THE IMPACT OF HOG PRODUCTION AND *STAPHYLOCOCCUS AUREUS* NASAL CARRIAGE ON THE MICROBIOME OF PIGS, PIGS WORKERS AND COMMUNITY RESIDENTS, NORTH CAROLINA, USA

By

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Abstract

Background: Industrial hog operation (IHO) densities have increased over the years in the United States with predominance in rural communities in Iowa and North Carolina. IHOs may serve as a reservoir for diverse microorganisms and create unique opportunities for microbial selection and adaptation in animal and human hosts. A critical concern lies in the sub-therapeutic, rather than therapeutic, use of antibiotics to enhance the growth of livestock, which can contribute substantially to selection of antimicrobial resistance (AMR) for medically important antibiotics. Emerging livestock-associated (LA-) strains of *S. aureus* (including methicillin-resistant *S. aureus* [MRSA]) have been isolated from livestock, including pigs, and are prevalent on IHOs, among IHO workers, their household contacts, and in communities with high densities of IHOs. It is unclear the degree to which AMR *S. aureus* strains in general—and AMR LA- *S. aureus* from IHOs in particular—impact other members of the bacterial community (microbiome) and whether occupational exposure to IHOs can influence and contribute LA-microbiota to human hosts.

Hypothesis: The overarching hypothesis of this dissertation is that pigs will demonstrate microbiome composition and diversity profiles that differ by mode of production and use of antimicrobials (IHO vs. antibiotic-free hog operations [AFHO]) and that there will be a transfer of the IHO pig microbiota to IHO workers and their household and community contacts. I further hypothesize that nasal microbiome composition and diversity profiles will differ by intensity of IHO work activities, *S. aureus* nasal carriage outcomes, and LA-microbial exposure markers.

Methods: Nasal swab samples from: 1) IHO pigs, IHO workers and children living in their households; 2) AFHO pigs and AFHO workers; and 3) community resident (CR) adults with no known livestock exposure and children living in their households were sequenced targeting the V4 region of the 16S rRNA gene. QIIME, a bioinformatics tool, was used to generate microbiome measures. We assessed differences in: 1) alpha diversity (Shannon diversity, observed OTUs, phylogenetic distance 2) beta diversity (weighted UniFrac, unweighted UniFrac, Bray Curtis, Binary Jaccard and Morisita-Horn); 3) and relative abundance and presence/absence of genera by participant type and *S. aureus* nasal carriage outcomes (*S. aureus*, multidrug-resistant *S. aureus* [MDRSA], and *scn*-negative *S. aureus* [a marker of livestock association]). Beta diversity differences were visualized spatially using non-metric dimensional scaling (NMDS). We used linear regression models and non-parametric Adonis methods to estimate associations of personal, household, and occupational characteristics and *S. aureus* nasal carriage outcomes with changes in alpha diversity, beta diversity, and IHO pig bacterial contributions. Additionally, bacterial taxa that were significantly different between participant types were identified and $\log_2$ transformed to display differences in relative abundance of taxa present in IHO pigs versus AFHO pigs and IHO workers versus AFHO workers. Lastly, bacterial taxa contributed from the pig were identified for each of the human participant groups' nasal microbiomes.

Results: The first aim showed that the microbiomes of IHO pigs and IHO workers demonstrated lower alpha diversity and a greater relative abundance of pathogenic bacterial taxa than AFHO pigs and AFHO workers. IHO pigs contributed a greater number of bacterial taxa to IHO workers than AFHO pigs contributed to AFHO workers.

Aim two demonstrated that IHO work activities and exposures and *S. aureus* nasal carriage outcome measures (*S. aureus,* MDRSA, and *scn*-negative *S. aureus*) correlated with bacterial community differences among IHO workers. IHO pig bacterial contributions correlated with beta diversity differences of IHO children and CR adults and CR children with no known livestock exposure. Finally, in aim three, we observed consistent positive associations between presence versus absence LA-microbial markers nasal carriage (*scn*-negative *S. aureus*, Pig-2-Bac, and IHO pig bacterial contributions) and beta diversity over time among IHO workers but not among children living in their households. **Conclusions:** Overall the results of this thesis support our hypotheses that microbiome composition and diversity profiles are impacted by mode of hog production and use of antimicrobial drugs. This may occur directly through the transfer of OTUs from the pig microbiota to hog workers via work activities and exposures or indirectly to members of IHO workers households and community residents with no known livestock exposure. This thesis suggests that the nasal microbiome may represent a useful exposure assessment tool to characterize the influence of hog production practices (e.g. antimicrobial use and confinement) on the microbiome of pigs, pig workers and their household contacts, and community residents living proximal to high-density hog production.

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"If I have ever seen further than others, it is by standing upon the shoulders of giants"

-Isaac Newton

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Chapter One: Introduction

Background and Scope

The shift from agrarian to industrial livestock production practices

Traditional agrarian lifestyles were an accepted way of farming for hundreds of years. Livestock were raised in pasture-based environments with the help of family and household members using an open-air growing cycle from birth to slaughter. This began to change in the 1890's. The term "factory farming" was first recorded in the 1890's and pig farming started becoming industrialized soon afterwards.[1] Shifts away from the traditional agrarian husbandry practice began in the 1920's, after discovering that the use of Vitamin A and D supplements allowed livestock to remain confined and indoors 12-months out of the calendar year.[1] The use of confinement and the number of pigs per operation within the corporate vertically-integrated and managed livestock production pushed local, small-scale family farmers out of the business.[1] Small-scale farmers could not compete with the onslaught of increases in the size of livestock operations, the number of head of livestock produced per farm, as well as falling prices as a result of economies of scale.[1] Farmers who remained in the livestock production business generally remained independent until they forced farmers to shift to the vertically-managed system, due to economic pressures. Livestock confinement brought new challenges.

Antimicrobial inputs in livestock production

Overcrowding is a risk factor for the spread of disease within populations – this was demonstrated elegantly during the investigation of The great plague in London,.[2] Confinement of livestock all year long led to the emergence of infectious disease

epidemics, which started on some livestock operations and then spread subsequently to different operations via herd relocation trucks and at relocation operations.[1] Mortality rates quickly rose, creating pressure on industries to identify interventions that could control such outbreaks.

Within this narrative, antimicrobial drugs were a saving grace of the industrial livestock production industry. Antimicrobial drugs were and still are used in livestock production to treat clinical disease (therapeutic), to prevent (prophylactic) and control disease outbreaks, and to promote livestock growth (non-therapeutic).[3] Reliable data do not exist to track the use of these drugs on livestock operations, however, the U.S. Food and Drug Administration (FDA) has been pushing for increased reporting by companies selling antimicrobial drugs to entities planning to administer drugs to food-producing animals.[4] Based on the most recent (2016) FDA report, 80% of medically important antimicrobial drugs in the U.S. are used in food-producing animals.[4] In 2013, the FDA released a voluntary guidance for industry to decrease the use of medically important antimicrobial drugs in food-producing animals;[4] however, the U.S. still lacks a federal mandatory directive that bans non-therapeutic antimicrobial drug use in livestock.[4]

Overall, 10 antimicrobial drug classes were approved for use in food-producing animals and actively marketed as of 2016.[4] These drug classes in decreasing order of use (by weight) include: tetracyclines, ionophores, penicillins, macrolides, sulfas, aminoglycosides, licosamides, cephalosporins, fluroquinolones, and those classes not individually reported because there were fewer than three distinct marketing sponsors (referred to as not individually reported [NIR]).[4] The Guidance for Industry documents, #152 and #213 state that the 10 antimicrobial drug classes included are considered

"medically important" in human medical therapy.[4–6] Usage of these drugs varies by livestock type.[4] Most medically important antimicrobial drugs are used (actively on the market) in swine (37%), followed by cattle (35%), turkeys (9%), and chickens (6%).[4]

Antimicrobial drugs are commonly used in pig operations, and this is a good setting to explore the possible impacts of these drugs on public health and pig health, by examining the microbial environments of workers and pigs. According to the FDA in 2016, hog production has recorded the use of 9 out of 10 of the approved antimicrobial drug classes.[4] The swine production sector's sales of these drugs reflect the following proportion of the total amounts sold in the U.S. (in descending order): lincosamides (83%), macrolides (61%), tetracyclines (43%), aminoglycosides (21%), sulfas (11%), penicillins (2%), and fluroquinolones and NIRs were not individually reported.[4]

Hospital-associated (HA-) antimicrobial resistant (AMR) microorganisms and infection emergence due to use of antimicrobial drugs in hospitals

Antimicrobial drugs are known to exert extensive selective pressures on the microbial communities of the host and therefore play a major role in the emergence of antimicrobial resistance (AMR).[7] The process of natural selection and adaptation to the selective pressure of antimicrobial drugs has been observed in hospitals with increased incidence of hospital-acquired (HA-) infections associated with patterns of antibiotic drug use in patients and hospital cleaning products.[7] Various microorganisms within the hospital setting are monitored to minimize the spread of HA-infections as they are major cause of morbidity and mortality.[8] Mechanisms of infection include ventilator-associated pneumonia (VAP), catheter-related bloodstream infection (CRBSI) and urinary tract

infections (UTI) and skin and soft tissue infection (SSTI).[8] Such microorganisms include *Staphylococcus aureus*, *Enterococcus* species, *Acinetobacter* species, *E. coli* species, and *Clostridium difficile.*[8] Hospitals have promoted antibiotic stewardship to limit the over-prescription of antibiotic drugs as they are aware of the increased probability of AMR in microorganisms and concomitant AMR bacterial infections.[8]

Community-associated (CA-) AMR microorganisms and infections

The use of antimicrobials within the general population (outside of hospitals) occurs for a range of illnesses, includes appropriate (for bacterial infections such as respiratory tract infections [RTIs], UTIs, and SSTIs) and inappropriate uses, which has heightened concerns about selective pressure on microorganisms that acquire AMR to drugs commonly used within non-hospital exposed popualtions.[9] This trend has been observed during cold and flu seasons.[10] Commonly highlighted respiratory pathogens include *Streptococcus pneumoniae*, *Haemophilus influenza*, *Mycoplasma pneumoniae* and certain Gram-negative pathogens like *Moraxella catarrhalis*, *Escherichia coli*, *Pseudomonas aeruginosa* (which is intrinsically drug-resistant), *Acinetobacter baumannii* and *Staphylococcus aureus*.[9] Strains of *S. pneumonia* resistant to macrolides have shown increasing prevalence globally among RTIs, especially in Asia and Europe while resistance to fluoroquinolones is a less significant problem in the treatment of RTIs.[11–13] Tetracycline resistance is so prevalent that this drug class is not a viable option for RTI treatment.[9] Strains of *H. influenzae* have been resistant to macrolides within community-acquired RTIs.[9] *M. catarrhalis* has been found to be resistant to beta-lactamase drugs.[9] *M. pneumoniae* has developed resistance to tetracyclines, macrolides, ketolides and

fluroquinolones with macrolide-resistant strains on the rise.[9,14] MRSA, a serious public health problem, has resulted from the use of broad-spectrum antimicrobial drugs.[9]

HA and CA infections and emergence of newly identified livestock-associated (LA-) strains of AMR bacteria

Pig farming uses both broad and narrow spectrum antimicrobial drugs[4], which raised critical questions about whether resistant strains have been increasing in prevalence on IHOs production operations as a result of their use.[15] By the early 2000s, a novel strain of MRSA, CC398 was discovered within pigs and pigs workers in the Europe Union (EU) with subsequent dissemination of this LA-MRSA CC398 strain into community and hospital settings.[15] The case of LA-MRSA CC398's emergence in the EU, provides an example of how IHOs should be studied as an important potential reservoir for emergence of AMR bacteria of human public health and clinical significance.[15]

Industrialized practices characterized by the act of raising a large number of animals on a small geographic footprint, high inventory and practices that maximize profits may have implications on animal welfare, soil nutrient balance, microbial and chemical water quality as well as food safety.[16]

A prime example of industrial-scale livestock production is the IHO mode for pigs production.[17] Densely packed conditions and the presence of feed, manure, urine and dead animals inside animal confinement buildings can promote microorganism growth and persistence.[17] Thus IHOs may serve as a microbial reservoir and source of human

exposure to diverse zoonotic microorganisms that possess numerous AMR patterns and mechanisms.[18,19]

The nasal cavity serves as reservoir for bacteria

The nasal passage acts as a filter against microorganisms and other particulates to prevent potential pathogenic organisms from entering the body and subsequently causing illness or infection. The nasal cavity may serve as a reservoir that captures transient bacteria from the environment, including from the hog production environment, leading to nasal acquisition and colonization.[20] The collection of these microorganisms makes up the nasal microbiome. Characterization of the nasal microbiome may provide insight into exposures and therefore serve as a tool for exposure assessment. To the best of my knowledge, there exist no prior studies that investigated the influence of pig production on the microbiome of other anatomical sites (e.g., skin, axilla, groin) to address whether the nasal microbiome is the best measure of a given pig production work exposure environment.

Staphylococcus aureus (S. aureus) on livestock operations

S. aureus, a gram-positive bacterium, colonizes the nares, oropharynx and skin of one third of the general human population.[21] *S. aureus* strains may additionally be characterized according to antimicrobial susceptibility phenotype or genotype, *e.g.* methicillin-sensitive *S. aureus* (MSSA), MRSA, multidrug-resistant *S. aureus* (MDRSA – defined as resistance to three or more antimicrobial drug classes). As an opportunistic bacterial species, *S. aureus* typically colonizes (*i.e.* adheres to the host and replicates in

number) the majority of its hosts asymptomatically. However, susceptible populations such as the young, the elderly, and those with weakened immune systems are at increased risk of *S. aureus* infection due to colonization.[22]

Annually in the U.S., MRSA causes more than 94,000 life-threatening infections and approximately 19,000 deaths with the majority (85%) being associated with hospital-associated MRSA (HA-MRSA).[19] However, since the 1980s, MRSA has emerged among healthy non-hospitalized individuals; now termed community-associated (CA-) MRSA. CA-MRSA prevalence has increased and is associated with contact sports teams, living in close quarters (*e.g.* military bases and prisons), children attending childcare, and residents of long-term care facilities.[23,24] Increasing prevalence of CA-MRSA in areas with high densities of human populations has heightened concerns about sources of exposure. These endemic CA-*S. aureus* (which tend to be USA300 or CC8 strains) are characterized by enhanced virulence, antibiotic resistance, colonization potential, and transmissibility.[23]

CC398: The emergence of livestock-associated S. aureus

S. aureus strains that have emerged among livestock production workers and in areas with high densities of pigs are termed livestock-associated (LA-) *S. aureus*, including LA-MRSA. LA-*S. aureus* strains, determined via specific genotypes or clonal complexes (CC), are dominated by CC398, CC9, and CC5.[15] LA-MRSA (dominated by CC398) in the US been found in industrial hog operations in Iowa.[25]

CC398 MRSA strains have also been observed in cattle, poultry, dogs, and humans in North and South America, Europe, Asia and Australia.[15] Genetic and

phenotypic markers associated with LA-*S. aureus* (SA) include: CC398 and other clonal complexes (*e.g.*, CC9), tetracycline resistance and the absence of the human immune evasion gene *scn*.[26] The origin and sources of LA-*S. aureus* exposure in U.S. livestock worker and general populations has been difficult to determine due to challenges with access to IHOs[15] to sample animals and the environment. Research by key groups determined CC398 MRSA began in humans as a *methicillin*-sensitive *S. aureus* (MSSA) clone.[27] The human adapted MSSA clone was transmitted to livestock, acquired methicillin-resistance, and lost the *scn* gene (human immune evasion gene) as well as phages that encode human innate immune modulators.[27] Furthermore, CC398 MRSA has consistently been found to possess resistance genes to tetracycline, which is commonly used antimicrobial drugs in livestock production globally and in the U.S.[27] CC398 *S. aureus* tends to be most prevalent among humans in areas with high pig densities and livestock production.[27]

Initially, LA-MRSA nasal carriage was thought to be transient.[28] Data demonstrated an increase in MRSA nasal carriage after livestock exposure followed by a clearing of MRSA nasal carriage within a 24-48 hour period after livestock work exposure activities ceased.[28] A longitudinal study by Wardyn et al. (2015) found individuals with livestock contact had higher prevalences of *S. aureus*, MRSA, tetracycline resistant *S. aureus* (TRSA), MDRSA and LA-SA compared to those without livestock exposures. The absence of pig exposure reduced *S. aureus,* TRSA, MDRSA and LA-SA colonization; however, this change was less pronounced in IHO workers with pig exposure only.[26]

Geographic regions with large numbers of IHOs have spatial clustering of TRSA and MDRSA.[26] In one study, IHO workers' self-reported face mask usage was a protective factor, with a 37% decrease LA-MRSA prevalence as well as MSSA carriage as an independent protective effect.[29] Disease burden was not assessed within this study. This suggests occupational interventions like face mask usage, for workers exposed to pigs and LA-SA at IHOs, could be a viable option to reduce the burden of microbial exposure and infectious disease burden in pig workers.

Utility of swine-specific microbial source tracking markers

Strains of *S. aureus* that have some degree of consensus as being associated with or serving as a marker of a livestock source include CC398 *S. aureus,* CC9 *S. aureus*, *scn*-negative *S. aureus*, tetracycline resistance, and MDRSA.[15,30–33] Other bacteria, including *Bacteroidales* species, have shown utility as fecal microbial source tracking markers. One swine-specific fecal *Bacteroidales*, Pig-2-Bac, has been used to track swine-specific fecal contamination within surface water.[34,35] Research has also identified nasal carriage of Pig-2-Bac as a biomarker of exposure to pigs and pig waste[36] and that Pig-2-Bac was positively associated with LA-*S. aureus* and MDRSA nasal carriage among IHO workers.[36]

QIIME's SourceTracker measure might be a novel useful source tracking biomarker tool.[37] SourceTracker identifies the bacterial taxa that are probabilistically derived from a source population and found in a sink population.[37] Its application to our study samples involves classifying pigs as the source population (the IHO pig and AFHO pig served as the source populations, respectively) and hog workers as the sink

population. Additionally, one can investigate IHO pigs as the source population to community resident (CR) populations as a sink. In this thesis, both markers, Pig-2-Bac and percent bacterial contributions from the pig, might serve as potentially useful biomarkers to assess the frequency, magnitude, and intensity of participants' exposure to bacterial taxa contributions directly from pigs and from pig-specific fecal matter.

IHOs as a source of S. aureus in nearby communities

In the U.S., during the past 15 years, there has been a 70% decrease in the number of hog operations with a steady inventory of pigs within the industry.[38] In the U.S., hog operations are geographically concentrated. The top two hog-producing U.S. states are Iowa and North Carolina. In North Carolina, IHOs are particularly geographically concentrated in the eastern region of the state, which is a coastal flood-plain. IHO are located in the backyards of surrounding North Carolina communities and have emerged as a source of AMR bacteria (i.e. MRSA) and zoonotic bacterial exposures.[15] Sources of human exposure to these bacteria can include secondary environmental exposure from hog operations[19,39,40] (e.g., dispersion of airborne particulate matter downwind of hog operation confinement building exhaust fans, airborne drift and surface water runoff of land applied hog lagoon waste as fertilizer) and occupational activities at hog operations, with the potential for secondary person-to-person spread to the surrounding household and community members.[15]

Significance of studying microbial communities rather than individual bacterial species

My dissertation research aims to characterize the nasal microbiome of pigs, pig workers and community residents. IHO workers have unique potential for exposure to AMR and LA microorganisms. Occupational health and safety research is needed to identify factors that could reduce IHO workers' burdens of nasal and dermal exposure to and risk of infection with AMR bacteria. Advancement in research of how the pig nasal microbiome and AMR and LA-*S. aureus* might impact the human nasal microbiome may lead elucidate novel exposure assessment tools and intervention strategies for the prevention of AMR LA-*S. aureus* nasal colonization and infection.

Innovation

IHOs have a well-known ability to serve as a reservoir for AMR microorganisms, however, the interplay of livestock-derived microbial communities with the human nasal microbiome is unclear. The majority of studies have focused on the microbiome composition and diversity profiles of workers who raise livestock other than pigs and few studies have characterized the microbiome of livestock animals, such as pigs.[41–43] No studies to my knowledge have examined whether differences in antimicrobial drug use (i.e., use vs. not) and confinement vs. pasture-based production of hogs, occupational exposure activities, and carriage of a specific LA-AMR pathogen (*i.e. S. aureus*) might influence the pig's and pig production worker's microbiome.

Investigation of the nasal microbiome of pigs, pigs workers and community residents in North Carolina could guide understanding of microbial exposures, including

exposure levels and transmission pathways between pigs, pig workers, household contacts, and community residents. Advances in this area research might inform recommendations about the use of personal protective equipment (PPE) and regulations to limit antimicrobial drug use on farms.

Hog production and human microbiome research

Two recent studies investigated the influence of IHO practices, including confinement and the use of antimicrobial drugs in the animal feed and water on the pig microbiome. Espinosa-Gongora et al. (2016) investigated the influence of *S. aureus* nasal carriage on the nasal microbiome of pig. Twenty OTUs, significantly associated with non-carriers of *S. aureus,* had known probiotic benefits and antimicrobial effects such as acid-producing and butyrate producing isolates (*Leuconostoc* spp. and some members of the *Lachnospiraceae* family). Five OTUs, significantly associated with *S. aureus* nasal carriage, were known pathogenic bacteria such as *Pasteurella multocida* and *Klebsiella* spp. This study showed *S. aureus* nasal carriage may have the capability to limit the number of OTUs observed in the nasal microbiome within pigs.

Weese et al. 2014 characterized the impact of MRSA nasal carriage on the nasal microbiome of slaughter-age pigs in China.[44] Significant increases in *Bacteroidetes* in feces microbial communities were observed among pigs exclusively liquid-fed and tylosin(antibiotic)-exposed; MRSA nasal carriers had significant increases in *Bacteroidetes*.[45] Pigs that were liquid-fed and tylosin-treated had significantly lower relative abundances of *Verrucomicrobia*.[45]

Two studies have investigated the effects of occupational exposures on the nasal microbiome of hog operation workers. Kates et al. 2017 investigated the nasal and oropharyngeal microbiome of healthy livestock workers and individuals with no known livestock exposure and individuals with no known livestock workers and found higher bacterial diversity in the nasal microbiomes of livestock workers.[46] There were no differences in nares ($p = 0.762$) and oropharynx ($p = 0.941$) alpha diversity across all animal types (cattle, poultry, swine, or more than one animal type). However, differences were observed in the bacterial community structure of the nares by animal type ($p= 0.009$); no differences were observed in the community structure of the oropharynx ($p= 0.297$).[46] There were 20 significantly differentially observed OTUs among livestock workers with swine exposure compared to all other animal types.[46] Livestock workers with swine exposure were likely to carry several pathogenic organisms in the oropharynx – these included *Dietzia*, *Prevotella*, *Streptococcus*, *Moraxella*, *Rothia* and *Oscillibacter*.[46]

Kraemer et al (2018) investigated the influence of pig farming on the human nasal microbiota in Switzerland.[47] Pig farming was strongly associated with increased alpha diversity (Shannon diversity and species richness) and differences in nasal microbiome community composition (with lower beta-diversity dispersion) (all $p < 0.001$), compared to non-exposed individuals.[47] This study concluded that pig farming had a strong influence on the nasal microbiome of pig farmers and leads to a more homogeneous microbial community structure.[47]

Research surrounding the pig's and pig worker's nasal microbiome is limited in its assessment of the influence of differences in mode of production as well as the influence of potentially antagonistic properties of *S. aureus* on the nasal microbiome.

Problem statement, hypotheses and specific aims

IHOs may serve as a reservoir for diverse microorganisms. A major concern lies in the sub-therapeutic, rather than therapeutic, use of antimicrobial drugs to enhance the growth of the pigs.

S. aureus, colonizes the nares, oropharynx, and skin of one third of the general U.S. population.[21] MRSA epidemiology has shifted from hospital-associated to community-associated strains, and human *S. aureus* nasal colonization has been found to increase *S. aureus* skin and soft tissue infection (SSTI) risk thus contributing to the economic and human burden of infectious disease.[26] Addition, LA-SA (including MRSA) have been isolated from livestock, including pigs, and are prevalent on IHOs and in communities with close proximity to IHOs.[15,27,48–51] What is not known is the degree to which *S. aureus* strains in general—and LA-MRSA strains in particular—interact with other members of the bacterial community (microbiome), and whether occupational exposure to livestock influences this relationship through contributions of animal-associated microbiota to human hosts.

In order to test the following hypotheses (underlined), I completed the following three aims:

Hypothesis 1: Pigs will harbor microbiome composition and diversity profiles that differ by mode of production and use of antimicrobials (IHO vs. AFHO). I further hypothesize that there will be direct transfer of key bacterial taxa from pigs to pig workers. I further hypothesize that the nasal microbiome composition and diversity profiles of pigs will differ by the intensity of the worker's hog production work activities, *S. aureus* nasal carriage outcomes, and livestock-associated microbial exposure markers.

To investigate this hypothesis I will, cross-sectionally, characterize and compare the microbiome diversity and composition of:

Aim 1a) Pigs (nasal and perineum) raised in the IHO and AFHO environment.

Aim 1b) Pigs raised in the IHO environment vs. IHO workers.

Aim 1c) Pigs raised in the AFHO environment vs. AFHO workers.

Hypothesis 2) There will be evidence of indirect transfer of key bacterial taxa from the IHO pig to members of the IHO workers' household and community residents with no known livestock exposure.

To investigate this hypothesis I will, cross-sectionally, characterize and compare the microbiome diversity and composition of:

Aim 2a) IHO workers and IHO children and community resident (CR) adults and CR children who have no known livestock exposure but live in top ten pig producing counties of North Carolina.

Aim 2b) Pigs raised in the IHO environment vs. IHO children and CR adults and CR children who have no known livestock exposure but live in top ten pig producing counties of North Carolina.

Hypothesis 3) Nasal microbiome differences will be observed when assessing IHO workers' occupational activities and LA- microbial exposures as a cumulative sum of exposure over the course of a 4-month follow up period.

To investigate this hypothesis I will, longitudinally, characterize and compare the nasal microbiome diversity and composition of:

Aim 3a) IHO workers and children living in their households;

Aim 3b) Determine microbial contributions from the IHO pig to IHO workers and children living in their households.

Within this thesis, I aimed to determine the influence of mode of hog production (IHO vs. AFHO) on the nasal and perineum microbiomes of pigs as well as its influences on the nasal microbiome of IHO workers and AFHO workers (**Aim 1**). We also aimed to determine whether occupational exposures at IHOs directly and indirectly, through cohabitation with pig workers (IHO child) were associated with differences in bacterial diversity and composition (**Aim 2)**. A sub-aim was to investigate the relation of personal, occupational and household exposure activities, *S. aureus* nasal carriage outcomes measures (*S. aureus*, MDRSA, and *scn* negative *S. aureus*) and the total percentage bacterial contributions from IHO pigs with changes in alpha diversity and beta diversity (**Aim 2**). Finally, we aimed to determine the stability of the nasal microbiome over time following direct occupational hog production exposure activities of IHO workers as well

as for children living in their households who may be indirectly exposed to microorganisms potentially brought into the household by IHO workers (**Aim 3**).

Chapter Two: Detailed Methods

This chapter contains a detailed write up of the epidemiological study sample collection and methods as well as the sequencing pipeline to prepare samples for downstream bioinformatics chapter-specific methods contained in chapters three, four and five. Study participants used to investigate the specific aims of this thesis are as follows: 1) Chapter three includes IHO pigs and IHO workers versus AFHO pigs and AFHO workers 2) Chapter four includes IHO worker-minor pairs compared to Community Referent (CR) adult-minor pairs and 3) Chapter five includes a 4-month longitudinal cohort study of IHO workers and household children with biweekly follow up visits (timepoints)

Detailed methods for chapter three: *S. aureus* nasal carriage outcomes and the microbiome of pigs and pig workers at industrial compared to antibiotic-free hog operations

Data for this study were collected in July 2015 with convenience sampling of hog production operations in North Carolina by principal investigators from JHSPH. One IHO and three AFHOs defined based on other groups prior evaluations.[33] Facilities were selected based on availability and the facility operator's interest in participating in the study.

The IHO was characterized as a conventional confinement hog operation that uses antimicrobial drugs for therapeutic and non-therapeutic purposes. We did not obtain information on the doses, frequency, and duration of antimicrobial drug use at the IHO. AFHOs raised hogs without use of antimicrobial drugs, which was confirmed by interviews with AFHO workers. AFHOs indicated that they would use antimicrobial

drugs for therapeutic treatment purposes and that those pigs receiving them would be quarantined and the meat from these pigs would not be sold to consumers. All AFHO herds sampled in this study did not receive antimicrobial drugs and were not in close contact with drug-treated pigs. Detailed sampling methods can be found in Davis et al, 2018.[52]

Animal sampling

Pigs were sampled on each facility (a priori, n=20 swine from one larger IHO, and 10 pig from each of the three smaller AFHOs, for a total n=30 AFHO swine). We collected samples from at least three animals from each of the five age groups, if present (e.g., farrow, sow, piglet, weaner, feeder pig) on each farm. Sampling was performed or supervised by a veterinarian. No restraints were used during sampling. Copan liquid Amies Elution swabs (Eswabs) (COPAN diagnostics, Murrieta, CA) were used for culture swabs and Catch-All (Epicentre Technologies, Madison, WI) swabs were used to sample the microbiome from the following anatomical sites: skin, nares, perineum and mouth of all pigs. Throughout the duration of pig sampling procedures, personnel wore disposable Tyvek™ Micro-Clean coveralls (DuPont, USA), Kleenguard boot covers (Kimberly-Clark, Roswell, GA, USA) and sterile gloves.

AFHO pig worker sampling and questionnaire

At the three AFHOs, we collected nasal swabs for culture (Copan Eswabs) and microbiome (Catch-All swabs) from AFHO workers for analysis. AFHO workers were interviewed about work history, contact with pigs, antibiotic use on animals, personal

antibiotic use prescribed by physicians and numerous other questions surrounding their health and common personal and household activities.

IHO pig worker sampling

For culture nasal swabs were obtained from IHO pig workers by rotating a sterile, a dual tipped BBL CultureSwab TM (BD, Sparks, MD) five times clockwise and five times counterclockwise in both nares. Swab transportation and storage, *S. aureus* characterization using one tip aseptically clipped swab, and antibiotic susceptibility of *S. aureus* testing using the Kirby–Bauer disk diffusion methods are previously detailed in Nadimpalli et al, 2016.[30]

Detailed methods for chapter four: *S. aureus* nasal carriage outcomes and the nasal microbiome among industrial hog operation workers, community residents, and children living in their households

Data for this study was collected between March and October 2014 in North Carolina 2014 by community organizers from the Rural Empowerment Association for Community Help (REACH) and principal investigators at JHSPH and collaborators at UNC Chapel Hill. IHO workers and children and CR adults and children (under 7 years of age) were recruited via a snowball approach. One adult, at least 18 years of age and a minor, were recruited from each household. CR adults and children (under 7 years of age) had no known livestock exposure in the last 12 months. IHO households consisted of an IHO worker who worked full time at the IHO at the time of the study or within the prior 3 months and did not have contact with any other livestock at work (i.e. dairy cows,

chickens). Households were ineligible if: adults worked in health care or child care setting, no children less than 7 years of age and study participant was unable to reply to questionnaire in English or Spanish. Eligibility criteria were assessed at the time of recruitment and again before beginning data and swab collection.

Study visit nasal swabs and questionnaires

Questionnaire queried the same information as previous described in chapter three along with additional information on household members contact with livestock and pets, health care exposures and child care attendance; greater focus on occupational exposures for IHO workers. The study included 200 adult IHO workers-child dyads and 200 CR adult-child dyads. Although for the purposes of this thesis, we focused on 20 IHO worker-child and 20 CR adult-child dyads.

Culture swabs were obtained from adult participants by rotating a sterile, a dual tipped BBL CultureSwab TM (BD, Sparks, MD) five times clockwise and five times counterclockwise within the nostril. Swab transportation and storage, *S. aureus* characterization, and antibiotic susceptibility of *S. aureus* testing using the Kirby–Bauer disk diffusion methods are detailed in Hatcher et al, 2017.[32]

Detailed methods for chapter five: Temporal relation of livestock-associated microbial nasal carriage outcomes and work activities with the nasal microbiome of industrial hog operation workers and children living in their households

Data for this study was collected between October 2013 and February 2014 by community organizers from the Rural Empowerment Association for Community Help

(REACH) that recruited volunteer IHO workers. IHO workers were recruited if they fit the following inclusion criteria: currently worked at an IHO resided in North Carolina, could speak English or Spanish, and were at least 18 years of age. Workers employed on other livestock animal farms and meat-processing facilities were excluded from this study. At least one adult and one minor within the household of IHO workers was invited to participate in the study if they were at least seven years old and spoke English or Spanish.

IHO workers attended a baseline enrollment session at the REACH location or within a community meet up space, lasting 2-3 hours. During these sessions, participants responded to baseline questionnaires assessing demographic information, household characteristics, work activities, risk factors for exposure to *S. aureus* and symptoms of SSTI and doctor-diagnosed *S. aureus* infection during the three months prior to enrollment. Additionally, participants performed a self-collected BBL CultureSwab (BD, Sparks, MD) from both of anterior nares under the surveillance of REACH volunteer and guided by instructional diagrams.

Names, phone numbers and addresses of participants were collected upon enrollment and recorded. Study participants were assigned study ID to maintain confidentiality. This study ID was used for materials collected including: nasal swabs and bi-weekly questionnaire data. Following consent, participants were enrolled in the study for a total follow-up of period 4 months. Nasal swab samples were collected from 103 adult (>=18 years of age) IHO workers and 80 of their household members over a 4-month follow up period. There were 54 child household members in the cohort.

Baseline and bi-weekly nasal swabs and questionnaires

Participants attended a training session to complete the baseline interview and were trained to collect of nasal and saliva swabs. Collection of swabs and administration of the interview were performed every two weeks with eight-follow up study visits after baseline. These follow up study visits were performed by research assistants from REACH for data collection at locations convenient to the participant (their homes or the REACH office). The data collected from each participant included self-collection of one nasal and one saliva swab with oversight by a research assistant and the completion of the bi-weekly questionnaire administered by a REACH research assistant in interview format. Oversight and administration of questionnaires by trained officials at REACH was done to ensure greater data quality. Over the course of the 4 months, nine in-person study visits were completed with each participant.

