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Getting ahead of antibiotic-resistant *Staphylococcus aureus* in U.S. hogs

Sarah Rhodes [a,1], Elizabeth Christenson [a,*,1], Allie Nguyen [a], Jesper Larsen [b], Lance B. Price [c], Jill Stewart [a]

[a] Gillings School of Global Public Health, Department of Environmental Sciences & Engineering, University of North Carolina at Chapel Hill, 27599, USA
[b] Department of Bacteria, Parasites and Fungi, Statens Serum Institut, Copenhagen, Denmark
[c] Milken Institute School of Public Health, Department of Environmental and Occupational Health, George Washington University, Washington DC, 20052, USA

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ABSTRACT

Antibiotic-resistant strains of *Staphylococcus aureus,* an opportunistic bacterial pathogen, have emerged in industrial livestock operations and agricultural settings. In the United States, there is limited access to industrial livestock operations and farm-level antibiotic use data. As a result, studies often rely on retail meat as a proxy for direct animal sampling. To move beyond this limitation and assess *S. aureus* colonization in hogs, we purchased the heads of recently-slaughtered hogs and compared *S. aureus* populations in those raised on industrial hog operations versus those raised without antibiotics. *S. aureus* isolates were analyzed for antibiotic resistance and putative genotypic markers of livestock adaptation. Although methicillin-resistant *S. aureus* (MRSA) was not detected in this study, all of the hogs from industrial hog operations (n = 9/9) carried multidrug-resistant *S. aureus* (MDRSA) with two livestock-adaptation markers (*scn*-negative and clonal complex (CC) 9 or 398) compared to 11% of hogs raised without antibiotics (n = 1/9). Hogs from industrial operations were 9.0 times (95% confidence interval (CI): 1.4–57.1) as likely to carry livestock-adapted *S. aureus* and 4.5 times (95% CI: 1.3–15.3) as likely to carry MDRSA as hogs raised without antibiotics. In contrast, the majority of antibiotic-free hogs (67%, n = 6/9) contained human-adapted *S. aureus* (i.e. *scn*-positive, CC1) compared to 11% (n = 1/9) of IHO hogs. These results indicate that antibiotic use in IHO hogs may make them more conducive hosts to antibiotic-resistant, livestock-adapted *S. aureus* strains when compared to hogs raised without antibiotics. Our results are important, as they provide strong evidence that antibiotic use practices influence the *S. aureus* populations carried by U.S. hogs, supporting the need for increased access to routine monitoring of hog operations for antibiotic resistance management using a One Health framework.

1. Introduction

Antibiotic resistance is a major threat to public health. The use and overuse of antibiotics in clinical, veterinary, and agricultural sectors have selected for antibiotic-resistant (ABR) microorganisms, some of which are known to cause human infections that are difficult and costly to treat (World Health Organization, 2015). The emergence of ABR pathogens threatens future effectiveness of routine medical care, including surgeries and childbirth (Interagency Coordination Group on Antimicrobial Resistance (IACG), 2019; O'Neil et al., 2016). To address this critical issue, health institutions worldwide are working to adopt a cross-disciplinary approach called 'One Health,' which includes calls for routine surveillance of major reservoirs of antibiotic resistance, including people, animals, and the environment (Interagency Coordination Group on Antimicrobial Resistance (IACG), 2019; National Academies of Sciences, 2017; O'Neil et al., 2016; Obama Administration, 2015).

The livestock industry is a leading consumer of antibiotics (Van Boeckel et al., 2015). Today, livestock are predominantly raised at high density in enclosed barns or feedlots. These industrial livestock operations administer antibiotics to animals for treatment and prevention of disease, and in some countries, for growth promotion (Marshall and Levy, 2011). As a result, ABR strains of *Staphylococcus aureus,* an opportunistic bacterial pathogen, have emerged in industrial livestock operations and agricultural settings (Bosch and Schouls, 2015; Price et al., 2012; Smith et al., 2018). Other key sources of antibiotic-resistant *S. aureus* include healthcare (e.g. hospitals) and community settings (e.g. gymnasiums) (Lakhundi and Zhang, 2018).

* Corresponding author. 1301 Michael Hooker Research Center, CB #7431, Chapel Hill, NC, 27599, USA.
E-mail address: eliz@alumni.unc.edu (E. Christenson).

[1] Elizabeth Christenson and Sarah Rhodes contributed equally to this work.

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To assess *S. aureus* emergence and transmission in animal production systems, European institutions have conducted comprehensive One Health studies (Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP), 2016; Grontvedt et al., 2016; Pletinckx et al., 2013; Sieber et al., 2018). These studies have honed the use of microbial markers to track *S. aureus* of livestock origin (i.e. livestock-adapted *S. aureus*) (Armand-Lefevre et al., 2005; Hasman et al., 2010; Hau et al., 2017; Price et al., 2012; Stegger et al., 2013) and have elucidated connections between *S. aureus* in hogs and *S. aureus* causing infections in humans with and without livestock contact (Larsen et al., 2017a; Sieber et al., 2018). They have also informed best practices for antibiotic use. For example, in 2006, the European Union banned the use of antibiotics for growth promotion, and countries like Denmark and the Netherlands have implemented strict regulations on antibiotic use, including caps on veterinary profit from antibiotic sales (Expert Commission on Addressing the Contribution of Livestock to the Antibiotic Resistance Crisis, 2017; U.S. Government Accountability Office (GAO), 2017). In these same countries, farm-specific antibiotic use data are publicly-available, and have been used to benchmark veterinary prescription rates and develop management strategies. Ultimately, these efforts have fostered more responsible antibiotic use and decreased antibiotic sales (Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP), 2016; U.S. Government Accountability Office (GAO), 2017).

