Occurrence of *Staphylococcus aureus* in swine and swine workplace environments on industrial and antibiotic-free hog operations in North Carolina, USA: A One Health pilot study

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**A R T I C L E   I N F O**

**Keywords:**
- *Staphylococcus aureus*
- Occupational health
- One Health
- Swine
- Antimicrobial resistance

**A B S T R A C T**

Occupational exposure to swine has been associated with increased *Staphylococcus aureus* carriage, including antimicrobial-resistant strains, and increased risk of infections. To characterize animal and environmental routes of worker exposure, we optimized methods to identify *S. aureus* on operations that raise swine in confinement with antibiotics (industrial hog operation: IHO) versus on pasture without antibiotics (antibiotic-free hog operation: AFHO). We associated findings from tested swine and environmental samples with those from personal inhalable air samplers on worker surrogates at one IHO and three AFHOs in North Carolina using a new One Health approach. We determined swine *S. aureus* carriage status by collecting swab samples from multiple anatomical sites, and we determined environmental positivity for airborne bioaerosols with inhalable and impinger samplers and a single-stage impactor (ambient air) cross-sectionally. All samples were analyzed for *S. aureus*, and isolates were tested for antimicrobial susceptibility, absence of *scn* (livestock marker), and *spa* type.

Seventeen of twenty (85%) swine sampled at the one IHO carried *S. aureus* at > 1 anatomical sites compared to none of 30 (0%) swine sampled at the three AFHOs. All *S. aureus* isolates recovered from IHO swine and air samples were *scn* negative and *spa* type t337; almost all isolates (62/63) were multidrug resistant.

*S. aureus* was recovered from eight of 14 (67%) ambient air and two (100%) worker surrogate personal air samples at the one IHO, whereas no *S. aureus* isolates were recovered from 19 ambient and six personal air samples at the three AFHOs. Personal worker surrogate inhalable sample findings were consistent with both swine and ambient air data, indicating the potential for workplace exposure. IHO swine and the one IHO environment could be a source of potential pathogen exposure to workers, as supported by the detection of multidrug-resistant *S. aureus* (MDRSA) with livestock-associated *spa* type t337 among swine, worker surrogate personal air samplers and environmental air samples at the one IHO but none of the three AFHOs sampled in this study. Concurrent sampling of swine, personal swine worker surrogate air, and ambient airborne dust demonstrated that IHO workers may be exposed through both direct (animal contact) and indirect (airborne) routes of transmission. Investigation of the effectiveness of contact and respiratory protections is warranted to prevent IHO worker exposure to multidrug-resistant livestock-associated *S. aureus* and other pathogens.

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1. Introduction

There is growing evidence that working with swine is associated with higher Staphylococcus aureus exposures, including carriage of methicillin-resistant S. aureus (MRSA) and multidrug-resistant S. aureus (MDRSA), and increased risk of clinical disease (Hatcher et al., 2017; Nadimpalli et al., 2015, 2016; Rinsky et al., 2013; Smith and Wardyn, 2015; Wardyn et al., 2015; Ye et al., 2016a). The majority of studies focused on S. aureus in swine worker populations have investigated the concordance of S. aureus strains from swine and workers (Cui et al., 2009; Denis et al., 2009; Dorado-Garcia et al., 2015; Hau et al., 2015; Khanna et al., 2008; Lewis et al., 2008; Oppliger et al., 2012; Sinlapasorn et al., 2015; Smith et al., 2009; van Cleef et al., 2014) and others have investigated environmental routes of contamination or dispersal of S. aureus within hog operations (Agerso et al., 2014; Bos et al., 2016; Ferguson et al., 2016; Friese et al., 2012; Gibbs et al., 2006; Hau et al., 2015; van Cleef et al., 2014). A number of prior studies have employed an ad hoc One Health approach, defined as an evaluation of animals, humans, and their shared environments at the same time (Grontvedt et al., 2016; Pletinckx et al., 2013; Schmithausen et al., 2015; van Cleef et al., 2011; van den Broek et al., 2009). While such an approach provides critical evidence for both direct and indirect routes of exposure to workers, to our knowledge, no prior U.S. study has concurrently evaluated S. aureus in swine and from farm environments in the context of a personal worker exposure assessment. In making this assessment, we applied a formal One Health approach using recently-developed standards for study design and reporting of evidence (Davis et al., 2017).

Occupational exposures to swine in the U.S. may occur in industrial settings that involve raising swine in high densities inside confinement buildings with non-therapeutic and therapeutic antibiotic inputs (hereafter, industrial hog operation [IHO]) or on open pasture in low densities without the use of antibiotics (hereafter, antibiotic-free hog operation [AFHO]), which serves an emerging consumer market for antibiotic-free pork. The AFHO workplace setting has not been well evaluated to date. Given the limited One Health data regarding occupational exposures to S. aureus and other microbial exposures among swine and personnel working at IHOs or AFHOs in the U.S., we aimed to characterize direct (animal) and indirect (environmental) routes of worker exposure to S. aureus of livestock origin (hereafter, livestock-associated S. aureus) on hog operations with differing antibiotic use practices (IHO vs. AFHO), and to optimize methods for sample collection on these operations.