The baseline questionnaire queried job-related activities (number and type of animals an individual is exposed to at work, hours per day and days per week of work, specific job tasks, etc.), household characteristics (number of individuals living in the household, household members with high risk jobs, etc.), personal activities (playing contact sports, cooking or preparing raw meat, hand washing, etc.), health (recent use of antibiotics, recent hospitalizations, previous *S. aureus* infections, respiratory symptoms, etc.), and demographics (age, gender, race, education, etc.). Bi-weekly interviews were administered every two weeks. This questionnaire recorded changes in work activities, personal activities, and health over the follow-up period.

For culture nasal swabs were obtained from adult participants by rotating a sterile, a dual tipped BBL CultureSwab TM (BD, Sparks, MD) five times clockwise and five times

counterclockwise in both nares. Swab transportation and storage, *S. aureus* characterization using one tip aseptically clipped swab, and antibiotic susceptibility of *S. aureus* testing using the Kirby–Bauer disk diffusion methods are previously detailed in Nadimpalli et al, 2016.[30]

Thesis sample selection

I have selected samples to be sequenced by collaborator Maria Dominguez-Bello's laboratory at Rutgers University in the Department of Biochemistry and Microbiology and the Department of Anthropology. Samples (n=310) were selected in total. Hog nasal and perineum samples were matched (as much as possible) on individual animal (within hog comparisons) and life stage (between hog comparisons). Human samples from IHO workers and household minors who experienced a skin and soft tissue infection (SSTI) were matched on age (within 2 years of age), sex and ethnicity. All AFHO workers were included within analysis. IHO pigs and AFHO pigs were selected to represent all age groups at each hog operation.

In total, sample sizes for each chapter are as follows: 1) chapter three contains 10 IHO pigs (with each contributing a nares and perineum sample), 10 AFHO (with each contributing a nares and perineum sample), 41 IHO workers and 7 AFHO workers (n=88); 2) chapter four contains 10 IHO worker-child dyads and 10 CR adult-child dyads (n=80); 3) chapter five contains 21 IHO workers and 21 children living in the households of IHO workers over three time points (n=126) (please note the total sample size will not sum to 310 samples as some study participants were analyzed multiple of Chapters 3-5).

The remainder of samples sequenced include: 1) four farm-specific blanks from the one IHO and three AFHOs in study 1; 2) 9 trip blanks collected during sample collection from study 3; 3) and 20 field blanks for respective IHO worker-child dyads and CR adult-child dyads (n=20) in study 2. These blanks were selected to serve as a measure of any bacterial background due to swab manufacturing and packaging.

Microbiome sequencing and sequence processing

16S rRNA gene amplicon library preparation and sequencing

Total DNA was extracted using the MoBio PowerSoil kit, the Earth Microbiome Project modified version (http://press.igsb.anl.gov/earthmicrobiome/protocols-and-standards/dna-extractionprotocol/). Polymerase chain reaction (PCR) amplified the V4 region of the 16S rRNA gene using degenerate primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) as well as an index primer. Amplicons were quantified using PicoGreen, and cleaned by QIAquick PCR purification kit**.** Cleaned DNA was quantified using the Qubit dsDNA HS assay kit (Life Technologies, Carlsbad, CA, USA) followed by pooling. Reagants for DNA extraction and PCR amplification were sequenced and served as negative controls. Libraries were sequenced on the Miseq platform (Genome Technology Center of NYU Medical Center) using the MiSeq V3 reagent kit with PhiX control (Illumina Inc., San Diego, CA, USA).

Bioinformatics sequence processing

Raw, paired-end 16S rRNA reads (V4 region) were merged into consensus fragments with FLASH[17]. Samples were subsequently filtered for quality (target error rate < 0.5%, windowsize=25) and length (minimum 200bp) using Trimmomatic and QIIME and QIIME.[53–55] Spurious hits to the PhiX control genome were identified using BLASTN and removed. Passing sequences were trimmed to remove primer sequences, evaluated for chimerism with UCLUST (de novo mode)[56]. To provide a comprehensive filter of host-associated contaminant sequences, Bowtie2 was utilized (non-default settings "--end-to-end --sensitive") to search reads against the NCBI Homo sapiens Annotation Release 106[57]. This was followed by a more sensitive BLASTN search against the GreenGenes 16S rRNA database[58]. Chloroplast and mitochondrial contaminants were detected and filtered using the RDP classifier[59] with a confidence threshold of 80%. High-quality 16S rRNA sequences were assigned to a high-resolution taxonomic lineage using Resphera Insight[60–63] (www.respherabio.com; Baltimore, MD). Sequence statistics are summarized in Table 1. De-plexing was performed using a Phred quality score of 20 and a maximum unacceptable Phred quality score threshold was set to 19 to allow reads to be trimmed at the 3' ends if errors in the last 100 bases exceed a threshold of greater than 1% errors in base calling.

Reference database for OTU and taxonomic assignment

High-quality 16S rRNA sequences were assigned to a high-resolution taxonomic lineage using Resphera Insight, a proprietary program developed to provide ultra-high-resolution taxonomic assignment of 16S rRNA sequences to species-level membership (http://www.respherabio.com/). This approach maintains a 99.9% sensitivity and >99.5%

species-level specificity for hundreds of bacterial pathogens. In the event of ambiguous membership, this approach accurately predicts consensus lineage (http://www.respherabio.com/). Refer to methods section for quality filtration parameters.

OTU contamination removal

Prior to OTU contaminant removal, 24,014 OTUs were assigned across 88 samples. OTUs assigned to any of the three DNA extraction reagent negative controls, three PCR reagent negative controls and nine trip blanks were removed from all samples. Taxa present within these samples were presumed contamination field sampling, laboratory processes and/or transport of samples to NYU for extraction, library preparation and sequencing. 1,642 unique OTUs were removed from sequences. 9,342 taxonomic observations were removed per sample.

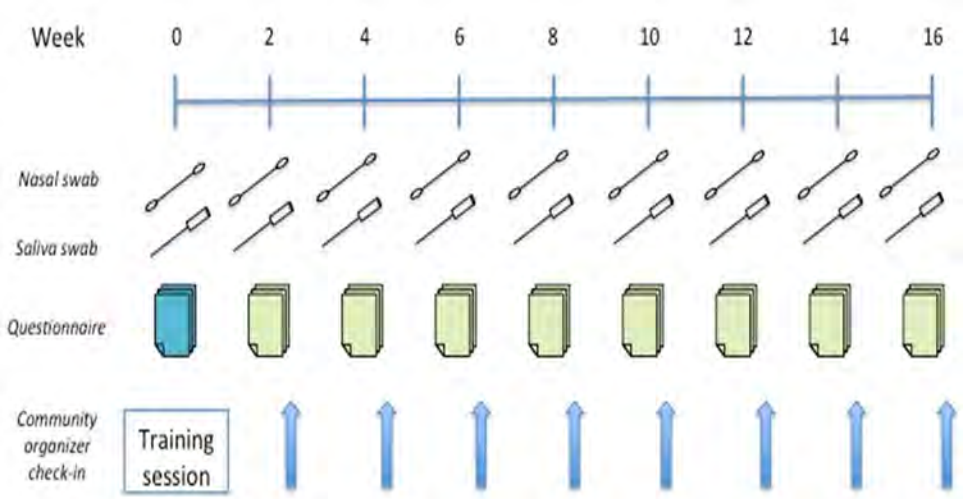

Figure 1: 4-month follow-up visit diagram for training and swab and questionnaire completion.

The darker "Questionnaire" in the diagram above represents the baseline questionnaire and the light green "Questionnaires" represent the bi-weekly interviews. Each arrow represents a follow-up study visit for data collection session between REACH research assistants and the study participant. Questionnaires and nasal swabs were directly transported to Dr. Heaney (EHE-JHSPH from the REACH office.) Diagram courtesy of Christopher Heaney.

Chapter Three: *S. aureus* nasal carriage outcomes and the microbiome of pigs and pig workers at industrial compared to antibiotic-free hog operations

ABSTRACT

Background: The impact of differences in modes of pig production, including antimicrobial use, and *Staphylococcus aureus* nasal carriage outcomes on pig and pig worker microbiomes remains unclear.

Objectives: To evaluate microbiome diversity and composition differences between pigs and pig workers at operations using confinement production with antimicrobial inputs (industrial hog operations [IHOs]) versus pasture-based production without antimicrobial inputs (antibiotic-free hog operations [AFHOs]) and by *S. aureus* nasal carriage outcomes.

Methods: Samples from pigs (nasal and perineum) and pig workers (nasal) at IHOs and AFHOs were sequenced targeting the V4 region of the 16S rRNA gene. We assessed differences in alpha and beta diversity, genera relative abundance, and $\log_2$ fold-change of genera that differed significantly between IHO and AFHO pigs and IHO and AFHO pig workers. We estimated the relation of personal, occupational activities, and household characteristics, and *S. aureus* nasal carriage outcomes with changes in alpha and beta diversity and bacterial contributions from the IHO pig using linear regression models and non-parametric analysis of variance using distance matrices (adonis methods).

Results: AFHO pigs and AFHO pig workers exhibited greater alpha diversity than IHO pigs and IHO pig workers, while IHO groups carried higher abundances of pathogenic genera (*e.g., Staphylococcus, Moraxella,* and *Corynebacterium*). Greater direct contact with pigs and pig fecal matter as well as *S. aureus* nasal carriage outcome positivity increased alpha diversity and bacterial contributions from IHO pigs and was correlated with bacterial community variations among IHO workers only.

Conclusion: Microbiome differences were observed by mode of pig production. The use of antimicrobials in pig production, pig workers' increased direct contact with pigs, and *S. aureus* nasal carriage outcome positivity shifted the nasal microbiome of IHO workers.

INTRODUCTION

Domestic and international demand for pork products has led to a trend of industrialization of pig production, with a predominance of large, mechanized, vertically-integrated operations.[1] Practices that are characteristic of such industrial hog operations (IHOs) include the production of hogs in exclusive confinement and the administration of antimicrobial drugs at non-therapeutic and therapeutic doses and durations, including in feed and/or water.[2] In contrast to this trend of industrialization there has also been increasing consumer demand for antibiotic-free pork, with some pork producers responding by removing antibiotics from their pig production practices.[3] At such AFHOs, pigs are raised with no antimicrobial drug inputs, typically outdoors on pasture-based environments.[2]

In 2013, the US Food and Drug Administration (FDA) issued Guidance for Industry 209 and 213, which called for the voluntary withdrawal of non-therapeutic antimicrobial uses in US livestock production.[4] Although this voluntary policy has coincided with announcements of U.S. corporate poultry producers' reduction or withdrawal of antimicrobial inputs[3], similar announcements of the withdrawal of antimicrobial drug use among U.S. corporate hog producers have been limited. The European Union (EU) banned non-therapeutic antibiotic uses in livestock production effective January 1, 2006,[5] in part based on the emergence in the early 2000s of a novel MRSA CC398 in pigs, pig workers, their families, and communities living proximal to areas of intensive pig production.[6] In 2017, the WHO issued similar guidance worldwide,[7] acknowledging that while certain antimicrobial uses are beneficial for animal health to control zoonotic infections (e.g., those caused by *Salmonella,*

Campylobacter, Escherichia coli, and *Enterococci*),[8] both therapeutic and non-therapeutic antimicrobial doses and delivery schedules in livestock production can be powerful drivers of antibiotic resistance via selective pressure.[9]

Most studies of the public health implications of using versus not using antimicrobial drugs in hog production have focused on characterization of exposure to specific antimicrobial resistant bacterial pathogens, including *S. aureus* (e.g., nasal carriage of methicillin-resistant *S. aureus*[2,10–20] or multidrug-resistant *S. aureus*[2,21–23]) among pigs, pig workers and their household contacts, and community residents. Some studies have focused on how *S. aureus* exposure, frequently assessed by measuring nasal carriage outcomes, is related to the risk of infection[19] and challenges that increasing antimicrobial drug resistance can create for treatment of *S. aureus* infections.[24]

Few studies have examined the role of antimicrobial drug use in hog production on microbial selective pressures within bacterial communities. Antimicrobial drugs used within hog production are known to alter antibiotic resistance genes (ARGs) in, air[18,25–29], water[15,30], soil[30,31], and animal[22,31,32] and human[32,33] anatomical sites. While studies of ARGs provide some insight into antimicrobial selective pressure, they do not sufficiently address knowledge gaps about how such antimicrobial selective pressures may affect microbial communities.[18,22,30,34] Sun et al. (2017), compared the fecal microbiome and ARGs among pigs, pig workers and villagers living in surrounding communities in Canada.[32] Pig workers' fecal microbiomes were less diverse compared to the local villagers and bacterial communities differed between pigs, pig workers, and local villagers.[32] Weese et al. (2014), characterized the impact of MRSA nasal carriage on the nasal microbiome of slaughter-age pigs in China.[35] Significant increases in *Bacteroidetes*

in the fecal microbiome were observed among exclusively liquid-fed/tylosin-exposed and MRSA carrying pigs.[36] Liquid-fed/tylosin-treated pigs had significantly lower relative abundances of *Verrucomicrobia*.[36] Kates et al. 2017, characterized the human nasal and oropharyngeal microbiomes of healthy livestock workers and healthy volunteer community residents in Iowa.[37] Compared to volunteer community residents, livestock workers' nasal and oropharyngeal microbiomes were more diverse.[37] Livestock workers' oropharyngeal microbiomes contained a greater relative abundance of several pathogenic operational taxonomic units (OTUs) compared to volunteer community residents (e.g., *Rothia* and *Streptococcus*).[37] These studies address some questions about microbiome alterations related to antimicrobials use and other aspects of pig production. To our knowledge, no studies have investigated microbiome differences between pigs and pig workers at operations that use versus do not use antimicrobial inputs and considered the influence of personal, occupational, and household characteristics, *S. aureus* nasal carriage, and percent pig OTU contributions to pig workers on alterations of the pig worker's nasal microbiome.[32,36,37]

The goals of this study were to: 1) determine whether differences in mode of hog production—non-therapeutic antimicrobial drug use and confinement (IHO) versus no antimicrobial drug use and pasture-based (AFHO)—were associated with differences in bacterial diversity and composition and relative abundance of potentially pathogenic bacteria OTUs among pigs and pig workers; 2) assess the influence of personal, occupational, and household characteristics, *S aureus* nasal carriage outcomes and bacterial contributions from the pigs on the alpha diversity of pig workers, by mode of production; and 3) determine if personal, occupational, and household characteristics, *S*

aureus nasal carriage outcomes and bacterial contributions from the pig were associated with changes in beta diversity of pig workers, by mode of production.

METHODS

Detailed methods for DNA extraction and amplification, library preparation and sequencing, bioinformatics sequence processing, taxonomic assignments, and OTU contamination removal methods are provided in Chapter 2. A diagram of the sample selection is provided in Supplemental Materials Figure S1.

Statistical analysis

The following alpha diversity measures were calculated as a reflection of the diversity of OTUs within each individual sample: Shannon diversity (overall bacterial diversity, taking into account OTU richness and evenness), phylogenetic distance (the diversity of lineages represented in OTUs), observed OTUs, and species evenness. These measures have been utilized in previous investigations of the influence of antimicrobial drug use on the fecal and nasal microbiome.[38–53] Rarefaction curves were created in MacQIIME 1.9.1 using the Shannon diversity index to determine adequate sampling of diversity.[54] Data was not rarefied to include all valid data available.[55]

The Student's t-test was used to assess differences in alpha diversity measures between two groups.[54] We first determined whether pig nasal and perineum sample alpha diversity differed or could be combined as an overall alpha diversity measure of a pig (Supplementary Materials Table S1). Once it was established that pig nasal and perineum samples could be combined, we assessed differences in alpha diversity measures between: 1) IHO pigs and AFHO pigs (including by pig lifestage); 2) IHO pigs and IHO

workers; 3) AFHO pig and AFHO workers and 4) by *S. aureus* nasal carriage outcome positivity among pig workers, by mode of production.

Normalized OTU tables were produced, using the DESeq2 tool within QIIME, prior to generating beta diversity measures to account for uneven sample sums as a result of sequencing techniques and possible low depth of coverage samples.[54,56] Beta diversity measures account for ecologic measures through adjustment by one or more of the following aspects of the microbiome: sequence presence/absence (qualitative), sequence abundance (quantitative), and/or sequence phylogeny. Bray-Curtis, Euclidean distance, Binary Jaccard, weighted UniFrac and unweighted UniFrac measures were used to investigate differences in bacterial community diversity between samples. An extended table of all comparisons of beta diversity distance measures within this paper can be found in the supplemental section (Supplementary Materials Table S2). Bray-Curtis measures were used to generate non-metric dimensional scaling (NMDS) using R studio (R Studio, Inc. Boston, MA).[57] Ellipses within figures were generated assuming a multivariate t-distribution as the population standard deviation is not known and sample sizes were small.

Relative abundances of OTUs were calculated using QIIME. Genera listed in each figure panel represent the top 19 most abundant genera and "other taxa" when conducting a pairwise analysis of two exposure groups (IHO vs. AFHO pig, IHO pig vs. IHO worker, AFHO pig vs. AFHO worker, and IHO vs. AFHO worker).

Variable transformations and regression analyses were performed using Stata version 13.0 (StataCorp, College Station, TX). Genera and species relative abundances generated in QIIME were $\log_2$ transformed, imputing half of the minimum non-zero

value for OTUs that were not detected in a sample. Using the Kruskal-Wallis test and the G-test of independence, we identified the number of OTUs (and genera to which they belonged) that statistically significantly differed in relative abundance (Kruskal-Wallis and presence/absence (G-test) between IHO and AFHO pigs using false discovery rate (FDR)[58] corrected *p*-values. In addition to the 47 genera identified via the G-test of independence, we investigated the $\log_2$-fold change for 44 additional genera because of: 1) *a priori* evidence of their pathogenic potential (21 genera)[36,37,46,50,59–64]; and 2) the previously reported increased relative abundance of carriage of 23 genera among those exposed to conventional pig production compared to healthy volunteer community residents[37]. We used generalized linear models (GLMs) to estimate the $\log_2$ fold-change of these three categories of OTUs between IHO and AFO pigs and IHO and AFHO pig workers.

SourceTracker, a tool within QIIME, was used to predict the contribution of microbial taxa present in at least 1% of samples from pig samples (specified as the source) to pig worker samples (specified as the sink), by mode of production (IHO; AFHO).[65,66] Species identified as source microbial taxa were then investigated to determine $\log_2$ fold changes of taxa between source and sink populations. Investigations excluded those bacterial assignments with non-specific OTU classifications.

We use generalized linear models (GLMs) to estimate the relation between personal, occupational and household characteristics and activities, bacterial contributions from the IHO pig, and *S. aureus* nasal carriage outcomes with changes in alpha diversity measures among pig workers, by mode of production (IHO; AFHO). We also examined whether percent pig OTU contributions were associated with changes in

alpha diversity, by mode of production. Similarly, the adonis non-parametric method was used to estimate how much variation in the Bray-Curtis distance measure was correlated with personal, occupational, and household characteristics and activities, *S. aureus* nasal carriage outcomes, changes in alpha diversity among pig workers, and percent of microbial taxa contributions from a pig to a worker, by mode of production.

RESULTS

Demographics

Table 1 presents the demographics of pig and pig worker study participants, overall and by mode of pig production (IHO vs. AFHO). One sample was collected from the nares and one from the perineum from each pig. Overall, we sampled all pig life stages except finishing pigs; however, the IHO pigs did not include stock and feeder pig life stages and the AFHO pigs did not include sows in gestation. IHO pigs were confined in farrowing, nursery and breeding barns, whereas AFHO pig lifestages tended to be mixed on pasture-based environments.

Forty four percent of IHO pig worker participants were male, 100% percent identified as Hispanic, and their mean age was 38 years (min: 18, max: 71). Forty three percent of AFHO workers were male, all identified as white, and their mean age was 34 years (min: 26; max: 49). Overall, 17% of pig workers reported being a current smoker (4/41 IHO; 4/7 AFHO) and one IHO worker reported asthma. All microbiome data analyzed for workers were recovered from nasal swab samples.

Microbiome sample pre-processing

We successfully merged 3,714,638 reads using the paired-end read protocol. On average 85.6% of reads pairs were successfully merged. Reads that did not merge were discarded. On average, each sample had 13,386 reads prior to pre-processing. Approximately, 6.7% of sequences were lost due to Phix, Chimeras, host contaminants, and other contaminant screening procedures. We observed an average of 661 chimeras per sample. The final number of clean reads per sample was 11,095, with an average length of 253 bp.

Microbiome diversity

Diversity was adequately sampled based upon rarefaction curves (see Supplementary Materials Figure S2), except among IHO workers although IHO workers had the largest sample size.

Alpha diversity

Alpha diversity was calculated for each sample. The pig host species nares and perineum samples were investigated for differences in alpha diversity (Table 2). Alpha and beta diversity of the pig nares and perineum were comparable and therefore anatomical site diversity measures were combined to create overall alpha diversity measures for IHO pigs and AFHO pigs (Table 3). Overall, AFHO pigs, regardless of anatomical site, were more diverse than IHO pigs. Compared to IHO pigs, AFHO pig Shannon diversity was statistically significantly higher (IHO pig mean standard deviation [SD]=6.44 [0.99], AFHO pig mean[SD]=7.60 [0.39]), phylogenetic distance, (IHO pig mean[SD]=40.2 [1.5], AFHO pig mean[SD]=65.0 [6.0]) and observed OTUs (IHO

mean[SD]=628 [27], AFHO mean[SD]=1167 [97]). IHO and AFHO pigs demonstrated similar species evenness (IHO mean[SD]=0.18 [0.03], AFHO mean[SD]=0.28 [0.06]).

Overall, IHO pigs were more diverse than IHO workers for all alpha diversity measures (all $p<0.001$), except species evenness (IHO pig mean [SD]= 0.18 [0.03], IHO worker mean [SD]= 0.52 [0.03]). IHO worker nares demonstrated greater species evenness. IHO pigs and IHO workers demonstrated similar Shannon diversity (IHO pig mean [SD]=6.44 [0.99], IHO worker=6.24 [0.18]) measures (Table 3). AFHO pigs and AFHO workers differed in alpha diversity when comparing phylogenetic distance, observed OTUs and species evenness (all $p<0.01$). Shannon diversity was not significantly different. Overall, AFHO pigs exhibited greater phylogenetic distance (mean [SD]=65.0 [6.0] and observed OTUs [AFHO pig mean[SD]=1167 [97]) compared to the AFHO worker (AFHO worker mean=42.4 [9]; 626 [75]). AFHO workers [mean[SD]=0.67 [0.07)])exhibited greater species evenness compared to AFHO pigs (mean[SD]=0.28 [0.06]).

Overall, regardless of mode of production, pigs had greater phylogenetic distance and observed OTUs than workers whereas worker microbiomes were more even than pigs. The alpha diversity differences between IHO pigs and IHO workers were orders of magnitude higher than differences between AFHO pigs and AFHO workers. The directionality and magnitude of these alpha diversity measures was agreeable across the IHO and AFHO modes of production. AFHO pig and AFHO worker diversity differed among the three AFHO facilities sampled, suggesting a facility-specific influence on the microbiota. Alpha diversity measures were statistically significantly different between facilities. Facility-specific influences were not examined for IHO pigs and IHO workers

because IHO pigs were sampled from only one facility and IHO workers were not asked whether they worked at the same facility as other IHO worker participants.

Beta Diversity

Beta diversity results are presented within Table 4. When assessing sequence presence/absence using unweighted UniFrac distance measures for microbial membership, statistically significant differences in the microbiome composition and diversity existed between IHO pigs and AFHO pigs, AFHO pigs and AFHO workers, and IHO pigs and IHO workers. IHO and AFHO workers were not statistically significantly different in their microbial membership and composition. When accounting for sequence abundance and phylogeny using weighted UniFrac measures, statistically significant differences were observed between IHO pigs and AFHO pigs, and AFHO pigs and AFHO workers. However, there were no statistically significant differences comparing IHO pigs to IHO workers and IHO workers to AFHO workers (Table 4).

Non-metric dimensional scaling (NMDS) plots

The beta diversity measure Bray Curtis, which accounts for sequence abundance, was used to visualize distances between samples within a two dimensional NMDS plot (Figure 1A and 1B). We observed separation between pigs and pig workers, by mode of production. IHO workers and AFHO workers carried a greater number of similar OTUs compared to IHO pigs and AFHO pigs.

Taxonomic characterization

Taxonomic composition and abundances at the genus level are displayed by participant type in Figure 1 (Panels C-F). For each pairwise characterization of the top 19 plus all other OTUs, we observed differences in the occurrence of potentially pathogenic bacteria as follows: 12/20 OTUs were potentially pathogenic among IHO pigs and AFHO pigs (Figure 1 panel A), 15/20 OTUs were potentially pathogenic among IHO pigs and IHO workers (Figure 1 Panel B), 12/20 OTUs were potentially pathogenic among AFHO pigs and AFHO workers (Figure 1 Panel C), and 9/20 OTUs were potentially pathogenic among IHO workers and AFHO workers (Figure 1 Panel D).

Overall, IHO pigs and IHO workers carried higher relative abundances of potentially pathogenic genera compared to their AFHO counterparts. *Staphylococcus* in particular, represented the most abundant genus in the IHO worker and AFHO worker groups, with similar carriage ($\log_2$ fold differences in relative abundance between IHO and AFHO workers: beta=-0.6295; p<0.354). Ninety-four OTUs differed significantly in relative abundance between the IHO pig and the AFHO pig. These 94 OTUs were classified into 53 genera. Twenty OTUs were statistically significantly higher among IHO pigs while 34 OTUs were higher among AFHO pigs (Figure 2). Sixteen of 20 genera associated with IHO pigs have been classified as potentially pathogenic (e.g., *Aerococcus, Rothia, Neisseria*) (Figure 2). Seven of 34 genera were observed to be potentially pathogenic among AFHO pigs (*e.g., Geobacter, Bryobacter, Parapedobacter*) (Figure 2), but have been found to have origin in soil environments and these OTUs have also been observed in AFHO workers.[67–71] Three of the 16 OTUs that were of higher relative abundance among IHO pigs compared to AFHO pigs tended to be carried at higher relative abundances among IHO workers compared to AFHO workers (Figure 3).

Eighty-four OTUs were found to be exclusive to one mode of production or the other (IHO or AFHO). Of these 84 OTUs, 19 were exclusively observed among IHO pigs and belonged to the following 10 genera: *Rothia, Moraxella, Pseudomonas, Corynebacterium, Bacteroides, Lactobacillus, Treponema, Aerococcus, Globicatella,* and *Staphylococcus*. Sixty-five OTUs were exclusively observed among AFHO pigs and belonged to the following 7 genera: *Prevotella*, *Moraxella, Massilia*, *Parapedobacter*, *Acinetobacter*, *Pasteurella*, and *Treponema*.

SourceTracker

Among OTUs contributing at least 1% abundance to the IHO worker's nasal microbiome, 90 OTUs appeared to be derived from the nares and 72 OTUs from feces/perineum of the IHO pig (Supplementary Materials File Figure S3). Among OTUs contributing at least 1% abundance to the AFHO worker's nasal microbiome, one OTU appeared to be derived from the AFHO pig nares and 4 OTUs from the AFHO pig feces/perineum (Supplementary Materials File Figure S3).

Epidemiologic analysis

IHO and AFHO pigs

Within the IHO group, sows carried greater numbers of OTUs compared to all other life stages combined (sow in gestation, weaned piglet, nursery piglet, piglet, stock, feeder) (all $p<0.05$) (data not shown). No statistically significant differences in alpha diversity were observed between IHO sows and all other IHO pig life stages (sows in gestation, piglets, stock, feeder) (all $p > 0.05$ using two sample student t-test). Older IHO

pigs (sows, sows in gestation, stock, feeder, stock) carried greater numbers of OTUs compared to young IHO pig life stages (piglets including nursery piglets and weaning piglets) (all $p < 0.05$).

Older AFHO pigs (sows and stocks) had statistically significantly higher Shannon ($p<0.05$), phylogenetic distance ($p < 0.001$), and observed OTUs ($p < 0.001$) than young AFHO pigs (feeders). AFHO sows (AFHO mean [SD]=1173 (350) carried a greater number of observed OTUs compared to the IHO sows (IHO mean [SD]=596 (207); $p<0.02$). In terms of bacteria composition, IHO pigs carried similar taxa when comparing sows to sows in gestation, young to old pigs, and sows to all other life stages (data not shown). AFHO pigs had similar observations. We observed no statistically significant differences in alpha diversity by *S. aureus* carriage (*S. aureus*, MDRSA, *scn*-negative *S. aureus*) among IHO pigs (data not shown). AFHO pigs did not carry *S. aureus.*

Factors associated with IHO workers' nasal microbiome diversity and composition

Table 5 summarizes the relation of personal and occupational exposures and *S. aureus* nasal carriage with alpha diversity and bacterial contributions from the IHO pig. Statistically significant increases in Shannon diversity (beta=0.08; 95% confidence interval (CI)=0.02, 0.14), phylogenetic distance (beta=0.64; 95% CI=0.07, 1.20), observed OTUs (beta=10.50; 95% CI=1.02, 19.98) and percent IHO pig contribution (beta=0.92; 95% CI=0.01, 1.83) were observed for each additional year working at any IHO (Table 5). Each additional 8-hour shift involving direct IHO pig contact was associated with increases in Shannon diversity (beta=0.22; 95% CI=-0.07, 0.50), phylogenetic distance (beta=3.05; 95% CI=1.23, 4.87), observed OTUs (beta=51.72; 95%

CI=22.03, 81.40) and percent IHO pig contribution (beta=3.65; 95% CI=0.12, 7.18). Each additional hour since the last IHO work shift was associated with increased Shannon diversity (0.10 (0.03, 0.17), phylogenetic distance (beta=1.00; 95% CI=0.55, 1.46) and observed OTUs (19.49 (12.08,26.90). Showering after work (beta=-66.80; 95% CI=-132.05, -1.55) and changing clothes after work (beta=-70.53; 95% CI=-138.81, -2.24) were associated with statistically significant decreases in the number of OTUs observed within the nasal microbiome of IHO workers. Although not significant, increasing face mask usage from never, sometimes to always was associated with decreasing Shannon diversity (-0.14 (-0.61, 0.33), phylogenetic distance (-1.92 (-6.62, 2.33), observed OTUs (-31.78 (-108, 44.8), species evenness (-0.01 (-0.07, 0.04) and percent IHO pig contribution (-2.44 (-9.83, 4.95) ($p > 0.05$).

For IHO workers raising between 500-8000 pigs, each additional 500 young IHO pigs at the facility (weaners and nursery pigs), were associated with increased Shannon diversity, phylogenetic distance, and observed OTUs ($p<0.001$) among IHO workers (Table 5). Personal antibiotic use, handling dead pigs, handing pig manure, current smoker and ownership of a pet were not associated with any differences in percent IHO pig contributions or alpha diversity measures (all $p >0.05$) (Table 5).

S. aureus nasal carriage versus non-carriage among IHO workers was associated with statistically significant increases in Shannon diversity (beta=0.71; 95% CI=0.08, 1.33), phylogenetic distance (beta=8.67; 95% CI=3.32, 14.01), and observed OTUs (beta=150.27; 95% CI=59.09, 241.45). MDRSA nasal carriage vs. no nasal carriage among IHO workers was associated with statistically significant increases in percent IHO pig contribution to the microbiome (beta=9.11; 95% CI=1.88, 16.35), phylogenetic

distance (beta=12.99; 95% CI=6.96, 19.01) and observed OTUs (beta=229.82; 95% CI=123.19, 336.45). *Scn*-negative *S. aureus* nasal carriage vs. no nasal carriage was associated with increases in percent IHO pig contributions (beta=9.85; 95% CI=2.95, 16.75), phylogenetic distance (beta=10.24; 95% CI=4.39, 16.09) and observed OTUs (beta=176.88; 95% CI=75.03, 278.74) among IHO workers.

Factors associated with AFHO workers' nasal microbiome diversity and composition

Table 6 summarizes the relationship between personal and occupation exposures and *S. aureus* nasal carriage and the alpha diversity and bacterial contributions from the IHO pig. On average for each additional year worked on any swine farm, AFHO workers carried 23 more OTUs within their nasal cavities (95% CI=11.96, 35.86). Working with various pig lifestages (weaners, feeders and finishers) resulted in statistically significant differences in percent that AFHO pigs contributed to the content and alpha diversity of AFHO workers' nasal microbiota (Table 6). However, on average, AFHO workers' nasal microbiome diversity decreased with greater exposure to pigs.

Frequency of hand washing significantly decreased alpha diversity trends observed in AFHO workers. Among AFHO workers, increased frequency of hand washing (by two additional times) statistically significantly decreased percent pig contribution (beta=-1.26; 95% CI=-1.70, -0.81) and observed OTUs (beta=-147.37; 95% CI=-269.73, -25.01). On average, nasal carriage of *S. aureus,* MDRSA and *scn*-negative *S. aureus* were associated with an increase in AFHO percent pig contribution and alpha diversity in the AFHO worker, although not statistically significant. Because all AFHO workers changed and showered after working with pigs, owned a household pet and did

not personally use antibiotics during the 3 months prior to the study visit, these variables could not be assessed in epidemiologic analyses.

Adonis results

Adonis analysis (Table 7) indicates that the following IHO worker activities contributed to variations in Bray-Curtis distance measures including: hours of direct contact with pigs per week, time since last work shift, increased frequency of facemask usage, drawing pigs' blood, changing clothes after work, the percent of IHO pig contribution to the IHO worker's nares, and two *S. aureus* nasal carriage outcomes (MDRSA and *scn*-negative *S. aureus*). AFHO worker personal and occupational activities, percent AFHO pig contributions, and *S. aureus* nasal carriage outcome status were not associated with significant variations of community membership and composition, via estimation by Bray-Curtis beta diversity measures.

DISCUSSION

Pig production involving antimicrobial drug inputs versus not and confinement versus pasture-based production environments was associated with alterations of pigs' and pig workers' nasal microbiomes. We observed that the microbiome of IHO pigs (combined nasal and perineum) and IHO workers (nasal) were less diverse and carried a greater number and relative abundance of potentially pathogenic OTUs compared to AFHO pigs and AFHO workers, respectively. Lower diversity signifies a lack of temporal stability and resilience and has been associated with increased odds of diminished health.[72,73] Such instability could be due to occupational exposures within

IHOs and routine antimicrobial exposures for pigs raised within the IHO pig confinement buildings. Antimicrobial drug residues may decrease the diversity of IHO workers' nasal microbes due to their bactericidal nature[72,74] Instability in the microbiome, defined by perturbation of the healthy microbiome, can also lead to colonization by potentially pathogenic organisms within the nasal cavity, which was exemplified by greater abundances of potentially pathogenic bacteria that we observed within IHO pigs and IHO worker.[72] IHO pigs carried significantly greater log_2-fold relative abundances of known opportunistic pathogenic genera *Streptococcus*, *Staphylococcus* and *Facklamia* that have a high capacity for development of antimicrobial resistance based on the literature.[63] With known antimicrobial drug usage, IHO pigs and IHO pig workers alike may experience exposure to bacteria that are responding to increased selective pressures and developing antimicrobial resistance.