The U.S. is the second-largest consumer of antibiotics for livestock production globally (Expert Commission on Addressing the Contribution of Livestock to the Antibiotic Resistance Crisis, 2017). In 2017, the U.S. banned the veterinary use of antibiotics for growth promotion; however, antibiotics are still sold for disease prevention and treatment, and 60% of these antibiotics belong to classes also used to treat humans (U.S. Food and Drug Administration, 2017). Relative to Europe, there are barriers to effective surveillance of ABR, livestock-adapted pathogens in U.S. livestock. For one, public and governmental access to on-site sampling of industrial livestock operations and their animals is largely restricted and farm-specific antibiotic use data are not publicly-available (U.S. Government Accountability Office (GAO), 2017). Governmental agencies must obtain producer consent to conduct on-farm investigations, even during foodborne disease outbreaks (U.S. Government Accountability Office (GAO), 2017). Consequently, far fewer U.S.-based One Health studies have been conducted (Davis et al., 2018; Mollenkopf et al., 2017; Smith et al., 2013, 2018; Waters et al., 2011) and knowledge is therefore limited on the *S. aureus* strain types circulating among livestock, workers, and the environment (Hatcher et al., 2016; Rinsky et al., 2013; Smith et al., 2018).

In this study, we developed an innovative monitoring approach to address our limited understanding of antibiotic resistance in U.S. livestock. We purchased heads of recently-slaughtered hogs raised on industrial hog operations (IHO) and antibiotic-free hog farms (AFHF) from a slaughterhouse facility in North Carolina, a top hog-producing state (U.S. Department of Agriculture, 2017), and sampled them for ABR, livestock-adapted *S. aureus*. This unique approach expands upon early characterizations of *S. aureus* circulating in hogs from IHOs and those raised without antibiotics in the U.S.

2. Material and methods

2.1. Sample description

S. aureus were isolated from the heads of recently-slaughtered hogs. Hog heads were purchased from a slaughterhouse in eastern North Carolina that processes hogs from both IHOs and AFHFs. The hogs classified as IHO could have been given antibiotics at some point in the production cycle, while AFHF hogs were USDA-certified as raised without antibiotics. The breed, age, sex, farm-of-origin, time-of-death, and antibiotic administration record (IHO group only) for the hog head samples were unknown. Before IHO and AFHF hog heads were sold, they underwent a cleaning process that involved submerging hog heads in boiling water, followed by a chilling process, to kill pathogens and to remove dirt and hair; however previous research has suggested that bacteria can survive this process (Dhup et al., 2015; Hadjirin et al., 2015; Waters et al., 2011). Nine IHO and nine AFHF heads were collected across three sampling dates between May 2015 and September 2015.

2.2. Detection of *S. aureus* and methicillin-resistant *S. aureus* (MRSA)

Once purchased, the hog heads were immediately transported on ice to the laboratory, where they were processed upon arrival, typically within 6–8 h of purchase. For each hog head, one snout swab and one mouth swab were collected. For hog snouts, a sterile double-tipped BD BBL™ CultureSwab™ was inserted into the left nostril and rotated along the inner lining of the nasal cavity (approximately 20 cm in length) from the innermost portion of the cavity until the nostril opening was reached. This process was repeated in the right nostril. Hog snout samples often contained blood and mucus. For hog mouths, the hog jaws were clenched tightly shut post-slaughter. To open the jaw for sample collection, stainless steel cell spreaders sterilized with 70% ethanol and flame were hooked under the front teeth of the upper and lower jaw to pry the mouth open. To ensure no contamination was present on the spreaders, a sterile PBS blank was prepared for each sampling run. Upon opening the hog mouth, a sterile double-tipped BD BBL™ CultureSwab™ was inserted. The top and bottom of the tongue, the upper and lower gums, and teeth were swabbed until swab saturation was reached. Hog mouth samples also contained blood, mucus and organic matter.

Each snout and mouth swab was aseptically clipped into 10 ml Mueller-Hinton Broth (MHB) supplemented with 6.5% NaCl and incubated overnight at 37 °C. After 24 h, a 10-μl loopful of inoculated MHB was streaked to isolation on CHROMagar™ Staph aureus (CA) media (BD, Franklin Lakes, NJ) and incubated at 37 °C for 24 h. To increase the number of *S. aureus* isolates recovered and to compare between media types, 10 μL of inoculated MHB was also streaked onto Baird-Parker with Egg Yolk Tellurite Enrichment (BP) and incubated at 37 °C for 48 h (Nadimpalli et al., 2013).

To detect methicillin-resistant *S. aureus* (MRSA), 1 mL of the inoculated MHB was transferred to 9 mL of MRSA-selective broth composed of Tryptic Soy Broth (TSB) containing 2.5% NaCl, 75 mg/L aztreonam, and 3.5 mg/L cefoxitin. The MRSA-selective broth was incubated overnight at 37 °C (Böcher et al., 2010; Larsen et al., 2017b). After 24 h, a 10-μL loopful of the inoculated MRSA-selective broth was streaked to isolation on CA and BP media and incubated at 37 °C for 24 and 48 h, respectively.

Up to five presumptive *S. aureus* colonies were selected from the CA (pink to mauve colonies) and BP (black to grey colonies with clear halo) plates streaked with inoculated MHB and also from CA and BP plates streaked with inoculated MRSA-selective TSB. Isolated colonies were streaked onto Trypticase Soy Agar (TSA) with 5% sheep blood (Remel Laboratories, Lenexa, KS) and incubated at 37 °C for 24 h.

