun, 2015 #219}. Of the human-associated strains, the ST80 representative was most susceptible to the phages sampled from swine farms (eight positive samples), with the USA300 strain yielding six positive samples. Of the swine-associated host strains, one of the two ST9 and ST398 representatives were most sensitive, with each strain sensitive to phages sampled from ten sites. We observed that *S. aureus* sequence type was not a strong predictor of phage sensitivity. Of the three ST398 strains used, one strain yielded plaques from ten farm samples, while the others yielded plaques from only one sample and three samples. Likewise, of the three ST9 strains used, one was positive for ten samples, one was positive for nine samples, and one was positive for three samples.

A total of 84 phage isolates were initially collected from farm samples and 51 of those survived *in vitro* subculturing and grew to titers high enough to provide useful amounts of DNA. Five distinct DraI enzyme restriction patterns were observed among these 51 isolated phage (Figure 2). Of the 51 phages examined, 19 isolates belonged to group 1, 14 isolates belgoned in group 2, 3 were in group 3, 11 were in group 4, and 5 were in group 5. The group 1 restriction phenotype is that of undigested DNA, indicating that the DNA of these phages cannot be cut by this enzyme.

In the first in-depth sampling at Farm 16, only one of 30 swabs was positive for phage on one of the six swine *S. aureus* panel strains. At the second sampling, six months later, seven swabs were positive for phage across four of the swine *S. aureus* strains (Table 3). These seven swabs yielded 12 phage isolates, eight of which have the same restriction pattern and morphology of the single phage isolated from the first sampling, suggesting this phage was persistent in the environment over the 6-month sampling interval. The other four

phage isolates display a group 1 (undigestible) restriction pattern. A subset of these phage genomes will be sequenced to determine their relatedness at the nucleotide level.

Transmission electron micrographs have taken of 13 of the isolated phage thus far, with TEM images revealing either myoviridae, podoviridae or siphoviridae morphology (Figure 3). Of the 13 phages studied by this method to date, two were of myophage, five were of siphophage and six were of podophage morphology. The two myophages observed all belonged to restriction group 1, the five siphophages to group 2, and the five podophages were placed in groups 3, 4 or 5, and one podophage was in group 1. *S. aureus* head diameter is also indicative of genome size, thus far all TEM images agree with genome size obtained from Illumina sequencing. Continued microscopy is underway for additional phages in the collection.

Fourteen of the isolated phages have been sequenced, with four of them having genomes of ~44 kb and the remaining ten, ~18 kb. The four ~44 kb genomes sequenced to date are identical at the DNA level and are related to known temperate *S. aureus* phage phiETA (Genbank AP001553) found in bacterial genome sequences. Several classes of these temperate phages are known to be able to transduce DNA between bacterial strains or act as helper phages to mobilize genomic elements resident in some *S. aureus* genomes [22]. The presence of these phages in some samples suggests that these natural processes are occurring in the swine production environment. The group of ten ~18 kb genomes are all related to known *S. aureus* virulent phages P68 and 44AHJD, which are part of a larger group of small, phi29-like phages infecting firmicutes including *Bacillus subtilis.* The ten sequenced podophages originated from four sites (farms 4, 7, 15, and 19) and preliminary analysis shows that phages originating from the same site are more closely related to each other than to phages from other sites, suggesting an ecological model of phage establishment followed by clonal spread within a site rather than co-colonization by multiple phages. The genomes of myophages belonging to restriction group 1 have thus far been refractory to multiple attempts at DNA sequencing on the Illumina platform; while genomic DNA can be extracted from these phages, usable sequence reads are not generated during sequencing. This phenomenon may be related to the indigestibility of this DNA by restriction enzymes.

DISCUSSION

In this study, 84 *S. aureus* phage were isolated against a panel of 10 different strains comprised of human and swine origin, resulting in a collection 51 phage isolates available for detailed characterization. The majority of farms sampled had *S. aureus* phage present with samples from farms 9 and 12 producing plaques on nine out of ten of the *S. aureus* hosts used.

Eight of the 12 phage-positive farms contained only one phage type based on DraI restriction digests. This could be indicative that a dominant phage group is usually present within a barn, a notion supported by the persistence of the same phage type in the first and second sampling of Farm 16. However, this would need to be further investigated with additional rounds of in-depth sampling. Factors such as the season, movement of people and animals, sanitation and many other factors may influence the movement of phage into new sites. Because phages are obligate predators of bacteria, the detection and culture of phages from the environment is dependent on the bacterial host strains used. In this study a diverse panel of 10 *S. aureus* strains in two separate enrichment panels was used to attempt to maximize the number and diversity of phages isolated. These 10 strains represented seven major lineages of *S. aureus*: human clinical isolates of USA300, USA800, ST80 and ST398, and swine-associated strains of ST5, ST9, and ST398. Despite these efforts however, the absence of phage-positive samples does not prove the absence of *S. aureus* phages at a given site. Phages may have been present at numbers too low to be detectable, at discrete sites not captured by swab sampling, or phages may have been present that were unable to replicate on the host strains used for this study.