The impact of the pig production environment on human health has been investigated with the hygiene and biodiversity hypotheses in mind.[75,76] Such patterns may be more supported by our findings among AFHO pigs and AFHO workers, where microbial exposures are high and selective pressures are likely minimal due to the absence of antimicrobial drug selection in the AFHO pigs. AFHO pigs exhibited significantly higher diversity compared to the IHO pig which is likely due both to exposure to more diverse external microbial communities present on AFHOs and minimal selective pressure due to the lack of antimicrobial drug use.[77,78] AFHO pigs also carried numerous soil-specific OTUs (*Bryobacter*, *Geobacter*, *Parapedobacter*, etc.) at higher log_2-fold relative abundances compared to IHO pigs.[67–71,79–82] Heightened carriage of soil-specific OTUs may be due to traditional pig behaviors including wallowing in the

mud and foraging throughout pastures.[83] IHO pigs, due to confinement, cannot perform such behaviors and therefore may lack exposure to such soil-specific OTUs.

Pig nares and perineum samples were comparable in their levels of bacterial diversity and composition (alpha and beta), similar to findings by Singh et al. within human participants.[84,85] Pig anatomy, physiology, immunology and development patterns are highly similar to that of a human.[86] These similarities of the nares and perineum microbiome, supported the decision to collapse these anatomical sites to one measure per individual pig. Sensitivity analyses were performed using one quarter, one half and three quarters of the minimum non-zero value to determine whether imputation of change relative abundances altered results. Such imputation did not alter interpretation and conclusions.

IHO pigs exhibited lower alpha diversity than AFHO pigs and this may indicate a less stable microbiome and probable increased likelihood for the carriage of opportunistically pathogenic OTUs.[87] Routine exposure to antimicrobial drugs through ingestion of feed and water as well as respiration of particulate matter with adsorbed antibiotics due to aerosolization, can apply selective pressures on bacteria in the nasal cavity of the IHO pigs.[30,34,88–90] Based on reports of antimicrobial use in food animals published by the U.S. Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM), in 2016, the percentage of domestic sales of products administered by feed was 72% and by water was 23%.[91] Pigs were identified as the second highest category for the use of medically important (22%) and non-medically important (3%) antimicrobial drugs compared to other food-producing animals (e.g. cattle, chicken, turkey).[91] The percentage of antimicrobial drug classes used in hog production compared

to all other food-producing animals (expressed as a proportion based upon the weight in kilograms) was: lincosamides (83%), macrolides (61%), tetracyclines (43%). aminoglycosides (21%), penicillins (2%), and sulfas (11%).[91] Aminoglycosides and tetracyclines are active against both Gram-positive and Gram-negative-bacteria, whether aerobic or anaerobic. Lincosamides, macrolides, penicillins and sulfas are narrow spectrum drug classes and therefore act against at least one of these bacteria types (Gram-positive, and Gram-negative).

Ciprofloxacin, a broad-spectrum antibiotic, has been found to cause significant declines in taxonomic richness, diversity and evenness within a short period of time, and can affect approximately 30% of bacteria within the human gut community.[44,45] Exposure to broad-spectrum antibiotics resulted in declines of *Faecalibacterium*, *Bacteroides*, *Alistipes*, *Porphyromonadaceae* and increases in a few OTUs within the genus *Bacteroides.*[45,92] Another study in China, found that in-feed antibiotic combinations among weaned pigs decreased observed OTUs after a 28-day trial; however, taxonomic changes were more complicated as they were dependent on antibiotic drug combinations.[93]

Gentamicin and duramycin, two broad-spectrum antibiotics, and Lincomix, a narrow-spectrum lincomycin, were used on the IHO farm sampled within this study. Gentamicin has similar influences on the microbiome as other broad-spectrum antimicrobial drugs, while Lincomix is primarily active against pathogenic genera *Streptococcus*, *Staphylococcus*, and *Mycoplasma*.[94] Such antimicrobial drugs may produce selective pressure whereby the microbiota diversity decreases and then returns to its pre-exposure diversity and composition. However, some suggest that complete

recovery may not be reached.[91] Lower microbial diversity observed in IHO pigs and IHO workers may be due to Lincomix and Gentamicin exposures at the hog operations. We were unable to distinguish between the influence of antibiotics and confinement due to lack of assess to antimicrobial drug use information including names, dosage and frequencies.

The healthy pig nasal passage tends to be composed of the genera *Moraxella, Psychrobacter*, *Pseudomonas*, and *Acinetobacter*, while *Janthinobacteirum*, *Clostridium sensu stricto*, *Lactobacillus*, *Aerococcus* and *Treponema* tend to be present in smaller quantities.[35] Similar relative abundances of *Acinetobacter* and *Clostridium sensu_stricto* were observed in IHO and AFHO pigs. AFHO pigs carried greater relative abundances of *Pseudomonas* and *Treponema* compared to IHO pigs, which is similar to results observed by others.[40,53] IHO pigs carried *Lactobacillus*, *Aerococcus*, *and Moraxella* at higher relative abundances than AFHO pigs, and this is consistent with studies showing that *Lactobacillus* is more frequently observed among pigs treated with Tylosin and other antibiotics.[40,49,51,95,96] *Lactobacillus* also tends to be associated with obesity among humans[97], which is consistent with the growth-promoting and improved feed conversion that is associated with the use of antibiotics in pig production.[98] Additional sources of *Lactobacillus* may be from probiotics used to improve gut health and nutrient utilization within industrial pig production.[99]

IHO pigs carried $\log_2$ fold higher relative abundances of *Moraxella, Rothia, Lactobacillus, Neisseria, Coprococcus, Globicatella* as well as other genera compared to AFHO pigs. Our findings are similar to Strube et al. (2018) of healthy pigs where they found that the skin and nose of pigs were dominated by *Aerococcus*, *Streptococcus,*

Lactobacillus, *Facklamia*, *Rothia* and *Staphylococcus*.[100] *Moraxella* species are known to cause respiratory infections and have been characterized as an air contaminant in poultry confinement buildings.[37] *Rothia* has been isolated from exhaust air of a pig barn as well as the gut, fecal and nasal microbiota of pigs along with *Lactobacillus, Neisseria, and Coprococcus.*[48,63,77,89,101] *Globicatella*, a *Streptococcus* organism, is known to cause bacteraemia in clinical settings.[102] *Aerococcus, Anaerococcus, Helcococcus and Corynebacterium* are commonly found on the human skin while *Methanobrevibacte*r has been found in the human gut.[63,86,103,104] *Dolosicoccus* has been observed in the cecal microbiome of broiler chickens.[43] AFHO workers carried greater amounts of *Actinobacteria* in their nares compared to IHO workers, which was similar to studies comparing healthy vs. inpatient nasal microbiomes where the highest diversity was observed among healthy adults.[105]

Staphylococcus was highly represented in IHO pigs and IHO workers compared to AFHO pigs and AFHO workers. This finding has been echoed in previous culture-based studies that found increased *S. aureus* nasal carriage among IHO workers (53%) compared to community residents (18%) with no known livestock exposure.[11] A systematic review of MRSA prevalence in people in contact with livestock by Liu at al. (2015) found that animal contact and the intensity of this contact was associated with increased risk of MRSA, with an average prevalence of 12.9% in North America.[106] The literature does not agree in terms of the influence of pig farming on MRSA nasal colonization.[106,107] There is greater consistency within the literature investigating the influence of livestock exposure on the nasal carriage of *S. aureus*, MDRSA and *scn* negative *S. aureus*. Wardyn et al. (2015), working in Iowa, compared individuals with

livestock contact to a community-based comparison group that had no livestock exposures, and found that individuals with livestock contact had increased odds of *S. aureus* nasal carriage (prevalence ratio (PR), 1.8; 95% CI, 1.4–2.2), MDRSA (PR, 6.1; 95% CI, 3.8–10.0), and LA-SA (PR, 5.8; 95% CI, 3.9–8.4) compared to those lacking livestock exposure.[107] Other studies found similar results for livestock and IHO workers.[2,19,108]

The present study found positive associations between *S. aureus,* MDRSA, and *scn*-negative *S. aureus* nasal carriage positivity and alpha diversity (among both IHO workers and AFHO workers) while positive associations with beta diversity changes were only observed among IHO workers.[84] This suggests that the presence of pathogenic bacteria could influence the nasal microbiome via ecological competition or the perturbation of the microbiome which may allow for the acquisition of IHO-associated taxa, such as *Staphylococcus*. A similar study in pigs found nasal colonization by *S. aureus* was associated with the presence of other *Staphylococcus* species in various pig lineages.[109]

Within the IHO environment, there is substantial concern about pathogenic bacteria acquiring resistance genes because of the potential for such genes to be transferred from one bacteria to another.[110] Such transfers are a cause for public health concern in part due to potential for dissemination of LA-*S. aureus* and other livestock-associated bacteria that may be harmful to humans between IHO pig workers and their household and community contacts. For example, IHO workers and AFHO workers differed significantly in 3 OTUs which included *Staphylococcus equorum/haemolyticus*

which is known to colonize pig lineages and this OUT was contributed by the IHO pig (source) to the IHO worker (sink) within our study.[109]

Taxonomic contributions from the pig perineum outweighed that of the pig nares, regardless of mode of production. This may be due to IHO workers' high exposure to fecal matter via the aerosolization of particulate matter (PM) comprised of bacteria sourced from pigs' fecal microbiota, (using the bacterial composition of pigs' perineal microbiota as a proxy).[111] Within this study, we used a novel marker of livestock-associated *S. aureus* (*scn*-negative *S. aureus*). The IHO pig contributed orders of magnitude higher numbers of OTUs to IHO workers than did AFHO pigs to the AFHO worker, which was consistent with trends seen in IHO work history and IHO occupational exposure activities. The frequency, magnitude and duration of swine direct contact with pigs during shifts was associated with increased IHO pig taxonomic contributions to the IHO worker, but an increased frequency of performing hygienic practices (showering after work, changing clothes after work, always wearing a face mask compared to sometimes and never mask users) may be protective. Increases in alpha diversity given a greater time away from IHO work was an unexpected results as we found increased exposures to antimicrobial drugs was associated with decreases in alpha and beta diversity. This increase may be due to the rebounding of the health of the microbiome following occupational exposures while at work.[72] These occupational activities and *S aureus* nasal carriage outcomes significantly altered beta diversity and may serve as points of control to minimize the influence of hog production practices on the nasal microbiome of pig workers.

This study had several limitations. Study-specific differences in nasal sample collection methods appeared to affect alpha diversity measures. To address this, we adjusted for study within regression models. All samples from the parent epidemiologic studies were not sequenced due to funding limitations, which means that our results may not be generalizable to all IHO workers and all pig participants in the parent studies. We sequenced only V4 region of the 16s rRNA gene in this study, which in general, tends to present challenges for distinguishing between genera and identifying *Staphylococcus* species, compared to other regions.[112,113] Advanced microbiome analysis methods (www.respherabio.com; Baltimore, MD); however, allowed us to distinguish genera and identify *Staphyloccocus* species. IHO pigs at one facility were relied on as proxies of IHO pig exposure for IHO workers who worked at different facilities in the same state (North Carolina). We believe this proxy is valid due to similar hog production practices at this one IHO compared to other IHOs in North Carolina. Interpretations of results are limited in this study due to small sample sizes. Future studies should include larger sample sizes to increase statistical power to identify important effects related to exposures (e.g. IHO vs. AFHO). This study did not sample the participants' household environment and/or household pets. Vestergaard et al. (2018), suggested that the airborne microbial community of pig stables and farmers' homes contained more diversity and abundant bacteria compared to suburban homes and also that these bacteria have been previously cited to enhance protective effects against respiratory conditions like asthma.[114] Such airborne pathogens have the potential to enter homes and remain as settled dust for subsequent exposures as there is evidence that household microbial communities may influence the nasal microbiome of humans.[115] Such samples would

allow quantification of external contributions (e.g. household air, household surfaces and pets) to the nasal microbiome of IHO workers and the AFHO workers. Lastly, this study was not able to measure antimicrobial drug residues in nasal and other anatomical site samples.

In future studies, there is a need for increased sample sizes involving multiple hog operations to obtain the most representative microbial profile and composition for the IHO pig, IHO worker, AFHO pig and AFHO worker. There is also a need to select an appropriate region of the 16s rRNA region depending on the bacteria of interest and to utilize metagenomic sequencing to investigate ARGs and functions of OTUs within the microbial community. Additional future studies should measure concentrations of antimicrobial drug residues present in IHO vs. AFHO production environments and pig and pig worker specimens. Lastly, future investigations should consider the associations of different LA-microbial markers (e.g., Pig-2-Bac[116], CC398[15], and CC9[117]) with the microbiome.

This study offered the rare opportunity in the United States to investigate the nasal microbiome of populations previously inaccessible to researchers (IHO pigs, IHO workers). This study is the first study, to our knowledge, in the United States, to compare the microbiome of IHO pigs (using a proxy IHO facility) and IHO workers as well as AFHO pigs and AFHO workers. The recruitment of AFHO pigs and AFHO workers as an unexposed group allowed us to investigate the role of antimicrobial drug use within IHOs compared to AFHOs. With epidemiologic data, we determined that well-known respiratory illnesses (asthma and allergies) were low among IHO workers and AFHO workers in our study.

The workers within our study were healthy, however there is still a need for increased personal protective equipment (PPE) for pig workers to minimize continued exposure via direct contact with swine and aerosolization of PM. There is also a need for intervening science policies to enact mandatory withdrawal of antimicrobial drugs from food-production animal markets and increase regulations for worker protection in the future.

CONCLUSION

Alterations to the microbiome due to antibiotic exposure has been shown to have lasting impacts on the stability of the microbiome as well as implications of health status.[72] Our study found key microbiome differences by mode of production and significant relationships between personal and occupational exposure activities and *S. aureus* nasal carriage outcomes in relation to microbiome outcomes (pig microbial contribution and changes in alpha diversity). Mode of pig production had implications on bacterial diversity and composition with clear differences observed between IHO workers and AFHO workers. Changes in IHO pig production practices, such as reductions of antimicrobial use and increased use of PPE, may be warranted in order to reduce microbial exposure burdens of IHO workers (including antimicrobial selective pressures to medically important drugs) and limit the emergence of pathogenic bacteria within the healthy human microbiome.

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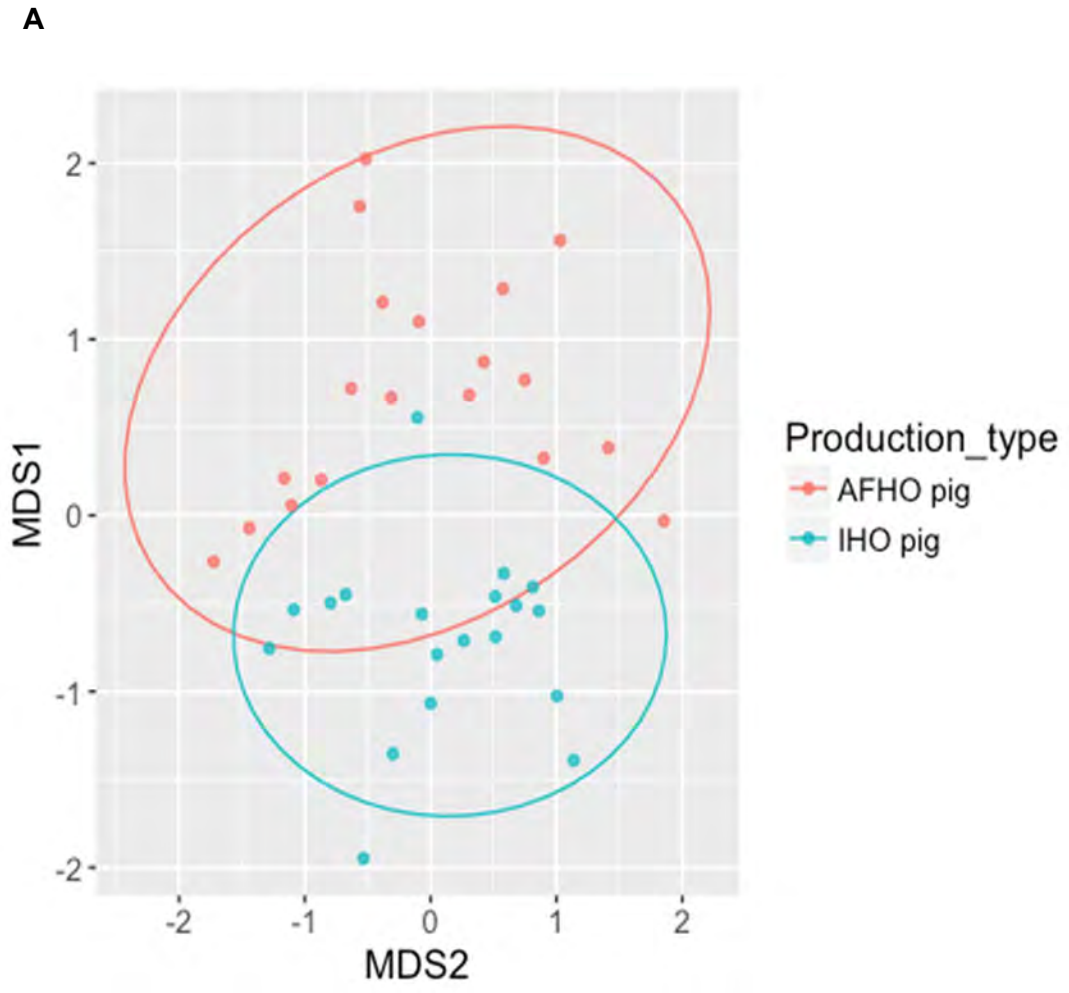
Figure 1. Relative abundance of the top 20 most abundant genera in 2-group comparisons (A-F).
Participant types represented by dots were colored as follows: IHO pigs (blue), AFHO pigs (red), IHO workers (purple) and AFHO workers (green). Panels A displays IHO and AFHO pigs within a non-metric dimensional scaling (NMDS) plot to compare differences in the microbial communities while maintaining accurate distance measures. Panel B displays an NMDS plot of all 4 exposure groups by mode of production and host species. Panels C, D, E and F display the top 20 most abundant genera for each 2-group comparisons (x-axis labels). The genera are displayed in descending order of relative abundance from bottom to top. IHO exposure groups were observed to carry greater abundances of pathogenic genera (signified by an asterisk). For example, *Staphylococcus* in more highly represented in both the IHO pig or IHO worker compared to their AFHO counterparts.



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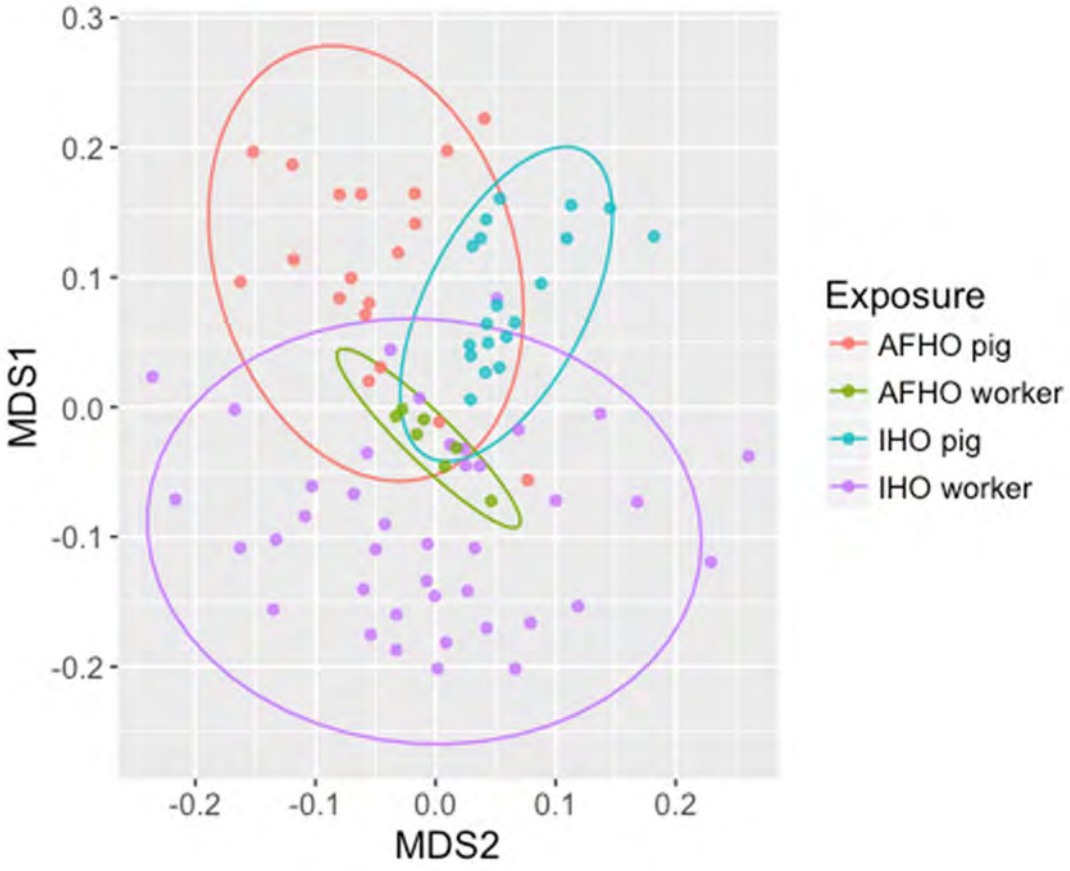
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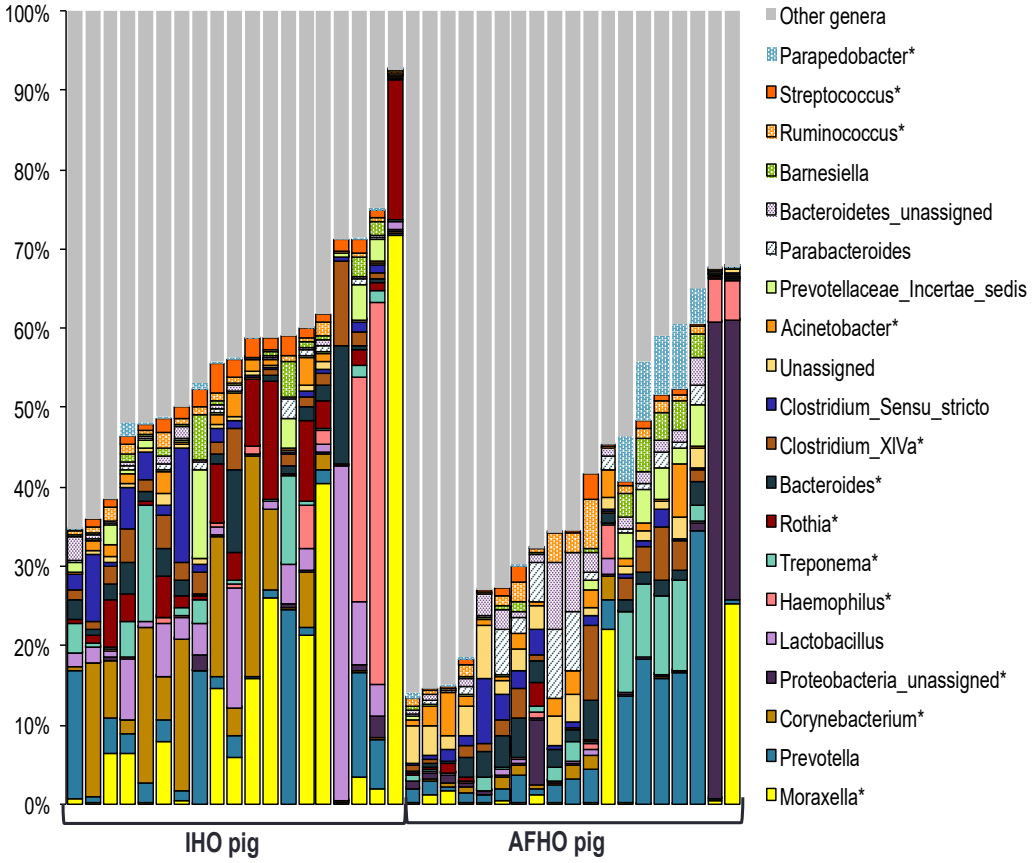
Exposure
AFHO pig
AFHO worker
IHO pig
IHO worker

C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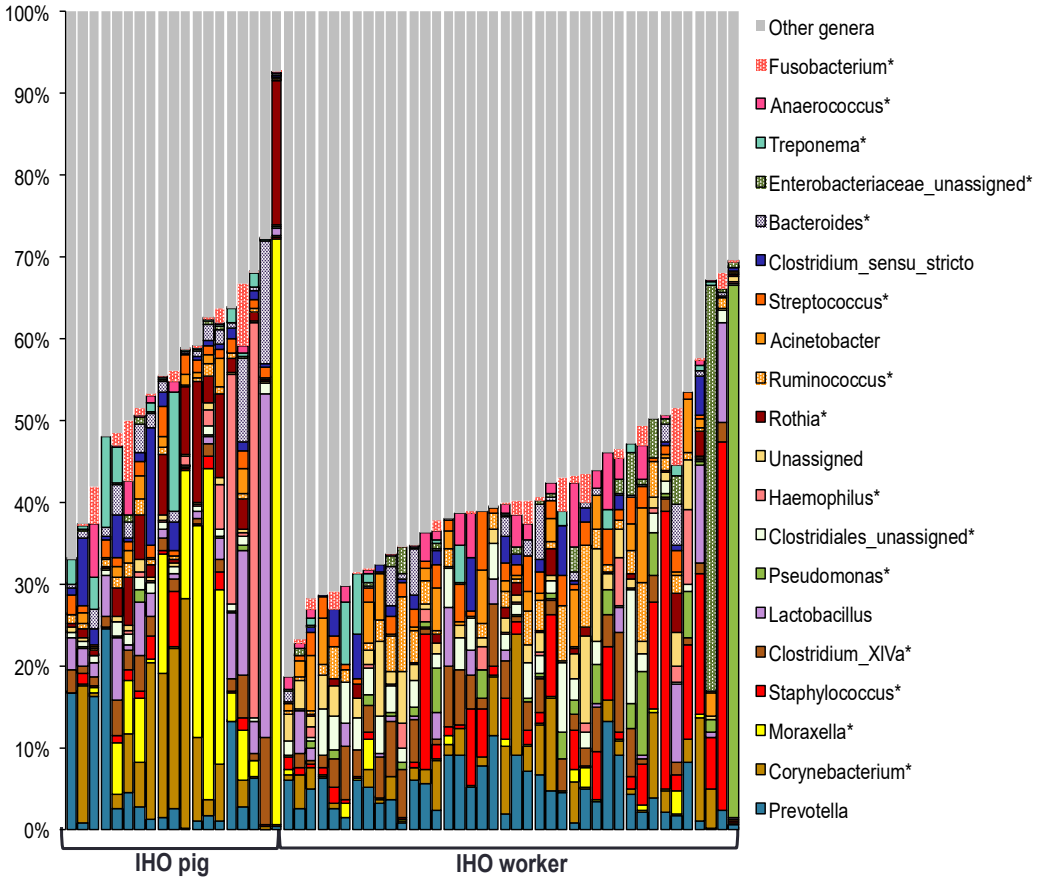
IHO pig
AFHO pig
Other genera
Parapedobacter*
Streptococcus*
Ruminococcus*
Barnesiella
Bacteroidetes_unassigned
Parabacteroides
Prevotellaceae_Incertae_sedis
Acinetobacter*
Unassigned
Clostridium_Sensu_stricto
Clostridium_XlVa*
Bacteroides*
Rothia*
Treponema*
Haemophilus*
Lactobacillus
Proteobacteria_unassigned*
Corynebacterium*
Prevotella
Moraxella*

D


100%
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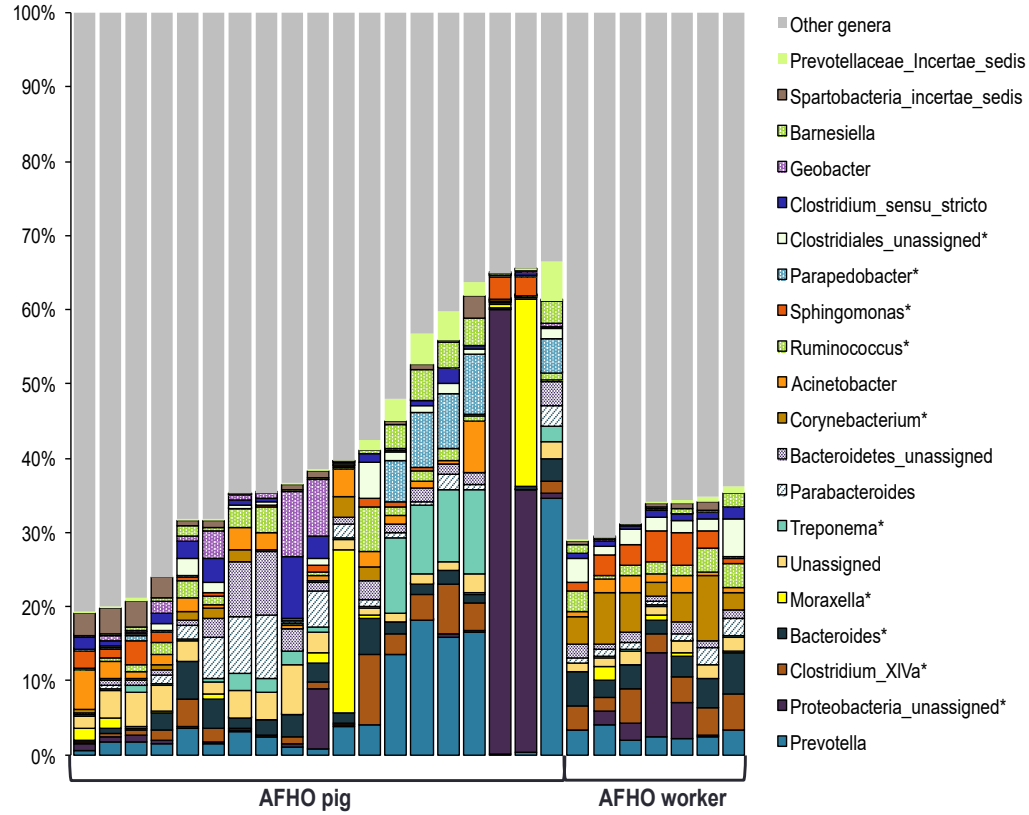
IHO pig
IHO worker
Other genera
Fusobacterium*
Anaerococcus*
Treponema*
Enterobacteriaceae_unassigned*
Bacteroides*
Clostridium_sensu_stricto
Streptococcus*
Acinetobacter
Ruminococcus*
Rothia*
Unassigned
Haemophilus*
Clostridiales_unassigned*
Pseudomonas*
Lactobacillus
Clostridium_XlVa*
Staphylococcus*
Moraxella*
Corynebacterium*
Prevotella

E


100%
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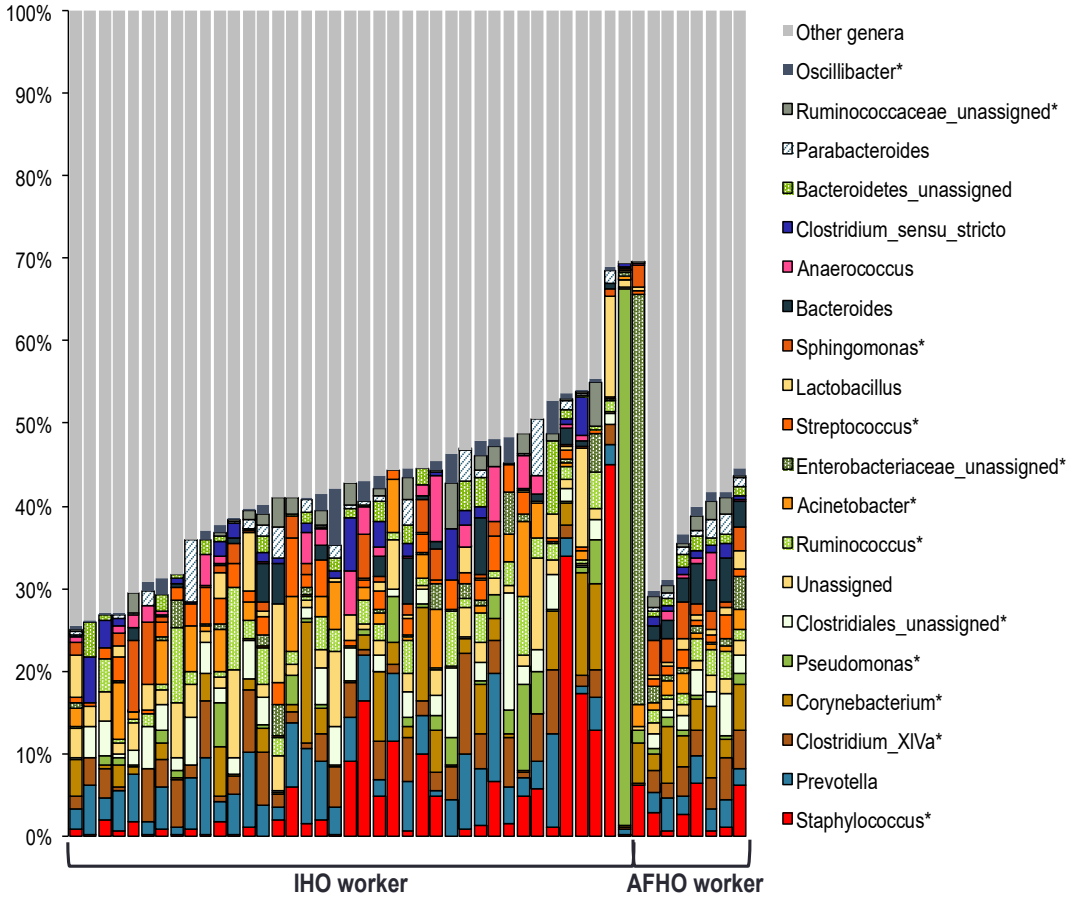
AFHO pig
AFHO worker
Other genera
Prevotellaceae_Incertae_sedis
Spartobacteria_incertae_sedis
Barnesiella
Geobacter
Clostridium_sensu_stricto
Clostridiales_unassigned*
Parapedobacter*
Sphingomonas*
Ruminococcus*
Acinetobacter
Corynebacterium*
Bacteroidetes_unassigned
Parabacteroides
Treponema*
Unassigned
Moraxella*
Bacteroides*
Clostridium_XlVa*
Proteobacteria_unassigned*
Prevotella

F


100%
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IHO worker
AFHO worker
Other genera
Oscillibacter*
Ruminococcaceae_unassigned*
Parabacteroides
Bacteroidetes_unassigned
Clostridium_sensu_stricto
Anaerococcus
Bacteroides
Sphingomonas*
Lactobacillus
Streptococcus*
Enterobacteriaceae_unassigned*
Acinetobacter*
Ruminococcus*
Unassigned
Clostridiales_unassigned*
Pseudomonas*
Corynebacterium*
Clostridium_XlVa*
Prevotella
Staphylococcus*

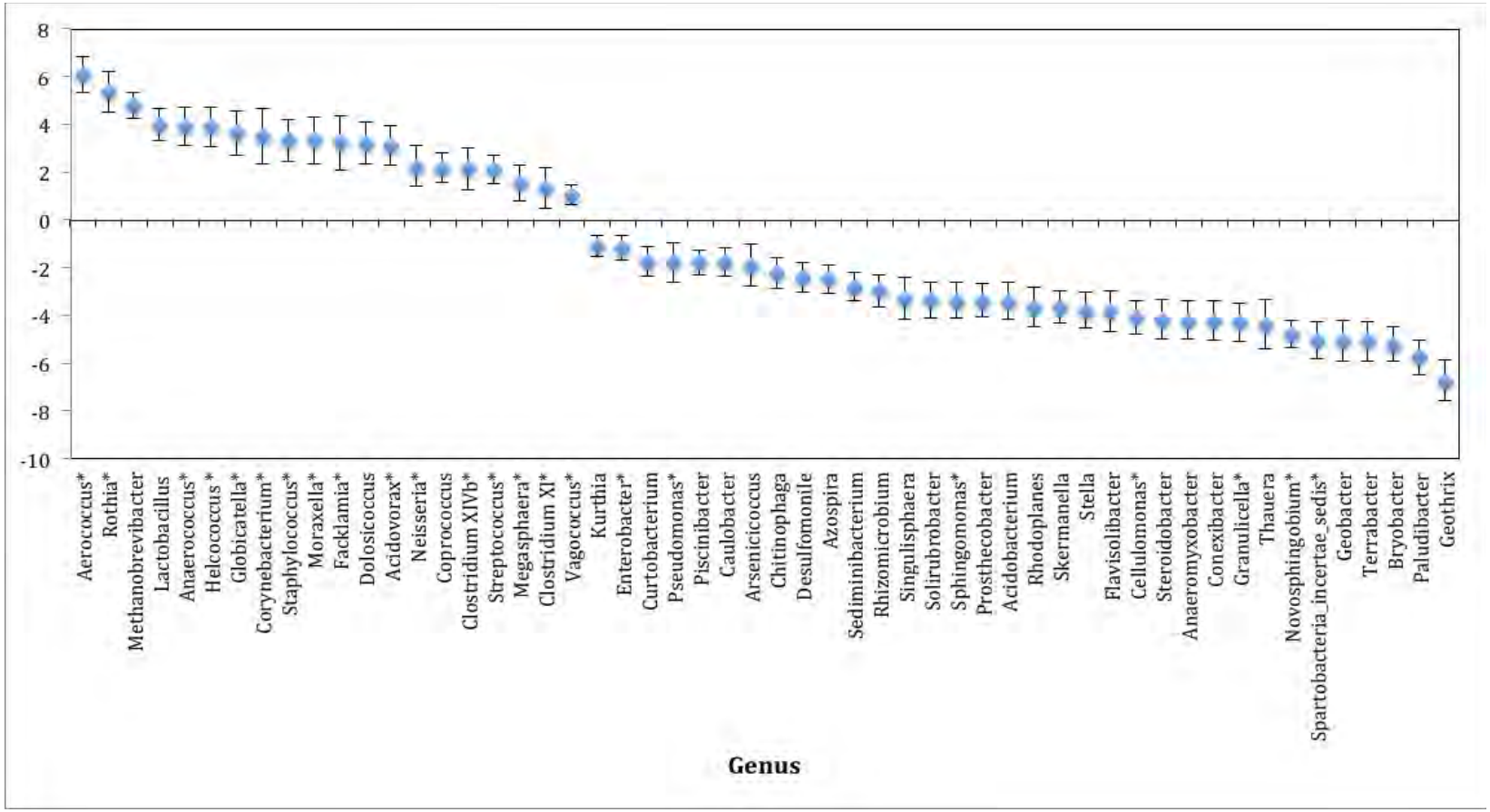


Figure 2: Log$_2$ fold-change in relative abundance of significantly different genera between pigs at industrial hog operations (IHO and antibiotic-free hog operations (AFHO).