To confirm *S. aureus* biochemical properties, presumptive *S. aureus* colonies were tested for catalase and coagulase positivity by aseptically transferring each colony to hydrogen peroxide or the Rabbit Plasma test (BD BBL™, Franklin Lakes, NJ), respectively. Presumptive *S. aureus* colonies from each hog snout and mouth were archived at −80 °C in 1 ml of Brain Heart Infusion Broth containing 15% glycerol for future molecular characterization.

2.3. Molecular characterization of *S. aureus* isolates

A crude DNA extraction was conducted on archived presumptive *S. aureus* isolates (Reischl et al., 2000). Multiplex polymerase chain reaction (PCR) was performed to amplify the *spa*, *mecA*, *mecC*, *pvl* and *scn* genes. LGA251 was used as a positive control for *mecC* and a clinical MRSA isolate was used as a positive control for *spa*, *mecA*, *pvl* and *scn* (Stegger et al., 2012). For *spa*-positive *S. aureus* isolates, the *spa* gene was

Table 1
Prevalence of livestock-adapted, multidrug-resistant *S. aureus* at the sample and unique isolate level in industrial hog operation (IHO) and antibiotic-free hog farm (AFHF) hog heads.

S. aureus Nasal Carriage Outcome	Industrial Hog Operation (IHO)				Antibiotic-Free Hog Farm (AFHF)			
	No. pos samples	% pos samples	No. pos unique isolates	% pos unique isolates	No. pos samples	% pos samples	No. pos unique isolates	% pos unique isolates
S. aureus	9/9	100%	30/108[a]	NA	6/9	67%	21/117[a]	NA[c]
scn-negative *S. aureus*	9	100%	29	97%	3	33%	4	19%
S. aureus CC9/CC398[b]	9	100%	28	93%	1	11%	2	10%
Livestock-adapted *S. aureus* (*scn*-negative *S. aureus* CC9/CC398)[b]	9	100%	28	93%	1	11%	2	10%
Human-adapted *S. aureus* (*scn*-positive *S. aureus* CC1)	1	11%	1	3%	6	67%	14	67%
Methicillin-resistant *S. aureus* (MRSA)	0	0%	0	0%	0	0%	0	0%
Multidrug-resistant *S. aureus* (MDRSA)	9	100%	25	83%	2	22%	3	14%
Livestock-adapted MDRSA (*scn*-negative CC9/CC398)	9	100%	25	83%	1	11%	2	10%
Human-adapted MDRSA (*scn*-positive CC1)	0	0%	0	0%	1	11%	1	5%

[a] A total of n = 108 (n = 30 unique, after accounting for clonal expansion) IHO isolates and n = 117 (n = 21 unique, after accounting for clonal expansion) AFHF isolates.
[b] All putative *S. aureus* clonal complex (CC) 9 and CC398 lacked the *scn* gene. No CC5 or CC30 were detected.
[c] NA = Not applicable.

sequenced and the quality of *spa* sequences was determined using Sequencher® version 5.4. *S. aureus* isolates were then characterized by *spa* typing using the Ridom StaphType software and the Ridom Spa-Server (http://spa.ridom.de/index.shtml). Multi-locus sequence typing (MLST) was not conducted but rather *spa* types were assigned to CCs based on *spa*-CC associations, as has been done previously (Hatcher et al., 2016; Nadimpalli et al., 2016). Isolates that lacked *spa* by PCR but that had morphological and biochemical characteristics of *S. aureus* were tested by PCR using an alternative *spa* primer pair and a primer pair that amplifies the *S. aureus*-specific *femA* gene (Institute DNF, 2009; Paule et al., 2004). Isolates that produced an alternative *spa* product or a *femA* product were considered *S. aureus*-positive. A sample was considered positive for *S. aureus* if the mouth, snout, or both sites, contained at least one isolate that was *S. aureus*-positive, even if the majority of isolates from the hog head were negative for *S. aureus*.

2.4. Putative markers of livestock adaptation

All isolates confirmed as *S. aureus* by phenotypic and genotypic characterization were tested for two putative markers of livestock adaptation, including: (1) lack of the *scn* gene and (2) *spa* types associated with clonal complexes CC5, CC9, CC30, or CC398. Lack of the *scn* gene was determined by multiplex PCR and CC was assigned based on *spa* type, as described above. *S. aureus* possessing both of these markers were classified as livestock-adapted *S. aureus*.

2.5. Antimicrobial susceptibility testing

All isolates confirmed to be *S. aureus*-positive were tested using standard Kirby-Bauer disk diffusion methods for resistance to fifteen antibiotics, representing eleven classes, which are approved for use in human medicine, food-producing animal medicine or both (see Supplementary Materials Table S1). Isolates were classified as resistant, susceptible or intermediately resistant (where applicable) according to the 2014 Clinical & Laboratory Standards Institute (CLSI) guidelines (Clinical Laboratory Standards Institute (CLSI), 2014), except for amoxicillin, where the 2012 CLSI guidelines (Clinical Laboratory Standards Institute (CLSI), 2012) were followed (amoxicillin cut-points were not available in the 2014 guidelines.) Isolates were defined as multidrug-resistant *S. aureus* (MDRSA) if they exhibited complete resistance to three or more classes of antibiotics (Magiorakos et al., 2012). Isolates were defined as MRSA if they were both (1) resistant to cefoxitin and (2) positive for either *mecA* or *mecC*. The D-zone test was used to detect induced clindamycin resistance in erythromycin-resistant isolates (Steward et al., 2005). It should be noted that standard methods for classifying resistance to spectinomycin and lincomycin in *S. aureus* isolates have not been developed. As such, spectinomycin resistance and lincomycin resistance were determined as binary outcomes, where resistance was defined as complete resistance and susceptible as any susceptibility (i.e. any diameter of non-growth). Therefore, we do not report any intermediate spectinomycin or lincomycin resistance.