2. Materials & methods

2.1. Study design

This was a pilot study conducted in July 2015 with convenience sampling of hog production operations in North Carolina, which is the second-largest hog producing state in the U.S. (NASS, 2015). One IHO and three AFHOs were selected on the basis of availability and operator interest in participation in this study. IHO and AFHO were defined in accordance with prior evaluation (Rinsky et al., 2013). Low-density, pasture-based hog operations that reported use of antibiotics in animals whose products were intended for consumer sale were excluded. AFHOs were included if antibiotics were never used or if antibiotics were only used in animals whose products were not intended for consumer sale. As confirmed by interviews with AFHO farmers, in cases where antibiotic treatment was used to maintain animal welfare, sick pigs would be quarantined for treatment purposes and meat from these pigs would not be sold to consumers. Therefore, all herds that were sampled in this study were neither administered antibiotics nor were they in close contact with treated pigs. The design and reporting of this study were performed in accordance with COHERE standards for One Health epidemiologic studies (Davis et al., 2017); the inference of the study was to the human health domain via surrogate worker data (personal airborne samples from investigators performing animal handling activities).

2.2. Characterization of facilities

Workers or hog operation managers were surveyed regarding whether and how antimicrobial drugs were used in their herds in order to confirm IHO (conventional) and AFHO (antibiotic-free) status. Specific information on the type, frequency, and dosage of antibiotics used on the IHO and AFHOs in this study was not available to the research team. Additionally, in the U.S., publicly-available antibiotic use data are only reported in aggregate at the federal level.

2.3. Animal sampling

To assess direct worker exposures from animal contact, swine were sampled on each facility (a priori, n = 20 swine from the larger IHO, and 10 swine from each of the smaller AFHOs, for a total n = 30 AFHO swine). At least three animals from each available swine age cohort (e.g. farrow sow, piglet, weaner, etc.) per facility were selected for sampling. Early discussions with potential producers suggested that use of animal handling equipment (such as chutes, boards or snares) could be a barrier to participation, as use of these items can cause stress to swine. Hence, swine restraint for sampling was limited on each farm to that suggested by each producer. A veterinarian conducted or directly supervised all sampling. Copan E-swabs were used for collection. Swine were swabbed in the right nare, right side of the mouth (lingual/palatal mucosa), skin behind the right ear, right perineal mucosa, and any observed skin lesion site (e.g. dermatitis, wound) to be consistent with strategies used in prior studies for animal sampling (Iversen et al., 2015). (The contralateral (left) side was sampled using other techniques for microbiome assessment; microbiome results are not reported here.) If other livestock were present and accessible in the vicinity of a swine cohort, these animals were sampled with farmer permission and according to IACUC protocol (JH SP13H232) in order to better characterize all potential animal (direct) sources of S. aureus to workers. Personnel wore disposable Tyvek™ Micro-Clean coveralls (DuPont, USA), Klee Vanguard boot covers (Kimberly-Clark, Roswell, GA, USA), and sterile gloves for sampling.

2.4. Settled dust sampling

To assess indirect surface exposures to workers, dry electrostatic cloths (Swiffer™ Proctor & Gamble) were used to collect settled dust from 30 x 30 cm horizontal or vertical surfaces inside barns or around pastures, as previously described (Davis et al., 2012; Peterson et al., 2012). Additional field blanks (cloths handled without sampling) were collected on each operation as a quality control step to ensure that handling alone did not contaminate the cloths.

2.5. Ambient air sampling

To assess indirect airborne worker exposures, ambient air was sampled at worker height (90–150 cm off the ground). Air samples were collected using three methods: inhalable sample cassettes (Button sampler®, SKC Inc.) loaded with 25 mm gelatin filters (Sartorius, Germany), sterile all-glass impingers (BioSampler®, SKC Inc and AGI-30, ACE glass Inc) with 20 mL sterile 1 x PBS as collection media, and a single stage Andersen impactor (N6, Thermo Scientific, Inc) with CHROMagar™ Staph aureus plates. Inhalable samples were run using personal sampling pumps (AirCheck 5000, SKC Inc) calibrated at 4 L/min. Air flow through the impingers (12.5 L/min) and impactor (28.3 L/min) was drawn through oil-less vacuum pumps (VP0435A, MEDO USA). All flow rates were calibrated before sampling, and confirmed at the end of the sampling period using an electronic flow calibrator (Bios Defender 530, SKC Inc). Inhalable button samplers are
design to collect aerosols smaller than 100 μm; impingers and impactors are designed to collect bioaerosols between ~0.5 μm and ~20 μm in aerodynamic diameter. All area samplers (inhaleable samplers, impingers and impactors) were placed side-by-side in a location between 3 and 6 m downwind of the ventilation exhaust fans (IHO) or swine pasture (AFHO). Two inhalable area button samplers were clipped to a lab stand or tree branch at a slightly downward angle (to avoid direct impaction) and were activated for between 80 and 100 min. Impingers were activated for between 40 and 60 min, while carefully monitoring that an adequate volume of 1x PBS (~10 mL) remained in the reservoir. Since we had no information on potential S. aureus concentrations in the air a priori, Andersen impactor samples were designated for subsequent collection at 5, 10 and 20 min.

2.6. Personal samples from worker surrogates

To assess personal worker exposures during performance of work-like activities, investigators conducting animal sampling served as worker surrogates and wore breathing-zone personal monitors during sampling. Inhalable button sampler filters with 25 mm gelatin filters were clipped to the front of a/on a personal backpack (CamelBak Products, LLC, Petaluma, CA) containing the personal sampling pump (AirCheck 5000, SKC Inc). The pump was calibrated at 4 L/min, wrapped in noise-dampening material (Acoustic Polyurethane foam, McMaster-Carr), and put inside the backpack before being worn by investigators. Personal samplers were activated for 120–150 min (2.25 h) according to the duration of activity by investigators conducting animal sampling.