Blue diamonds represent the mean log$_2$ fold change of significantly different genera relative abundance (FDR-corrected Kruskal-Wallis test) comparing the IHO pig to the AFHO pig. Error bars represent the standard errors. Point estimate above the zero line were found to be significantly associated with the IHO pig while those under the zero line were found to be significantly associated with the AFHO pig. Pathogenic genera (signified by an asterisk) were overrepresented in the IHO pig (16/53) vs. the AFHO pig (7/53). We observed common pig genera in AFHO pigs as well as genera found to be associated with soil environment microbiota, which may be due to the pasture-based practices on AFHOs.

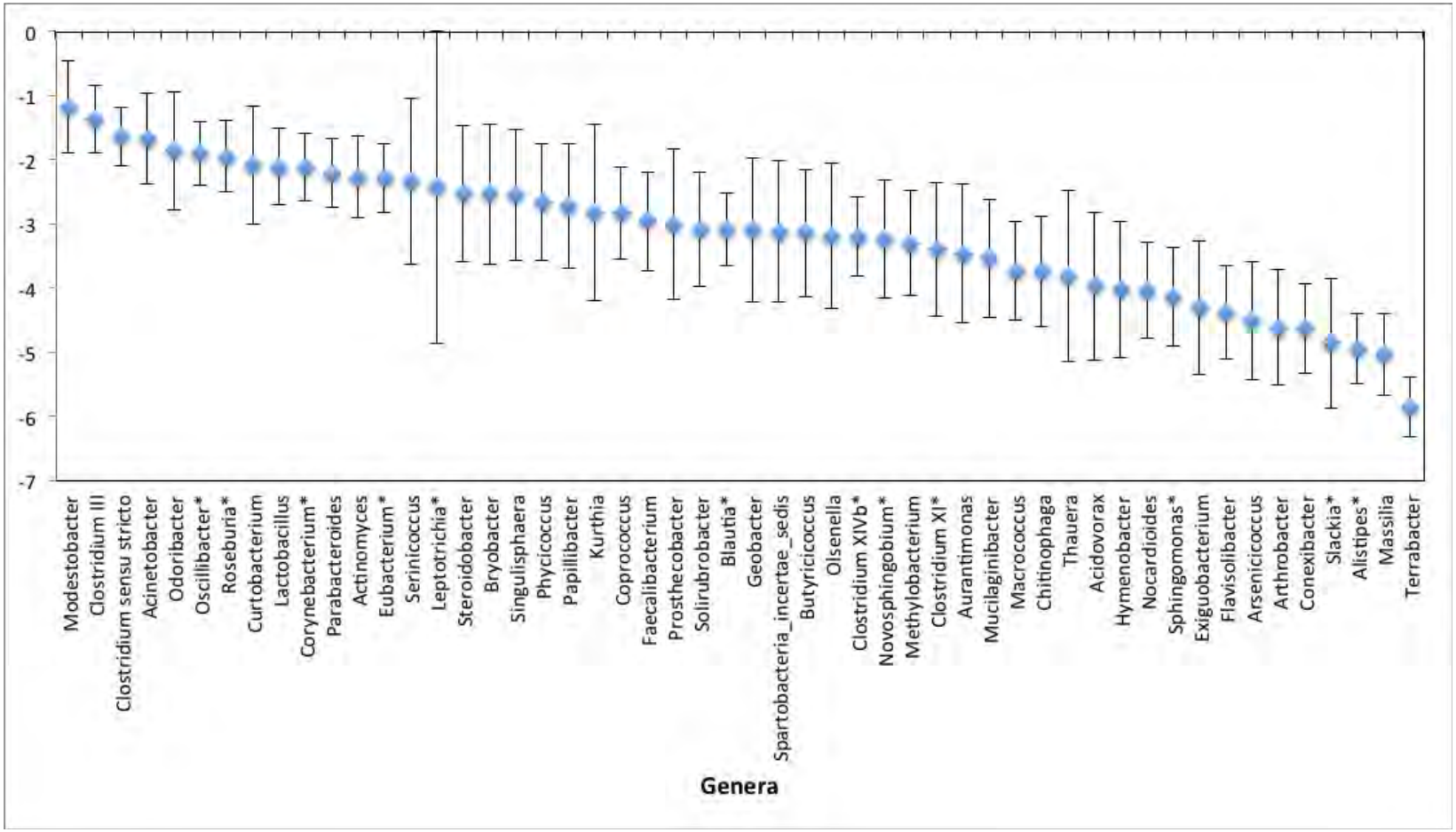


Figure 3: Log_2 fold-change in relative abundance of significantly different genera between pig workers at industrial hog operations (IHO and antibiotic-free hog operations (AFHO).

Blue diamonds represent the mean log_2 fold change of significantly different genera relative abundance (FDR-corrected Kruskal-Wallis test) comparing the IHO workers to AFHO workers. Error bars represent the standard errors. All significantly different OTUs between these two comparison groups were associated with greater relative abundance in the AFHO worker (represented below the zero line). Probable human pathogenic genera (signified by an asterisk) were overrepresented in the AFHO worker (12/52), which were all presumably sourced from swine fecal matter or gut microbiota (6) as well as soil microbiome (6).

Table 1. Characteristics of pigs and pig workers at IHO and AFHO, 2013-2015, North Carolina, USA.

	Overall	IHO	AFHO
Pigs			
Anatomical site, n (%)			
Nares	20 (50)	10 (50)	10 (50)
Perineum	20 (50)	10 (50)	10 (50)
Lifestage, n (%)			
Sow	12 (30)	2 (10)	4 (20)
Sow gestation	6 (15)	3 (15)	0 (0)
Piglet	15 (35)	4 (20)	2 (10)
Stock	4 (10)	0 (0)	2 (10)
Feeder	4 (10)	0 (0)	2 (10)
Type of barn, n (%)			
Farrowing barn	10 (50)	10 (50)	-
Nursery barn	4 (25)	4 (20)	-
Breeding barn	6 (30)	6 (30)	-
Workers			
Anatomical site, n (%)			
Nares	48 (100)	41 (85)	7 (15)
Male, n (%)	24 (50)	21 (51)	3 (43)
Race/ethnicity, n (%)			
Hispanic	41 (85)	41 (100)	0 (0)
African American	0 (0)	0 (0)	0 (0)
White	7 (15)	0 (0)	7 (100)
Age in years, mean (range)	38 (18-71)	38 (18-71)	34 (26-49)
Smoking, n (%)	8 (20)	4 (10)	4 (57)
Asthma, n (%)	1 (2)	1 (2)	0 (0)
***S. aureus* nasal carriage outcomes, n (%)**			
S. aureus	27 (56)	22(54)	5(71)
MDRSA	14 (29)	12(29)	2(29)
scn-negative *S. aureus*	12 (25)	12(29)	0(0)

Note. IHO, industrial hog operation. AFHO, antibiotic-free hog operation. MDRSA=multidrug-resistant *S. aureus*.

Characteristics presented in this table represent a subs-ample of the study populations from Nadimpalli et al., 2016[19], Hatcher et al., 2017[11] and Davis et al. ,2018[23].

Table 2. Differences in alpha diversity between IHO and AFHO pigs, by anatomical site, 2015, North Carolina, USA.

	IHO pig			AFHO pig		
	Nares	**Perineum**		**Nares**	**Perineum**	
	Mean (SD)	Mean (SD)	*p*-value[a]	Mean (SD)	Mean (SD)	*p*-value[a]
Alpha Diversity Measures						
Shannon	6.43 (1.65)	6.63 (1.65)	0.760	7.40 (2.01)	7.915 (0.90)	0.453
Phylogenetic distance	41.34 (8.87)	40.66 (7.26)	0.859	69.78 (25.20)	59.04 (18.40)	0.308
Observed OTUs	644.5 (194.0)	629.22 (169.6)	0.858	1342.1 (613.6)	958.44 (426.3)	0.136
Species evenness	0.19 (0.160)	0.176 (0.072)	0.822	0.194 (0.147)	0.394 (0.313)	0.088

Note. IHO, industrial hog operation. AFHO, antibiotic-free hog operation.

[a]*p*-values estimated from t-test comparing differences in alpha diversity measures within each of the modes of production, by anatomical site.

Table 3. Differences in alpha diversity between pigs and pig workers at IHOs and AFHOs, 2013-2015, North Carolina, USA.

	IHO pig	**AFHO pig**	**IHO worker**	**AFHO worker**				
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	*p*-value[a]	*p*-value[b]	*p*-value[c]	*p*-value[d]
Alpha Diversity Measures								
Shannon	6.44 (0.99)	7.60 (0.39)	6.24 (0.18)	8.61 (0.14)	0.033	0.001	0.620	0.055
Phylogenetic distance	40.2 (1.5)	65.0 (6.0)	17.6 (1.6)	42.4 (3.3)	0.001	0.001	<0.001	0.011
Observed OTUs	628 (27)	1167 (97)	210 (27)	626 (75)	0.000	0.040	<0.001	0.004
Species evenness	0.18 (0.03)	0.28 (0.06)	0.52 (0.03)	0.67 (0.07)	0.121	0.001	<0.001	<0.001

Note. IHO, industrial hog operation. AFHO, antibiotic-free hog operation. SD, standard deviation.

[a] *p*-values estimated from t-test comparing differences in alpha diversity measures overall of pigs (average of both pig anatomical sites) between IHO and AFHO pigs.

[b] *p*-values estimated from t-test comparing differences in alpha diversity measures between IHO workers and AFHO workers .

[c] *p*-values estimated from t-test comparing differences in alpha diversity measures between the IHO pigs and the IHO workers.

[d] *p*-values estimated from t-test comparing differences in alpha diversity measures between the AFHO pigs and the AFHO workers.

Table 4: Differences in beta diversity among pigs and pig workers, by mode of production (IHO vs. AFHO), 2013-2015, North Carolina, USA.

Comparison category	**Beta diversity metric**	***R* statistic**	***p-value***
IHO pig (combined) vs. AFHO pig (combined)	Weighted UniFrac	0.485	0.001
	Unweighted UniFrac	0.526	0.001
	Bray-Curtis	0.603	0.001
	Binary Jaccard	0.629	0.001
IHO pig (combined) vs. IHO worker			
	Weighted UniFrac	0.143	0.012
	Unweighted UniFrac	0.392	0.001
	Bray-Curtis	0.258	0.001
	Binary Jaccard	0.264	0.001
AFHO pig (combined) vs. AFHO worker	Weighted UniFrac	0.214	0.023
	Unweighted UniFrac	0.281	0.009
	Bray-Curtis	0.336	0.004
	Binary Jaccard	0.363	0.002
IHO worker nares vs. AFHO worker nares	Weighted UniFrac	-0.214	0.962
	Unweighted UniFrac	0.002	0.482
	Bray-Curtis	-0.113	0.839
	Binary Jaccard	-0.033	0.601

Note. IHO, industrial hog operation. AFHO, antibiotic-free hog operation. Combined, average of nares and perineum.

p-values estimated from R-statistic comparing beta diversity measures within our comparison categories.

Table 5. Relation of occupational swine production and personal exposure activities with pig contributions to and alpha diversity measures of the IHO worker's nasal microbiome, 2013-2015, North Carolina, USA.

	IHO worker				
	% pig contributions [a]	**Shannon diversity**	**Phylogenetic distance**	**Observed OTUs**	**Species evenness**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Demographics					
Age (per year)	0.33 (-0.02, 0.68)	**0.03 (0.001, 0.06)**	0.21 (-0.03, 0.45)	3.74 (-0.71, 8.20)	0.001 (-0.001, 0.003)
Sex (male as referent group)	-4.77 (-14.50, 4.97)	-0.32 (-0.96, 0.32)	-4.89 (-10.6, 0.79)	-82.3 (-181, 16.0)	0.03 (-0.07, 0.13)
Swine work history					
Years worked at any swine farm (per year)	**0.92 (0.01, 1.83)**	**0.08 (0.02, 0.14)**	**0.64 (0.07, 1.20)**	**10.5 (1.02, 20.0)**	0.002 (-0.006, 0.011)
Occupation exposures					
Hours of direct contact per week (per 8-hour shift)	**3.65 (0.12, 7.18)**	**0.22 (-0.07, 0.50)**	**3.05 (1.23, 4.87)**	**51.7 (220, 81.4)**	0.01 (-0.03, 0.04)
Time since last work shift (per 8-hour shift)	0.09 (-0.84, 1.02)	**0.10 (0.03, 0.17)**	**1.00 (0.55, 1.46)**	**19.5 (12.1, 26.9)**	-0.01 (-0.02, 0.01)
Mask usage[b]	-2.44 (-9.83, 4.95)	-0.14 (-0.61, 0.33)	-1.92 (-6.62, 2.33)	-31.8 (-108, 44.8)	-0.01 (-0.07, 0.04)
Gave pigs antibiotics (Yes/No)	1.61 (-11.7, 14.9)	0.41 (-0.34, 1.15)	0.04 (-7.03, 7.11)	-7.32 (-133, 118)	0.06 (-0.05, 0.17)
Handled dead pigs (Yes/No)	**-0.50 (-9.70, -8.77)**	-0.11 (-0.95, 0.74)	2.20 (-5.46, 9.87)	38.4 (-96.2, 173)	-0.08 (-0.17, 0.16)
Drew pig blood (Yes/No)	12.0 (-18.8, 42.8)	**0.75 (0.08, 1.41)**	7.01 (-5.18, 19.20)	12 (-111, 355)	0.02 (-1.56, 0.19)
Lifestages working with (per 500 animals)					
Sows	0.26 (-3.69, 4.21)	-0.06 (-0.19, 0.07)	-0.98 (-1.99, 0.04)	-17.2 (-36.1, 1.76)	0.01 (-0.004, 0.03)
Farrowing pigs	-1.12 (-2.65, 0,43)	-0.03 (-0.11, 0.05)	-0.78 (-1.50, -0.06)	-12.5 (-25.7, 0.72)	0.01 (0.001, 0.02)
Weaned piglets	**8.01 (0.49, 15.5)**	**-0.61 (-1.17, -0.06)**	**-3.25 (-5.41, -1.10)**	**-46.4 (-84.5, -8.33)**	-0.07 (-0.18, 0.04)
Nursery piglets	-0.09 (-0.76, 0.58)	**0.11 (0.07, 0.15)**	**0.86 (0.60, 1.12)**	**13.0 (8.11, 17.9)**	0.01 (-0.001, 0.01)
Feeder pigs	**80.4 (53.1,108)**	**6.23 (4.87, 7.59)**	**38.1 (26.8, 49.5)**	**675 (464, 885)**	**0.28 (0.10, 0.47)**
Finisher pigs	-0.87 (-2.02, 0.27)	-0.06 (-0.10, 0.02)	**1.28 (0.43, 2.13)**	**23.7 (8.94, 38.4)**	**-0.03 (-0.04, -0.02)**

Hygienic practices					
Frequency of hand washing (every 2 addtl. washes)	0.87 (-9.21, 10.95)	0.12 (-0.53, 0.77)	-2.07 (-10.1, 5.96)	-32.7 (-173, 108)	0.07 (-0.12, 0.25)
Showered after work[b]	-6.65 (-15.1, 1.84)	-0.29 (-0.71, 0.14)	-1.90 (-5.44, 1.63)	**-66.8 (-132, -1.55)**	-0.13 (-0.07, 0.04)
Changed clothes after work[b]	-7.66 (-16.36, 1.04)	-0.30 (-0.75, 0.15)	-2.07 (-5.78, 1.64)	**-70.5 (-139, -2.24)**	-0.01 (-0.07, 0.05)
Personal exposures					
Number of household members	3.34 (-1.01, 7.69)	0.03 (-0.27, 0.23)	0.83 (-1.62, 3.29)	14.7 (-30.4, 59.7)	-0.02 (-0.05, 0.01)
Household pet (Yes/No)	-0.95 (-11.0, 9.07)	0.09 (-0.57, 0.75)	4.60 (-1.23, 10.43)	74.1 (-26.7, 175)	-0.06 (-0.17, 0.05)
Current Smoker (Yes/No)	23.4 (-2.25, 49.0)	0.07 (-0.99, 1.13)	2.82 (-12.47, 18.11)	73.3 (-203, 350)	-0.05 (-0.22, 0.11)
Personal antibiotic use within last 3 months (Yes/No)	10.9 (-31.4, 53.2)	0.01 (-1.11, 1.13)	5.74 (-11.43, 22.90)	120 (-199, 440)	-0.09 (-0.25, 0.07)
***S. aureus* outcomes**					
S. aureus (Yes/No)	5.69 (-0.73, 12.11)	**0.71 (0.08, 1.33)**	**8.67 (3.32, 14.0)**	**150 (59.1, 241)**	0.01 (-0.09, 0.11)
MDRSA (Yes/No)	**9.11 (1.88, 16.4)**	0.80 (-0.04, 1.64)	**13.0 (6.96, 19.0)**	**230 (123, 336)**	-0.05 (-0.20, 0.10)
scn negative (Yes/No)	**9.85 (2.95, 16.8)**	0.76 (-0.07, 1.59)	**10.2 (4.39, 16.1)**	**177 (75.0, 279)**	-0.01 (-0.16, 0.13)
SourceTracker					
Pig contributions (%)[a]	--	**0 .03 (0.02-0.05)**	**0.28 (0.12, 0.44)**	**5.12 (2.09, 8.16)**	0.001 (-0.004, -0.005)

Note. IHO, industrial hog operation. CI, confidence interval. Beta (95% CI) estimated from linear regression models, adjusted for the study in which individuals participated.

[a]% pig contributions defined as the percentage of OTUs contributed to the worker from the pig's total microbiome (nares and perineum) estimated via SourceTracker tool in QIIME -- SourceTracker is designed to predict the source (IHO or AFHO pig) of microbial communities in a set of sink samples (IHO or AFHO worker)

[b]Reported as: 0 = Always (≥ 80%), 1 = Sometimes (11-79%); 2 = Never (0-10%)

Dashes indicate an insufficient number of observations for that work activity.

Table 6. Relation of occupational swine production and personal exposure activities with pig contributions to and alpha diversity measures of the AFHO worker's nasal microbiome, 2013-2015, North Carolina, USA.

	AFHO worker				
	% pig contributions [a] β (95% CI)	**Shannon diversity β (95% CI)**	**Phylogenetic distance β (95% CI)**	**Observed OTUs β (95% CI)**	**Species evenness β (95% CI)**
Demographics					
Age (per year)	0.08 (-0.2, 0.19)	0.01 (-0.02, 0.04)	0.75 (0.21, 1.29)	**17.8 (5.95, 29.7)**	**-0.14 (-0.03, -0.002)**
Sex (male as referent group)	-1.15 (-3.06, 0.75)	0.09 (-0.54, 0.72)	-0.13 (-14.59, 14.33)	-22.8 (-34.4, 302)	0.01 (-0.25, 0.27)
Swine work history					
Years worked at any swine farm (per year)	0.02 (-0.09, 0.12)	0.01 (-0.02, 0.05)	1.03 (0.48, 1.58)	**22.9 (12.0, 35.9)**	-0.01 (-0.04, 0.01)
Occupation exposures					
Hours of direct contact per week (per 8-hour shift)	--	--	--	--	--
Time since last work shift (per 8-hour shift)	**-3.66 (-7.90, 0.58)**	**-0.43 (-.1.31, 0.44)**	**-19.8 (-58.0, 18.4)**	**-425 (-1299, 448)**	0.498 (-0.16, 1.15)
Mask usage[b]	0.92 (-0.90, 2.74)	0.40 (0.02, 0.78)	11.5 (0.28, 22.8)	249 (-2.92, 501)	-0.12 (-0.44, 0.19)
Gave pigs antibiotics (Yes/No)	1.38 (-0.20, 2.95)	-0.09 (-0.69, 0.50)	2.53 (-8.29, 13.4)	103 (-140, 346)	-0.09 (-0.36, 0.18)
Handled dead pigs (Yes/No)	0.39 (-0.90, 1.67)	0.07 (-0.37, 0.52)	5.41 (-4.08, 14.9)	110 (-95.2, 315)	-0.04 (-0.29, 0.21)
Drew pig blood (Yes/No)	--	--	--	--	--
Lifestages working with (per 500 animals)					
Sows	250 (-225, 726)	-101 (-183, -18.1)	86.6 (-2158, 2331)	8794 (-47515, 65103)	**-55.7 (-86.9, -24.5)**
Farrowing pigs	--	--	--	--	--
Weaned piglets	--	--	--	--	--
Nursery piglets	--	--	--	--	--
Feeder pigs	**-42.1 (-64.9, -19.3)**	-8.28 (-18.5, 1.94)	**-321 (-410, -232)**	**-7507 (-9096, -5918)**	**5.55 (2.87, 8.23)**

Finisher pigs	**-69.54 (-122, -16.9)**	**-20.0 (-34.3, -5.69)**	**-712 (877, 548)**	**-16021 (-19744, -12298)**	**11.4 (9.12, 13.69)**
Hygienic practices					
Frequency of hand washing (every 2 addtl. washes)	**-1.26 (-1.70, -0.81)**	-0.14 (-0.49, 0.21)	-5.65 (-12.1, 0.78)	**-147 (-270, -25.0)**	**0.12 (0.03, 0.20)**
Showered after work[b]	--	--	--	--	--
Changed clothes after work [b]	--	--	--	--	--
Personal exposures					
Number of household members	-0.30 (-1.12, 0.52)	0.11 (-0.05, 0.28)	1.65 (-3.97, 7.28)	33.1 (-100, 166)	0.01 (-0.12, 0.15)
Household pet (Yes/No)	--	--	--	--	--
Current Smoker (Yes/No)	-1.17 (-2.86, 0.53)	-0.39 (-0.86, 0.08)	-7.31 (-19.3, 4.70)	-191 (-448, 65.7)	0.04 (-0.22, 0.29)
Personal antibiotic use within last 3 months (Yes/No)	--	--	--	--	--
***S. aureus* outcomes**					
S. aureus (Yes/No)	0.39 (-0.90, 1.67)	0.07 (-0.38, 0.52)	5.41 (-4.08, 14.9)	110 (-95.2, 315)	-0.04 (-0.29, 0.21)
MDRSA (Yes/No)	1.32 (-0.41, 3.04)	-0.17 (-0.57, 0.23)	7.64 (-1.11, 16. 4)	188 (-2.90, 379)	-0.28 (-0.39, -0.17)
scn negative (Yes/No)	--	--	--	--	--
SourceTracker					
Pig contributions (%)[a]	--	0.10 (-0.20, 0.40)	4.80 (0.32, 9.29)	121 (33.0, 209)	-0.09 (-0.16, -0.03)

Note. IHO, industrial hog operation. CI, confidence interval. Beta (95% CI) estimated from linear regression models, adjusted for the study in which individuals participated.

[a]% pig contributions defined as the percentage of OTUs contributed to the worker from the pig's total microbiome (nares and perineum) estimated via SourceTracker tool in QIIME -- SourceTracker is designed to predict the source (IHO or AFHO pig) of microbial communities in a set of sink samples (IHO or AFHO worker)

[b]Reported as: 0 = Always (≥ 80%), 1 = Sometimes (11-79%); 2 = Never (0-10%)

Dashes indicate an insufficient number of observations for that work activity.

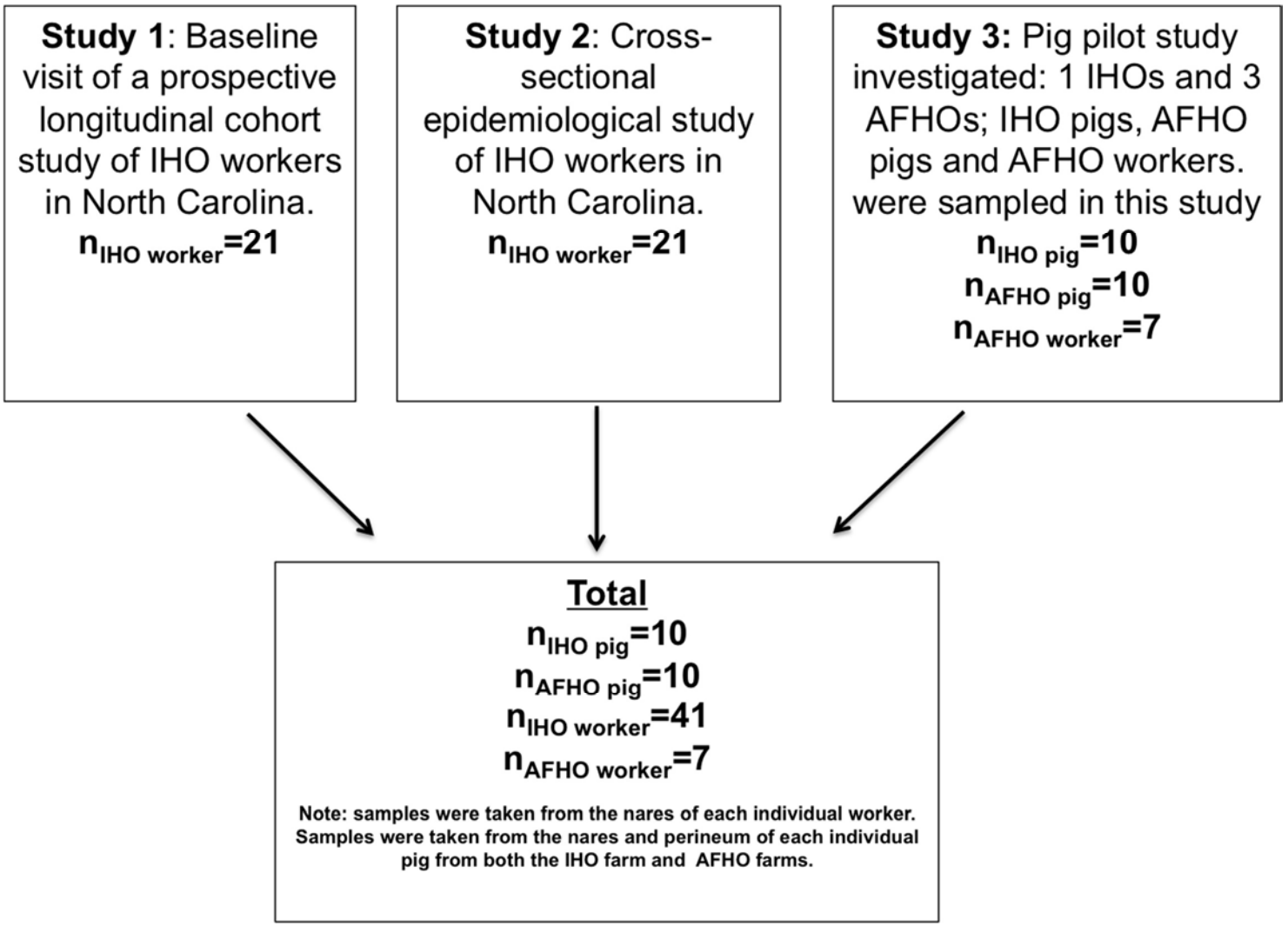
Table 7. Correlation of personal exposures and occupational swine production activities with nasal microbiome Bray-Curtis beta diversity, 2013-2015, North Carolina, USA.

	IHO worker R^2 (p-value)	AFHO worker R^2 (p-value)
Demographics		
Age (per year)	0.03 (0.135)	0.17 (0.269)
Sex (male as referent group)	0.03 (0.224)	0.16 (0.600)
Swine work history		
Years worked at any swine farm (per year)	0.54 (0.263)	0.16 (0.799)
Occupation exposures		
Hours of direct contact per week (per additional hour)	**0.03 (0.015)**	0.31 (0.847)
Time since last work shift (per additional hour)	**0.70 (0.036)**	0.51 (0.324)
Mask usage[a]	**0.03 (0.007)**	0.33 (0.550)
Gave pigs antibiotics (Yes/No)	0.02 (0.730)	0.34 (0.272)
Handled dead pigs (Yes/No)	0.02 (0.950)	0.17 (0.436)
Drew pig blood (Yes/No)	**0.06 (0.002)**	--
Hygienic practices		
Frequency of hand washing (for every 2 addtl. washes)	0.08 (0.351)	0.19 (0.100)
Showered after work[c]	0.03 (0.289)	0.17 (0.434)
Changed clothes after work [c]	**0.06 (0.044)**	--
Personal exposures		
Number of household members	0.02 (0.673)	0.15 (0.824)
Household pet (Yes/No)	0.03 (0.434)	0.17 (0.311)
Current Smoker (Yes/No)	0.05 (0.146)	0.15 (0.939)
Personal antibiotic use within last 3 months (Yes/No)	**0.06 (0.043)**	0.17 (0.277)
***S. aureus* outcomes**		
S. aureus (Yes/No)	0.03 (0.083)	0.17 (0.435)
MDRSA (Yes/No)	**0.03 (0.005)**	0.17 (0.276)

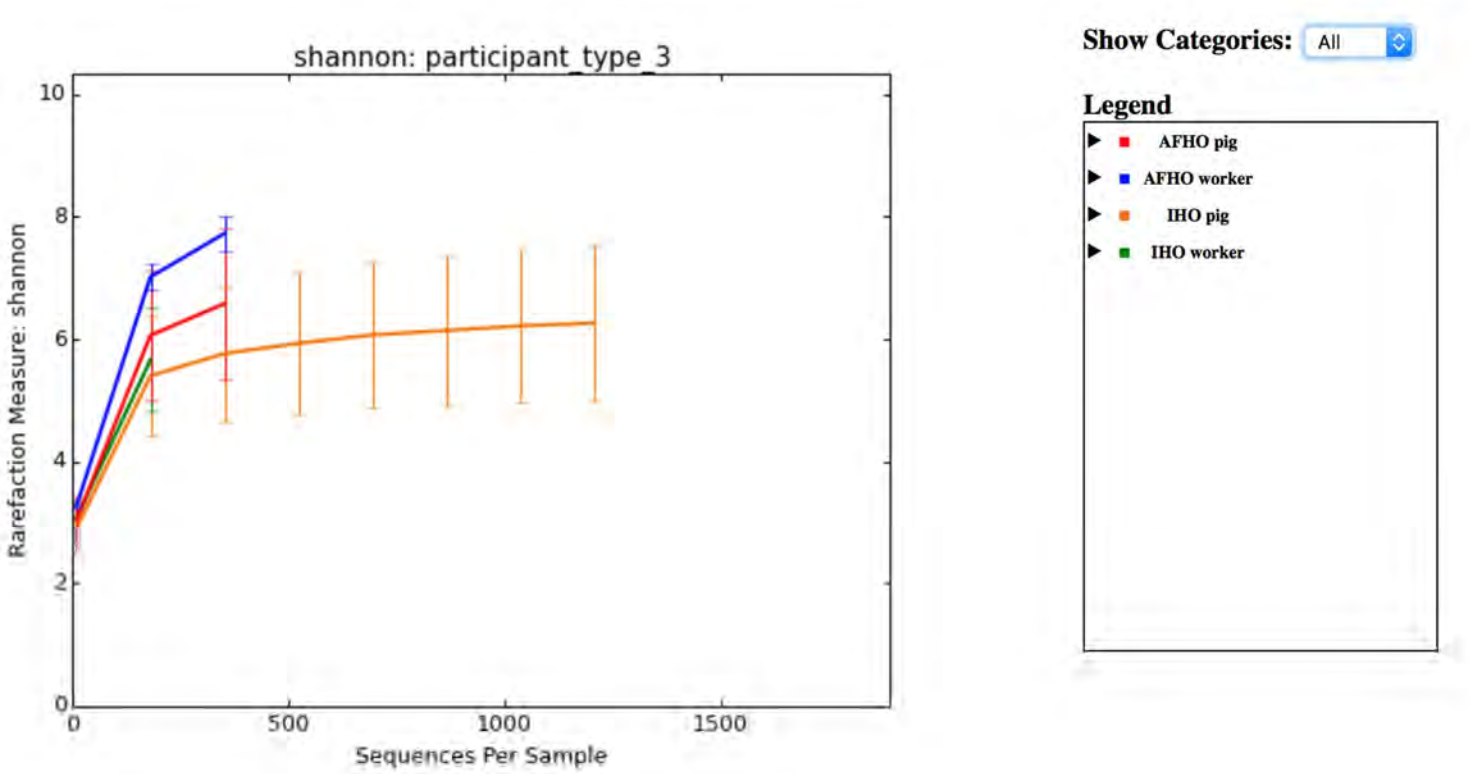
scn negative (Yes/No)	**0.03 (0.012)**	--
SourceTracker		
% pig contributions [b]	**0.03 (0.002)**	0.67 (0.492)

Note. IHO, industrial hog operation. AFHO, antibiotic-free hog operation.