2.6. Statistical analysis

All statistical analyses were conducted in SAS version 9.4 (SAS Institute Inc., Cary, NC). Sample-level prevalence ratios (PRs) and 95% confidence intervals (CIs) were calculated to compare the proportion of IHO hog heads that were positive for (1) *S. aureus*, (2) livestock-adapted *S. aureus*, and (3) MDRSA relative to that in AFHF hogs.

In addition, to account for clonal expansion in the enrichment process, the frequency of unique isolate profiles (based on a unique combination of antibiotic resistance, *scn* status, and *spa* type) was calculated and is illustrated in Table 1 and Fig. 1. Unique isolate-level PRs with 95% CIs were calculated to compare the proportion of unique *S. aureus* isolates that were resistant to individual antibiotics. The corresponding p-values are not presented, as they are often misinterpreted in non-

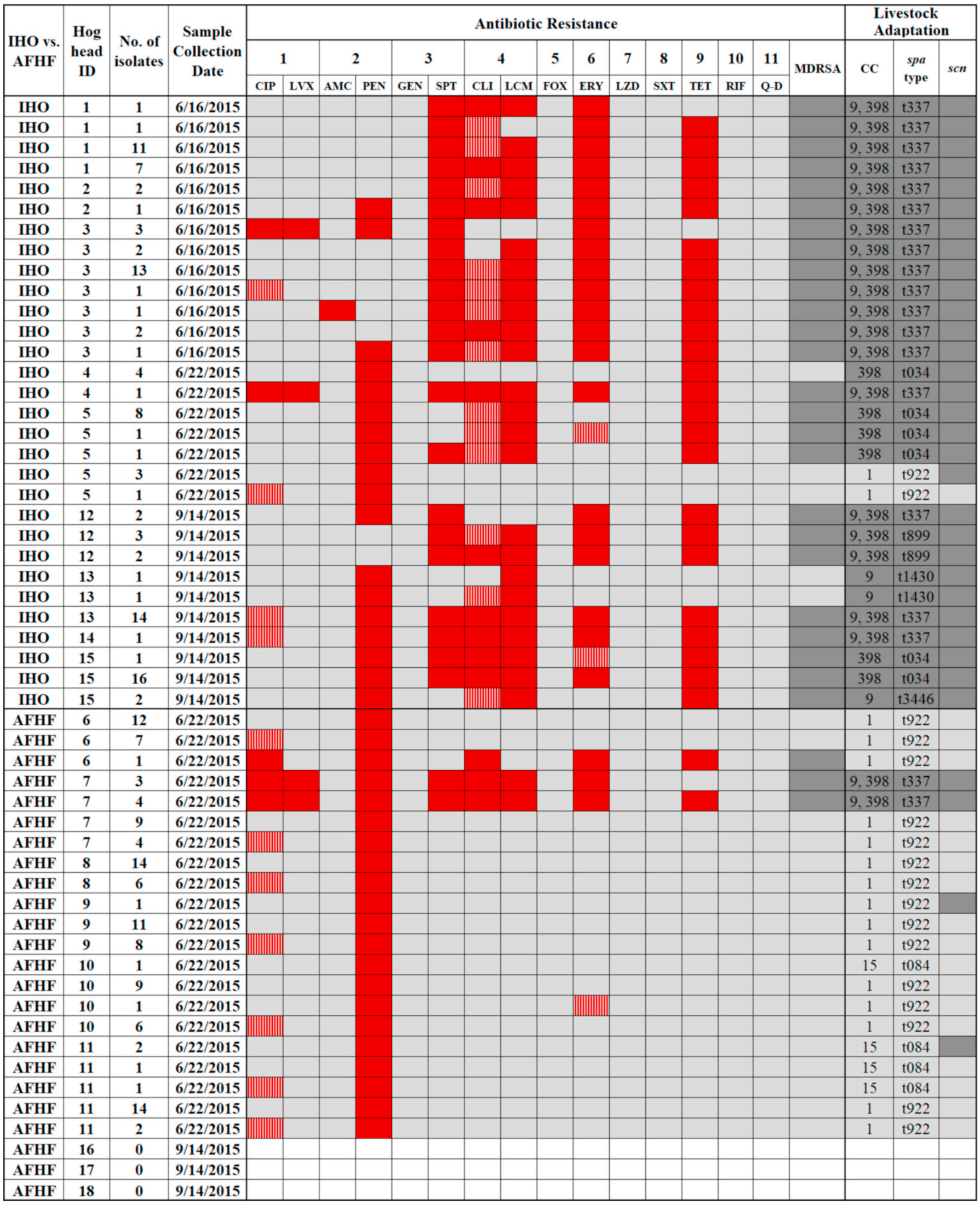

Fig. 1. Frequency of unique *S. aureus* isolate profiles, based on phenotypic antibiotic resistance, multidrug-resistant *S. aureus* (MDRSA), clonal complex (CC), *scn*, and *spa* type, from IHO and AFHF hogs. Note: Red = complete antibiotic resistance. Striped red/grey = intermediate resistance. Light grey = antibiotic susceptibility, lack of a livestock-adapted CC or *spa* type, or *scn*-positivity. Dark grey = positivity for MDRSA, livestock-adapted CC or *spa* type, or *scn*-negativity. Antibiotic class codes are as follows: quinolones class (1), penicillins (2), aminoglycosides (3), lincosamides (4), cephems (5), macrolides (6), oxazolidinones (7), folate pathway inhibitors (8), tetracyclines (9), ancamycins (10), streptogrammins (11). Individual antibiotic abbreviations are as follows: ciprofloxacin (CIP), levofloxacin (LVX), amoxicillin with clavulanic acid (AMC), penicillin (PEN), gentamycin (GEN), spectinomycin (SPT), clindamycin (CLI), lincomycin (LCM), cefoxitin (FOX), erythromycin (ERY), linezolid (LZD), trimethoprim-sulfamethoxazole (SXT), tetracycline (TET), rifampin (RIF), quinopristin/dalfopristin (Q–D). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