2.7. Air sample field processing

At the termination of each run (within 10 min), all inhalable button sampler filters were aseptically placed in containers containing 6 mL sterile 1x PBS, the volume of remaining impinger fluid was recorded and pipetted into sterile tubes, and agar plates from the single stage impactor were capped and sealed with parafilm. All plates were stored in a iced-pack-chilled cooler and transported to the laboratory at the University of North Carolina (UNC), and analyzed within five hours.

2.8. Microbial culture of isolates from swine swabs and electrostatic cloths

Swine swabs and electrostatic cloths of settled dust were subjected to double-enrichment broth culture using a method previously described and validated for harmonized assessment of human, animal and environmental samples (Davis et al., 2012, 2016). This method provides selective enrichment for both methicillin-susceptible (MSS) and methicillin-resistant staphylococci (MRSA), with identification selective enrichment for both methicillin-susceptible (MSS) and methicillin-resistant staphylococci (MRS). With identification of coagulase-positive staphylococci (CPS) according to tellurite reduction and lecithinase activity on Baird-Parker agar.

2.9. Characterization of isolates from swine swabs and electrostatic cloths

S. aureus species was confirmed using a multiplex PCR assay that amplifies species-specific segments of the nuc gene (nuc) (Sasaki et al., 2010). Isolates were screened for presence of a universal meca/C sequence, with ATCC43300 used as mecaA positive and LGA251 used as meccA positive controls (Garcia Alvarez et al., 2011) and for presence of the scn gene (van Wamel et al., 2006). Absence of the scn gene is considered a marker of livestock-association (Price et al., 2011). Confirmed S. aureus isolates were spa-typed as previously described (DTU, 2009; Shospin et al., 1999). Antimicrobial susceptibility testing, including to gentamicin, ampicillin, oxacillin, penicillin, cefoxitin, moxifloxacin, vancomycin, clindamycin, daptomycin, erythromycin, nitrofurantoin, linezolid, rifampin, quinupristin/dalfopristin, sulfamethoxazole/trimethoprim, tetracycline and minocycline, was performed using the BD Phoenix system (BD Diagnostics, Sparks, MD).

2.10. Microbial culture of isolates from air samples

Upon arrival at the laboratory, inhalable button sampler and impinger samples were processed by membrane filtration and direct spread-plating. CHROMagar™ Staph aureus plates from Andersen impactors were immediately incubated at 37 °C for 24 h. Prior to filtration, gelatin filters from inhalable button samplers were dissolved in 6 mL of 1x PBS by placing the storage tubes in a water bath (35 °C) for 5 min and vortexing to mix, based on the manufacturer's instructions (SKC). Approximately 5 mL of 1x PBS from each inhalable button sampler and impinger sample was then filtered through 47 mm diameter, 0.45 μm pore size polycarbonate filters (HTTP, Millipore Corporation, Bedford, MA) using sterile filter funnels, a six-place filter manifold, and sterile 1x PBS as a laboratory blank. All filters were transferred onto CHROMagar™ Staph aureus plates and incubated for 24 h at 37 °C. After incubation, if inhalable button sampler or impinger plates were overgrown or uncountable, the sample was diluted by either filtering 1 mL of the remaining sample (stored overnight at 4 °C) or by directly plating up to 100 μL of remaining sample onto CHROMagar™ Staph aureus using a steel cell spreader sterilized with 70% ethanol and flame. Presumptive S. aureus colonies (pink to mauve in color) that grew from inhalable button sampler, impinger and impactor plates were counted and streaked to isolation until pure. Pure cultures were then streaked onto Tryptic Soy Agar (TSA) with 5% sheep blood (Remel Laboratories, Lenexa, KS) and incubated at 37 °C for 24 h. Finally, all pure colonies were stored in 1 mL Brain Heart Infusion Broth (BHB) supplemented with 15% glycerol at ~80 °C for future molecular analyses and antibiotic resistance testing.

2.11. Characterization of isolates from air samples

DNA was extracted from archived presumptive S. aureus isolates using a crude DNA extraction (Reischl et al., 2000). Multiplex polymerase chain reaction (PCR) was performed to amplify five genes of interest, including: spa, meca, mecc, pvl ( lukF-PV) and scn. Positive controls included LGA251 (positive for meccA) and a clinical MRSA isolate (positive for spa, meca, pvl and scn) (Stegger et al., 2012). The spa gene was sequenced and spa typing was performed using the Ridom StaphType software and the Ridom SpaServer (http://spa.ridom.de/index.shtml), and spa-negative isolates were re-tested by PCR using a primer set that detects the species-specific femA gene (Paule et al., 2010), similar to Hatcher et al. (2017). If an isolate was positive for spa or femA, it was considered S. aureus.