[a]Reported as: 0 = Always (≥ 80%), 1 = Sometimes (11-79%); 2 = Never (0-10%)
[b] % pig contributions defined as the percentage of OTUs contributed to the worker from the pig's total microbiome (nares and perineum) estimated via SourceTracker tool in QIIME -- SourceTracker is designed to predict the source (IHO or AFHO pig) of microbial communities in a set of sink samples (IHO or AFHO worker)
[c]Reported as: 0 = Always, 1 = Sometimes, 2 = Never



Supplemental Materials Figure S1. Sample selection for microbiome sequencing. *Note.* IHO, industrial hog operation. AFHO, antibiotic-free hog operation. Diagram outlines samples selected from parents studies for microbiome sequencing.



Supplemental Materials Figure S2. Rarefaction curve of Shannon diversity across all participant types. Panel displays that IHO pig have adequate coverage. AFHO pigs and AFHO workers are approach adequate depth of coverage. IHO workers did not reach asymptote however we are confident in our results due to sample size. *Note.* IHO, industrial hog operation. AFHO, antibiotic-free hog operation.

Supplemental Materials Table S1: Differences in alpha diversity between IHO and AFHO pigs, by anatomical site, 2013-2015, North Carolina: USA.

	Nares			Perineum		
	IHO pig	**AFHO pig**		**IHO pig**	**AFHO pig**	
	Mean (SD)	Mean (SD)	p-value[a]	Mean (SD)	Mean (SD)	p-value[a]
Alpha Diversity Measures						
Shannon	6.43 (0.521)	7.40 (0.635)	0.255	6.63 (0.34)	7.96 (0.299)	0.010
Phylogenetic distance	41.34 (2.81)	69.78 (7.70)	0.003	40.66 (2.42)	59.04 (6.13)	0.013
Observed OTUs	644.5 (61.35)	1342.1 (194.04)	0.003	629.22 (56.2)	958.44 (142.1)	0.047
Species evenness	0.189 (0.051)	0.194 (0.046)	0.951	0.176 (0.024)	0.394 (0.104)	0.059

Note. IHO, industrial hog operation. AFHO, antibiotic-free hog operation.
[a]p-value estimated from t-test comparing differences in alpha diversity measures of the IHO pig and AFHO pig, by anatomical site.

Supplemental Materials Table S2. Differences in beta diversity among pigs and workers, by mode of hog production (IHO vs. AFHO), 2013-2015, North Carolina, USA.

Comparison category	Beta diversity metric	*R* statistic	*p-value*
IHO pig nares vs. AFHO pig nares	Weighted UniFrac	0.638	0.001
	Unweighted UniFrac	0.685	0.001
	Bray-Curtis	0.789	0.001
	Binary Jaccard	0.819	0.001
IHO pig perineum vs. AFHO pig perineum	Weighted UniFrac	0.396	0.002
	Unweighted UniFrac	0.415	0.002
	Bray-Curtis	0.477	0.001
	Binary Jaccard	0.474	0.001
IHO pig nares vs. IHO worker nares	Weighted UniFrac	0.083	0.216
	Unweighted UniFrac	0.260	0.002
	Bray-Curtis	0.128	0.083
	Binary Jaccard	0.142	0.068
IHO pig perineum vs. IHO worker nares	Weighted UniFrac	0.116	0.144
	Unweighted UniFrac	0.393	0.001
	Bray-Curtis	0.190	0.026
	Binary Jaccard	0.185	0.047
AFHO pig nares vs. AFHO worker nares	Weighted UniFrac	0.502	0.001
	Unweighted UniFrac	0.581	0.001
	Bray-Curtis	0.618	0.002
	Binary Jaccard	0.618	0.001
AFHO pig perineum vs. AFHO worker nares	Weighted UniFrac	0.308	0.009
	Unweighted UniFrac	0.272	0.021
	Bray-Curtis	0.331	0.006
	Binary Jaccard	0.336	0.003
IHO worker nares vs. AFHO worker nares	Weighted UniFrac	-0.214	0.962
	Unweighted UniFrac	0.002	0.482
	Bray-Curtis	-0.113	0.839
	Binary Jaccard	-0.033	0.601

Note. IHO, industrial hog operation. AFHO, antibiotic-free hog operation.

Supplementary Materials Figure S3. OTU contributions to the pig worker's nasal microbiome by the pig nares and/or perineum anatomical site, 2013-2015, North Carolina, USA.

Source population	**OTUs contributed to the IHO worker by the IHO pig**
IHO pig nares	-*Janibacter* (unclassified species) -*Acinetobacter* (unclassified species) -Unclassified genera with similarity to *Staphylococcus hyicus* -*Abiotrophia defectiva* among others -85 additional OTUs not listed here)
IHO pig perineum	-*Lactobacillus* (*gasseri, hominis, johnsonii* or *taiwanensis)* -*Parabacteroides distasonis* -*Facklamia tabacinasalis* -*Clostridium (baratii* or *sardiniense)* -*Staphylococcus (carnosus, condiment, haemolyticus, piscifermentans,* or *simulans)* -*Anaerococcus (prevotti* or *tetradius).*
Source population	**OTUs contributed to the AFHO worker by the AFHO pig**
AFHO pig nares	-*Acinetobacter (bouvetti, johnsonii, junii or schingleri).*
AFHO pig perineum	-Acinetobacter (*calcoaceticus, nosocomialis, pittii or rhizosphaerae)* -*Acinetobacter brisouii* -*Aggregatibacter segnis* -*Aeromonas (allosaccharophila, aquariorum, bivalvium, caviae, hydrophilajandaei, media, molluscorum, piscocola, popoffi, rivuli,salmonicia, sobria, veronni).*

Note. IHO, industrial hog operation. AFHO, antibiotic-free hog operation.
IHO pig bacterial taxa contributions that are greater than or equal to 1% of relative abundance.

Chapter Four: *S. aureus* nasal carriage outcomes and the nasal microbiome of industrial hog operation workers, community residents, and children living in their households: North Carolina, USA

ABSTRACT

Background: The impact of direct (occupational) and indirect (household or community) exposure to industrial hog operations (IHOs) and *S. aureus* nasal carriage outcomes, including livestock-associated (LA) and antimicrobial-resistant (AMR) strains, on the human nasal microbiome remains unclear.

Objectives: To investigate differences in nasal microbiome diversity and composition among adults with versus without occupational exposure and children living in their households.

Methods: Nasal samples of IHO workers and community resident (CR) adults and children living in their households were sequenced targeting the V4 region of the 16S rRNA gene. We assessed differences in alpha (Shannon diversity, observed OTUs, and phylogenetic distance) and beta (unweighted UniFrac and Bray Curtis) diversity, relative abundance and presence/absence of genera by participant type and *S. aureus* nasal carriage outcomes (*S. aureus*, *scn*-negative *S. aureus* [marker of livestock association], and multidrug-resistant *S. aureus* [MDRSA]). Linear regression models and non-parametric Adonis methods were employed to estimate associations of personal, household, and occupational characteristics and *S. aureus* nasal carriage outcomes with changes in alpha diversity, beta diversity, and bacterial contributions from IHO pigs (source) to human participant groups (sink).

Results. Intensive pig contact, all *S. aureus* nasal carriage outcomes (*S. aureus*, MDRSA, and *scn*-negative *S. aureus*), and bacterial contributions from the IHO pig were positively associated with alpha diversity and altered beta diversity among IHO workers. Decreases in alpha diversity among children living in IHO worker households, CR adults and their

children were associated with MDRSA and *scn*-negative *S. aureus* nasal carriage positivity. Greater bacterial contributions from IHO pigs decreased alpha diversity and altered beta diversity among children living in IHO worker households, CR adults and their children.

Conclusion. Results suggest IHO work exposures and AMR and LA-*S. aureus* strains may alter the nasal microbiome structure of IHO workers while percent IHO pig bacterial taxa contributions may alter the nasal microbiome structure of indirectly exposed individuals (IHO children, CR adults and CR children).

INTRODUCTION

S. aureus, a commensal Gram-positive bacterium, colonizes the nares, oropharynx, and/or skin of roughly one third of the general U.S. population.[1,2] *S. aureus* strains may be categorized according to antimicrobial susceptibility phenotypes or genotypes (*e.g.* methicillin-sensitive *S. aureus* [MSSA], methicillin-resistant *S. aureus* [MRSA], and multidrug resistant *S. aureus* [MDRSA]). Antibiotic-resistant (ABR) *S. aureus* was once limited to hospital settings in association with antibiotic use for the treatment of illnesses and infections.[3] However, in recent decades, the epidemiology of ABR *S. aureus* has shifted from hospital-associated (HA-) to community-associated (CA-) *S. aureus*.[4,5] Within CA-*S. aureus*, livestock-associated (LA) *S. aureus*, including LA-MRSA, have emerged in livestock (particularly pig) workers, and among community residents who live in close proximity to high-density livestock production.[5–10] Such LA-*S. aureus* strains have been characterized using several markers: clonal complex (CC) 398, CC9*,* tetracycline resistance, and absence of the human immune evasion cluster gene *scn*.[11–14] Increased prevalence of LA-*S. aureus* nasal carriage, including MRSA and MDRSA, has also been observed among children living in households with adults who have occupational exposure to IHOs.[5,15–21]

Although the global epidemiology of LA-*S. aureus* strains is evolving, it remains unclear how the changes in livestock production impacts human nasal colonization with emerging LA-*S. aureus* strains and how such strains may interact with other members of the nasal bacterial community (microbiome). It is also unclear whether specific occupational exposure activities related to livestock production, specifically production

of pigs in the IHO settings,[7,8,22] can influence LA-*S. aureus* exposure through contributions of livestock-associated microorganisms to human hosts.

In this study we determined the influence of IHO occupational exposure activities and *S. aureus* nasal carriage on the nasal microbiome of the IHO workers, their child household members, and community resident (CR) adult and their child household members.

METHODS

Please refer to the detailed methodology (Chapter two) for DNA extraction and amplification, library preparation and sequencing, bioinformatics sequence processing, taxonomic assignments, and OTU contamination removal methods.

Statistical analysis

Following pre-processing, singletons were filtered prior to downstream analysis. Operational taxonomic units (OTUs) found within field blanks, trip blanks, laboratory processing controls, and DNA negative controls were filtered from sequence data to remove contaminant OTUs prior to downstream analysis.

Analysis of alpha diversity by participant type

The following alpha (within sample) diversity measures were calculated using MacQIIME 1.9.1: 1) Shannon diversity (a measure of overall bacterial diversity, taking into account OTU richness and evenness), 2) observed OTUs, and 3) phylogenetic

distance (the diversity of phylogenetic lineages represented in OTUs). Differences in alpha diversity measures were determined using the Student's t-test between the following groups: 1) IHO workers and their children, 2) CR adults and their children, 3) IHO workers and CR adults, 4) IHO worker's children and CR adult's children.

Analysis of beta diversity by participant type

We used the DESeq2 variance stabilization tool within MacQIIME to generate normalized beta diversity distance measures (unweighted UniFrac and Bray Curtis) of bacterial community membership and composition. Beta diversity differences were investigated using a nonparametric analysis of similarity (ANOSIM) test by participant type. Bray-Curtis, a beta diversity measure taking into account both community membership (what bacteria are present) and composition (how much of each bacteria are present), was used to generate non-metric dimensional scaling (NMDS) plots within R Studio (R Studio, Inc. Boston, MA).[23]

Analysis of alpha and beta diversity differences by S. aureus *nasal carriage outcomes within participant types*

The Student's t-test was used to investigate alpha and beta diversity differences by *S. aureus* nasal carriage outcomes (*S. aureus*, MDRSA, and *scn*-negative *S. aureus*) within each participant group (i.e., IHO workers, IHO worker's children, CR adults, CR adult's children). Visualization of differences in Bray-Curtis distance was examined by *S. aureus* nasal carriage outcomes using NMDS plots.

Analysis of percent OTU contributions from IHO pigs to each participant type

SourceTracker, a tool within MacQIIME 1.9.1, is a Bayesian approach to estimate the proportion of OTUs in a given participant type that originate from a source environment—in this case from IHO pig (sampled and analyzed for microbiome data through a separate study).[24] We used the SourceTracker tool to predict the taxonomic contributions from IHO pig samples (specified as the source from our previous study)[25], to IHO worker, IHO child, CR adult and CR child samples (specified as the sink).[24,26] Bacterial contributions from the IHO pig were limited to those contributing at least 1% relative abundance to the sink population/participant types samples. These

Relation of personal, occupational, and household activities and S. aureus *nasal carriage with outcomes of alpha diversity and IHO pig bacterial contributions*

Generalized linear models (GLMs) were used to estimate the relation of personal, IHO occupational, and household activities and characteristics, and *S. aureus* nasal carriage with: 1) alpha diversity measures (Shannon diversity index, observed OTUs and phylogenetic distance); and 2) percent IHO pig bacterial contributions. Percent bacterial contributions from the IHO pig were also investigated as an exposure (independent) variable to estimate its association with alpha diversity measures (Shannon diversity index, observed OTUs and phylogenetic distance).

Relation of personal, occupational, and household activities, S. aureus *nasal carriage, and IHO pig bacterial contributions with beta diversity measures*

The adonis method, a non-parametric test, partitions a distance matrix among sources of variation to determine the strength and significance that a given exposure variable (categorical or continuous) has contributed to the variation in the beta diversity distance measure. We estimated how much variation in the Bray-Curtis distance measure was correlated with personal, occupational and household exposures, *S. aureus* nasal carriage outcomes, and IHO pig SourceTracker within each participant type.

Analysis of bacterial taxa (OTUs) unique to S. aureus *nasal carriage and IHO pig bacterial contributions*

Using the Kruskal-Wallis test (relative abundance) and the G-test of independence (presence/absence), we first identified the number of OTUs (and genera to which they belonged) that differed by relative abundances within participant types by *S. aureus* carriage status (carrier versus not of *S. aureus*, MDRSA, and *scn*-negative *S. aureus*) using false discovery rate (FDR)[27] corrected *p*-values. Finally, we identified the specific bacterial taxa (and the genera to which they belonged) that were sourced from the IHO pig (nares and perineum) to each of the participant types (IHO workers, IHO children, CR adults, and CR children).

RESULTS

Read statistics are summarized within Supplementary Material Table S1. All study participants were Hispanic, and Table 1 shows demographics, personal antibiotic use, self-reported asthma, current smoking, physical activity, IHO-related activities,

household characteristics and *S. aureus* nasal carriage outcomes in the 20 IHO worker-child and 20 CR adult-child pairs.

On average, IHO workers were 38 years of age and 50% male. IHO worker's children were 5 years of age and 65% male; CR adults were 33 years of age and 35% male; CR adult's children were 4 years of age and 60% male. Fifty percent of IHO workers and children carried *S. aureus* in the nares. Fourty-five percent of CR adults and their children carried *S. aureus*. Twenty-five percent of IHO workers carried MDRSA. 5% of IHO worker's children, CR adults and CR adult's children carried MDRSA. Thirty percent of IHO workers carried *scn*-negative *S. aureus.* Five percent of CR adults and their children carried *scn*-negative *S. aureus*. IHO worker's children did not carry *scn*-negative *S. aureus.*

Variability of nasal bacterial alpha diversity by participant type

We used the Student's t-test to determine whether differences in alpha diversity measures (Shannon diversity index, observed OTUs and phylogenetic distance) are statistically significant (Table 2). IHO workers and their children, CR adults and their children, IHO workers and CR adult's children, and CR adults and CR children had similar alpha diversity values (Table 2).

Bacterial community structure differs between adults and their children

Bacterial community membership (Unweighted UniFrac) and composition (Bray-Curtis) differences are summarized in Table 3. Overall, we observed similar beta diversity between IHO workers and CR adults (all $p>0.05$) and between IHO worker's

children and CR adult's children (all $p>0.05$). IHO workers and their children carried similar bacterial taxa (Unweighted UniFrac R-statistic [p-value]: 0.054 [$p<0.065$]); however, their bacterial composition differed (Bray-Curtis: 0.131; [$p<0.01$]). Differences were also observed between CR adults and their children for Unweighted UniFrac (R-statistic value: 0.064 [$p<0.04$]) and Bray-Curtis (R-statistic value: 0.135 [$p<0.01$]). NMDS plots visualized the community structure of IHO workers (purple dots) in relation to separately sampled IHO pigs (blue dots), IHO worker's children (green dots), CR adults (pink dots), and CR adult's children (light green dots) (Figure 1). Ellipses encircling the exposure groups show that IHO pigs (blue line) cluster in close proximity to IHO workers (purple line). The participant groups that represent individuals who had indirect exposure to IHOs (IHO worker's children who lived in an IHO worker household; and CR adults and their children who live in a region with a high density of IHOs) all appeared to cluster together separately (black dotted line) from IHO workers and IHO pigs (Figure 1). The IHO workers' nasal microbiome was dominated by *Firmicutes, Proteobacteria*, and *Actinobacteria* whereas *Firmicutes, Proteobacteria*, and *Bacteroidetes* dominated the nasal microbiome of IHO worker's children, CR adults and their children. IHO workers carried lower relative abundances of *Bacteroidetes*.

S. aureus nasal carriage influences alpha diversity and beta diversity

We observed that the IHO workers who carried *S. aureus* outcomes had higher alpha diversity compared to those that did not carry *S. aureus* nasal carriage outcomes (all $p<0.01$), including for MDRSA (all $p<0.02$) and *scn*-negative *S. aureus* (all $p<0.01$) (Table 4). Alpha diversity values of IHO worker's children, CR adults and their children

were not lower among those who were nasal carriage positive versus not for each *S. aureus* outcome (all $p>0.05$) (Table 4). IHO pigs demonstrated similar, if not greater, alpha diversity than IHO workers and other participant types (IHO worker's children, CR adults and their children) (Supplementary Materials Figure S1). We observed lower dispersion of beta diversity measures for those IHO workers carrying MDRSA and *scn*-negative *S. aureus* versus those who do not.

The nasal carriage of *S. aureus* outcomes (*S. aureus*, MDRSA, and *scn*-negative *S. aureus*) in IHO workers was correlated with changes in community membership and composition of the nasal microbiome (Table 5). Also, among IHO workers who carried *S. aureus,* MDRSA, and *scn*-negative *S. aureus,* we observed exclusive carriage of the following 3 bacterial taxa: 1) *Staphylococcus equorum:Staphylococcus haemolyticus* (could not distinguish species), *2) Cobetia crustatorum,* and *3) Halomonas halodenitrificans* (all $p<0.004$) (Supplementary Material, Table S2). IHO workers who did not carry *S. aureus,* MDRSA, and *scn*-negative *S. aureus* carriers carried one bacterial taxa exclusively (all $p<0.0001$) (Supplementary Material, Table S2). There were no bacterial taxa consistently observed in IHO worker's children, CR adults and their children who carried *S. aureus* outcomes.

The nasal carriage of *S. aureus* did not correlate with clustering within the NMDS (Supplemental Material, Figure S2, Panel A). Clustering was observed when stratified by MDRSA and *scn*-negative *S. aureus* nasal carriage (Supplemental Materials Figure S2 Panels B and C).

IHO pig bacterial contributions were higheest among IHO workers

On average, it was estimated that IHO pigs contributed 13% of the bacterial relative abundance for IHO workers' nasal microbiomes, but contributed less than 2% for IHO children, CR adults and their children. Sixteen, 5, 7 and 8 OTUs were uniquely contributed by the IHO pigs to IHO workers, IHO worker's children, CR adults, and their children, respectively (Supplemental Materials Table S3).

Relation of personal, household, and occupational activities and S. aureus nasal carriage outcomes with alpha diversity and IHO pig bacterial contributions

IHO workers

IHO workers' demographics and personal and household characteristics and activities were not consistently related to changes in alpha diversity or the bacterial contributions from the IHO pig (Table 6). Each additional 8 hours of direct contact with IHO pigs per week was associated with a four percent increase in IHO pig bacterial contributions to the IHO workers nasal microbiome (95% confidence interval [CI]=0.22, 7.88) and increases in all alpha diversity measures (all $p<0.05$).

The carriage of *S. aureus* in IHO workers was associated with a 7% increase in IHO pig bacterial contributions (beta= 7.31, 95% CI =-3.97, 18.5). The carriage of MDRSA in IHO workers was associated with a 10% increase in IHO pig bacterial contributions (beta= 9.73, 95% CI= 4.75, 24.2). The carriage of *scn*-negative *S. aureus* by IHO workers was associated with a 16% increased in IHO pig bacterial contributions (beta= 16.4; 95 % CI= 2.53, 30.26). Increases in IHO pig bacterial contribution was associated with an increase in Shannon diversity (beta= 0.07; 95 % CI= 0.04, 0.10),

observed OTUs (beta= 6.70; 95 % CI= 1.02, 12.4) and phylogenetic distances (beta= 0.42; 95 % CI= 0.07, 0.77) in IHO workers.

IHO children

IHO children's demographics, personal characteristics and household activities were not consistently related to changes in alpha diversity and IHO pig bacterial contributions (Table 7). Age was associated with increased in alpha diversity within IHO worker's children. Children in a household where an IHO worker gave versus did not give pigs shots had a lower alpha diversity. Personal antibiotics use and bringing work gear home was associated with an increase in alpha diversity. The carriage of MDRSA versus not among IHO children was associated with an increase in IHO pig bacterial contributions by 1% (beta= 0.06, 95% CI= 0.05, 0.08). The carriage of MDRSA among IHO children was associated with decreases in Shannon diversity (beta= -1.76; 95 % CI= -2.39, -1.13), observed OTUs (beta= -60.4 (95 % CI = -153, 32.34), and phylogenetic distance (beta= -5.63 (95 % CI = -11.6, 0.36). Increased IHO pig bacterial contributions were associated with decreases in Shannon diversity (beta= -11.7; 95 % CI= -21.2, -2.17), observed OTUs (beta= -1260; 95 % CI= -2264, -257) and phylogenetic distance (beta= -87.5; 95 % CI= -152, -22.8) among IHO children.

CR adults

CR adults' demographics, personal characteristics and household activities were not consistently related to changes in alpha diversity and IHO pig bacterial contributions (Table 8). CR adults' alpha diversity increased with age, increased frequency of

handwashing and household size (all $p > 0.05$). Women had lower alpha diversity than men ($p < 0.05$). Household pet ownership and smoking was associated with decreases in alpha diversity within CR adults ($p > 0.05$). The carriage of *S. aureus* in CR adults, was associated with an increase in alpha diversity values (all $p > 0.05$). The carriage of MDRSA and *scn*-negative *S. aureus*, was associated with decreases in Shannon diversity (beta= -0.86, 95% CI= -1.38, -0.35), observed OTUs (beta= -126; 95% CI= -196, -57), and phylogenetic distance (beta= -8.51, 95% CI= -12.9, -4.09). Increased IHO pig bacterial contribution was associated with a decrease in Shannon diversity (beta= -13.8; 95 % CI= --21.5, -6.21), observed OTUs (beta= -1930; 95% CI= -2955, -904) and phylogenetic distance (beta= -144; 95% CI= -209, -79.3).

CR children

CR children's demographics, personal characteristics and household activities were not consistently related to changes in alpha diversity and IHO pig bacterial contributions (Table 9). Age was associated with increased alpha diversity within CR children. The nasal microbiomes of female CR children were less diverse than males. The carriage of *S. aureus* in CR children, versus those who did not carry *S. aureus*, was associated with a decrease in Shannon diversity (beta= -0.23; 95% CI= -1.25, 0.79), observed OTUs (beta= -4.05 95% CI= -12.9, 4.78), and phylogenetic distance (beta= -73.98; 95% CI= -212, 64.2). CR children who carried MDRSA and *scn*-negative *S. aureus* were associated with a decrease in Shannon diversity (beta= 1.00, 95% CI= -1.56, -0.44), observed OTUs (beta= -7.75, 95% CI= -12.7, -2.79), and phylogenetic distance (beta= -120, 95% CI= -198, -41.4). Increasing IHO pig bacterial contributions was

associated with a decrease in Shannon diversity (beta= -12.5; 95 % CI= -28.8, 3.71), observed OTUs (beta= -1951; 95 % CI= -3914, 12.3) and phylogenetic distance (beta= -123; 95 % CI= -248, 1.91).

Relation of personal, household, and occupational activities with beta diversity and IHO pig bacterial contributions

Beta diversity differences tested via the adonis method are summarized in Table 10. Beta diversity is referred to hereafter as community structure. Among IHO workers, changes in bacterial communities structure correlates with the number of years working at any swine farm ($p< 0.06$) and giving pigs shots ($p<0.09$), although statistical significance was not reached (Table 10). The carriage of *S. aureus (*$p<0.05$), MDRSA ($p<0.001$), and *scn*-negative *S. aureus* ($p<0.001$) in IHO workers was associated with differences observed in the bacterial community structure (Table 10).

The carriage of MDRSA versus not among IHO children was associated with differences in the bacterial community structure ($p<0.05$) (Table 8). Bacterial contributions from the IHO pig present in the nasal microbiome of IHO children was correlated with differences in the bacterial community structure ($p<0.03$). Sex was correlated with differences in bacterial community structure ($p<0.004$) while being a current smoker ($p<0.06$) and IHO pig bacterial contributions ($p<0.08$) were not as strongly associated. IHO pig bacterial contributions to the nasal microbiome of CR children was correlated with differences in the bacterial community structure ($p<0.04$) (Table 10).

DISCUSSION

S. aureus nasal carriage, particularly of LA- and AMR *S. aureus* strains, was associated with increased alpha diversity and greater bacterial taxa contributions from the IHO pigs among IHO workers. This was not observed among participant groups who did not have direct exposure to IHOs. An opposite trend of decreased alpha diversity given the nasal carriage of *S. aureus* was observed among children living with IHO workers and CR adults. This may reflect a unique bacterial community contribution that accompanies *S. aureus* nasal carriage positivity among IHO workers or that there are possible differences in the ecological dynamics within a child's microbiome compared to an adult's. Khamash et al. (2018) found that neonates within a hospital setting who carried *S. aureus* had lower biodiversity and more unevenly distributed bacterial communities than that of non-carriers.[28] This setting may not speak to otherwise healthy children; however, it has informed understanding of ecological dynamics that exist in a child as possessing extreme microbiome plasticity. These potential ecological dynamics of a child's nasal microbiome could lead to decrease diversity in the presence of *S. aureus* colonization.

Beta diversity differences were observed only between adult and child participants, regardless of exposure group (IHO or CR). Beta diversity similarities observed among IHO workers, IHO worker's children, CR adults and their children may be influenced by living close to one another and experiencing similar environmental and bacterial exposures, which could include particulate matter that drifts from IHO facilities containing bacteria adsorbed to aerosols.[29,30]

The nasal carriage of *S. aureus*, MDRSA, and *scn*-negative *S. aureus* was significantly associated with increased alpha diversity and correlated with variations in bacterial community composition among IHO workers. For IHO children, CR adults, and CR children, alpha diversity did not change by *S. aureus* nasal carriage outcomes; however, variations in bacterial community composition were significantly correlated with the presence of IHO pig bacterial contributions. We expected to observe that nasal carriage of *S. aureus* outcome measures would be related to alpha diversity and beta diversity consistently, regardless of participant type. However, our results suggest that the IHO work environment and associated LA and AMR *S. aureus* strains may influence the nasal bacterial alpha diversity and community composition and structure of IHO workers in different ways than among those who do not have direct IHO exposure.

Two studies investigated the influence of *S. aureus* nasal carriage on the nasal microbiome. Singh et al. (2016), observed that *S. aureus* carriers were more diverse and had greater observed OTUs, compared to non-carriers, among military trainees.[31] The microbiome community composition was altered by the nasal carriage of MSSA and MRSA as well as among those who experience a skin and soft tissue infection (SSTI) [31] Similarly Johnson et al. (2015), found *S. aureus* nasal carriage was associated with significant differences in microbiome community composition (beta diversity).[31,32] Our results agree with previous findings as we found MDRSA and *scn*-negative *S. aureus* nasal carriers were more diverse and differed in community membership and composition than those who did not carry MDRSA and *scn*-negative *S. aureus*.

The prior literatures on *S. aureus* nasal carriage outcomes and the nasal microbiome may suggest that IHO workers carry unique nasal communities of *S. aureus*

and other bacteria that accompany *S. aureus* exposure, which might be related to their direct occupational exposure activities (which IHO children and community residents with no known livestock exposure would not experience). *S. aureus* and other pathogenic bacteria may have disruptive and/or adverse effects on the nasal microbiome and increase the risk of nasal colonization by foreign bacteria due to the disruption of the normal microbiome,[33] which might be manifested by a greater diversity among IHO worker *S. aureus* nasal carriers versus non-carriers in this study.

Three OTUs were exclusively observed in IHO workers who carried one of the three *S. aureus* nasal carriage outcomes (*S. aureus*, MDRSA or *scn*-negative *S. aureus*). These OTUs include and have known sources from the following: 1) *Staphylococcus equorum:Staphylococcus haemolyticus* is found in healthy horses[34], cows with mastitis[35] and other animals[36,37], 2) *Cobetia crustatorum* is found in fermented food products[38] and 3) *Halomonas halodenitrificans* is found in fermented food products & resembles a *Moraxella*-like bacteria.[38,39] Fermented liquid feed, the mixing of feed with water at a specific ratio, allows for the proliferation of lactic acid bacteria and yeasts with subsequent production of lactic acid, acetic acid and ethanol to inhibit the growth of pathogenic organisms by reducing pH.[40] This feed also inhibits the growth of pathogenic bacteria in the stomach and gut of pigs.[40] We do not have information on the use of fermented liquid feeds on farms sampled however this may explain the presence of fermentation related bacteria in the nasal microbiome of IHO workers.

The presence of *Staphylococcus equorum* and *S. haemolyticus* were noted in a recent study in 2018 of LA-MRSA in stable dust from pig farms.[18] With a half life of 4 days, *S. equorum* and *S. haemolyticus* are a public health concern as they have been

shown to cause human blood stream infections.[18] In all samples, *S. equorum* concentrations were at least 10-fold higher than the concentrations of *S. aureus.*[18] Feld et al found a significant association between the concentration of LA-MRSA in the stable air of the pig farm and the risk of nasal LA-MRSA nasal carriage in people who were in direct contact with animals and those in close proximity to pig pens.[18] This association was independent of the number of hand facial touches which argued the primary source of LA-MRSA was bioaerosols.[18,41] The presence of *Staphylococci*, specifically LA-SA, highlights the increased risk of nasal colonization when in direct contact with IHO pigs or while cleaning the operation due to the rather long half life of LA-MRSA, *S. equorum and S. haemolyticus*. Additionally, Strube et al. (2018), investigated the core microbiome of the health pig's skin and nose and also found that the *Staphylococcus* genus was dominated by *S. equorum*.[42] In our study, the observation of *S. equorum* among IHO workers and also in IHO pigs and may suggest an increased likelihood that *S. equorum* may be contributed by the IHO pig to the IHO worker.

The nasal cavity serves as a filter and point of deposition for particulate matter to which bacteria—including but not limited to *S. aureus*--may be adhered. Because of this, direct IHO occupational exposure may also strongly influence on the IHO worker's nasal microbiome through nasal high bacterial loading from the IHO environment and IHO pigs.[43] The airborne exposure pathway is a critical route of exposure to LA-*S. aureus* among individuals working inside IHOs.[22]

Kraemer et al. (2017), investigated the influence of pig farming on the nasal microbiome of pigs and pig workers compared to dairy farmers and non-exposed adults. They observed lower beta diversity dispersion among pig workers compared to cow

farmers and adults not exposed to animals.[44] They argued pig farming created a homogeneous pressure, decreasing the dispersion of beta diversity and making the nasal microbiome community structure of pig workers more similar to one another.[44] Such homogeneity may lead to greater similarities in nasal bacteria given occupational IHO exposures, which is consistent with the Bray-Curtis clustering that we observed for IHO pig (nares and perineum) and IHO worker nasal microbial communities. This may suggest a homogeneous pressure from hog production activities on IHO workers nasal microbiomes.