randomized studies (Greenland, 1990).

As a sensitivity analysis, clustered logistic regression was conducted to account for any dependence among unique isolates recovered from the same hog head sample. The clustered logistic regression (clustered by hog head) assessed the association between antibiotic use (IHO vs. AFHF) and antibiotic resistance characteristics among unique *S. aureus* isolates. This supplemental analysis was conducted for antibiotics where more than five isolates were resistant, which included penicillin, erythromycin, tetracycline, spectinomycin, lincomycin, and clindamycin. Odds ratios (ORs) and 95% CIs were calculated and are presented in Supplementary Material Table S2.

3. Results

3.1. Prevalence of S. aureus

All IHO hog heads (100%, n = 9/9) were positive for *S. aureus* compared to 67% (n = 6/9) of AFHF hogs (sample-level PR: 1.5; 95% CI:

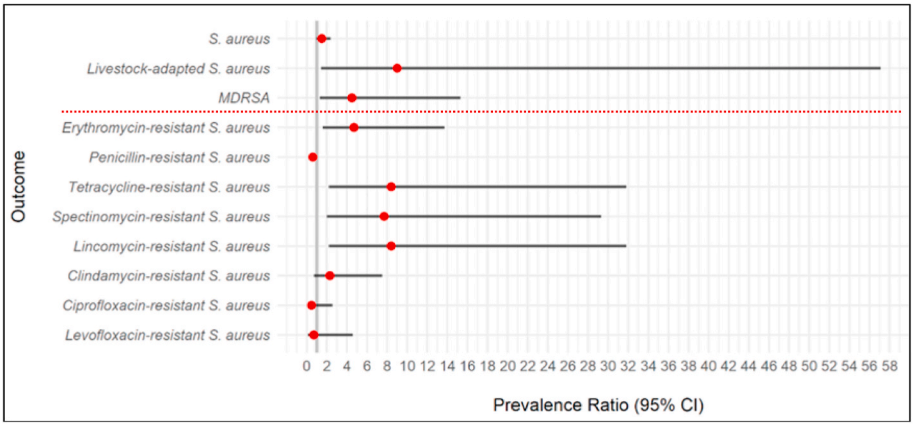

Fig. 2. Sample-level prevalence ratio (PR) and 95% confidence interval (CI) of *S. aureus*, livestock-adapted *S. aureus*, and MDRSA; Unique isolate-level PR and 95% CI of *S. aureus* resistant to specific antibiotics in hogs from industrial hog operations relative to antibiotic-free hog farms. Red dotted line distinguishes sample-level vs. isolate-level prevalence ratios. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

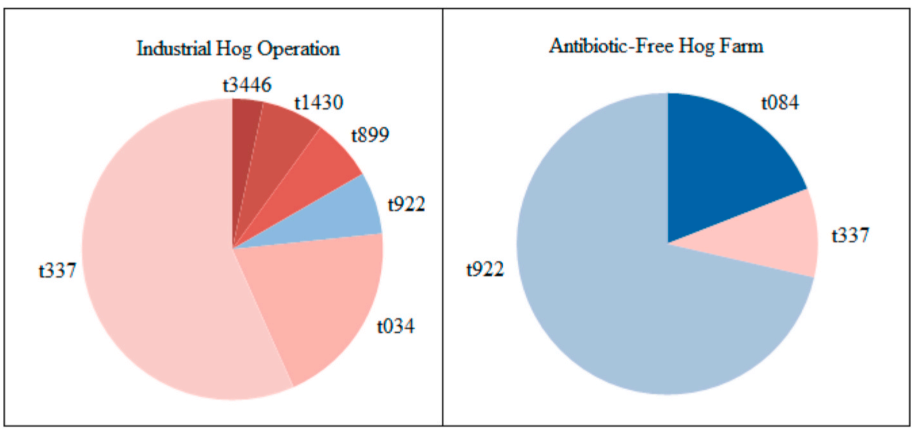

Fig. 3. Distribution of *spa* types for unique isolates from the heads of hogs raised on industrial hog operation (IHO) and antibiotic-free hog farms (AFHF). Shades of red = livestock-adapted *spa* types associated with CC9 only (t1430, t3446), CC398 only (t034), or both CC9 and CC398 (t337, t899). Shades of blue = *spa* types associated with CC1 (t922) or CC15 (t084). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

0.95–2.38). Thirty unique *S. aureus* isolates (of total n = 108) were identified from IHO hog heads and 21 unique isolates (of total n = 117) from AFHF hog heads (Table 1; Fig. 1).