Prior to this study, three major classes of *S. aureus* phages had been described: large (~140 kb) virulent K-like myophages, small (~18 kb) virulent phi29-like podophages, and a slightly more diverse group of temperate siphophages with genomes of ~45 kb [16]. Phages that appear to belong to each of these three groups were isolated from swine production environments in this study. The sequences of 14 phage thus far have been obtained, with ten podophages with genomes of approximately 18 kb and four siphophages with genomes of approximately 45 kb. Attempts to sequence myophage genomic DNA have been unsuccessful.

TEM imaging thus far has revealed six podophages, five siphophages and two myophages and is in agreement with genome size sequencing data obtained so far.

Of the 54 phage isolates, only five distinct DraI restriction enzyme patterns were found. This low diversity is somewhat surprising as the phage were isolated from widely different geographic locations. The DNA of the large myophages of restriction group 1 has thus far been refractory to both restriction digestion and DNA sequencing, and additional diversity may be contained within this group. While the DNA of other K-like myophages infecting *S. aureus* have been sequenced by conventional methods, there are known to be more distantly-related myophages that infect other members of the Firmicutes that are refractory to DNA sequencing due to the presence of highly modified bases in the phage DNA. Perhaps the most striking recent example of this is *Bacillus subtilis* phage CP51, which contains hydroxymethyluracil (hmU) in place of thymidine (T) in its DNA; this substitution rendered the phage DNA resistant to most restriction enzymes and Illumina and Sanger sequencing approaches [23]. This phage was eventually sequenced by using the PacBio platform, which was able to process this modified DNA, and this approach will be attempted for the unsequencable group 1 phages in our collection. We hypothesize that the group 1 phages in our collection represent a novel subtype of K-like myophage infecting *S. aureus.* To our knowledge this would be the first incidence of *S. aureus* phage containing such hypermodified DNA. Other phage such as *Bacillus* phage SPO1 have been documented to modify their DNA as well but this has not yet been recorded in *S. aureus* phages [16]. Work to obtain the genome sequences for these phage is currently ongoing.

Sequencing thus far has revealed that the small podoviridae are very similar at the DNA level, with the most dissimilar pair thus far having 82% identity. Phages from a single site share high DNA sequence identity (>99%), while inter-farm identity is lower (~82% - 95%), suggesting each site is dominated by a single phage clone. It must be noted however that only a small portion of the entire phage collection has been sequenced to date, and greater resolution will be gained by sequencing more isolates. Phage DNA sequencing, annotation, evaluation of host range and biofilm clearing ability by these phage is currently ongoing.