To our knowledge, ours is the first study to investigate nasal microbiome differences by MDRSA and LA-*S. aureus* nasal carriage outcomes among IHO workers and individuals (including children under 7 years of age) with indirect IHO exposure and those with no known livestock exposure. Interestingly, the occupational activities (handling dead pigs, giving pig shots) and *S. aureus* nasal carriage outcome positivity of the IHO worker decreased the diversity of the IHO child's nasal microbiome. The strong influence of occupational activities and IHO pig bacterial contributions (as an exposure measure) on the alpha diversity of IHO children may indicate a greater risk for the nasal acquisition of LA-bacteria when living in a household with an IHO worker[45]

Several strengths of this study included the ability to use information about the IHO pig microbiome (nasal and perineum) from a previous study (Brown et al., In submission) in order to relate the taxonomic contributions from IHO pigs to IHO workers and individuals without direct IHO exposure. This revealed associations for IHO pig bacterial contributions in the expected direction (positive) for IHO work activities involving intensive pig contact among IHO workers but in an unexpected (negative)

direction among IHO children, CR adults, and CR children. Without access to specimens and data from child household members and community residents (adults and children) we would not have been able to examine the influence of living in the same home as an IHO worker or living in a region with a high density of IHOs on alpha and beta diversity and bacterial taxa carriage associated with IHO pigs.

To our knowledge, there are two studies that have investigated the pattern of exchange and similarity of bacterial among animals, humans and the environment in pig farms. Kraemer et al. (2017), suggested hog farming could create homogeneous pressures on the nasal microbiome that lead to similar alterations to the microbiome of pig workers compared to cow farmers and adults not exposed to animals.[44] Vestergaard et al. (2018), investigated the diversity of airborne bacteria in pig stables, pig farmers' homes and suburban homes.[46] This study suggested that airborne bacteria were more diversity and abundance in pig stable and farmers' homes compared to suburban homes.[46] Additionally, with known protective effects in asthmatic individuals (*e.g. Prevotellaceae*, *Lachnospiraceae, Ruminococcaceae*, *Ruminiclostridium*, and *Lactobacillus*) were found in higher absolute and relative abundances within pig stables and farmers' homes although there was no clear evidence of direct transfer from pig stable to the farmers' home.[46]

Another strength of the present analysis is the integration of sequencing data on *Staphylococcus spp.* abundance with culture-based and molecularly characterized *S. aureus* nasal carriage outcomes (*S. aureus,* MDRSA and *scn*-negative *S. aureus*). Without the integration of these data the alpha and beta diversity and IHO pig bacterial

contributions differences among IHO workers by *S. aureus* nasal carriage status would not have been observable.

Several limitations of this study should be considered. The study design was cross-sectional, which limits assignment of directional or temporal relationships between nasal microbiota and *S. aureus* nasal carriage outcomes among IHO workers, IHO worker's children, CR adults and their children. Future longitudinal analyses would allow the investigation of questions surrounding the temporal dynamics of nasal microbiome alpha diversity and community membership and composition in relation to IHO occupational exposure activities and *S. aureus* nasal carriage outcomes (including LA-*S. aureus* strains) and among IHO workers and children living within IHO worker households. Second, our study was limited to a rural setting with a large density of hog operations proximal to both IHO worker and community resident homes. Therefore, there is a need to include suburban and/or urban populations of similar age, race and ethnicity, who do not live proximal to IHO facilities, to understand these dynamics in a truly unexposed population similar to Vestergaard et al. (2018).[46] Finally, all participants of the present study were healthy volunteers who did not report any health outcomes typically associated with *S. aureus* exposure (e.g., SSTIs). Thus there is a need in microbiome studies of IHO work and community exposures to move beyond measures of nasal carriage (exposure) in order to understand how antimicrobial selective pressures may impact in the nasal microbiome in ways that alter progression toward infection rather than just exposure.

CONCLUSION

The relationship between IHO occupational activities and *S. aureus* nasal carriage (*S. aureus*, MDRSA and *scn*–negative *S. aureus*) with changes in bacterial diversity, community structure and composition, and percent IHO pig bacterial contributions among IHO workers suggest that they may be exposed to and colonized by different populations of microbes, including *S. aureus*, compared to individuals who do not have direct IHO exposures (IHO worker's children, CR adults and their children). The presence of *S. equorum and haemolyticus* in the nasal microbiome of IHO workers with *S. aureus* nasal carriage positive outcomes suggest IHOs can be a primary and persistent source of exposure to bacteria that are capable of causing bacteremia and other health issues. Occupational exposures of the IHO worker, including cleaning activities and the persistence of microbes in the IHO environment, highlight the need for further investigation of the influence of hog production on the microbiome of pigs, pig workers and residents in surrounding communities. This will improve our understanding not only of potential exposure risks, but also of potential human health risks. IHO workers may require improved protections (e.g., more extensive use of personal protective equipment [PPE], especially during cleaning activities to minimize inhalation exposure; changing of their clothing before and after work; improved ventilation) to reduce IHO-associated microbial exposure burdens that could potentially represent a health hazard.

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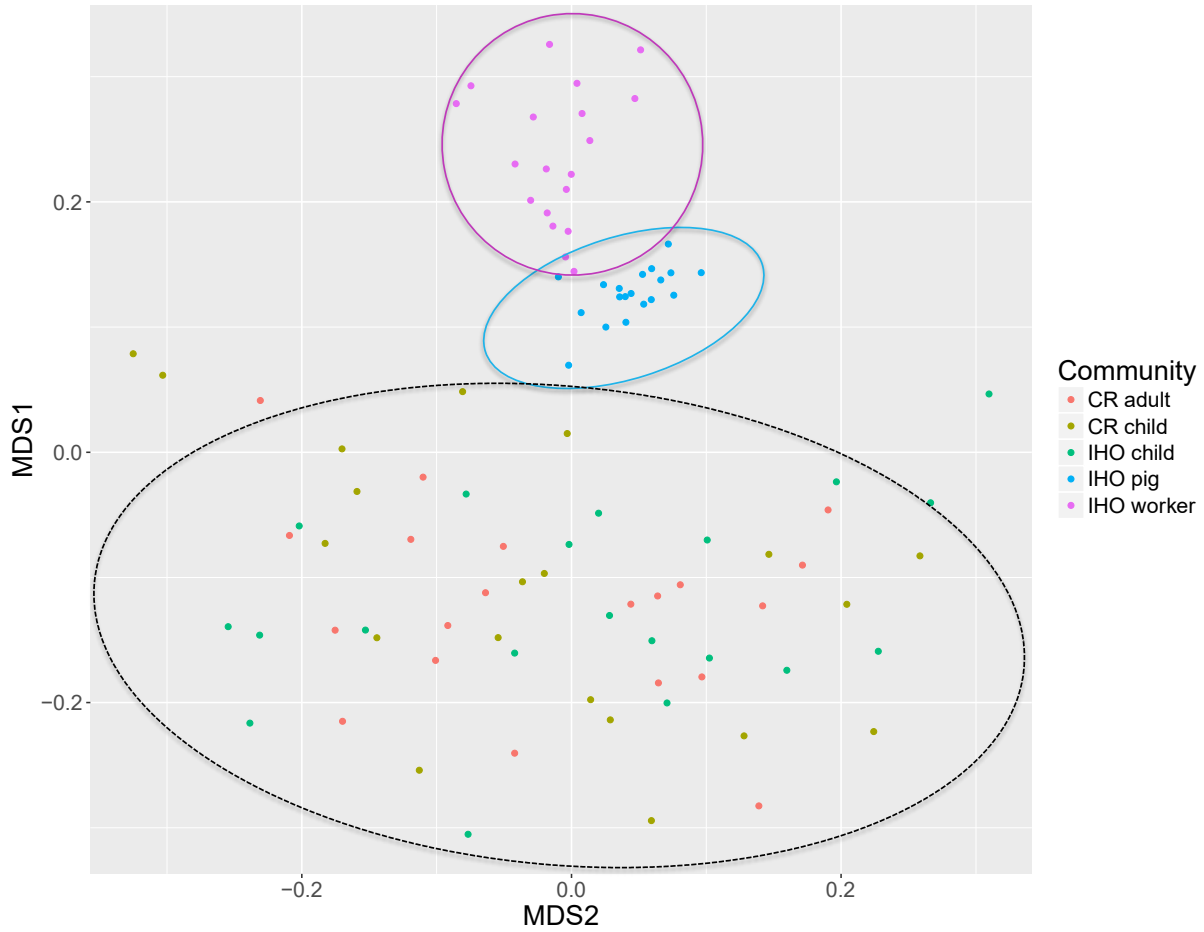

Figure 1. Non-metric dimensional scaling (NMDS) of microbial communities in nasal samples from of CR adults, CR children, IHO children, IHO pigs and IHO workers.

Panel displays all four comparison groups (CR adults, CR adult's children, IHO worker's children, IHO pigs and IHO workers) within a non-metric dimensional scaling (NMDS) plot to compare differences in the microbial communities while maintaining accurate distance measures. No clustering observed by participant type. However, we do observed clustering by direct vs. indirectly exposed individual. The NMDS demonstrates clustering among IHO pigs (encircled in blue) and IHO workers (in purple) both directly exposed to the IHO facility cluster together while CR adults, CR adult's children and IHO worker's children (encircled in the dotted line), with no known direct IHO exposures, clustered together.

Table 1. Characteristics of industrial hog operation (IHO) worker-child and community resident (CR) adult-child pairs, 2014, North Carolina, USA.

	IHO		CR	
	Worker	**Child**	**Adult**	**Child**
	n=20	n=20	n=20	n=20
Age in years, mean (range)	38 (18-71)	5 (1-6.4)	33 (20-43)	4 (2-6)
Male, n (%)	10 (50)	13 (65)	7 (35)	12 (60)
Race/ethnicity, n (%)				
Hispanic	20 (100)	20 (100)	20 (100)	20
Antibiotic use in the last 3 months, n (%)	0 (0)	1 (5)	0 (0)	0 (0)
Self-reported asthma, n (%)	1 (5)	0 (0)	0 (0)	0 (0)
Current smoker, n (%)	3 (15)	--	3 (15)	--
Gym in the last 3 months (Yes/No)	1 (5)	--	2 (10)	0 (0)
Sports in the last 3 months (Yes/No)	1 (5)	1 (5)	0 (0)	0 (0)
IHO-related activities				
Took IHO PPE home, n (%) (Yes/No)	6 (30)	--	--	--
Washed IHO PPE with household laundry	0 (0)	--	--	--
Mask usage[a]				
Always	11 (55)	--	--	--
Sometimes	7 (35)	--	--	--
Never	2 (10)	--	--	--
Household characteristics				
Number of HH members, mean (range)	5 (3-8)	--	4 (2-8)	--
Owned a pet (Yes/No)	8 (40)	--	3 (15)	--
***S. aureus* nasal carriage (Yes/No)**				
S. aureus	10 (50)	10 (50)	9 (45)	9 (45)
MDRSA	5 (25)	1 (5)	1 (5)	1 (5)
scn-negative	6 (30)	0 (0)	1 (5)	1 (5)

Note. IHO, industrial hog operation. CR, community resident. PPE, Personal protective equipment.
[a]Mask usage: 0=always (80% or greater), 1=sometimes (10-79%); 2=never (less than 10%).
Demographic characteristics presented in this table represent a sub-sample from the study population from Hatcher et al. (2017)[15].

Table 2. Differences in alpha diversity by participant type (adults and children) and IHO and CR household types. 2014, North Carolina, USA.

	IHO worker	**IHO child**	**CR adult**	**CR child**				
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	*p*-value[a]	*p*-value[b]	*p*-value[c]	*p*-value[d]
Alpha Diversity								
Shannon	6.76 (1.17)	6.75 (1.43)	6.53 (1.31)	6.73 (1.23)	0.851	0.590	0.526	0.957
Observed OTUs	261 (190)	243 (202)	200 (153)	218 (172)	0.298	0.732	0.275	0.688
Phylogenetic distance	21.2 (11.4)	20.4 (13.0)	17.6 (9.75)	18.8 (10.9)	0.400	0.703	0.296	0.686

Note. IHO, industrial hog operation. CR, community resident.

[a] *p*-values estimated from t-test comparing differences in alpha diversity measures between IHO workers and IHO children.

[b] *p*-values estimated from t-test comparing differences in alpha diversity measures between CR adults and CR children.

[c] *p*-values estimated from t-test comparing differences in alpha diversity measures between IHO workers and CR adults.

[d] *p*-values estimated from t-test comparing differences in alpha diversity measures between IHO children and CR children.

Table 3: Differences in beta diversity between participant (adults and children) and IHO and CR household types, 2014, North Carolina, USA.

Comparison category	Beta diversity metric	*R* statistic	*p*-value
IHO worker vs. IHO child	Unweighted UniFrac	0.054	0.065
	Bray-Curtis	0.131	0.008
CR adult vs. CR child	Unweighted UniFrac	0.064	0.037
	Bray-Curtis	0.135	0.006
IHO worker vs. CR adult	Unweighted UniFrac	0.005	0.374
	Bray-Curtis	-0.009	0.570
IHO child vs. CR child	Unweighted UniFrac	0.009	0.279
	Bray-Curtis	0.049	0.067

Note. IHO, industrial hog operation. CR, community resident.

p-values estimated from R-statistic comparing beta diversity distance measures between participant types.

Table 4. Differences in alpha diversity by S. aureus nasal carriage outcome measures among industrial hog operation (IHO) worker-child and community resident (CR), 2014, North Carolina, USA.

	S. aureus			**MDRSA**			***scn*-negative *S. aureus***		
IHO worker	**Carriers**	**Non-carriers**	***p*-value**	**Carriers**	**Non-carriers**	***p*-value**	**Carriers**	**Non-carriers**	***p*-value**
Shannon	7.47 (0.52)	6.13 (0.46)	0.008	7.91 (1.41)	6.46 (0.93)	0.023	7.86 (1.23)	6.37 (0.91)	0.011
Observed OTUs	387 (41.3)	148 (20.0)	0.003	537 (67.0)	188 (133)	0.0001	503 (95.9)	175 (129)	0.0001
Phylogenetic distance	28.7 (3.05)	14.4 (1.34)	0.003	37.9 (2.65)	16.7 (7.95)	0.0001	35.5 (4.91)	16.0 (7.82)	0.0001
IHO child	**Carriers**	**Non-carriers**	***p*-value**	**Carriers**	**Non-carriers**	***p*-value**	**Carriers**	**Non-carriers**	***p*-value**
Shannon	6.41 (1.31)	7.09 (1.53)	0.299	--	--	--	--	--	--
Observed OTUs	203 (181)	284 (223)	0.384	--	--	--	--	--	--
Phylogenetic distance	17.8 (11.5)	22.9 (14.6)	0.399	--	--	--	--	--	--
CR adult	**Carriers**	**Non-carriers**	***p*-value**	**Carriers**	**Non-carriers**	***p*-value**	**Carriers**	**Non-carriers**	***p*-value**
Shannon	6.91 (1.30)	6.21 (0.91)	0.176	--	--	--	--	--	--
Observed OTUs	234 (186)	172 (123)	0.382	--	--	--	--	--	--
Phylogenetic distance	19.6 (12.0)	15.9 (7.66)	0.423	--	--	--	--	--	--
CR child	**Carriers**	**Non-carriers**	***p*-value**	**Carriers**	**Non-carriers**	***p*-value**	**Carriers**	**Non-carriers**	***p*-value**
Shannon	6.60 (0.27)	6.83 (0.46)	0.689	--	--	--	--	--	--
Observed OTUs	177 (31.9)	251 (64.8)	0.353	--	--	--	--	--	--
Phylogenetic distance	16.6 (2.19)	20.7 (4.07)	0.186	--	--	--	--	--	--

Note. IHO, industrial hog operation. CR, community resident.
Sample size too small to conduct analysis (--)

p-values estimated from Student's t-tests comparing beta diversity distance measures between participant types.

Table 5. Differences in beta diversity by *S. aureus* nasal carriage outcomes (carrier vs. non-carrier) among participant types, 2014, North Carolina, USA.

		S. aureus		**MDRSA**		***scn*-negative *S. aureus***	
Comparison category	**Beta diversity metric**	***R* statistic**	***p*-value**	***R* statistic**	***p*-value**	***R* statistic**	***p*-value**
IHO worker carrier vs. non-carrier	Unweighted UniFrac	0.046	0.232	0.092	0.247	0.008	0.426
	Bray-Curtis	0.184	0.031	0.303	0.015	0.269	0.023
IHO child carrier vs. non-carrier	Unweighted UniFrac	-0.077	0.954	-0.143	0.673	---	---
	Bray-Curtis	-0.069	0.848	0.035	0.416	---	---
CR adult carrier vs. non-carrier	Unweighted UniFrac	-0.033	0.670	0.014	0.487	---	---
	Bray-Curtis	-0.046	0.748	0.199	0.318	---	---
CR child carrier vs. non-carrier	Unweighted UniFrac	0.115	0.042	-0.077	0.469	---	---
	Bray-Curtis	0.036	0.250	0.024	0.558	---	---

Note. IHO, industrial hog operation. CR, community resident.
p-values estimated from R-statistic comparing beta diversity distance measures between participant types by *S. aureus* nasal carriage.

Table 6. Relation of IHO occupational and personal activities to alpha diversity measures and IHO pig contributions of the IHO worker's and nasal microbiome. 2014, North Carolina, USA.

	IHO worker			
	Shannon	**Observed OTUs**	**Phylogenetic distance**	**% pig**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Demographics				
Age (by year)	0.02 (-0.01, 0.05)	2.42 (-3.06, 7.90)	0.15 (-0.14, 0.45)	0.38 (-0.10, 0.86)
Sex (Male=reference)	-0.12 (-1.18,	-30.18 (-200, 139)	-2.62 (-12.77, 7.53)	-3.09 (-14.2, 8.0)
Personal and household				
Personal antibiotic use within last 3	--	--	--	--
Number of household members	0.06 (-0.21, 0.34)	0.5 (-44, 45.4)	0.28 (-2.37, 2.93)	1.20 (-1.36, 3.77)
Household pet (Yes/No)	-0.11 (-1.20,	85.5 (-86, 257.1)	5.14 (-5.22, 15.49)	-1.59 (-13.4, 10.2)
Current Smoker (Yes/No)	-0.31 (-1.52,	-86.8 (-265, 91.2)	-5.94 (-16.16, 4.27)	9.30 (-9.96, 28.6)
Occupation exposures				
Years worked at any swine farm	0.08 (-0.04, 0.19)	15.12 (-3.83, 34.1)	1.00 (-0.13, 2.13)	0.75 (-0.09, 1.58)
Hours of direct contact per week (by 8-	**0.41 (0.10, 0.73)**	**71.8 (26.0, 117.6)**	**4.25 (1.33, 7.16)**	**4.05 (0.22, 7.88)**
Time since last work shift (by 8-hour	-0.05 (-0.41,	20.4 (-48.8, 89.5)	1.28 (-2.78, 5.34)	0.19 (-4.76, 5.14)
Mask usage[b]	-0.16 (-0.83,	-42.0 (-141, 57.1)	-1.63 (-7.60, 4.33)	-3.17 (-11.2, 4.86)
Gave pigs antibiotics (Yes/No)	-0.09 (-1.36,	50.8 (-150, 251)	3.38 (-8.59, 15.36)	6.83 (-4.47, 18.1)
Handled dead pigs (Yes/No)	0.17 (-0.93, 1.27)	94.7 (-80, 270)	5.56 (-5.06, 16.17)	4.28 (-4.89, 13.5)
Gave pigs shots (Yes/No)	0.39 (-0.58, 1.35)	**138 (-9.88, 285)**	**7.84 (-0.74, 16.42)**	0.74 (-15.1, 16.6)
Frequency of hand washing at work (for	0.12 (-0.54, 0.78)	-32.7 (-175, 110)	-2.07 (-10.21, 6.08)	0.87 (-9.35, 11.1)
Showered after work[C]	**-0.67 (-1.26, -**	**-147 (-240, -53.7)**	**-6.51 (-12.15, -0.87)**	**-6.47 (-12.8, -**
Take IHO gear home (Yes/No)	0.42 (-0.66, 1.50)	-6.11 (-184, 172.0)	-0.93 (-11.39, 9.52)	2.20 (-13.5,
***S. aureus* nasal carriage outcomes**				
S. aureus (Yes/No)	**1.34 (0.46, 2.23)**	**240 (104, 375)**	**14.3 (6.1, 22.5)**	7.31 (-3.97, 18.6)
MDRSA (Yes/No)	**1.46 (0.14, 2.77)**	**349 (260, 438)**	**21.3 (16.6, 25.9)**	9.73 (-4.75, 24.2)
scn negative (Yes/No)	**1.48 (0.39, 2.58)**	**328 (226, 430)**	**19.5 (13.2, 25.7)**	**16.4 (2.53, 30.3)**
SourceTracker nasal carriage				
% pig contributions[a]	**0.07 (0.04, 0.10)**	**6.70 (1.02, 12.4)**	**0.42 (0.07, 0.77)**	--

Note. IHO, industrial hog operation. CI, confidence interval. Beta (95% CI) estimated from linear regression models.

[a]% pig contributions defined as the percentage of OTUs contributed to the worker from the pig's total microbiome (nares and perineum) estimated via

[b]Reported as: 0 = Always (≥ 80%), 1 = Sometimes (11-79%); 2 = Never (0-10%).

[c]Reported as: 0 = Always, 1 = Sometimes, 2 = Never. Dashes indicate an insufficient number of observations for that work activity

"--"; Not investigated due to limited data available

Table 7. Relation of IHO occupational and personal activities to alpha diversity measures and IHO pig contributions of the IHO child's nasal microbiome. 2014, North Carolina, USA.

	IHO child			
	Shannon	**Observed OTUs**	**Phylogenetic distance**	**% pig**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Demographics				
Age (by year)	**0.30 (0.05, 0.55)**	**37.0 (-0.87, 74.8)**	**2.39 (-0.03, 4.81)**	-0.01 (-0.02 ,0.00)
Sex (Male=reference)	-0.41 (-1.59, 0.76)	-103 (-266, 59.9)	-7.10 (-17.6, 3.43)	-0.01 (-0.03 ,0.02)
Personal and household				
Personal antibiotic use within last 3	**2.09 (1.47, 2.71)**	**291 (203, 379)**	**19.3 (13.6, 25.0)**	-0.01 (-0.03 ,0.00)
Number of household members	-0.04 (-0.39, 0.32)	-17.1 (-62.8, 28.7)	-0.89 (-3.95, 2.17)	-0.01 (-0.02 ,0.00)
Household pet (Yes/No)	0.78 (-0.43, 1.98)	66.6 (-114, 247)	4.21 (-7.42, 15.8)	-0.01 (-0.04 ,0.01)
Current Smoker (Yes/No)	--	--	--	--
Occupation exposures				
Years worked at any swine farm	0.10 (-0.01, 0.21)	11.0 (-6.30, 28.31)	0.72 (-0.40, 1.83)	0.00 (0.00 ,0.00)
Hours of direct contact per week	0.28 (-0.28, 0.85)	37.7 (-47.1, 123)	2.55 (-2.84, 7.94)	0.00 (0.00 ,0.01)
Time since last work shift (by 8-	-0.11 (-0.59, 0.37)	-3.2 (-70.4, 63.9)	-0.11 (-4.34, 4.12)	0.00 (-0.01 ,0.01)
Mask usage[b]	-0.04 (-0.77, 0.68)	-32.1 (-132, 67.9)	-1.92 (-8.37, 4.53)	-0.01 (-0.03 ,0.00)
Gave pigs antibiotics (Yes/No)	-0.15 (-1.70, 1.40)	-25.9 (-239, 187)	-2.15 (-16.2, 12.0)	0.02 (0.00 ,0.04)
Handled dead pigs (Yes/No)	0.07 (-1.24, 1.38)	11.6 (-180, 203)	1.39 (-10.83, 13.62)	0.02 (0.00 ,0.04)
Gave pigs shots (Yes/No)	**1.54 (0.63, 2.46)**	**221 (104, 339)**	**14.2 (6.36, 022)**	**0.01 (0.00 ,0.02)**
Frequency of hand washing at	-0.51 (-1.58, 0.57)	11.6 (-180, 203)	-2.99 (-13.5, 7.55)	0.01 (0.00 ,0.02)
Showered after work[C]	--	--	--	--
Take IHO gear home (Yes/No)	-0.24 (-1.69, 1.22)	11.6 (-180, 203)	1.42 (-11.1, 13.90)	0.00 (0.00 ,0.03)
***S. aureus* nasal carriage outcomes**				
S. aureus (Yes/No)	-0.68 (-1.89, 0.53)	-80.8 (-254, 92.06)	-5.07 (-16.3, 6.12)	0.02 (-0.01 ,0.04)
MDRSA (Yes/No)	**-1.76 (-2.39, -1.13)**	-60.4 (-153, 32.34)	-5.63 (-11.6, 0.36)	**0.06 (0.05 ,0.08)**
scn negative (Yes/No)	--	--	--	--
SourceTracker nasal carriage				
% pig contributions[a]	**-11.7 (-21.2, -2.17)**	**-1260 (-2264,-257)**	**-87.5 (-152, -22.8)**	--

Note. IHO, industrial hog operation. CI, confidence interval. Beta (95% CI) estimated from linear regression models.

[a]% pig contributions defined as the percentage of OTUs contributed to the worker from the pig's total microbiome (nares and perineum) estimated

[b]Reported as: 0 = Always (≥ 80%), 1 = Sometimes (11-79%); 2 = Never (0-10%).

Dashes indicate an insufficient number of observations for that work activity

[c]Reported as: 0 = Always, 1 = Sometimes, 2 = Never.

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"--"; Not investigated due to limited data available

Table 8. Relation of CR personal activities and S. aureus nasal carriage outcome measures to alpha diversity measures and IHO pig contributions of the CR adult's nasal microbiome. 2014, North Carolina, USA.

	CR adult			
	Shannon diversity	**Observed OTUs**	**Phylogenetic distance**	**% pig contributions[a]**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Demographics				
Age (by year)	0.02 (-0.03, 0.08)	6.27 (-0.85, 13.38)	0.41 (-0.05, 0.87)	-0.001 (-0.002, 0.001)
Sex (Male=reference)	**-1.76 (-2.62, -0.90)**	**-260 (-364, -155)**	**-16.8 (-23.2, -10.5)**	0.01 (-0.005, 0.016)
Personal and household exposure/activities				
Frequency of hand washing at work (for every 2 addtl. washes)	0.45 (-0.10, 1.01)	37.23 (-56.2, 131)	2.80 (-3.1, 8.7)	-0.002 (-0.008, 0.004)
Number of household members (per additional member)	0.08 (-0.27, 0.43)	18.9 (-25.8, 63.6)	1.43 (-1.39, 4.25)	-0.002 (-0.005, 0.002)
Household pet (Yes/No)	-0.33 (-0.96, 0.31)	-19.0 (-162, 124)	-1.85 (-11.1, 7.41)	-0.004 (-0.013, 0.004)
Current Smoker (Yes/No)	**-0.99 (-1.85, -0.12)**	-15.4 (-157, 126)	-1.64 (-10.8, 7.50)	-0.004 (-0.012, 0.004)
***S. aureus* nasal carriage outcomes**				
S. aureus (Yes/No)	0.70 (-0.28, 1.68)	61.93 (-75.1, 199)	3.62 (-5.17, 12.42)	0.01 (-0.01, 0.02)
MDRSA (Yes/No)	**-0.86 (-1.38, -0.35)**	**-126 (-196, -57.0)**	**-8.51 (-12.9, -4.09)**	0.004 (-0.01, 0.003)
scn negative (Yes/No)	**-0.85 (-1.38, -0.35)**	**-126 (-196, -57.0)**	**-8.51 (-12.9, -4.09)**	0.004 (-0.011, 0.003)
SourceTracker nasal carriage				
% pig contributions[a]	**-13. 8 (-21.5, -6.21)**	**-1930 (-2955, -904)**	**-144 (-209, -79.3)**	--

Note. IHO, industrial hog operation. CI, confidence interval. Beta (95% CI) estimated from linear regression models.

[a]% pig contributions defined as the percentage of OTUs contributed to the worker from the pig's total microbiome (nares and perineum) estimated via SourceTracker tool in QIIME -- SourceTracker is designed to predict the source (IHO or AFHO pig) of microbial communities in a set of sink samples (IHO or AFHO worker)

Dashes indicate an insufficient number of observations for that work activity.

Table 9. Relation of CR personal activities and S. aureus nasal carriage outcome measures to alpha diversity measures and IHO pig contributions of the CR child's nasal microbiome. 2014, North Carolina, USA.

	CR child			
	Shannon diversity	**Observed OTUs**	**Phylogenetic distance**	**% pig contributions[a]**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Demographics				
Age (by year)	0.11 (-0.18, 0.39)	0.98 (-1.52, 3.47)	18.5 (-20.9, 57.9)	0.001 (-0.01, 0.01)
Sex (Male=reference)	**-1.02 (-2.01, -0.03)**	-6.62 (-15.8, 2.55)	-97.0 (-245, 50.9)	-0.005 (-0.03, 0.02)
Personal and household exposure/activities				
Frequency of hand washing at work (for every 2 addtl. washes)	0.42 (-0.30, 1.15)	3.68 (-2.93, 10.29)	62.2 (-37.2, 162)	-0.01 (-0.03, 0.01)
Number of household members (per additional member)	0.27 (-0.10, 0.64)	2.46 (-1.37, 6.28)	41.8 (-19.4, 103)	-0.001 (-0.01, 0.01)
Household pet (Yes/No)	-0.26 (-1.60, 1.09)	-0.73 (-11.6, 10.1)	-23.7 (-177, 130)	0.002 (-0.03, 0.03)
Current Smoker (Yes/No)	--	--	--	--
***S. aureus* nasal carriage outcomes**				
S. aureus (Yes/No)	-0.23 (-1.25, 0.79)	-4.05 (-12.9,4,78)	-73.98 (-212, 64.2)	0.01 (-0.01, 0.03)
MDRSA (Yes/No)	**-1.00 (-1.56, -0.44)**	**-7.75 (-12.7, -2.79)**	**-120 (-198, -41,4)**	**-0.02 (-0.03, -0.004)**
scn negative (Yes/No)	**-1.00 (-1.56, -0.44)**	**-7.75 (-12.7, -2,79)**	**-120 (-198, -41,4)**	**-0.02 (-0.03, -0.003)**
SourceTracker nasal carriage				
Pig contributions (%)[a]	-12.5 (-28.8, 3.71)	-1951 (-3914, 12.3)	-123 (-248, 1.91)	--

Note. IHO, industrial hog operation. CI, confidence interval. Beta (95% CI) estimated from linear regression models.
[a]% pig contributions defined as the percentage of OTUs contributed to the worker from the pig's total microbiome (nares and perineum) estimated via SourceTracker tool in QIIME -- SourceTracker is designed to predict the source (IHO or AFHO pig) of microbial communities in a set of sink samples (IHO or AFHO worker)

Dashes indicate an insufficient number of observations for that work activity.

Table 10. The significance of the proportion of variation in Bray-Curtis measures (bacterial community membership and composition of the nasal microbiome) in relation to occupational swine production and personal exposure activities, 2014, North Carolina, USA.

	IHO worker Bray-Curtis	IHO child Bray-Curtis	CR adult Bray-Curtis	CR child Bray-Curtis
	R^2 (p-value)	R^2 (p-value)	R^2 (p-value)	R^2 (p-value)
Season	0.1170 (0.209)	0.1128 (0.195)	0.0610 (0.070)	0.0533 (0.359)
Demographics				
Age (per year)	0.0582 (0.291)	0.0606 (0.125)	0.0544 (0.269)	0.0542 (0.304)
Sex (male as referent group)	0.0537 (0.543)	0.0540 (0.310)	0.0766 (0.004)	0.0556 (0.235)
Personal and household exposure/activities				
Number of household members	0.0583 (0.257)	0.0491 (0.679)	0.0540 (0.341)	--
Household pet (Yes/No)	0.0525 (0.665)	0.0502 (0.525)	0.1020 (0.690)	0.0538 (0.381)
Current Smoker (Yes/No)	0.0516 (0.758)	0.0614 (0.097)	0.0592 (0.053)	0.0538 (0.371)
Personal antibiotic use within last 3 months (Yes/No)	--	0.0476 (0.856)	--	--
Occupation exposures				
Years worked at any swine farm (per year)	0.0645 (0.060)	0.0484 (0.726)	--	--
Hours of direct contact (per 8 hour shift)	0.0612 (0.150)	0.0457 (0.871)	--	--
Time since last work shift (per 8 hour shift)	0.7279 (0.269)	0.7462 (0.235)	--	--
Mask usage[b]	0.1747 (0.156)	0.1016 (0.602)	--	--
Gave pigs antibiotics (Yes/No)	0.0492 (0.892)	0.0492 (0.635)	--	--
Handled dead pigs (Yes/No)	0.0530 (0.617)	0.0485 (0.713)	--	--
Give pig shots (Yes/No)	0.1205 (0.099)	0.1192 (0.069)	--	--
Frequency of hand washing (for every 2 addtl. washes)	0.7743 (0.649)	0.0650 (0.022)	0.0564 (0.069)	0.0641 (0.037)
Showered after work[C]	--	--	--	--
Changed clothes after work [C]	0.0537 (0.632)	0.0475 (0.800)	0.1543 (0.644)	0.1717 (0.080)
Take IHO gear home (Yes/No)	0.1149 (0.153)	0.1069 (0.643)	--	--
***S. aureus* nasal carriage outcomes**				
S. aureus (Yes/No)	0.0730 (0.020)	0.0465 (0.842)	0.0510 (0.558)	0.0639 (0.055)
MDRSA (Yes/No)	0.0852 (0.001)	0.0595 (0.053)	0.0550 (0.305)	0.0546 (0.309)
scn negative (Yes/No)	0.0794 (0.006)	--	0.0550 (0.309)	0.0546 (0.307)
SourceTracker nasal carriage				
Pig contributions (%)[a]	0.7743 (0.662)	0.0650 (0.031)	0.0565 (0.070)	0.0641 (0.027)

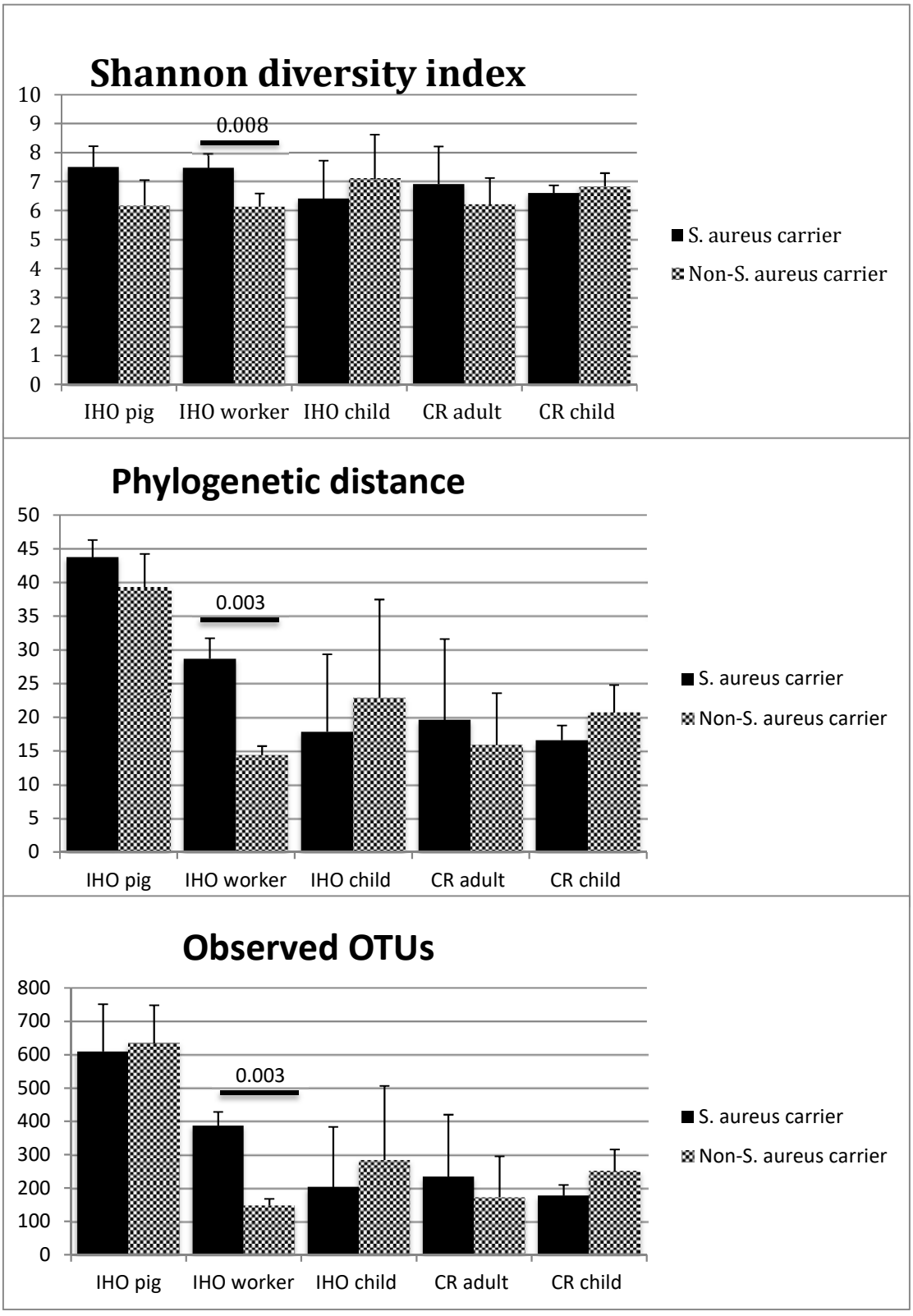
Note. IHO, industrial hog operation. CI, confidence interval. Beta (95% CI) estimated from linear regression models.