3.2. Livestock-vs. human-adapted S. aureus

S. aureus that were positive for two putative genotypic markers of livestock adaptation (*scn*-negative and *spa* type associated with CC9 or CC398) were classified as livestock-adapted *S. aureus*. All *S. aureus* with *spa* type associated with livestock-adapted CC9 or CC398 were *scn*-negative (Fig. 1). In the IHO group, 100% (n = 9/9) of samples and 93% (n = 28/30) of unique IHO isolates were livestock-adapted, compared to 11% (n = 1/9) of AFHF samples and 10% (n = 2/21) of unique AFHF isolates (Table 1). The sample-level prevalence of livestock-adapted *S. aureus* in IHO hogs was 9 times that in AFHF hogs (PR: 9.0; 95% CI: 1.4–57.1) (Fig. 2). The most common *spa* type among the unique IHO isolates was *spa* type t337, which is associated with livestock-adapted CC9 and CC398 (Davis et al., 2018) (Fig. 3). Of note, *spa* types associated with livestock-adapted CC30 and CC5 were not identified in this study (Fig. 3).

In contrast, all *S. aureus*-positive AFHF hog heads (67%, n = 6/9) and the majority of unique AFHF isolates (67%, n = 14/21) were considered human-adapted (*scn*-positive and of *spa* type t922) compared to 11% (n = 1/9) of IHO samples and 3% (n = 1/30) of unique IHO isolates (Table 1). *Spa* type t922 is associated with human-adapted *S. aureus* CC1 (Rijnders et al., 2009) (Figs. 1 and 3). The sample-level prevalence of human-adapted *S. aureus* in AFHF hogs was 6 times that in AFHF hogs compared to IHO hogs (PR: 6.0; 95% CI: 0.89–40.3). The remaining 22% (n = 2/9) of AFHF hog head samples and 19% (n = 4/21) of unique AFHF isolates were positive for *spa* type t084, which is associated with CC15. CC15 has been identified as a healthcare-associated strain and is less commonly reported in pigs, dogs, and other animals (Cuny et al., 2015; Ho et al., 2012; Kosecka-Strojek et al., 2016). None of the IHO samples were positive for *spa* type t084 (CC15).

3.3. Antibiotic resistance

All IHO samples (100%, n = 9/9) and 83% of unique IHO isolates (n = 25/30) were positive for MDRSA compared to 22% (n = 2/9) of AFHF samples and 14% (n = 3/21) of unique AFHF isolates (Table 1). The sample-level prevalence of MDRSA carriage in IHO hogs was 4.5 times that in AFHF hogs (PR: 4.5; 95% CI: 1.3–15.3). MRSA were not detected in this study.

The proportion of unique *S. aureus* isolates in IHO hogs exhibiting resistance to fifteen antibiotics was compared to that in AFHF hogs (Fig. 2; Fig. 4). All IHO and AFHF isolates were susceptible to the following antibiotics: gentamycin, trimethoprim-sulfamethoxazole, linezolid, rifampin, and quinopristin/dalfopristin. For the remaining antibiotics of interest, unique isolate-level PRs and 95% CIs were calculated and are presented in Fig. 2. Compared with unique AFHF isolates, unique IHO isolates were more commonly resistant to tetracycline (PR: 8.4; 95% CI: 2.2–31.8), lincomycin (PR: 8.4; 95% CI: 2.2–31.8), spectinomycin (PR: 7.7; 95% CI: 2.0–29.3), and erythromycin (PR: 4.7; 95% CI: 1.6–13.7). Sixty percent of unique IHO isolates were

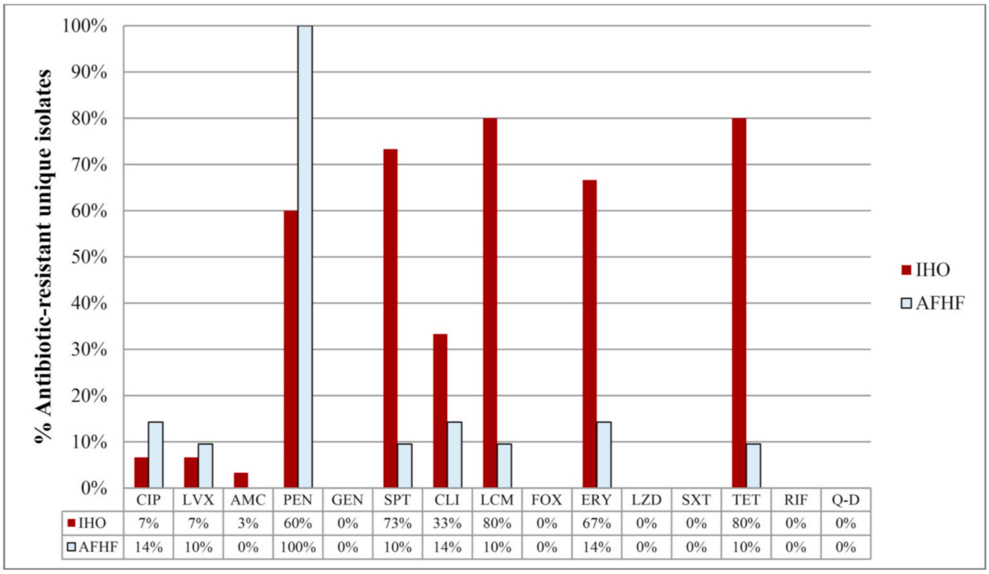

Fig. 4. Prevalence of complete antibiotic resistance amongst unique *S. aureus* isolates from hogs raised on industrial hog operations (IHOs) and antibiotic-free hog farms (AFHFs). Intermediate resistance was classified as susceptible in prevalence calculations. Antibiotic abbreviations are as follows: ciprofloxacin (CIP), levofloxacin (LVX), amoxicillin with clavulanic acid (AMC), penicillin (PEN), gentamycin (GEN), spectinomycin (SPT), clindamycin (CLI), lincomycin (LCM), cefoxitin (FOX), erythromycin (ERY), linezolid (LZD), trimethoprim-sulfamethoxazole (SXT), tetracycline (TET), rifampin (RIF), quinopristin/dalfopristin (Q–D).

