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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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Abstract

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

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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.

Keywords

Staphylococcus aureus; occupational health; One Health; swine; antimicrobial resistance

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. 2016; Nadimpalli, et al. 2015; Nadimpalli, et al. 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 (Iverson, 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), Kleenguard 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 30x30cm 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 (90–150cm off the ground). Air samples were collected using three methods: inhalable sample cassettes (Button sampler®, SKC Inc.) loaded with 25mm gelatin filters (Sartorius, Germany), sterile all-glass impingers (BioSampler®, SKC Inc and AGI-30, ACE glass Inc) with 20 ml sterile 1× PBS as collection media, and a single stage Andersen impactor (N6, Thermo Scientific, Inc) with CHROMagar™ Staph aureus plates. Inhalable samplers 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 designed 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 (inhalable samplers, impingers and impactors) were placed side-by-side in a location between 3 and 6

meters 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 minutes. Impingers were activated for between 40 and 60 minutes, while carefully monitoring that an adequate volume of 1x PBS (~10 mL) remained in the reservoir. Since we had no information of potential *S. aureus* concentrations in the air *a priori*, Andersen impactor samples were designated for sequential collection at 5, 10 and 20 minutes.

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 samplers loaded with 25 mm gelatin filters were clipped to the front strap of a personal backpack (CamelBak Products, LLC, Petaluma, CA) containing the personal sampling pump (AirCheck 5000, SKC Inc). The pump was calibrated at 4 L/m, 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 to 150 minutes (2–2.5 hours) 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 tubes containing 6ml 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 samples were stored in an ice-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; Davis, et al. 2016). This method provides 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 nuclease gene (*nuc*) (Sasaki, et al. 2010). Isolates were screened for presence of a universal *mecA/C* sequence, with ATCC43300 used as *mecA* positive and LGA251 used as *mecC* 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; Shopsin, 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 hours. 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 minutes 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 hours at 37°C. After incubation, if inhalable button sampler or impinger plates were overgrown or uncountable, the sample was diluted by either filtering 1mL 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 colonies were then streaked onto Tryptic Soy Agar (TSA) with 5% sheep blood (Remel Laboratories, Lenexa, KS) and incubated at 37°C for 24 hours. Finally, all pure colonies were stored in 1 mL Brain Heart Infusion Broth (BHIB) 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 *mecC)* 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 *fem*A gene (Paule, et al. 2010), similar to Hatcher *et al.* (Hatcher, et al. 2016). If an isolate was positive for *spa* or *fem*A, 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, gentamycin, levofloxacin, lincomycin, linezolid, penicillin, quinuprisin/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 intermediately resistant) or as susceptible per the CLSI guidelines (CLSI 2012; CLSI 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 in interpretation, non-susceptible (resistant or intermediately-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—included 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 underslat flush system that was flushed every 4 hours 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 (<3m) 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 antimicrobials, and all positive swine carried MDRSA. Rates of resistance (non-susceptibility) were: clindamycin, 100%; erythromycin, 100%; ampicillin, 97%; penicillin, 97%; tetracycline, 43%; gentamicin, 3%; cefoxitin, 0%; daptomycin, 0%, linezolid, 0%, minocycline, 0%; moxifloxacin, 0%; nitrofurantoin, 0%; oxacillin, 0%; quinupristin-dalfopristin, 0%; rifampin, 0%; trimethoprim-sulfamethoxazole, 0%; and vancomycin, 0%. No MRSA were identified, as indicated by 0% resistance to cefoxitin and PCR confirmation.

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*.

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-suceptibility) 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 outdoor *S. aureus* concentrations (61 CFU/m^3) were measured with the Andersen single stage impactor during the afternoon sampling outside between 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 minutes 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/m^3).

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/m^3 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 *scn* gene, belonged to *spa* type t337, and exhibited resistance (non-susceptibility) to erythromycin, penicillin, and spectinomycin (100%, 2/2). However, both of the isolates were susceptible to tetracycline (see Table 3).

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 337 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. 2016; 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 (nares and mouth) provided enhanced sensitivity, classifying 87% of positive swine correctly. Site-specific sensitivity calculated from the reported data of Linhares *et al.*, who 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.* (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 arm as well as an antimicrobial-selective enrichment arm and was adapted from EFSA guidelines for identification of MRSA from swine confinement facilities (Davis, et al. 2012; Davis, et al. 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 MDRSA than for MRSA strain detection under settings where other staphylococcal species are prevalent and possibly dominant. Numerous non-*aureus* staphylococci, 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* staphylococci 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 minutes 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/m^3 in ambient air downwind of barn ventilation exhaust is similar to the mean MRSA concentrations of 63 CFU/m^3 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 25m to 150m downwind of a 1,000-sow confinement operation (Gibbs, et al. 2006). A prior Danish study reported use of a filter-based technique and a 15-minute 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/m^3 MRSA at 50-150m 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. 2016; Nadimpalli, et al. 2015; Nadimpalli, et al. 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 *scn*-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 livestock-associated *S. aureus* on AFHOs require future studies that systematically investigate a larger number of IHOs and AFHOs. Given that we detected airborne MDRSA 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. IHO provides insights into potential occupational exposure routes and may guide future studies to identify worker protections to reduce the potential for *S. aureus* exposure.

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Highlights

- We characterized worker exposure on 1 industrial and 3 antibiotic-free hog operations
- Drug-resistant *S. aureus* was detected in hogs and air on the industrial hog operation
- Drug-resistant *S. aureus* was not detected on the antibiotic-free hog operations
- Future One Health studies should target worker safety strategies to reduce exposure

Table 1

Characteristics of hog facilities, swine, and target worker populations.