[a]% pig contributions defined as the percentage of OTUs contributed to the worker from the pig's total microbiome (nares and perineum) estimated

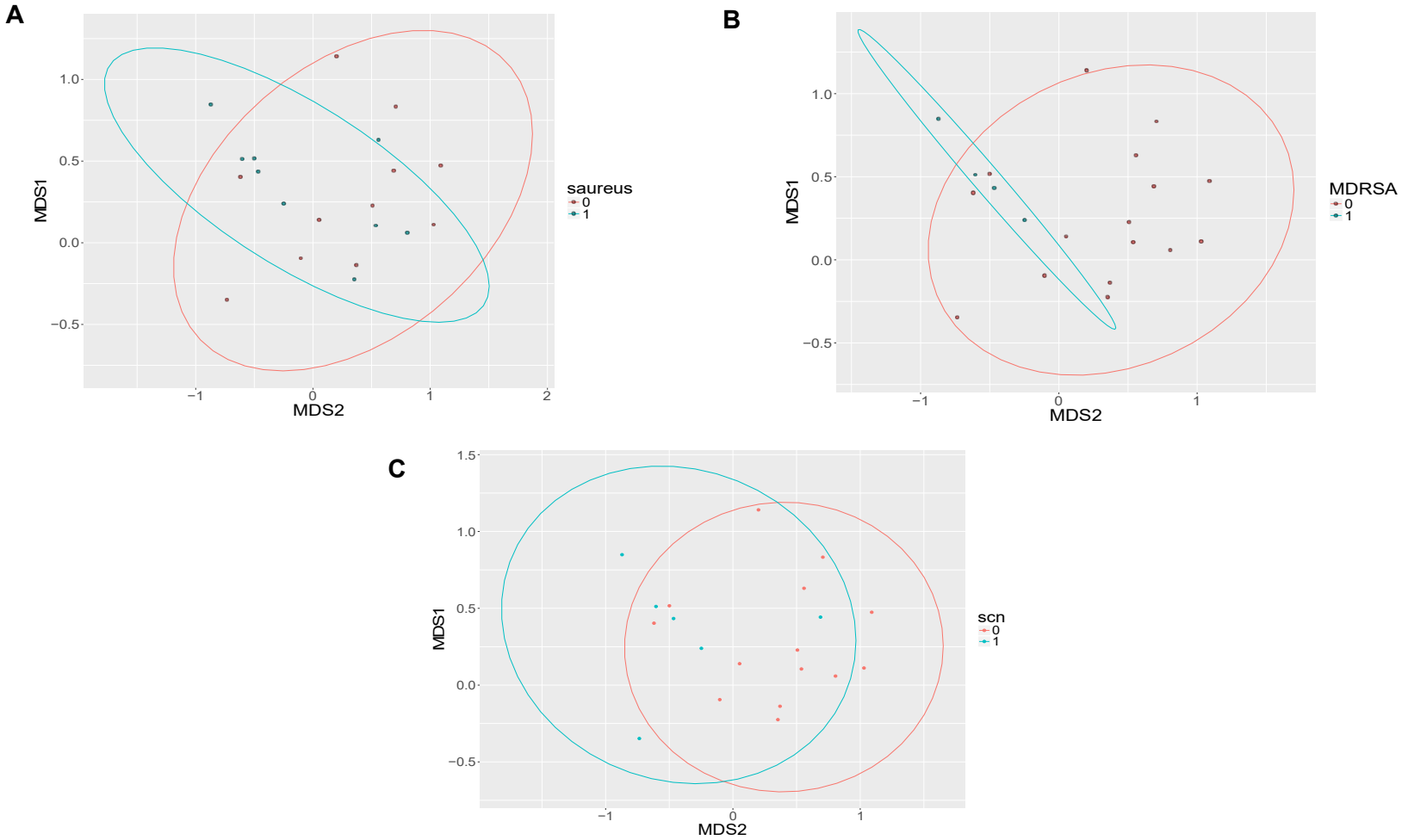
[b]Reported as: 0 = Always (≥ 80%), 1 = Sometimes (11-79%); 2 = Never (0-10%).

Dashes indicate an insufficient number of observations for that work activity

.



Supplementary Materials Figure S1. Alpha diversity of IHO pigs, IHO workers, IHO children, CR adults and CR children by S. aureus nasal carriage (carrier vs. non-carrier). Each vertical bar represents the average within-group Shannon diversity index, observed OTUs and phylogenetic distance. Error bars indicate the standard deviation (SD) of each alpha diversity measure.



Supplementary Material Figure S2. Non-metric dimensional scaling (NMDS) plots. Panels display IHO workers stratified by the following *S. aureus* nasal carriage outcome measures: A) shows *S. aureus* nasal carriage status; B) shows multidrug-resistant *S. aureus* (MDRSA) nasal carriage; and C) shows *scn*-negative *S. aureus* nasal carriage. We see lower dispersion for those IHO workers carrying MDRSA and *scn*-negative *S. aureus* versus those who do not.

Supplementary Materials Table S1. Read statistics for participant types (IHO workers, IHO children, CR adults and CR children), 2014, North Carolina, USA.

Raw reads	1,202,029
Successfully merged read-pairs	1,033,739
Average reads/sample pre-processing	13,977
Average successful paired reads/sample:	12,020
Average high quality reads/sample:	12,017
Average number of chimeras/sample	764
Average final clean reads/sample:	11,113
Average read length:	253

Pre- and post-processing parameters are outlined in methods section

Supplementary Materials Table S2. Significantly different OTUs (presence/absence) that were exclusively carried among S. aureus outcome nasal carriers and not among non-carriers among industrial hog operation (IHO) workers, 2014, North Carolina, USA.

		p-value		
Operational taxonomic unit (OTUs)	**Exclusive to**	***S. aureus***	**MDRSA**	***scn*-negative**
IHO worker				
*Staphylococcus equorum:Staphylococcus haemolyticus**	Carrier	0.000	0.000	0.000
*Cobetia crustatorum**	Carrier	0.000	0.000	0.000
Halomonas halodenitrificans	Carrier	0.004	0.000	0.000
Cronobacter malonaticus:Cronobacter sakazakii:Escherichia albertii:Escherichia coli:Escherichia fergusonii:Pantoea dispersa:Shigella boydii:Shigella dysenteriae:Shigella flexneri:Shigella sonnei	Non-carrier	0.000	0.000	0.000

Note. IHO, industrial hog operation. CR, community resident.

p-values estimated from G-test of independence identifying OTUs exclusively carried by one category of *S. aureus* nasal carriage outcome measures (*S. aureus*, MDRSA, *scn*-negative *S. aureus)* among IHO workers.

* = bacterial taxa exclusively carried by IHO workers, regardless of *S. aureus* nasal carriage outcome.

Supplemental Materials Table S3. Operational taxonomic units (OTUs) sourced from the IHO pig (nares and perineum) to IHO workers, IHO children, CR adults and CR children. North Carolina,

Source	Sink (n_{OTUs})	Operational taxonomic units (OTUs)
	IHO	*-Acinetobacter bouvetii:Acinetobacter*
		-Acinetobacter lwoffii:Prolinoborus fasciculus
		-Aerococcus urinaeequi:Aerococcus viridans
		-Clostridium baratii:Clostridium sardiniense
		-Corynebacterium freiburgense:Corynebacterium variabile
		-Corynebacterium freneyi:Corynebacterium xerosis
		-Facklamia tabacinasalis
		-Kocuria atrinae:Kocuria carniphila:Kocuria marina
		-Lactobacillus acidophilus:Lactobacillus amylovorus
		-Lactobacillus reuteri
		-Moraxella lacunata ◆
		-otu16326:Alloiococcus otitis:Facklamia tabacinasalis
		-otu18310:Bacteroides salanitronis:Barnesiella intestinihominis:
		-otu9045:Fulvimarina pelagi:Thiofaba tepidiphila
		-Rothia nasimurium
		-Staphylococcus carnosus:Staphylococcus condimenti:
	IHO child	*-Corynebacterium amycolatum:Corynebacterium lactis*
		-Corynebacterium stationis
		-Helcobacillus massiliensis
		-Moraxella lacunata ◆
		-otu13948:Leptotrichia goodfellowii
	CR adult (7)	*-Corynebacterium camporealensis:Corynebacterium glutamicum*
		-Corynebacterium confusum
		-Corynebacterium jeikeium
		-Facklamia tabacinasalis
		-Lactobacillus reuteri
		-Moraxella pluranimalium
		-Staphylococcus pettenkoferi
	CR child (8)	*-otu12199:Butyrivibrio fibrisolvens:Ruminococcus lactaris*
		-Kocuria atrinae:Kocuria carniphila:Kocuria marina
		-Moraxella lacunata ◆
		-Moraxella pluranimalium
		-Moraxella porci
		-otu13948:Leptotrichia goodfellowii
		-Rothia arfidiae:Rothia endophytica
		-Selenomonas bovis

SourceTracker is designed to predict the source (IHO pig) of microbial communities in a set of

◆ Observed in IHO workers, IHO children and CR children

 Observed in IHO children and CR children

 Observed in CR adults and CR children

Chapter Five: Temporal relation of livestock-associated microbial nasal carriage exposure measures and work activities with the nasal microbiome of industrial hog operation workers and children living in their households, North Carolina, USA

ABSTRACT

Background: Within industrial hog operations (IHOs), the exchange of livestock-associated [LA-] bacteria between pigs and humans can occur via airborne and direct contact pathways. Although cross-sectional analyses suggest a positive association of IHO work activities and LA-*S. aureus* nasal carriage outcomes with IHO workers' nasal microbiome diversity and community structure, the temporal variability of these associations remains unclear.

Objectives: To investigate the temporal relation of changes in IHO work activities and LA-microbial nasal carriage exposure measures (including LA-*S. aureus*) with changes in alpha diversity, beta diversity, and community composition among IHO workers and children living in their households (hereafter referred to as "IHO workers' children").

Methods: Nasal swab samples from baseline, midpoint, and endpoint study visits (representing a four month follow-up period) from IHO workers and IHO workers' children were sequenced targeting the V4 region of the 16S rRNA gene. We assessed differences in alpha (Shannon diversity, observed OTUs, and phylogenetic distance) and beta diversity (Morisita-Horn index) over time. We used generalized estimating equation (GEE) regression models to examine associations using two different classifications of exposure temporality: 1) transient time-varying exposure across study time points; and 2) an accumulating sum of exposure over time. For each exposure classification we investigated the association of IHO work activities and LA-microbial nasal carriage exposure measures with alpha diversity and beta diversity. LA-microbial nasal carriage exposure measures were examined as the single presence of one or a combined LA-microbial nasal carriage (exposure) index score of one or more of the following: *scn*-

negative *S. aureus*, presence of Pig-2-Bac (a swine-specific fecal microbial source tracking marker), or any bacterial contributions from IHO pigs. The G-test for independence was used to determine bacterial OTUs that were exclusively observed for specific *S. aureus* nasal carriage outcomes and we determined the overlap of OTUs contributed from the IHO pig to IHO workers and IHO workers' children.

Results: Presence vs. absence of multidrug-resistant *S. aureus* (MDRSA) and LA-microbial nasal carriage exposure measures (single presence and combined LA-microbial nasal carriage (exposure) index scores) were positively associated with alpha diversity over time. For the transient exposure classification, hours worked at the IHO per week, decreased mask usage, and *scn*-negative *S. aureus* nasal carriage positivity increased similarity of community structure over time among IHO workers and IHO workers' children. For the accumulation exposure classification, hours since last IHO work shift, hours worked at the IHO facility, hours of direct contact with IHO pigs, and a greater proportions of total work hours spent in direct contact with IHO pigs increased similarity of community structure over time among IHO workers and IHO workers' children.

Conclusion: Results suggest transient and accumulating IHO occupational exposures over time may have persistent impacts on the nasal microbiome of IHO workers and children living in their households, making it more similar over time. Use of facemasks and other personal protective equipment at IHOs should be investigated as a means to minimize the potential influence of LA-microbial exposure burdens on the nasal microbiome of IHO workers and children living in their households.

INTRODUCTION

The nasal cavity can serve as reservoir for microorganisms as it filters microbes attached to particulate matter from air that is inhaled.[1] The IHO environment contains diverse mixtures of microorganisms from environmental, animal, and human inputs.[2] There is evidence of transmission of microorganisms between pigs produced in IHOs and IHO workers.[3–6] Studies primarily have focused on culture-based investigations of *S. aureus* using markers of livestock-association (LA) in *S. aureus* (strains lacking the human immune evasion cluster gene *scn*) and antimicrobial resistance – e.g., MRSA and MDRSA.[3–6] LA-*S. aureus*, LA-MRSA and LA-MDRSA nasal carriage has been shown to more prevalent among IHO workers compared to unexposed community residents[3–6] and livestock workers without pig contact.[7,8]

Pisanic et al. in 2015 investigated the use of a novel microbial source tracking biomarker that is specific to pig fecal matter.[9] They demonstrated the utility of a swine-specific fecal *Bacteroidales* microbial source-tracking DNA marker (Pig-2-Bac) as a biomarker of livestock-associated microbe nasal carriage among IHO workers.[9] This suggested working at IHOs may create exposures to a mixture of diverse microbes derived from and/or accompanied by microbes found in pig fecal matter. Pisanic et. al., 2015, demonstration of a positive relation between Pig-2-Bac and LA-*S. aureus* nasal carriage among IHO workers suggests that a microbial marker of pig-specific fecal bacteria might improve understanding of the source and temporal dynamics of exposure to LA- and AMR bacteria of human health significance.

The potential for microbiome-based source tracking measures to advance our understanding of these complex exposure dynamics remains unclear. The influence of

hog production on the composition of the human nasal microbiome is limited to two studies.[2,10] Kates et al. (2017) found that pig farming led to an increased prevalence of potentially pathogenic OTUs in pig production workers.[10] Kraemer et al. (2017) found the pig farming environments led to a more homogeneous nasal microbiome (via the lower dispersion of the beta-diversity) among pig farmers compared to both diary farmers and non-exposed adults.[2] However, there are no studies, to our knowledge, have investigated temporal variations in alpha diversity, beta diversity, and the influences of LA-microbial nasal carriage exposure measures (e.g., LA-*S. aureus* and Pig-2-Bac) on the nasal microbiome of IHO workers and how this carriage might impact the nasal microbiomes of children living in their households.

The aims of the present study were to: 1) to determine the temporal variability in alpha diversity, bacterial contributions from the IHO pig, and beta diversity among IHO workers and children living in their households (hereafter referred to as "IHO workers' children"; 2) to estimate the time-varying relations between frequency and intensity of IHO work exposures and LA-microbial nasal carriage exposure measures (*scn*-negative *S. aureus*, Pig-2-Bac, and bacterial contributions from the IHO pig) with alpha diversity and beta diversity among IHO workers and children living in their households; 3) characterize temporal variability in bacterial community composition that was unique and/or overlapping between IHO pigs, IHO workers, and IHO workers' children, by LA-microbial nasal carriage exposure measures.

METHODS

Please refer to the detailed methodology (Chapter two) for DNA extraction and

amplification, library preparation and sequencing, bioinformatics sequence processing, taxonomic assignments, and OTU contamination removal methods.

Definitions of derived variables from questionnaire data

Within this study, we have derived binary and categorical variables from questionnaire data for both IHO workers and IHO workers' children. Face mask usage was defined as a categorical variable as always (80% or greater mask usage; reference), sometimes (10-79% mask usage) and never (less than 10% mask usage). In analyses of indirect IHO exposure for IHO workers' children, the occupational exposures of the IHO worker were assigned to the child living in their same household. Binary variables were coded based on the presence (coded as 1) and absence (coded as 0) for the Pig-2-Bac.

A LA-microbial nasal carriage exposure measures was evaluated using the presence/absence of each of the following as stand-alone measures: *scn*-negative *S. aureus*, Pig-2-Bac, or any bacterial contribution from IHO pigs. We also created a LA-microbial nasal carriage (exposure) index score based on the presence/absence of each of the above measures. This index variable's range was between 0 (none were present) and 3 (all 3 were present). We considered two hypotheses: 1) Transient time-varying changes (from time point to time point) in the intensity of hog production work activities, *S. aureus* nasal carriage outcomes, and LA-microbial nasal carriage exposure measures will be related to changes in the nasal microbiome composition and diversity profiles over time; and 2) Changes in the accumulation of exposure over time (incremental sum of exposure from time point to time point) for intensity of hog production work activities, *S. aureus* nasal carriage outcomes, and LA-microbial nasal carriage exposure measures will

be related to changes in alpha diversity and will be related to an increasingly homogeneous nasal microbiome of IHO workers and their household members.

Statistical analysis

Following pre-processing and quality control, singletons were filtered before downstream analysis. OTUs found within field blanks, trip blanks, laboratory processing controls, and DNA negative controls were filtered from sequence data to remove contaminant OTUs from the field, transportation, JHU laboratory and laboratory processing prior to downstream analysis.

The following alpha diversity measures were calculated as a reflection of the bacterial diversity within each individual sample: Shannon diversity (overall bacterial diversity, taking into account OTU richness and evenness), phylogenetic distance (the diversity of lineages represented in OTUs) and observed OTUs. These measures have been utilized in previous investigations of the influence of antimicrobial drug use on the fecal and nasal microbiome.[11–26] Data were not rarefied to include all valid data available.[27] The Student's t-test was used to assess differences in alpha diversity measures.

Normalized OTU tables were produced, using the DESeq2 tool within QIIME, prior to generating beta diversity measures to account for uneven sample sums as a result of sequencing techniques and possible low depth of coverage samples.[28,29] We assessed changes in beta diversity over time using the Morisita-Horn dissimilarity index. This index takes into account sequence presence/absence and relative abundance of bacterial taxa present within the microbial community (ranging from 0 to 1). A value of 0

indicates there is not similarity between two bacterial communities. A value of 1 indicates complete similarity between two bacterial communities.

SourceTracker, a tool within MacQIIME 1.9.1, is a Bayesian approach to estimate the proportion of OTUs in a given community/participant type that originate from the IHO pig.[30] We used this tool to predict the taxonomic contributions from IHO pig (specified as the source population from our previous study)[31], to IHO workers and children in their household (specified as the sink).[30,32]

I ran both fixed effects[33] and GEE regression models. We present the results of within-person GEE regression model beta coefficients because they were consistent with the fixed effects model beta coefficients. We used GEE regression models adjusted for repeated measures within individuals[33] to estimate the relation of transient time-varying occupational exposures and LA-microbial nasal carriage exposure measures (*scn*-negative *S. aureus*, Pig-2-Bac, and bacterial contributions from an IHO pig) with alpha diversity measures and the Morisita-Horn index. Next, we used GEE models to estimate the influence of time-varying accumulating occupational exposures and LA-microbial nasal carriage exposure measures (*scn*-negative *S. aureus*, Pig-2-Bac, and bacterial contributions from an IHO pig) with alpha diversity measures and the Morisita-Horn index.

We characterized the bacterial contributions from the IHO pig to IHO workers and to IHO workers' children. We also assessed the bacterial OTUs exclusively carried by IHO workers and children living in their household and by *S. aureus* nasal carriage outcomes and determined the overlap of these OTUs with those contributed by the IHO pigs in both IHO workers and children living in their household.

RESULTS

Read statistics

Supplementary Materials Table S1 outlines the read statistics for IHO workers and children living in their household. On average, 13,602 reads were assigned to each sample. After quality control and contaminants as well as chimera removal, an average of 11,310 clean reads were assigned to each sample. The average read length was 253 base pairs.

Time-invariant baseline personal demographics and activities

Population demographics including age, ethnicity, sex, personal antibiotic use in the last 3 months, self-reported asthma, current smoker status and going to the gym or playing sports in the last 3 months is reported within Table 1. Household units were comprised of an IHO worker and a child living in their household. A total of 43% of (n=9) households owned a pet. Average household size within this study was 4, with a range from 1-7 household members.

IHO workers were on average 38 years of age. All IHO workers were Hispanic and 52% were male (11/21). Sixteen percent (3/21) of IHO workers used personal antibiotic drugs within the 3 months prior to study enrollment. None of the IHO workers self-reported having asthma. Five percent (1/21) of IHO workers were current smokers and 14 percent (3/21) went to the gym or played sports in the 3 months prior to study enrollment.

IHO workers' children were on average 10 years of age. All children were Hispanic and 67% were (14/21) male. Five percent of IHO workers' children (1/21) used personal antibiotic drugs in the 3 months prior to study enrollment. Nineteen percent (4/21) of IHO workers' children self-reported asthma. Eighty-six percent (18/21) of IHO workers' children went to the gym or played sports in the last 3 months prior to study enrollment.

Occurrence of transient time-varying facemask usage, hygienic practices and S. aureus nasal carriage outcomes over time

Transient time-varying occupational exposures, personal facemask usage at work, *S. aureus* and LA microbial exposure markers by timepoint is summarized in Table 2. Timepoint 0 is the baseline visit, timepoint 1 is the mid-point visit, and timepoint 2 is the end-point visit. Occupational exposures are scaled by the 8-hour work shift. The majority of IHO workers spent the majority of their work shifts in direct contact with IHO pigs. On average, IHO workers used face masks 62% of the time across the three timepoints. Categorical mask usage, *S. aureus* and LA-*S. aureus* varied widely between timepoints. In our study, the number of times an IHO worker washed their hands was not associated with *S. aureus* nasal carriage, specifically LA-*S. aureus* (Supplementary Materials Table S2).

Alpha diversity changes over time

Figure 1 displays the temporal changes in alpha diversity measures (Shannon diversity, phylogenetic distance and observed OTUs) along with standard errors to

determine significant differences between IHO workers and IHO workers' children. On average, IHO workers were more diverse compared to their children. Shannon diversity over time was not significantly different (p>0.05) however average phylogenetic distance (p <0.004) and average observed OTUs (p <0.003) were significantly higher in IHO workers compared to IHO workers' children.

Alpha diversity changes over time by S. aureus nasal carriage outcomes

IHO workers who were *S. aureus* nasal carriage outcome positive versus negative were more diverse in measures of phylogenetic distance and observed OTUs. Shannon diversity in IHO workers did not differ between *S. aureus* outcome carriers and non-carriers (p>0.05). The microbiome of IHO workers' children who carried versus did not *S. aureus* were less diverse over time (Figure 3).

Transient occupational activities and S. aureus nasal carriage outcomes associated with changes in alpha diversity, bacterial contributions from the IHO pig and beta diversity

IHO workers

Transient time-varying changes in IHO workers' occupational exposures, *S. aureus* nasal carriage and LA microbial exposure marker nasal carriage outcomes were associated with increased alpha diversity and increased IHO pig bacterial taxa contributions (Table 3). Time since last work shift significantly increased Shannon diversity (beta= 0.15, 95% CI =0.10, 0.28). IHO workers who carried MDRSA were more diverse in phylogenetic distance measures (beta= 10.2, 95% CI =2.69, 17.7) and

observed OTUs (beta= 204, 95% CI =80.8, 328) than those who did not carry MDRSA. We observed a 40% increase in bacterial contributions from the IHO pig for those IHO workers who carried MDRSA vs. those who did not (beta= 39.5, 95% CI =15.7, 63.4). The nasal carriage vs. not of Pig-2-Bac was associated with an increase in observed OTUs ((beta= 98.8, 95% CI =11.4, 186) and an 18% increase in bacterial contributions from the IHO pig (beta= 18.1, 95% CI =1.11, 35.16). Bacterial contributions from the IHO pig, investigated as an independent variable (transient exposure measure) was associated with significant increases in measures of Shannon diversity (beta= 0.02, 95% CI =0.001, 0.03), phylogenetic distance (beta=0.21, 95% CI =0.14, 0.28), and observed OTUs (beta= 3.62, 95% CI =2.46, 4.78). A one-unit increase in the LA-microbial nasal carriage (exposure) index score was associated with increases in Shannon diversity (beta= 0.76, 95% CI = 0.34, 1.19), phylogenetic distance (beta= 4.65, 95% CI = 2.12, 7.18) and observed OTUs (beta= 81.5, 95% CI = 38.6, 124). Every 17% (95% CI = 9.04, 25.1) increase in bacterial contributions from the IHO pig was associated with a one-unit increase in the LA microbial exposure index score. In table 3, the nasal microbiome community structure of IHO workers became more similar over time with each additional 8-hour shift at the IHO facility (beta= 0.03, 95% CI =0.01, 0.03) and decreased face mask usage (beta= 0.40, 95% CI =0.19, 0.62).

IHO children

Transient time-varying IHO occupational exposures of the household IHO worker were not consistently related to changes in alpha diversity, bacterial contributions from the IHO pig, or beta diversity among IHO workers' children (Table 4). Children living

with IHO workers who had frequent direct contact with pigs were observed to have decreased alpha diversity. *S. aureus* and LA-microbial nasal carriage exposure marker positivity among IHO workers' children was associated with increased alpha diversity and decreased bacterial contributions from the IHO pig. In IHO workers' children, the nasal carriage of *scn*-negative *S. aureus* was associated with increases in Shannon diversity (beta: 1.52; 95% CI: 0.14, 2.90). A one-unit increase in the LA microbial exposure marker index score was associated with an increase in bacterial contributions from the IHO pig (beta= 1.79, 95% CI = 0.39, 3.19).

The nasal microbiome community structure of IHO workers' children became more similar over time with each additional 8-hour shift at the IHO facility (beta= 0.02, 95% CI =0.01, 0.02). Face mask usage of the household IHO worker did not influence the similarity of the nasal microbiome of IHO workers' children between two adjacent timepoints.

Accumulating occupational exposures is associated with greater similarity in bacterial community structure over time

Table 5 models the influence of accumulating occupational exposures (scaled by an 8-hour shift) and persistent/accumulating *S. aureus* nasal carriage and LA microbial exposure index scores on alpha diversity, bacterial contributions from the IHO pig and bacterial community structure (Morisita-Horn index) in IHO workers.

IHO workers

An accumulation of more frequent exposures to pigs within the IHO facility environment over time was associated with decreases in alpha diversity and bacterial contributions from the IHO pig as well as a greater similarity of the nasal microbiome structure of IHO workers. We observed greater similarity in the current bacterial community structure (between a subsequent timepoint compared to its previous and adjacent timepoint) for every additional 8-hours since the last IHO work shift (beta: 0.15; 95% CI: 0.04, 0.26), for every additional 8-hours worked at the IHO facility (beta: 0.005; 95% CI: 0.003, 0.01), and for every additional 8-hours of direct contact with IHO pigs (beta: 0.01; 95% CI: 0.004, 0.02). An increase in the proportion of time spent in direct contact with IHO pigs during work was associated with a strong similarity of the bacterial community membership and composition between a subsequent compared to its previous and adjacent timepoint (beta: 0.48; 95% CI: 0.35, 0.60).

A one-unit increase in the accumulated LA microbial exposure index score was associated with decreased alpha diversity and increased bacterial contributions from the IHO pig. A one-unit increase in the accumulated LA microbial exposure index was associated with a strong similarity of a subsequent compared to its previous and adjacent timepoint's current bacterial community membership and composition (beta= 0.24, 95% CI = 0.15, 0.33).

IHO workers' children

Table 6 shows results of models of the influence of persistent/accumulating occupational exposures (scaled by an 8-hour shift) and persistent/accumulating *S. aureus*

nasal carriage and LA microbial exposure index scores on alpha diversity, bacterial contributions from the IHO pig and bacterial community structure (Morisita-Horn index) among IHO workers' children. More frequent exposure to pigs within the IHO facility environment decreased alpha diversity and bacterial contributions from the IHO pig (all $p > 0.05$). More frequent exposure to pigs within the IHO facility environment was associated with greater similarity in the nasal microbiome community structure over time (all $p < 0.05$).

We observed greater similarity in the current bacterial community structure to the previous timepoint for every additional 8-hours since your last work shift (beta: 1.08; 95% CI: -0.07, 2.22), for every additional 8-hour shift at the IHO facility (beta: 0.005; 95% CI: 0.001, 0.01), and for every additional 8-hour shift in direct contact with IHO pigs (beta: 0.01; 95% CI: 0.003, 0.02). An increase in the proportion of time spent in direct contact with pigs during their work was associated with a strong similarity of the bacterial community membership and composition between a subsequent compared to its previous and adjacent timepoint (beta: 0.45; 95% CI: 0.14, 0.77).

In IHO workers' children, a one-unit increase in the accumulated LA microbial exposure marker index score was associated with an increase in alpha diversity and a decrease in bacterial contributions from the IHO pig. A one-unit increase in the accumulated LA microbial exposure marker index was associated with a strong similarity of the bacterial community membership and composition between a subsequent compared to its previous and adjacent timepoint (beta= 0.64, 95% CI = 0.27, 1.01).

OTUs contributed to the IHO worker and IHO workers' children from the IHO pig and overlap between participant types

The QIIME SourceTracker tool allowed for the enumeration of the OTUs contributed (in relative abundances greater than 1%) by the IHO pig to the nasal microbiome of IHO workers and IHO workers' children (Table 7). IHO pigs over the course of the study, contributed 43 OTUs to the IHO worker's nasal microbiome. Three of these 43 OTUs were *Staphylococcus* OTUs (*Staphylococcus carnosus: Staphylococcus condimenti: Staphylococcus haemolyticus:Staphylococcus piscifermentans:Staphylococcus simulans, Staphylococcus koferi, and Staphylococcus sciuri*). Over the course of the study, IHO pigs contributed 14 OTUs to the IHO workers' children's nasal microbiome. Only one *Staphylococcus* OTU was contributed to the IHO worker's child (*Staphylococcus pettenkoferi*). Six OTUs overlapped between IHO workers and the IHO workers' children.

OTUs shared by S. aureus outcome carriers in IHO workers and IHO workers' children and overlap with OTUs contributed by the IHO pig

The G-test for independence confirmed the OTUs exclusively observed in carriers of *S. aureus* nasal carriage outcome (*S. aureus,* MDRSA, and *scn*-negative *S. aureus*) in IHO workers and IHO children (Tables 8 and 9). Six OTUs are shared by all three *S. aureus* nasal carriage outcomes, six OTUs shared between *S. aureus* and MDRSA, and five OTUs shared between MDRSA and *scn*-negative *S. aureus* carriers. OTUs contributed by the IHO pig that overlap with *S. aureus* nasal carriage outcomes include: two OTUs exclusively observed in *S. aureus* carrying IHO workers versus non-carriers,

eight OTUs exclusively observed in MDRSA carrying IHO workers versus non-carriers and five OTUs exclusively observed in *scn*-negative *S. aureus* carrying IHO workers versus non-carriers. In IHO workers' children, two OTUs are shared by all three *S. aureus* nasal carriage outcomes, two OTUs shared between *S. aureus* and MDRSA, and two OTUs shared between MDRSA and *scn*-negative *S. aureus* carriers. OTUs contributed by the IHO pig do not overlap with *S. aureus* nasal carriage outcomes in IHO workers' children. IHO worker and IHO workers' children who carried *S. aureus* and MDRSA have no overlap in exclusive OTUs (Table 9 and 10). *Scn*-negative *S. aureus* nasal carriers in IHO workers and IHO workers' children exclusively carried one OTU (Table 9 and 10).

DISCUSSION

The present study demonstrated that IHO work activities, *S. aureus* nasal carriage outcomes, and LA-microbial nasal carriage exposure measures (considered as stand-alone microbial measures and as a combined index score) were associated with higher nasal microbiome alpha diversity and increased homogeneity of the nasal bacterial community structure among IHO workers and their children over time. We also observed an overall trend of higher alpha diversity among IHO workers compared to children living in their households. There was consistency in these findings for both exposure classification approaches that were considered – i.e., transient time-varying as well as an accumulation of IHO occupational activity, *S. aureus* nasal carriage, and LA-microbial nasal carriage exposures over time.