resistant to penicillin, whereas all unique AFHF isolates were penicillin-resistant (PR: 0.6; 95% CI: 0.45–0.80). For amoxicillin, the prevalence of resistance was similar between the two groups, with only one unique IHO isolate exhibiting resistance (Fig. 2; Fig. 4). Resistance to ciprofloxacin and levofloxacin was uncommon in both groups and the prevalence of clindamycin resistance was similar among unique isolates from IHO and AFHF hogs (PR: 2.3; 95% CI: 0.70–7.5).

The results of the sensitivity analysis (clustered logistic regression) aligned with those represented by PRs. Odds ratios and 95% CIs are presented in supplementary material (Supplementary Materials Table S2).

4. Discussion

Our team developed a novel hog head sampling method to provide one of the first comparisons of ABR, livestock-adapted *S. aureus* in hogs raised on both IHOs and AFHFs in North Carolina. All of the hog head samples were collected from the same slaughterhouse facility. Although the slaughterhouse used sterilization processes designed to kill bacteria, we successfully cultured *S. aureus* in the hogs, which supports previous studies suggesting that *S. aureus* can be carried through the slaughter process to retail meat products (Dhup et al., 2015; Hadjirin et al., 2015; Waters et al., 2011). We found that IHO hogs more commonly carried multidrug-resistant, livestock-adapted *S. aureus* (*scn*-negative and a *spa* type associated with CC9 or CC398), whereas AFHF hogs predominantly carried a previously described human-adapted, *scn*-positive *S. aureus* CC1 strain (Rijnders et al., 2009). Results suggest that antibiotic use by IHOs contributes to increased prevalence of ABR, livestock-adapted *S. aureus* in IHO hogs, and also that AFHF hogs may be more susceptible to colonization by human-adapted strains. Increased surveillance is needed to track trends in resistance patterns and acquisition of virulence traits among *S. aureus* and other pathogens over time in hogs, people, and the environment.

No MRSA were detected in this study. However, a recent study found that the MRSA-selective enrichment broth used here may lead to false negative results (Larsen et al., 2017b). Despite this potential limitation, our results agree with the few studies that have characterized ABR *S. aureus* in industrial (Frana et al., 2013; Osadebe et al., 2013; Smith et al., 2009, 2013; Sun et al., 2015) and antibiotic-free (Smith et al., 2013) hogs in the U.S. To date, the majority of existing studies have observed either a low (<20%) or 0% prevalence of MRSA in U.S. hog herds, and have not detected MRSA in the few NC hogs sampled. A significant limitation of all current studies is that the prevalence of multi-drug resistance among methicillin-susceptible *S. aureus* was not reported (Smith et al., 2018). Although MRSA prevalence is low in IHOs in the United States, MRSA CC398 transmission from livestock to humans is well-documented in Europe (Hartmeyer et al., 2010; Smith and Pearson, 2011; Voss et al., 2005).

IHO hogs sampled in this study more commonly carried livestock-adapted MDRSA CC9/CC398 than AFHF hogs. Higher prevalence of MDRSA in IHO hogs is likely a result of selection pressure from antibiotic use. These results are in agreement with the current literature. The predominant strain types observed in previous U.S. hog sampling studies were MRSA CC9, CC398, and CC5 (Frana et al., 2013; Osadebe et al., 2013; Smith et al., 2013; Sun et al., 2015). A recent study conducted by Davis et al. (2017) was the first study in NC to assess the prevalence of livestock-adapted MDRSA in hogs raised with and without antibiotics. Davis et al. predominantly recovered *scn*-negative, methicillin-susceptible, MDRSA t337 (associated with CC9/CC398 (Hasman et al., 2010; Larsen et al., 2012; Sun, 2016)) from hogs, the air, and worker surrogates on one NC IHO, and did not recover *S. aureus* from the three NC AFHF herds sampled (Davis et al., 2017). Similarly, in our study, *scn*-negative MDRSA t337 were predominant in the IHO hogs sampled, however, unlike in Davis et al. (2017) and in Smith et al. (2013), *S. aureus* were recovered in AFHF hog heads.

AFHF hog heads were predominantly colonized by human-adapted *scn*-positive, methicillin-susceptible *S. aureus* t922 (CC1), a well-defined healthcare-associated strain (Rijnders et al., 2009). Isolates from AFHF hogs were primarily resistant to penicillin alone, and penicillin resistance is largely ubiquitous in *S. aureus* isolated from humans. The majority of unique t922 isolates in AFHF hogs were *scn*-positive (13/14), whereas half of the unique IHO isolates of *spa* type t922 were *scn*-negative (1/2) (Fig. 1). These results indicate that the lack of antibiotic use in AFHF hogs may make them more conducive hosts to antibiotic-sensitive, human-adapted strains compared to IHO hogs with antibiotic selection pressure. Carriage of MSSA CC1 by AFHF hogs may be the result of human contamination either at the slaughterhouse or prior to arrival at the slaughterhouse (i.e. at the AFHF or in transit) or that these strains are more prevalent among AFHF compared to IHO hogs. To our knowledge, *S. aureus* have not been characterized in U.S. AFHF hogs and these data provide insight into the strains these animals may carry.