Antimicrobial susceptibility testing was conducted at UNC by Kirby Bauer disc diffusion for the following antibiotics: amoxicillin, ciprofloxacin, cefoxitin, clindamycin, erythromycin, gentamicin, levofloxacin, lincomycin, linezolid, penicillin, quinupristin/dalfopristin, rifampin, spectinomycin, sulfamethoxazole/trimethoprim, and tetracycline. The panel, which differed slightly from that used for testing of swine isolates, included antibiotics that are sold exclusively for use in humans (e.g., levofloxacin, rifampin), food-producing animals (e.g., spectinomycin, lincomycin), or both (e.g., tetracycline) (FDA, 2016). Isolates were classified as non-susceptible (resistant or intermediate resistant) or as susceptible per the CLSI guidelines (CLSI, 2012, 2014). For spectinomycin and lincomycin, CLSI guidelines do not currently exist, and therefore isolates were classified as non-susceptible to these antibiotics if they exhibited complete resistance (S. aureus growth up to the edge of the disc) and as susceptible otherwise. To detect erythromycin-resistant isolates with induced clindamycin resistance, the D-zone test was used (Steward et al., 2005). For ease of interpretation, non-susceptible (resistant or intermediate-resistant) isolates are reported in the results and tables as resistant.

2.12. Definition of MDRSA and MRSA

S. aureus isolates were classified as multidrug resistant S. aureus (MDRSA) if they were completely resistant to three or more classes of
antibiotics (Magiorakos et al., 2014) and as methicillin-resistant S. aureus (MRSA) if they were positive for either mecA or mecC or phenotypically non-susceptible to cefoxitin according to the Clinical and Laboratory Standards Institute (CLSI) Approved Standard M100-S23.

2.13. Statistical analysis

Descriptive and comparative analyses were performed in Stata 13.1 (Stata Corp, College Station, TX). Calculation of single and combination anatomical site test sensitivity was conducted as previously described (Iverson et al., 2015). Briefly, swine were defined as positive if a confirmed S. aureus was recovered from any anatomical site; any site positive was used as the gold standard for calculation of test sensitivity for each individual site and lesion sites were excluded from this analysis.

2.14. Study team

The study team—who contributed to the design, conduct, analysis and interpretation of the work—including experts in occupational and environmental health, environmental microbiology, industrial hygiene, veterinary medicine, and molecular epidemiology. The team consulted extensively with participants and related stakeholders from both conventional and antibiotic-free swine facilities before, during, and after sampling.

2.15. Regulatory oversight

Johns Hopkins University and North Carolina State University IACUC boards approved this study. Johns Hopkins Bloomberg School of Public Health Institutional Review Board approved the human sampling in this study (IRB00005253).

3. Results

3.1. Facility characteristics

Table 1 provides an overview of the one IHO and three AFHOs, including total numbers of animals in each production stage. Antibiotic use was not reported among sampled swine on the AFHOs. All animal and environmental sampling was performed on the same day for each operation, and all operations (the one IHO and three AFHOs) were sampled during the same week under nearly-identical weather conditions.

3.2. IHO environment

In the morning, IHO air monitoring was conducted in and around a mechanically-ventilated farrowing barn; each farrowing room consisted of 12 individual, bow-bar farrowing crates that measured 1.5 m wide by 2.5 m long. The operation of one 14″ variable speed fan; two 18″ single speed fans; a propane heater; and the cool cell were coordinated through a single control panel with the goal of keeping the ambient temperature at 72 °F within the room. Waste was removed via an underslab flush system that was flushed every 4 h with fresh water. In the afternoon, IHO air monitoring was conducted outside between the farrow barn and an adjacent, mechanically-ventilated nursery barn; nursery rooms consisted of six elevated pens measuring 1.82 m × 1.82 m on each side of a central walkway. The operation of one 14″ variable speed fan; one 18″ single speed fan; and the propane heater was coordinated through a single control panel that modified the indoor temperature according to the age of the swine. All sampling was performed during daytime hours with sunny weather conditions and no strong prevailing winds.

3.3. AFHO environments

AFHO air monitoring was conducted immediately adjacent (< 3 m) to the fence of an outdoor pasture designated by the producer to be a typical or primary cohort for the operation. On two of three AFHOs, the sampling occurred in a semi-forested environment. No fans or other forms of mechanical ventilation were in use. All sampling was performed during daytime hours with sunny weather conditions and no strong prevailing winds.

3.4. Animal S. aureus results

According to animal disposition and availability of restraint options, samples were obtained from one or more sites from 20 swine at the one IHO and 30 swine, one ovine (sheep), one bovine (steer), three gallines (chickens), and four canines (dogs) from the three AFHO farms. Typically, no restraint was used for AFHO swine sampling. Considering the core anatomical sites sampled (nares, mouth, ear skin, perineum), 176 of an expected 200 swabs (88%) were collected from 50 swine. An additional five swabs from lesion sites also were collected. From all swabs, 37 confirmed S. aureus isolates were recovered representing 28 unique anatomical sites and 17 unique swine. All S. aureus-positive swine were from the one IHO and all swine S. aureus isolates belonged to spa type t337. All but one S. aureus isolate were scn-negative (three scn-negative and one scn-positive S. aureus isolates were recovered from one swine). One AFHO ovine (sheep) was positive for S. aureus spa type t034 at the mouth site. No swine, other livestock or canines were S. aureus-positive on the AFHOs; however the bovine (steer) and gallines (poultry) were sampled at only one site each, i.e. the nares and cloaca, respectively.

Table 2 illustrates the anatomical site-specific prevalence and anatomical site sensitivity for S. aureus carriage among swine and indicates that the mouth was the most sensitive single site for sampling to determine if a swine was positive. However, sampling the mouth alone classified only 53% of positive swine correctly. Sampling the nares and mouth was the most sensitive combination of two sites (87% sensitivity); the second-most sensitive combination of two sites was the mouth and perineum (80% sensitivity).