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FIGURES AND TABLES

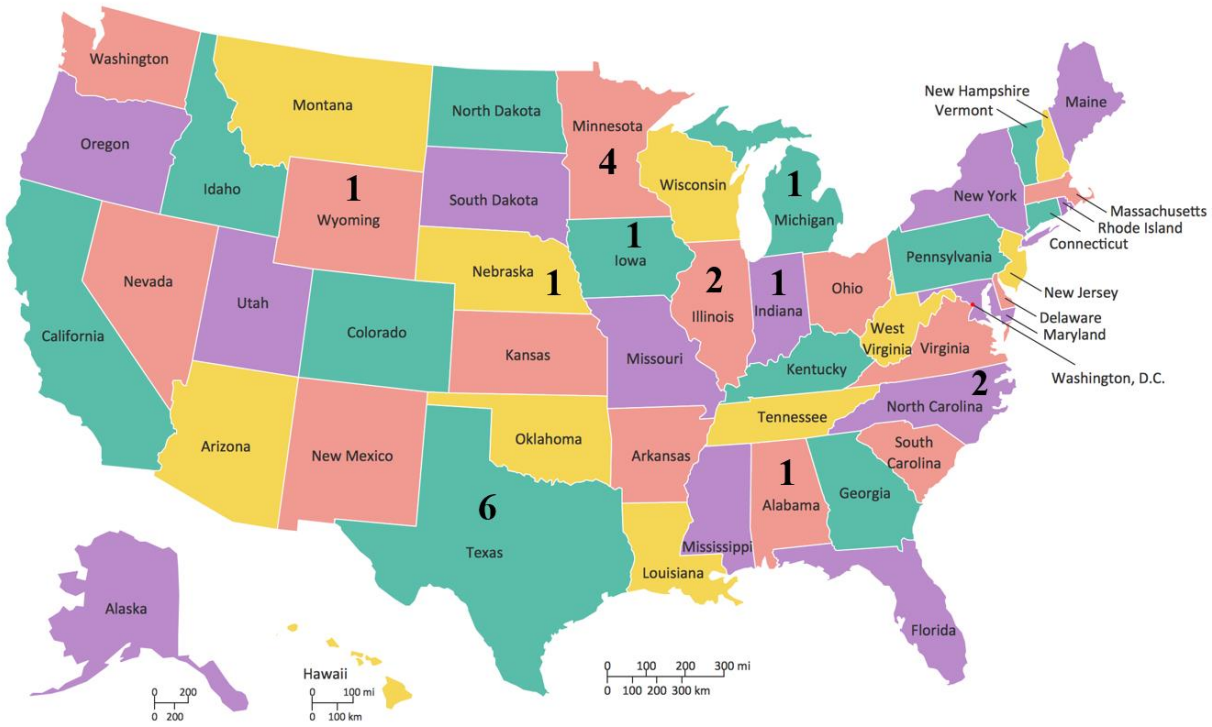


Figure 1. State-level locations of farms sampled. Numbers indicate how many farms were sampled within each state.

Table 1. Bacterial strains used in this study.

Strain No.	Strain Name	ST	CC or PFGE type	SCCmec	Geographic region	Isolation source
NRS384	USA300-0114	8	USA300	IV	United States, Mississippi	Human Clinical
NRS255	HT2002 0371	80	80	IV	France	Human Clinical
NRS253	HT2002 0354	398	398	NONE	France	Human Clinical
NRS653	CA-513	5	USA800	IV	United States, California	Human Clinical
N/A	PD6	9	N/A	NONE	United States, Illinois	Swine Nasal
N/A	PD10	398	N/A	YES	United States, Iowa	Swine Nasal
N/A	PD17	398	N/A	NONE	United States, Texas	Swine Nasal
N/A	PD18	9	N/A	NONE	United States, North Carolina	Swine Nasal
N/A	PD19	5	N/A	NONE	United States, North Carolina	Swine Nasal
N/A	PD32	9	N/A	NONE	United States, Alabama	Swine Nasal

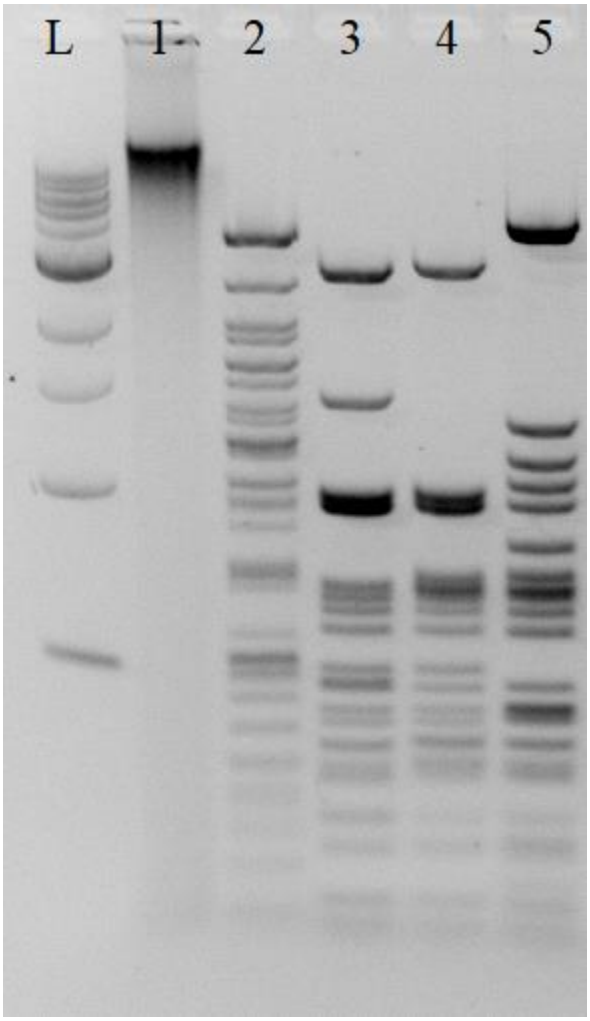
Table 2. Prevalence of *S. aureus* phage in swine environmental samples for individual farms. Samples were collected from sources listed and enriched for phage using different mixed-host enrichment strain panels. Presence of phage capable of infecting a given *S. aureus* strain is denoted with a "+" sign. Farm samples that were negative for phage on a respective strain are indicted by a blank space. Farm 16 is excluded from this table as in-depth sampling was obtained for that farm and is displayed separately in Table 3.