This hog head sampling methodology has a few key limitations. Most notably, our sample size was restricted by the cost of hog head incineration prior to disposal, which resulted in imprecise effect estimates. Further, the generalizability of our results are limited due to our reliance on convenience sampling at one slaughterhouse. Additionally, although all AFHF samples were USDA-certified as "raised without antibiotics," the hog operation(s) of origin are unknown and we could not obtain information about the antibiotics administered to IHO hogs. Thus, we could not connect resistance profiles to farm-specific antibiotic use practices. However, hog heads were collected across three sampling dates, increasing the likelihood that they came from different operations (Fig. 1). Finally, although were able to isolate MDRSA, we did not assess virulence and risk to public health from meat consumption. Although foodborne illness from MDRSA is understudied, it is not seen as a high risk to public health (Waters et al., 2011; Wendlandt, 2013).

Despite these limitations, *S. aureus* were detected in both IHO and AFHF hogs, and significant differences in the prevalence of antibiotic resistance and of livestock adaptation among *S. aureus* from each group were observed. In addition, given slaughterhouse sterilization practices, it is probable that the *S. aureus* prevalence reported here is an underestimate. Because IHO and AFHF hog head samples were collected from the same slaughterhouse facility, any differences in the *S. aureus* populations observed between the two *S. aureus* populations are not likely attributable to contamination from the slaughterhouse environment, i.e. transfer of *S. aureus* from the slaughter line or slaughterhouse workers to the hogs. Rather, large differences in the prevalence of antibiotic resistant, livestock-adapted *S. aureus* between the two groups are likely attributable to on-farm exposures. On the other hand, similarities between the two groups may be attributable to contamination that occurred in the slaughterhouse. For example, cross contamination may have occurred on June 22, 2015. One IHO hog sample (04) and one AFHF sample (07) collected on that day carried multi-drug resistant, livestock-adapted (t337) *S. aureus* that were resistant to the same eight antibiotics (Fig. 1). Additionally, all AFHF hogs heads positive for MSSA CC1 (samples 06–11) were collected on June 22, as was the only IHO sample carrying MSSA CC1 (sample 05) (Fig. 1). These occurrences may be indicative of human contamination in the slaughterhouse or cross-contamination between IHO and AFHF hogs in the slaughterhouse environment. Most importantly, despite the potential for contamination in the slaughterhouse, notable differences in antibiotic resistance and livestock adaptation were observed among the *S. aureus* detected in IHO and AFHF hog head samples in this study.

To assess the extent of public health risks of livestock-adapted *S. aureus* in the U.S., we must characterize the strains circulating in domestic hogs, determine how antimicrobial susceptibility relates to on-farm antimicrobial use, and then quantify how often livestock-adapted strains transmit to humans and cause disease. Restricted access to U.S. hog operations has limited the ability of researchers to sample hogs directly, leading many to use meat, environmental, and hog operation worker samples as proxies for what may be circulating among the animals. In the U.S, there is limited characterization of *S. aureus* in the hog population and existing studies on animal-to-human transmission have primarily targeted detection of MRSA CC398 in humans, which is common in Europe (Feingold et al., 2019). Thus, by design, these U.S. studies may have missed important livestock-adapted strains circulating in humans. Previous hog worker studies have provided indirect but convincing evidence that methicillin-susceptible, livestock-adapted MDRSA can disseminate from hog operations and result in nasal carriage and/or infection among workers, their household members, and community members without direct livestock exposure (Hatcher et al., 2016; Nadimpalli et al., 2016, 2018; Rinsky et al., 2013; Wardyn et al., 2015). In this study, we have developed a novel collection method that generates *S. aureus* isolates that likely reflect the strains circulating among animals within specific hog operations. This method may be employed to further define the public health risks of hog production in the U.S. Concurrent One Health sampling of hog operations, workers, neighboring communities, and the environment would improve our ability to characterize *S. aureus* transmission dynamics for the protection of public health.

5. Conclusions

Overall, this case study provides one of the first comparisons of antibiotic resistant *S. aureus* from IHO and AFHF hogs in the United States and highlights major differences in multidrug-resistance and livestock adaptation among the *S. aureus* detected in hogs raised on industrial hog operations compared to antibiotic-free hog operations in North Carolina. Our results indicate that antibiotic use in IHO hogs may make them more conducive hosts to antibiotic-resistant, livestock-adapted *S. aureus* strains when compared to hogs raised without antibiotics. Given these striking results, this study highlights the essential need for increased access to sampling of IHO, AFHF, and slaughterhouse environments, animals, and food production workers, not only in the event of foodborne outbreaks, but also in routine monitoring programs to track the emergence and spread of multidrug-resistant bacteria in livestock production using a One Health framework.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. - Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.110954.

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Author contribution

Sarah Rhodes and Elizabeth Christenson contributed equally to this work. Sarah Rhodes: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data Curation, Writing – Original Draft, Visualization. Elizabeth Christenson: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing – Original Draft, Visualization. Allie Nguyen: Investigation. Jesper Larsen: Methodology, Software, Investigation, Resources, Writing – Review & Editing. Lance Price: Conceptualization, Resources, Writing – Review & Editing, Supervision. Jill Stewart: Conceptualization, Resources, Supervision, Writing –

Review & Editing, Funding acquisition, Project administration.

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