Nearly all swine isolates (97% of 37) were multi-drug resistant S. aureus (MDRSA), defined as resistance to three or more classes of

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of hog facilities, swine, and target worker populations.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>Industrial Hog Operation (IHO)</td>
</tr>
<tr>
<td>Number of facilities</td>
<td>1</td>
</tr>
<tr>
<td>Type of facility</td>
<td>Confinement</td>
</tr>
<tr>
<td>Total number of swine, average [range]</td>
<td>789</td>
</tr>
<tr>
<td>Sows, average [range]</td>
<td>259</td>
</tr>
<tr>
<td>Nursery/Weaner, average [range]</td>
<td>530</td>
</tr>
<tr>
<td>Number of barns</td>
<td>4</td>
</tr>
<tr>
<td>Number of workers, average [range]</td>
<td>3</td>
</tr>
</tbody>
</table>

samples were collected from one or more anatomical sites given swine disposition and availability of restraint options:

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outdoor S. aureus MRSA were present. Table 4 provides sampling times, air volumes, and antibiotics in the panel, including cefoxitin, which indicates that no tetracycline (21%, 5/24). Air isolates were fully susceptible to all other antibiotics: erythromycin (100%, 24/24), penicillin (100%, 24/24), spectinomycin (100%, 24/24), clindamycin (67%, 16/24) and tetracycline (49%, 24/24). Several isolates (20%, 4/20) inhibited growth and led to an underestimation of S. aureus growth and led to an underestimation of S. aureus concentrations (CFU/m3).

3.6. Ambient air S. aureus results

Air samples collected at the one IHO facility were positive for S. aureus, while no air samples from the AFHO facilities were positive for S. aureus (see Table 3a). All S. aureus isolates (n = 24 isolates) recovered from ambient air at the one IHO were multidrug resistant (MDRSA), lacked the scn gene and belonged to spa type t337 (see Table 3b). Ambient air isolates exhibited resistance (non-susceptibility) to the following antibiotics: erythromycin (100%, 24/24), penicillin (100%, 24/24), spectinomycin (100%, 24/24), clindamycin (67%, 16/24) and tetracycline (21%, 5/24). Air isolates were fully susceptible to all other antibiotics in the panel, including cefoxitin, which indicates that no MRSA were present. Table 4 provides sampling times, air volumes, and S. aureus concentration ranges for each type of air sample. The highest concentration ranges (61 CFU/m3) were measured with the Andersen single stage impactor during the afternoon sampling outside the farrowing and nursery barns. Running the Andersen impactor for five minutes was determined to be adequate under existing conditions to yield samples above the limit of detection of the method. However, because the CHROMagar™ Staph aureus plates were unable to fully inhibit growth of non-S. aureus bacteria, samples run for 10–20 min often yielded plates with an ideal countable range of S. aureus colonies (20–200 CFU), but plates that were simultaneously overgrown (> 300 CFUs) with non-S. aureus colonies, which may have inhibited S. aureus growth and led to an underestimation of S. aureus concentrations (CFU/m3).

3.5. Settled dust S. aureus results

Nine environmental surface samples were collected from the conventional farm, and 13 were collected among the three pasture farms (three, six, and four, respectively) based on availability of surfaces to sample. None of these surfaces was positive for S. aureus.

Table 2
Staphylococcus aureus carriage and anatomical site-level sensitivity among swine.

<table>
<thead>
<tr>
<th>S. aureus carriage, N (%)</th>
<th>Combined n=50</th>
<th>IHO swine&lt;sup&gt;a&lt;/sup&gt; n=20</th>
<th>AFHO swine&lt;sup&gt;b&lt;/sup&gt; n=30</th>
<th>IHO swine&lt;sup&gt;c&lt;/sup&gt; n=15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall (swine-level)</td>
<td>17 (34%)</td>
<td>17 (85%)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0 (0%)</td>
<td>100% (ref)</td>
</tr>
<tr>
<td>Nares</td>
<td>5 (12%)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5 (28%)</td>
<td>0 (0%)</td>
<td>33%</td>
</tr>
<tr>
<td>Mouth</td>
<td>8 (20%)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8 (42%)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0 (0%)&lt;sup&gt;j&lt;/sup&gt;</td>
<td>53%</td>
</tr>
<tr>
<td>Ear skin</td>
<td>8 (13%)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>8 (40%)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>0 (0%)&lt;sup&gt;j&lt;/sup&gt;</td>
<td>40%</td>
</tr>
<tr>
<td>Perineum</td>
<td>6 (13%)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6 (30%)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>0 (0%)&lt;sup&gt;j&lt;/sup&gt;</td>
<td>40%</td>
</tr>
<tr>
<td>Skin lesion</td>
<td>1 (20%)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1 (25%)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>0 (0%)&lt;sup&gt;j&lt;/sup&gt;</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Samples were collected from one or more anatomical sites given swine disposition and availability of restraint options:

<sup>a</sup> n = 42 swabs.
<sup>b</sup> n = 41 swabs.
<sup>c</sup> n = 45 swabs.
<sup>d</sup> n = 48 swabs.
<sup>e</sup> n = 5 swabs.
<sup>f</sup> n = 18 swabs.
<sup>g</sup> n = 19 swabs.
<sup>h</sup> n = 20 swabs.
<sup>i</sup> n = 4 swabs.
<sup>j</sup> n = 24 swabs.
<sup>k</sup> n = 22 swabs.
<sup>l</sup> n = 25 swabs.
<sup>m</sup> n = 28 swabs.
<sup>n</sup> n = 1 swab.
<sup>o</sup> Acronyms: IHO, Industrial Hog Operation (confinement) and AFHO, Antibiotic-Free Hog Operation (pasture).
<sup>p</sup> 37 S. aureus isolates were identified from 28 anatomical sites and all S. aureus were scn-negative t337 strains.
<sup>q</sup> Limited to positive swine (n = 15) with all sites tested (excluding the lesion site); the sensitivity for each anatomic site was calculated by dividing the number of animals positive at a single anatomic site by the number of animals positive at any anatomical site; only IHO swine were positive.