Sampling Site	State	Date Sampled	Animals Housed at Site	Production Stage of Site	Bacterial strain and sequence type									
					4011 USA300	4016 ST80	4018 ST398	4023 USA800	PD6 ST9	PD10 ST398	PD17 ST398	PD18 ST9	PD19 ST5	PD32 ST9
1	TX	7/2016	120	Gestation	+	+								
2	TX	7/2016	100	Finishing										
3	TX	7/2016	500	Gestation		+					+	+	+	+
4	TX	7/2016	700	Gestation	+	+			+		+	+	+	+
5	TX	7/2016	14	Cull Barn										
6	TX	7/2016	100	Finishing										
7	MN	8/2016	1,100	Finishing							+	+		+
8	IL	8/2016	12,000	Farrowing										+
9	NC	8/2016	200	Gestation	+	+		+	+	+	+	+	+	+
10	IN	8/2016	2,100	Finishing										
11	MN	8/2016	826	Finishing	+	+					+			+
12	NE	8/2016	6,000	Farrowing/Gestation	+	+	+	+	+		+	+	+	+
13	NC	9/2016	450	Finishing										
14	IA	9/2016	1,750	Gestation	+	+					+	+		+
15	AL	10/2016	72	Nursery		+	+	+			+	+		+
17	MN	11/2016	1,000	Finishing							+	+		
18	MI	12/2016	4,182	Finishing										
19	MN	12/2016	1,200	Finishing			+	+			+	+		+
20	IL	4/2017	1,200	Wean to Finish										

Table 3. 30 individual swab phage prevalence for two separate sampling dates of Farm 16. Presence of phage capable of infecting a given *S. aureus* strain is denoted with a "+" sign. Swab samples that were negative for phage on a respective strain are indicted by a blank space. Only swabs that were positive for one of the two sampling times are shown.

	Swabs collected 11/18/2016						**Swabs collected 5/11/2017**					
Swab	PD6	PD10	PD17	PD18	PD19	PD32	PD6	PD10	PD17	PD18	PD19	PD32
	ST9	ST398	ST398	ST9	ST5	ST9	ST9	ST398	ST398	ST9	ST5	ST9
1										+		
4										+		
5									+		+	
10											+	
11				+					+	+		
12												
13									+	+		+
21									+	+		

Figure 2. Restriction digest groups based on restriction enzyme DraI. Phage genomic DNA was digested with DraI and the fragments analyzed by gel electrophoresis. Left panel: representative patterns for the five restriction groups found among 51 phage isolates; L: NEB 1 kb DNA size marker. Right panel: distribution of phage restriction group totals for the 51 isolated phage.



DraI Restriction Group	Number of Phage
Group 1	19
Group 2	14
Group 3	3
Group 4	11
Group 5	5
TOTAL	**51**

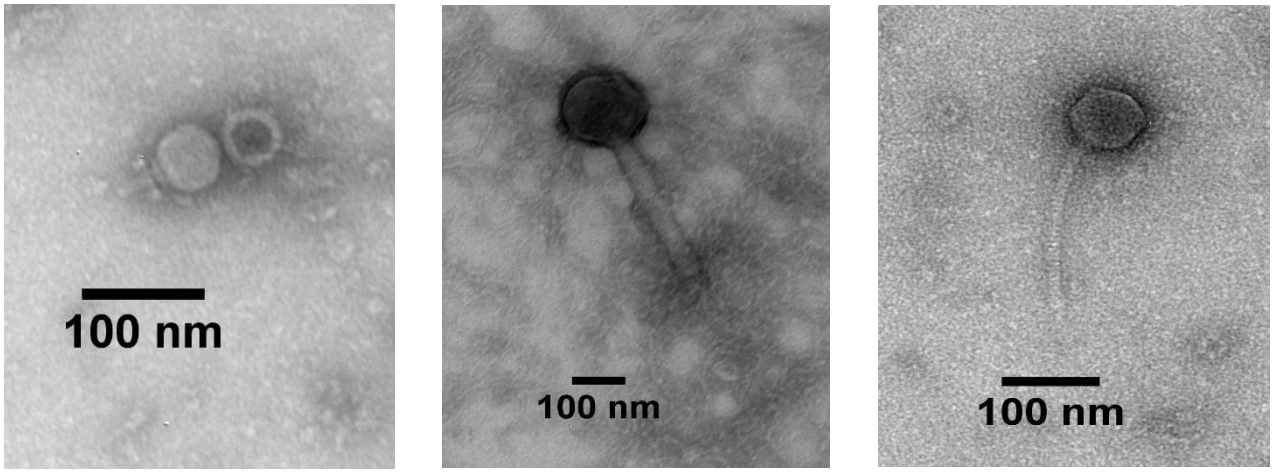


Figure 3. Transmission electron micrographs of phages isolated from swine environments. The three main morphological types seen thus far. Left: the podoviridae group which display a short, non-contractile tail; center: the myoviridae group which display a non-flexible but contractile tail; right: the siphoviridae group which display a flexible non-contractile tail.