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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 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 (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 30 × 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× 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