Table 3a
Prevalence of S. aureus (sample and isolate level) collected from IHO and AFHO swine, facility environments, and worker surrogate samples.

<table>
<thead>
<tr>
<th>Sample/Isolate level</th>
<th>Sample N</th>
<th>S. aureus n (%)</th>
<th>Isolate N</th>
<th>S. aureus n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHO swine</td>
<td>20</td>
<td>17 (85%)</td>
<td>203</td>
<td>37 (18%)</td>
</tr>
<tr>
<td>IHO environment</td>
<td>14</td>
<td>8 (57%)</td>
<td>40</td>
<td>24 (60%)</td>
</tr>
<tr>
<td>Area airborne&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9</td>
<td>0 (0%)</td>
<td>21&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Settled dust</td>
<td>2</td>
<td>2 (100%)</td>
<td>10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>AFHO swine</td>
<td>30</td>
<td>0 (0%)</td>
<td>162&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Area airborne&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19</td>
<td>0 (0%)</td>
<td>56&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Settled dust</td>
<td>13</td>
<td>0 (0%)</td>
<td>37&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>AFHO worker surrogate</td>
<td>6</td>
<td>0 (0%)</td>
<td>33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

<sup>a</sup> IHO: Industrial Hog Operation; AFHO: Antibiotic-Free Hog Operation.
<sup>b</sup> MDRSA: multidrug resistant S. aureus; tet-R: tetracycline resistant (non-susceptible).
<sup>d</sup> Area airborne: Anderson, Inhalable (Button) Area, and Impinger (SKC and AGI-30) samplers; Personal airborne: Inhalable (Button) Personal samplers only; N.B. S. aureus was recovered from all sampler types at the one IHO.
<sup>e</sup> A sample was considered S. aureus positive if at least one spa positive isolate was recovered from that sample.
<sup>f</sup> Total number of coagulase-positive staphylococci identified on Baird-Parker agar following broth-enrichment culture.
<sup>g</sup> Total number of target and non-target isolates identified using CHROMagar Staph aureus plates, without enrichment.
Table 3b
Characteristics of Staphylococcus aureus isolates collected from swine anatomical sites, hog facility environments, and worker surrogate samples.

<table>
<thead>
<tr>
<th>Isolate level</th>
<th>S. aureus isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isolate N</td>
</tr>
<tr>
<td>IHO swine</td>
<td>203</td>
</tr>
<tr>
<td>IHO environment</td>
<td></td>
</tr>
<tr>
<td>Area airborne</td>
<td>40</td>
</tr>
<tr>
<td>Settled dust</td>
<td>21</td>
</tr>
<tr>
<td>IHO worker surrogate</td>
<td></td>
</tr>
<tr>
<td>Personal airborne</td>
<td>10</td>
</tr>
</tbody>
</table>


3.7. Personal air sample

S. aureus results from worker surrogates: Filters from the personal inhalable samplers were run during the period of sample collection from swine, which was approximately equivalent to two hours on each operation. Both personal air samples collected at the one IHO were positive for S. aureus (7 and 9 CFU/m³ respectively); none of the personal air samples (two each on three operations) from the AFHOs were positive for S. aureus. Similar to the ambient air samples, S. aureus isolates recovered from personal inhalable samplers at the one IHO were also multidrug-resistant, lacked the spa gene, belonged to spa type t337, and exhibited resistance (non-susceptibility) to erythromycin (100%, 2/2), penicillin (100%, 2/2), spectinomycin (100%, 2/2), and clindamycin (50%, 1/2). However, both of the isolates were susceptible to tetracycline (see Table 3b).

4. Discussion

Findings from this cross-sectional pilot study, which employed a One Health approach, suggest that routes of exposure to S. aureus for U.S. hog workers can include direct swine contact and indirect exposure via air inside (personal worker surrogate air samples) and directly outside (ambient air samples) confinement barns. S. aureus with spa type t337 was identified among 85% (17 of 20) swine on the one IHO but not among any of the swine on the three pasture-based AFHOs. Ambient and personal air samples from the one IHO also were positive for S. aureus with spa type t337, which matched the spa type of S. aureus found among the swine. S. aureus with spa type t337 has previously been associated with multi locus-sequence type clonal complex (CC) 9 (Larsen et al., 2012) and with CC398 (Sun, 2016). Both CC9 and CC398 are well-characterized livestock-associated S. aureus lineages (Hasman et al., 2010; Price et al., 2011; van Loo et al., 2007; Ye et al., 2016b). None of the swine or air samples collected at the AFHO facilities were positive for S. aureus, which improves the evidence for test specificity (individual samples negative on an operation where all samples were negative). MDRSA spa type t337 strains were identified in both swine and air samples on the one IHO.