Characteristic	Industrial Hog Operation (IHO)	Antibiotic-Free Hog Operation (AFHO)
Number of facilities	1	3
Type of facility	Confinement	Pasture-based
Total number of swine, average [range]	789	54 [21–75]
Sows, average [range]	259	10 [3–18]
Nursery/Weaner, average [range]	280	0
Feeder/Finish, average [range]	0	42 [18–55]
Number of barns	4	0
Number of workers, average [range]	3	2 [2–3]

Table 2

Staphylococcus aureus

carriage and anatomical site-level sensitivity among swine.

	***S. aureus* carriage, N (%)**			**Test sensitivity[q]**
	Combined *n*=50	**AFHO swine[o] *n*=20**	**IHO swine[o] *n*=30**	**IHO swine[o] *n*=15**
Overall (swine-level)	17 (34%)	17 (85%)[p]	0 (0%)	100% *(ref)*
Nares	5 (12%)[a]	5 (28%)[f]	0 (0%)[j]	33%
Mouth	8 (20%)[b]	8 (42%)[g]	0 (0%)[k]	53%
Ear skin	8 (13%)[c]	8 (40%)[h]	0 (0%)[l]	40%
Perineum	6 (13%)[d]	6 (30%)[h]	0 (0%)[m]	40%
Skin lesion	1 (20%)[e]	1 (25%)[i]	0 (0%)[n]	*n/a*

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

[a] n=42 swabs;

[b] n=41 swabs;

[c] n=45 swabs;

[d] n=48 swabs;

[e] n=5 swabs;

[f] n=18 swabs;

[g] n=19 swabs;

[h] n=20 swabs;

[i] n=4 swabs;

[j] n=24 swabs;

[k] n=22 swabs;

[l] n=25 swabs;

[m] n=28 swabs;

[n] n=1 swab;

[o] Acronyms: IHO, Industrial Hog Operation (confinement) and AFHO, Antibiotic-Free Hog Operation (pasture);

[p] 37 *S. aureus* isolates were identified from 28 anatomical sites and all *S. aureus* were *scn*-negative t337 strains;

[q] 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

	Sample level[b]		Isolate level	
	Sample N	***S. aureus* n (%)**	**Isolate N**	***S. aureus* n (%)**
IHO swine	20	17 (85%)	203[d]	37 (18%)
IHO environment				
Area airborne[a]	14	8 (57%)	40[e]	24 (60%)
Settled dust	9	0 (0%)	21[d]	0 (0%)
IHO worker surrogate				
Personal airborne[a]	2	2 (100%)	10[e]	2 (20%)
AFHO swine	30	0 (0%)	162[d]	0 (0%)
AFHO environment				
Area airborne[a]	19	0 (0%)	56[e]	0 (0%)
Settled dust	13	0 (0%)	37[d]	0 (0%)
AFHO worker surrogate				
Personal airborne[a]	6	0 (0%)	33[e]	0 (0%)

IHO: Industrial Hog Operation; AFHO: Antibiotic-Free Hog Operation

[a] 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.

[b] A sample was considered *S. aureus* positive if at least one *spa* positive isolate was recovered from that sample;

[c] MDRSA: multidrug resistant *S. aureus*; tet-R: tetracycline resistant (non-susceptible);

[d] Total number of coagulase-positive staphylococci identified on Baird-Parker agar following broth-enrichment culture;

[e] 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.

	Isolate level			Characteristics of *S. aureus* isolates		
	Isolate N	***S. aureus* n (%)**	***spa* type t337 n (%)**	***scn* negative t337 n (%)**	**MDRSA t337[c] n (%)**	**tet-R t337[c] n (%)**
IHO swine	203[d]	37 (18%)	37 (100% of 37)	37 (100% of 37)	36 (97% of 37)	16 (43% of 37)
IHO environment						
Area airborne[a]	40[e]	24 (60%)	24 (100% of 24)	24 (100% of 24)	24 (100% of 24)	5 (21% of 24)
Settled dust	21[d]	0 (0%)	No isolates	No isolates	No isolates	No isolates
IHO worker surrogate						
Personal airborne[a]	10[e]	2 (20%)	2 (100% of 2)	2 (100% of 2)	2 (100% of 2)	0 (0% of 2)

IHO: Industrial Hog Operation; AFHO: Antibiotic-Free Hog Operation

[a]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.

[b]A sample was considered *S. aureus* positive if at least one *spa* positive isolate was recovered from that sample;

[c]MDRSA: multidrug resistant *S. aureus*; tet-R: tetracycline resistant (non-susceptible);

[d]Total number of coagulase-positive staphylococci identified on Baird-Parker agar following broth-enrichment culture;

[e]Total number of target and non-target isolates identified using CHROMagar Staph aureus plates, without enrichment

Table 4

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

	Avg. sampling duration (min)	Avg. volume (L)	Standard deviation (L)	Range (L)	Coefficient of variation (%)	CFU/m^3 range[a]
Button sampler[a]						
Personal inhalable airborne	138	559	36	520–607	6%	<L OD - 9.10
Area inhalable airborne	103	431	97	330–571	22%	<L OD - 2.10
Impinger						
	48	611	120	346–805	20%	<L OD - 8.60
Andersen impactor						
And-5 min	5	153	17	140–170	11%	<L OD - 28.5
And - 10 min	10	293	16	275–312	6%	<L OD - 38.8
And-20 min	20	574	33	530–620	6%	<L OD - 61.3

Avg: average

<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