Given that MDRSA spa type t337 strains also were recovered from personal inhalable air samplers, which represent the breathing zone of worker surrogates conducting sampling activities with the swine, the work environment is a likely source of livestock-associated S. aureus and MDRSA for workers at this facility. This conclusion is further supported by prior studies which identified that 10–30% of IHO workers carried t337 at one or more time points, whereas only 0–1% of household members or community referents carried this strain (Hatcher et al., 2017; Nadimpalli et al., 2016; Rinsky et al., 2013). Further, all isolates were scn-negative, indicating likely animal (rather than human) adaptation and implicating swine as the source of S. aureus exposure to workers. Prior studies have linked environmental contamination of swine production facilities to higher risk for nasal carriage of livestock-associated, antimicrobial-resistant S. aureus among swine production workers (Bos et al., 2016; Schmithausen et al., 2015). Given that this pilot study was cross-sectional, which limits causal inference, future studies will need to employ longitudinal evaluation to assess whether exposure results in transmission of S. aureus to worker populations.

A key aim of this work was to optimize methods for animal and environmental sampling for future studies in this population. For animal sampling, the mouth was the most sensitive single site for detection of S. aureus but classified only 53% of positive pigs correctly. A combination of two sites (nasal and mouth) provided enhanced sensitivity, classifying 87% of positive swine correctly. Site-specific sensitivity calculated from the reported data of Linhares et al., who

Table 4
Sampling times, air volumes, and recovery of Staphylococcus aureus colony-forming units (CFUs) from air samplers.

<table>
<thead>
<tr>
<th>Button sampler</th>
<th>Avg. sampling duration (min)</th>
<th>Avg. volume (L)</th>
<th>Standard deviation (L)</th>
<th>Range (L)</th>
<th>Coefficient of variation (%)</th>
<th>CFU/m³ range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personal inhalable</td>
<td>138</td>
<td>559</td>
<td>36</td>
<td>520–607</td>
<td>6%</td>
<td>&lt; LOD – 9.10</td>
</tr>
<tr>
<td>Area inhalable</td>
<td>102</td>
<td>431</td>
<td>97</td>
<td>330–571</td>
<td>2%</td>
<td>&lt; LOD – 2.10</td>
</tr>
<tr>
<td>Impinger</td>
<td>48</td>
<td>611</td>
<td>120</td>
<td>346–805</td>
<td>20%</td>
<td>&lt; LOD – 8.60</td>
</tr>
<tr>
<td>Andersen impactor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>And-5 min</td>
<td>5</td>
<td>153</td>
<td>17</td>
<td>140–170</td>
<td>11%</td>
<td>&lt; LOD – 28.5</td>
</tr>
<tr>
<td>And-10 min</td>
<td>10</td>
<td>293</td>
<td>16</td>
<td>275–312</td>
<td>6%</td>
<td>&lt; LOD – 38.8</td>
</tr>
<tr>
<td>And-20 min</td>
<td>20</td>
<td>574</td>
<td>33</td>
<td>530–620</td>
<td>6%</td>
<td>&lt; LOD – 61.3</td>
</tr>
</tbody>
</table>

< LOD: Below the limit of detection.

*a* Dissolution of gelatin filters was incomplete and eluate was further diluted; this may result in underestimation of CFU counts.
evaluated swine on two IHOs in Minnesota, suggested that nares alone classified 75% of swine correctly, and skin and tonsil sites each classified 68% of swine correctly; combination-site sensitivity could not be calculated from the reported data (Linhares et al., 2014). Single anatomical site-specific carriage prevalence rates in our pilot study were slightly lower than those of Linhares et al. (2014). We sampled multiple anatomical sites per animal for swine and found that swine carried the same strain regardless of anatomical site sampled. We only detected one spa-type from one farm in our study, and it is possible that anatomical carriage of S. aureus on swine may vary according to both strain and host (swine) characteristics. It is also possible that pig herds that share the same confinement and environment also share one dominant S. aureus strain and that—if we had sampled additional IHOs—we would have found different dominant strains on different IHOs. Further, swine, like humans, may carry S. aureus intermittently (Espinosa-Gongora et al., 2015). Therefore, it is important for future studies that may be carried out among different swine breeds or may identify different S. aureus strains both cross-sectionally and over time to confirm whether the finding of the mouth as the most sensitive site for S. aureus recovery from positive swine remains consistent.

None of the environmental surface samples was positive for S. aureus. This was unexpected given the literature on potential for settled dust and/or surfaces in the vicinity of positive animals to be contaminated (Agerso et al., 2014; Bos et al., 2016; Broens et al., 2011; Friese et al., 2012; Peterson et al., 2012; Pletinckx et al., 2013) and given that the protocol used in this study employs a non-selective enrichment medium as well as an antimicrobial-selective enrichment medium and was adapted from EFSA guidelines for identification of MRSA from swine confinement facilities (Davis et al., 2012, 2016; EFSA, 2010).

False-negatives have been noted previously with the antimicrobial-selective enrichment arm in the context of MRSA detection (Larsen et al., 2017). In addition, it is possible that the protocol performance is weaker for S. aureus including MRDRA than for MRSA strain detection under settings where other staphylococcal species are prevalent and possibly dominant. Numerous non-aureus staphyloccoci, including S. sciuri, S. epidermidis, S. simulans, and S. lentus were identified from IHO swine (data not shown), which may be shed into the environment. If these more resistant non-S. aureus staphyloccoci are differentially selected during broth-enrichment culture of surface dust samples, rarer S. aureus may be missed. Future studies may consider elution of the cloth samples into a buffered non-enrichment solution to allow for direct plating of aliquots via dilution or may consider microfluidic technologies or metagenomic techniques to address this potential challenge. Alternately, another inhibitor, such as a bactericidal chemical, could have contributed to this finding. Finally, it is possible, although unlikely given the positive air samples, that settled dust in the one IHO environment did not harbor viable S. aureus.

For ambient air sampling under IHO conditions, five minutes was determined to be sufficient to recover airborne S. aureus and to have total colonies of all bacteria countable without dilution when using an Andersen impactor, in which air is mechanically directed onto the surface of an agar plate. No S. aureus isolates were recovered from any samples at the AFHOs, however a sample time of longer than 20 min for the Andersen Impactor is not recommended due to drying of the agar. Two prior U.S. studies have evaluated airborne S. aureus or MRSA using similar equipment (Ferguson et al., 2016; Gibbs et al., 2006). Our finding of concentrations of S. aureus as high as 61.3 CFU/m³ in ambient air downwind of barn ventilation exhaust is similar to the mean MRSA concentrations of 63 CFU/m³ observed in similar downwind samples collected as part of a recent study outside a Midwestern U.S. nursery-grower swine production facility (Ferguson et al., 2016). The Midwestern U.S. study also used short sampling times, from 5 s to 5 min, and identified that antimicrobial-resistant S. aureus followed a declining gradient from 25 m to 150 m downwind of a 1000-sow confinement operation (Gibbs et al., 2006). A prior Danish study reported use of a filter-based technique and a 15-min sampling window; however this study produced only binary detection versus non-detection outcomes, not CFU concentrations (Agerso et al., 2014). A prior German study did not report sampling times but did add glycerol to the impinger solution to "extend the sampling time" and detected a median of 151 CFU/m³ MRSA at 50–150 m downwind (Schulz et al., 2012).

The major limitation of this work is that animal and environmental specimens were collected from a convenience group of operations and not from the facilities where IHO workers were employed in our prior epidemiologic studies of IHO and AFHO workers (Hatcher et al., 2017; Nadimpalli et al., 2015, 2016; Rinsky et al., 2013). In these studies, given the potential for employer retribution against workers participating in research studies, de-coupling of worker-workplace sampling was necessary to ensure worker anonymity. Further, animal and environmental specimens were collected here to perform pilot assessment of One Health methods to characterize the potential for hog worker S. aureus exposures; therefore, results are based on limited sampling at a small number of operations and may not be generalizable to other facilities. The lack of S. aureus among swine and environmental samples collected at AFHO facilities could represent low prevalence of S. aureus among AFHO swine or could be due to selection bias. For example, it is possible that AFHOs less likely to be impacted by S. aureus were more likely to volunteer for this study. It is also possible that differences in our microbial culture techniques for air samples (no enrichment) compared to our swine and settled dust samples (double-enrichment) could have biased results, particularly for recovery of resistant organisms. Such bias could explain differences in prevalence of tetracycline resistance (non-susceptibility) between air and swine samples. Therefore, a key finding of this pilot study was the need for better harmonization of culture techniques among the different types of samples (e.g., subjecting an aliquot of medium from air samples to double-enrichment culture in parallel to the CFU technique described here, or direct plating of swine and surface sample aliquots prior to enrichment). Finally, lack of overlap in the distribution of swine age cohorts across the operations, as shown in Table 1, prevented an analysis of potential confounding by age cohort, which could represent another potential source of bias. Regardless, this work demonstrates the feasibility for collection of animal, air, environmental surface, and personal worker monitoring samples on IHO and pasture-based AFHOs.

IHO workers may be exposed to S. aureus via airborne routes on positive operations, and multidrug-resistant strains can be detected in the worker breathing zone. Given that a prior study in the NC IHO worker population found that consistent mask use was associated with lower nasal carriage of sccm-negative S. aureus (Nadimpalli et al., 2016), further investigation of risk factors for transmission and the effectiveness of both contact and respiratory protections to prevent livestock-associated S. aureus and other pathogen exposure among IHO hog workers is warranted. Although our findings are consistent with other studies that suggest the importance of air and direct animal contact as pathways of IHO worker S. aureus exposure, our study was small. Confirmation of this finding and determination of a potential lower risk of workplace exposures to live-stock-associated S. aureus on AFHOs require future studies that systematically investigate a larger number of IHOs and AFHOs. Given that we detected airborne MRDSA with spa type t337 near the outflow of existing barn vents, future studies also are needed to investigate the potential for community exposure via airborne environmental pathways.

5. One Health contribution

The One Health approach to characterize hog worker exposures allowed concurrent assessment of both direct and indirect routes of livestock-associated S. aureus transmission. Because S. aureus carriage and environmental contamination can be time-varying, concurrent assessment reduced bias. Compared to the alternative, which would have been conduct of two separate studies (worker-animal and worker-environment), concurrent assessment resulted in time savings of four days for at least three study personnel (including the PI), resulted in time...
savings of one day each for farmers, and resulted in cost savings for travel. The One Health approach, bringing together multiple disciplines, also required joint leadership and coordination among several institutions and organizations. The study team concluded that while this required more extensive communication and planning, the scientific benefits outweighed the cost.

6. Conclusion

Our recovery of a specific strain of S. aureus (MDRSA with spa type t337) from animals, ambient air, and worker breathing zone samples collected at one U.S. site provides insights into potential occupational exposure routes and may guide future studies to identify worker protection to reduce the potential for S. aureus exposure.

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