In models of transient, time-varying exposure, IHO work activities (particularly greater hours worked at the IHO per week, less frequent use of a face mask, and hours spent in direct contact with IHO pigs) and positivity of *S. aureus* nasal carriage outcomes (*S. aureus,* MDRSA and *scn*-negative *S. aureus*) were associated with increases in IHO worker alpha diversity. Not all of the transient time-varying IHO work exposures were consistently positively associated with changes in alpha diversity or bacterial contributions from IHO pigs. For example, unexpectedly, increasing hours of direct contact with IHO pigs per week (and a higher proportion of the IHO work shift spent in direct contact with IHO pigs) were associated with decreases in percent bacterial contributions from IHO pigs among both IHO workers and their children. We were unable to explain these trends observed.

We assessed the influence of IHOs on the nasal microbiome of IHO workers and their children with the knowledge that occupational exposures to the IHO and IHO pigs has been associated with increased *S. aureus* carriage.[3–6] MDRSA has been positively associated with exposures at the IHO environment and from the IHO pig from studies employing a single-pathogen culture-based measurement approach.[3,6,35] Higher alpha diversity among *S. aureus* carriage positive individuals is similar to previous findings by Singh et al., (2016).[34] Increased percent bacterial contributions from IHO pigs to IHO workers were also associated with MDRSA (transient time-varying exposure measures). IHO workers' children with *S. aureus* nasal carriage outcome positivity and those who carried greater contributions from the IHO pig were less diverse in transient time-varying exposure models. Contrary to this, was our observation that transient time-varying nasal carriage of *scn*-negative *S. aureus*, a marker of LA-*S. aureus*,[4] in IHO workers' children

was significantly associated with increases in alpha diversity and percent bacterial taxa contributions from the IHO pig. The IHO worker living in their households as well as bioaerosols downwind of the IHO may represent a source for *scn*-negative *S. aureus* exposure and bacterial contributions from the IHO pig.[36–39] Only exposure to the IHOs, IHO pigs and/or LA microbial exposure markers tended to correlate with increased diversity in the nasal microbiome of IHO workers and their children alike.

Transient time-varying LA-microbial nasal carriage exposure measures (*e.g. scn*-negative *S. aureus*, Pig-2-Bac, and total percent bacterial contributions from the IHO pig) were positively associated with changes in alpha diversity measures within IHO workers and their children. This finding argues that the presence of LA-bacteria may have an ability to change the alpha diversity of IHO worker's and their children, at least transiently. Among IHO workers, a greater LA-microbial nasal carriage marker index score (0-3) was associated with greater alpha diversity as well as percent bacterial contributions from IHO pigs. Within IHO workers' children, a greater time-varying LA-microbial nasal carriage marker index score was associated with an increase in the percent bacterial contributions from IHO pigs. These findings suggest that IHO pigs' microbiomes may impact the nasal microbiome of IHO workers and their children by increasing alpha diversity and opportunities for LA-microbial nasal colonization.

Within previous literature, Pisanic et al. (2015), determined the utility of Pig-2-Bac, a swine-specific fecal *Bacteroidales* source-tracking marker, to help identify sources of *S. aureus* carriage within IHO workers.[9] This study suggested the transient time-varying nasal carriage of Pig-2-Bac was positively associated with *S. aureus* and MDRSA nasal carriage.[9] The novel use of specific LA-microbial nasal carriage exposure

measures (*e.g. scn*-negative *S. aureus*, Pig-2-Bac, and total percent bacterial contributions from the IHO pig) was spurred by these findings. Our study found that transient time-varying Pig-2-Bac was positively associated with the total percent bacterial contributions from IHO pigs to IHO workers. As previously discussed, the OTUs contributed from IHO pigs were dominated by those derived from IHO pigs' perineum samples. The positive associations observed between the percent OTUs contributed from IHO pigs to IHO workers and Pig-2-Bac carriage may be explained as these two measures serve proxies for the IHO workers' exposure to IHO pigs' fecal matter. Analogous positive associations were observed among IHO workers' children, however the magnitude was 100 times lower compared associations for IHO workers.

In transient time-varying exposure classification models, we also observed that an increased frequency of direct contact with IHO pigs and the IHO environment were consistently associated with an increase in nasal bacterial community structure homogeneity at adjacent timepoints among IHO workers and their children. These findings are consistent with a recent study by Kraemer et al. (2017), which demonstrated that exposure to the IHO environment resulted in a more homogenous bacterial composition and less dispersion of beta diversity among pig workers' nasal microbiomes.[2]

In accumulating time-varying exposure classification models, we observed that a greater accumulation of occupational exposures over time was associated with decreased alpha diversity and bacterial contributions from IHO pigs to IHO workers and their children. This may be due to a maximum level of nasally acquired foreign bacterial taxa or that the bacterial taxa already contributed by IHO pigs and present in the nasal

microbiome of IHO workers may have inhibitory ecological effects on new bacteria and therefore limit further OTU acquisition over time. Contrary to the observed trends for accumulating IHO work activity exposures, the accumulation of the LA-microbial nasal carriage (exposure) index score over time was associated with increased alpha diversity and decreased percent bacterial contributions from IHO pigs. This finding suggests the persistence of LA-microbial nasal carriage exposure measures (a larger index score) may influence the diversity of the nasal microbiome for workers and children to become more unstable with a greater risk for the nasal acquisition of foreign bacterial taxa.[12,40] The newly acquired foreign taxa as well as the selective pressures from antimicrobial drug use may then further perturb the nasal microbiome.[12]

However, when investigating the association of accumulating occupational exposures (involving increased direct contact with pigs) with beta diversity, we found that such measures increased the similarity of beta diversity (comparing midpoint and endpoint to the previous adjacent timepoint) among IHO workers and children living in their households. Additionally, the accumulation of the LA-microbial nasal carriage (exposure) index score—meaning the greater the number of LA-microbes present over time—the more likely the bacterial community structure was to remain the same over time. These results suggest there is a homogenous microbial pressure present at IHOs over time and that the LA-microbial nasal carriage index may serve as a proxy to determine the presence and magnitude of IHO pig-related microbial signature's impact on the nasal microbiome of both IHO workers and children living in their households.

IHO pigs were estimated to contribute a greater number of OTUs to IHO workers (n=43) compared to IHO workers' children (n=14). OTUs contributed from the IHO pig

to IHO workers have previously been found in pigs (*Aerococcus urinaeequi:Aerococcus viridans*[41], *Moraxella pluranimalium*[42], and *Moraxella porci*[43]), found in bacteremic pigs (*Staphylococcus pettenkoferi*[44,45]) and are associated with the use of prebiotics and probiotics within the pig diet (*Lactobacillus* species[46,47] and *Parabacteroides distasonis*[48]). Additionally, MDRSA and *scn*-negative *S. aureus* positive IHO workers exclusively carried greater numbers of bacterial OTUs contributed from IHO pigs. Both MDRSA and *scn*-negative *S. aureus* have been largely found in the IHO environment support the use of these two strains of *S. aureus* as livestock-associated markers.[3,4,6] We observed greater overlap in the OTUs exclusively observed in MDRSA and *scn*-negative *S. aureus* positive IHO workers and OTUs contributed by IHO pigs to IHO workers.

Two of the three *Staphylococcus* OTUs contributed from the IHO pig to the IHO worker were found within the pig farms stable dust (*Staphylococcus carnosus: Staphylococcus condiment: Staphylococcus haemolyticus:Staphylococcus piscifermentans:Staphylococcus simulans*, and *Staphylococcus sciuri*).[37] *Staphylococcus pettenkoferi* contributed from the IHO pig to the nasal microbiome of IHO workers' children was also found in stable dust within pig farms.[37] Both IHO workers and children living in their households were colonized by *Staphylococci* species primarily sourced from the IHO pig, presumably via direct contact with pigs and via inhalation of bioaerosols in the form of stable IHO dust.[37] In our study, we found the number of times an IHO worker washed their hands was not associated with *S. aureus* nasal carriage, specifically LA-*S. aureus*. This finding is similar to Feld et al. (2018) who found that hand washing was not associated with increased prevalence of nasal carriage of LA-MRSA [37] However, because our sample sizes are small the confidence in these

associations is not strong. Consistent results in a future larger study may signify that inhalation may be the primary source of exposure to these LA-bacteria.

Within this study, transient time-varying facemask usage in IHO workers protected the ability of the nasal microbiome to go through its natural fluctuations in community diversity and structure (beta diversity). The use of a facemask can act as a barrier and filtration system for the respiratory tract instead of the nasal cavity acting as the primary filter. Typically, the nose functions as an efficient filter, however, with such high bacterial loads present at IHOs there is a great need for the use of personal protective equipment (PPE) for better protection against IHO-sourced bacteria that are not as well understood currently within the literature.

The strengths of this study include the access to IHO workers as well as their household children. Access to workers allows us to assess the influence of IHO occupational exposures on the nasal microbiome. Access to the children of IHO workers allowed us to assess the influence of indirect IHO exposures through IHO worker on the nasal microbiome of the IHO workers' children. The use of the SourceTracker tool allowed us to assess the bacterial taxa contributed to the nasal microbiome of IHO workers and IHO workers' children from the source of IHO pigs, as a proxy for the IHO facility environment. The greatest strength of this study is the longitudinal exposure-outcome analysis that allowed for the investigation of temporal variability (using two different assumptions of exposure temporality—transient and accumulating time-varying) of alpha diversity, beta diversity and bacterial contributions from IHO pigs on the nasal microbiome.

Our study had several important limitations. For future studies, there is a need to sample IHO environments (air, settled dust, surfaces, wastes), IHO pigs, IHO pig workers as well as IHO workers' household environments in order to distinguish between the influence of the IHO environment, IHO pig, and the household environment on the nasal microbiome of IHO workers' and their household children over time. Kraemer et al. (2017) suggested IHOs need to continue to be investigated as potential sources of homogenous microbial pressure on the nasal microbiomes of pig workers. Vestergaard et al. (2018), observed the airborne bacterial communities of pig stables and farmers' homes to have similar diversity and abundances of bacterial taxa compared to suburban homes.[49] Stables and homes also were observed to have greater absolute and relative abundances of taxa known to have protective effects against respiratory illnesses (*e.g.* asthma).[49] Although there was no direct evidence of transfer between the stables and homes observed by Vestergaard et al. (2018), Feld et al. (2018) found evidence of shared taxa between pigs and pig workers while investigating the survival of LA-MRSA in dust from swine farms.[37,49] Feld et al. (2018) determined that there is a risk of LA-MRSA nasal colonization from farm dust due to occupational farm environment exposures, that may also be transported to other environments through dust and other particulate matter.[37]

The selection of the V4 region of the 16S rRNA gene is less distinguishing of *S. aureus* strains compared to the V1-V3 region.[50] The requirement to select three out of the nine follow up visits within the four month study due to funding limitations contributed to a lack of large sample sizes for given exposure groups. This limited sample size may impact the certainty of conclusions made within this paper although to our knowledge we are the first group to perform longitudinal analysis of the influence of IHO facilities on

the nasal microbiome of IHO workers and their household children. For future studies, there is an ongoing need to determine the most meaningful occupational activities that correlate directly with direct IHO pig exposures and large inputs of bacterial loads sourced from the IHO environment. In future studies, the use of IHO exposure index scores to quantify an IHO worker's magnitude of IHO sourced bacterial influx to the nasal cavity of the IHO worker.

CONCLUSION

Our study found key differences in the alpha diversity of IHO workers' and IHO workers' children's nasal microbiomes over time which varied according to differences in transient time-varying hours at an IHO per week, direct contact with IHO pigs, MDRSA nasal carriage, and LA-nasal carriage microbial exposure marker presence versus absence. Accumulating IHO exposures over time as well as direct contact with IHO pigs was associated with homogeneity in the nasal microbiome community structure among IHO workers and IHO workers' children. The overlap in OTUs observed among those who carried *S. aureus* intranasally (versus those who did not) and OTUs contributed from IHO pigs with evidence of persistence in the IHO pig confinement building dust and air of suggests the need for further investigation of the ecological dynamics of these bacterial exposure burdens, including persistence of the nasal microbiome of IHO workers and their sources. The use of facemasks may mitigate the homogeneous pressure from IHOs and IHO pigs on the nasal microbiome of IHO workers and their children to become more similar to their previous timepoint. Improved surveillance and an emphasis on improved guidance for PPE use at IHOs appears

necessary to limit this homogeneous exposure pressure on the nasal microbiome of IHO workers as well as children living in their households.

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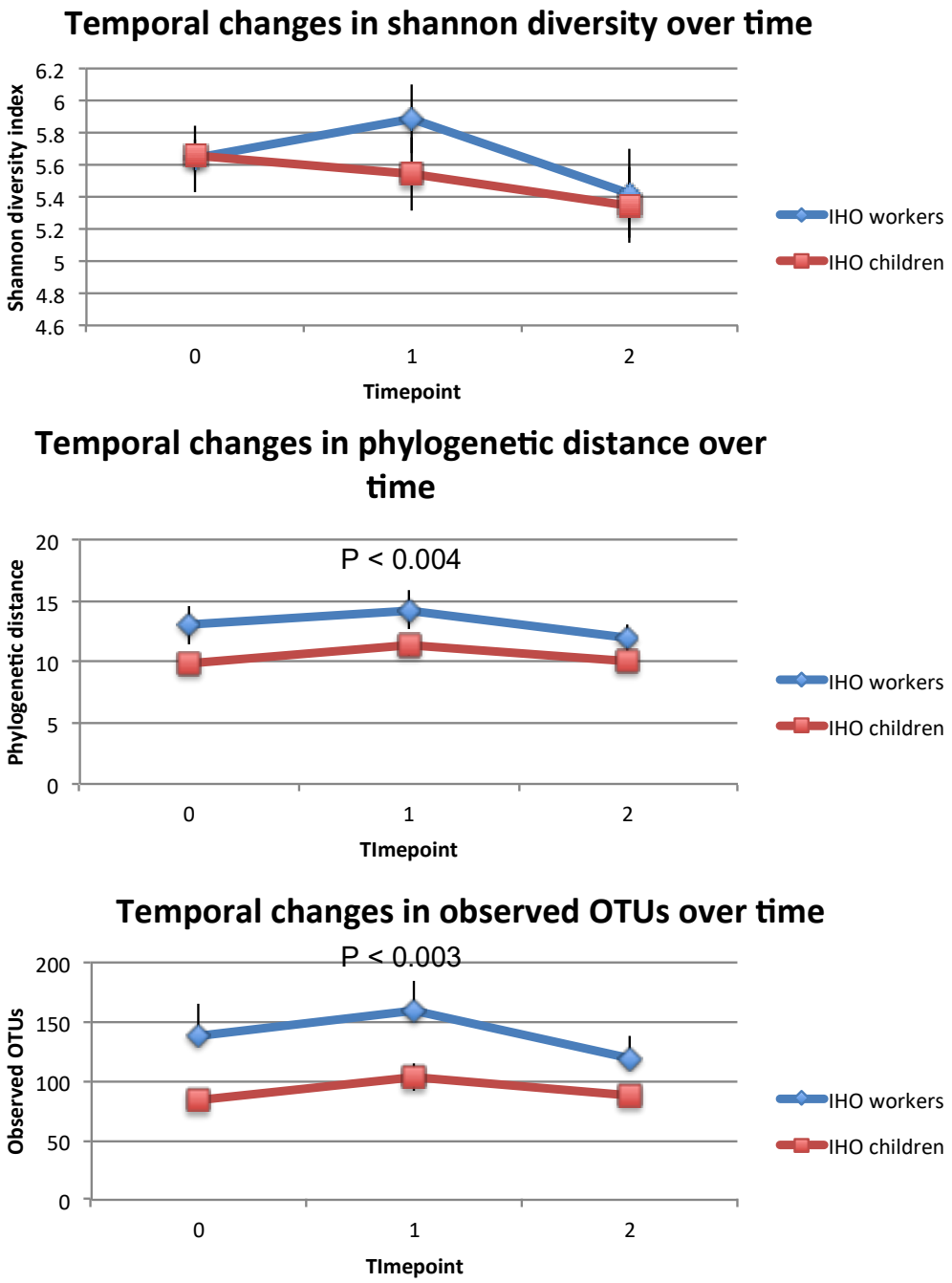


Figure 1. Temporal variability in alpha diversity measures between IHO workers and children living in their households**.**
Panel displays the temporal changes in alpha diversity measures over time in IHO workers and IHO workers' children using a Student's t-test. On average, we observed higher alpha diversity in workers compared to minors. We observed significant differences in phylogenetic distance and observed OTUs.

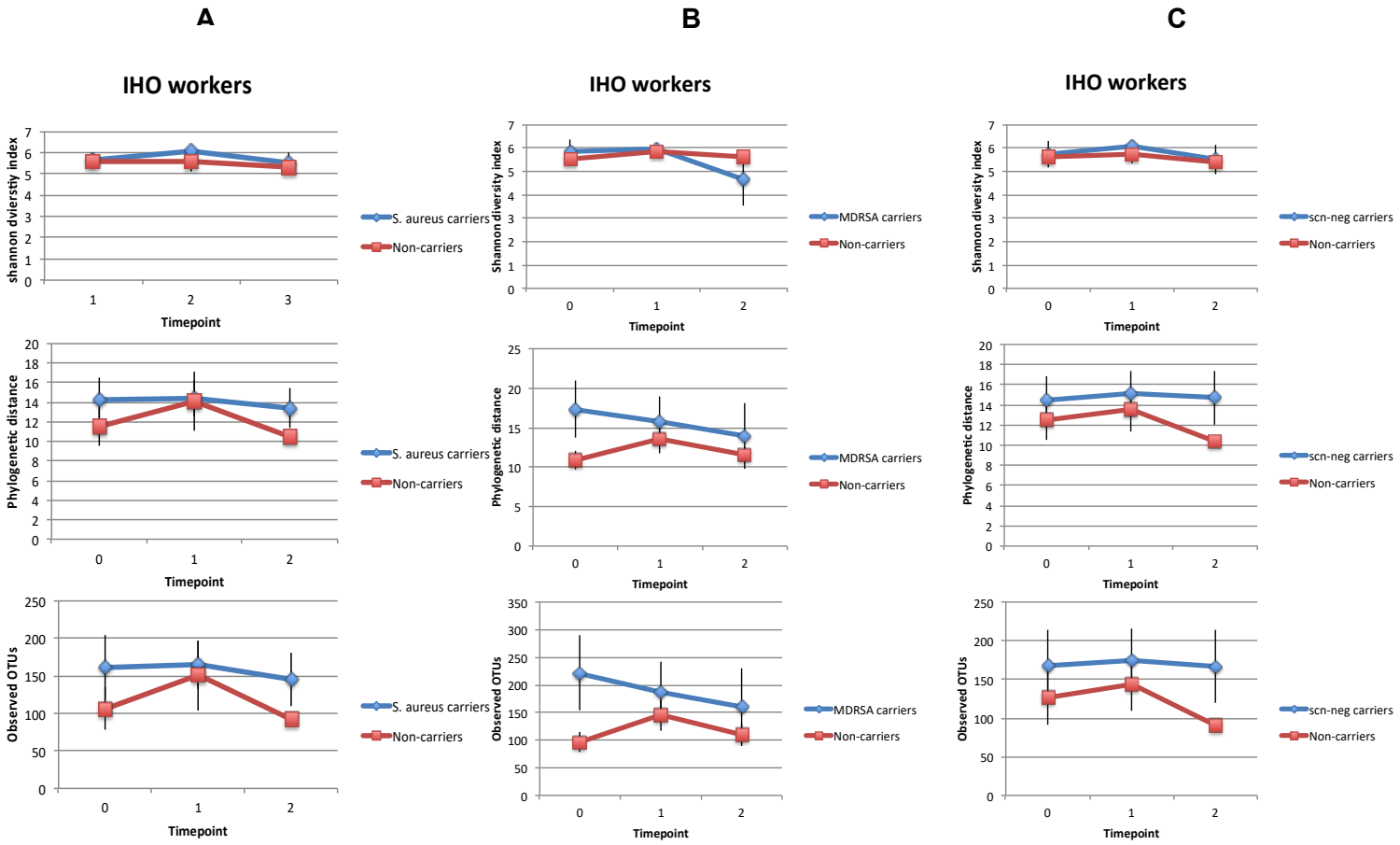


Figure 2. Temporal trends of alpha diversity (Shannon diversity index, phylogenetic distance and observed OTUs) in IHO workers by *S. aureus* nasal carriage outcomes.
Panels display the temporal changes in alpha diversity measures over time in IHO workers by *S. aureus* nasal carriage outcome strata (A: *S. aureus,* B*:* MDRSA, and C. *scn*-negative *S. aureus*) using a Student's t-test. On average, we observed *S. aureus* nasal carriage outcome carriers were more diversity compared to non-carriers.

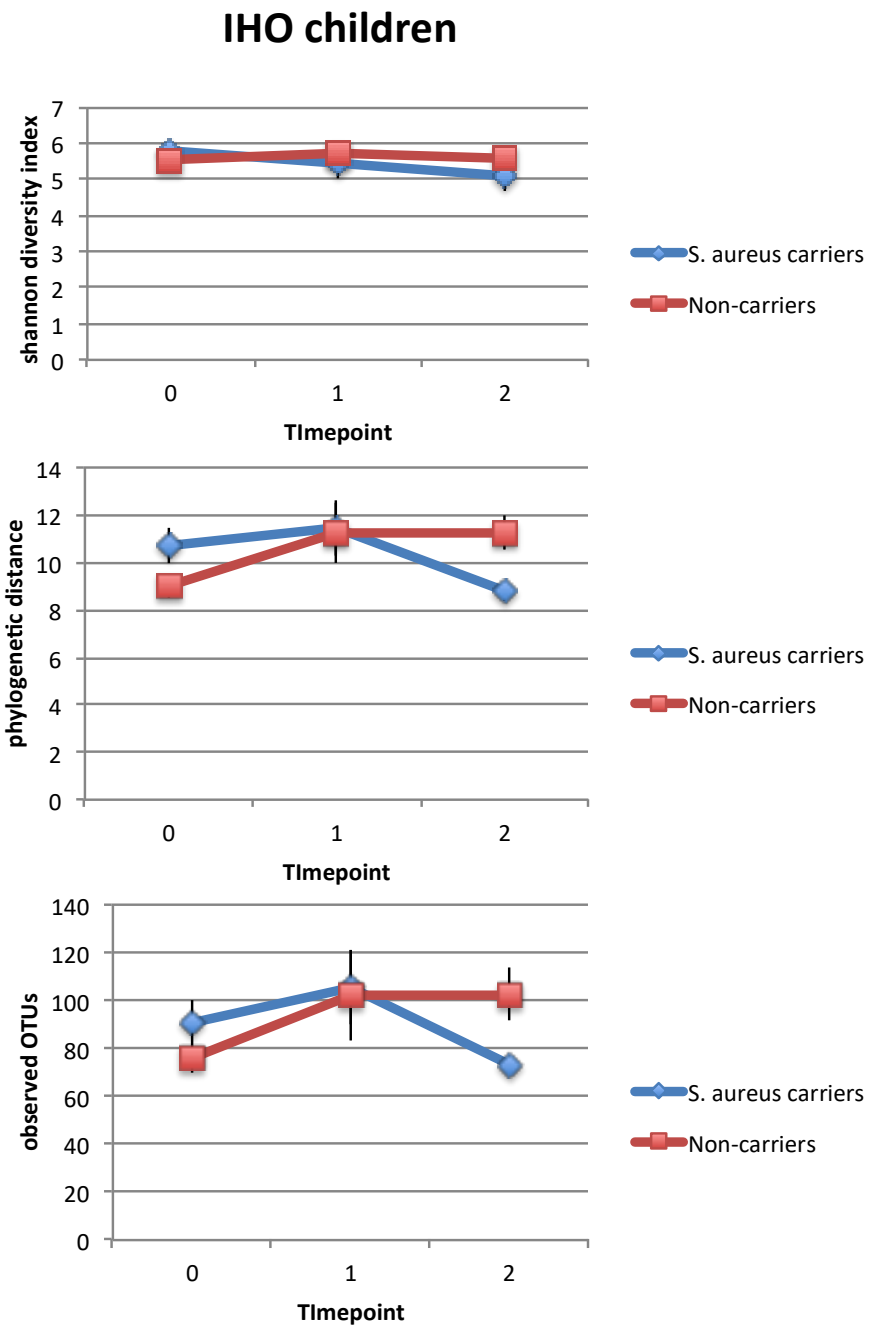


Figure 3. Temporal trends of alpha diversity (Shannon diversity index, phylogenetic distance and observed OTUs) in children living in the IHO worker's household by *S. aureus* nasal carriage. Panel displays the temporal changes in alpha diversity measures over time in IHO workers' children by *S. aureus* nasal carriage using a Student's t-test. On average, we observed *S. aureus* nasal carriers became less diverse over time compared to non-carriers.

Table 1. Baseline characteristics of industrial hog operations (IHO) workers and children living in their households, 2013-2014, North Carolina, USA.

	IHO	
	Worker	**Child**
Age in years, mean (range)	38 (25-56)	10 (7-14)
Male, n (%)	11 (52)	14 (67)
Race/ethnicity, n (%)		
Hispanic	21 (100)	21 (100)
Antibiotic use, n (%)	3 (16)	1 (5)
Self-reported asthma, n (%)	0 (0)	4 (19)
Current smoker, n (%)	1 (8)	0 (0)
Gym or sports in the last 3 months (Yes/No)	3 (14)	18 (86)
Household characteristics		
Number of household members, mean (range)	4 (1-7)	--
Owned a pet (Yes/No)	9 (43)	--

Note. IHO, industrial hog operation.
Characteristics in this table represent a subsample of the study population from Nadimpalli et al. (2016)[4]

Table 2. Description of occupational exposures, mask usage and *S. aureus* and livestock-associated microbial exposure marker nasal carriage over time among industrial hog operations (IHO) workers and children living in their households, 2013-2014, North Carolina, USA.

	Timepoint 0		Timepoint 1		Timepoint 2	
	IHO Worker	**IHO Child**	**IHO Worker**	**IHO Child**	**IHO Worker**	**IHO Child**
Occupational exposures						
Time since last IHO work shift (8-hour shift)	2.00(0.20- 18.0)	--	0.60 (0.10-3.25)	--	1.20 (0.06-9.06)	--
Hours at IHO per week (8-hour shift)	6.10 (3.50-7.88)	--	11.2 (2.50-	--	11.4 (2.00-15.0)	--
Hours of direct contact with IHO pigs per week (8-hour shift)	5.33 (0.50-7.80)	--	5.20 (1.23-7.50)	--	5.15 (1.00-7.50)	--
Proportion of IHO work shift in direct contact with pigs	0.88 (0.10-1.15)	--	0.94 (0.73-1.00)	--	0.97 (0.42-1.24)	--
IHO-related activities						
Mask percent usage, mean (range)	57 (1-100)	--	61 (1-100)	--	68 (1-100)	--
Mask usage[a], n (%)						
Always	4 (19)	--	14 (67)	--	13 (69)	--
Sometimes	13 (62)	--	3 (14)	--	6 (29)	--
Never	4 (19)	--	4 (19)	--	2 (10)	--
S. *aureus* nasal carriage outcomes						
S. aureus, n (%)	12 (57)	11 (52)	13 (62)	12 (57)	11 (52)	10 (48)
MDRSA, n (%)	7 (33)	1 (5)	7 (33)	1 (5)	7 (33)	0 (0)
Livestock-associated microbial nasal carriage exposure markers						
scn-negative *S. aureus (Presence/Absence)*, n (%)	6 (29)	0 (0)	10 (48)	1 (5)	8 (38)	1 (5)
Tetracycline resistance *S. aureus* (Presence/Absence), n (%)	7 (58)	0 (0)	6 (46)	1 (8)	4 (36)	0 (0)
S. aureus CC398 qPCR (Presence/Absence), n (%)	1 (5)	0 (0)	3 (14)	0 (0)	2 (10)	0 (0)

Pig-2-Bac qPCR (Presence/Absence), n (%)	10 (48)	2 (10)	9(42)	0 (0)	6 (29)	1 (5)
% pig contributions[b], mean (range)	5.60 (0-73)	1.11 (0-14)	17.6 (0-94)	0.54 (0- 9)	4.24 (0-84)	0.16 (0-3)

Note. IHO = industrial hog operation. CI = confidence interval.

[a]0 = always (80% or greater), 1 = Sometimes (10-79%); 2 = Never (less than 10%)

Table 3. Relation of occupational exposures and protective activities and *S. aureus* nasal carriage with alpha diversity measure and bacterial contributions from the IHO pig to the IHO workers nasal microbiome over time, 2013-2014, North Carolina, USA.

	IHO worker				
	Shannon diversity	**Phylogenetic distance**	**Observed OTUs**	**% pig contributions [b]**	**Morisita-Horn[d]**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Occupational exposures					
Time since last IHO work shift (per 8-hours)	**0.15 (0.01, 0.28)**	0.46 (-0.10, 1.03)	9.45 (-0.37, 19.3)	-0.62 (-2.67, 1.44)	-0.03 (-0.10, 0.03)
Hours at IHO per week (per 8-hours)	-0.004 (-0.02, 0.02)	-0.03 (-0.13, 0.08)	-0.40 (-2.21, 1.4)	-0.11 (-0.46, 0.25)	**0.02 (0.01,0.03)**
Hours of IHO pig direct contact per week (8-hour shift)	0.01 (-0.07, 0.10)	-0.002 (-0.69, 0.68)	0.48 (-11.8, 12.7)	-0.47 (-2.24, 1.30)	0.03 (-0.03, 0.08)
Proportion of time in direct contact with pigs at work	0.60 (-1.65, 2.85)	0.75 (-11.8, 13.3)	10.2 (-207, 227)	-9.55 (-51.8, 32.7)	0.81 (-0.30, 1.92)
Occupational protective activities					
Mask usage[a] (Reference: always mask usage)	0.02 (-0.48, 0.52)	1.58 (-1.36, 4.51)	21.6 (-28.7, 72.0)	6.10 (-3.54, 15.8)	**0.40 (0.19, 0.62)**
***S. aureus* nasal carriage outcomes**					
S. aureus (Yes/No)	0.09 (-1.49, 1.67)	2.45 (-6.92, 11.81)	48.5 (-112, 209)	15.6 (-15.1, 46.3)	0.00 (-0.79, 0.78)
MDRSA (Yes/No)	0.22 (-1.14, 1.59)	**10.2 (2.69, 17.7)**	**204 (80.8, 328)**	**39.5 (15.7, 63.4)**	-0.32 (-1.00, 0.35)
Livestock-associated microbial nasal carriage exposure markers					
scn-negative *S. aureus* (Yes/No)	0.15 (-1.07, 1.37)	1.50 (-5.77, 8.76)	27.9 (-96.3, 152)	5.83 (-18.2, 29.9)	**0.57 (-0.02, 1.15)**
Pig-2-bac qPCR (Presence/Absence)	**0.66 (-0.22, 1.55)**	**4.77 (-0.44, 9.99)**	**98.8 (11.4, 186)**	**18.1 (1.11, 35.2)**	-0.26 (-0.70, 0.19)
Percent pig contributions[b]	**0.02 (0.00, 0.03)**	**0.21 (0.14, 0.28)**	**3.62 (2.46, 4.78)**	--	0.00 (-0.01,0.01)
Index score of livestock-associated microbial exposure markers[c]	**0.76 (0.34, 1.19)**	**4.65 (2.12, 7.18)**	**81.5 (38.6, 124)**	**17.1 (9.04, 25.1)**	0.01 (-0.23,0.26)

Note. IHO = industrial hog operation. CI = confidence interval.
All beta coefficients were estimated using conditional fixed effects linear regression models.
[a]0 = always (80% or greater), 1 = Sometimes (10-79%); 2 = Never (less than 10%)

[b]% pig contributions defined as the percentage of OTUs contributed to the worker from the pig's total microbiome (nares and perineum) estimated via SourceTracker tool in QIIME -- SourceTracker is designed to predict the source (IHO pig) of microbial communities in a set of sink samples (IHO worker).
[c]Index score between 0 and 3 was calculated based on presence of one or more of livestock-associated microbial nasal carriage exposure markers: 1) *scn*-negative *S. aureus* (yes/no); 2) Pig-2-bac qPCR (presence/absence); 3) Total percent pig contributions (presence/absence).
[d]Morisita-horn index, defined as a a dissimilarity index ranging between 0 and 1; Index was calculated based on the similarity in the nasal microbiome

Table 4. Relation of occupational exposures and protective activities and S. aureus nasal carriage with alpha diversity measure and bacterial contributions from the IHO pig to children living in IHO worker's households nasal microbiome over time, 2013-2014, North Carolina, USA.

	IHO child				
	Shannon diversity	**Phylogenetic distance**	**Observed OTUs**	**% pig contributions [b]**	**Morisita-Horn[d]**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Occupational exposures					
Time since last IHO work shift	0.26 (-0.99, 1.51)	-0.99 (-2.73, 0.75)	-9.24 (-29.5, 11.1)	0.00 (-0.06, 0.05)	-0.28 (-0.69, 0.12)
Hours at IHO per week (per 8-	-0.01 (-0.03, 0.01)	0.01 (-0.04, 0.06)	0.16 (-0.34, 0.67)	0.00 (-0.01, 0.02)	**0.02 (0.01, 0.02)**
Hours of IHO pig direct contact	-0.03 (-0.15, 0.09)	-0.15 (-0.41, 0.10)	-1.18 (-3.85, 1.48)	-0.01 (-0.08, 0.05)	0.04 (-0.02, 0.09)
Proportion of time at IHO spent	1.86 (-11.6, 15.3)	-1.20 (-31.2, 28.8)	-121 (-413, 171)	-0.45 (-7.50, 6.59)	-0.31 (-7.12, 6.51)
Occupational protective activities					
Mask usage[a] (Reference: always	-0.32 (-1.87, 1.23)	-2.34 (-5.47, 0.80)	-11.3 (-44.4, 23.9)	0.02 (-0.77, 0.82)	0.50 (-0.19, 1.18)
***S. aureus* nasal carriage outcomes**					
S. aureus (Yes/No)	0.05 (-0.73, 0.84)	2.19 (-0.13, 4.52)	17.0 (-16.5, 50.5)	0.27 (-1.98, 2.52)	0.04 (-0.47, 0.56)
MDRSA (Yes/No)	0.48 (-0.97, 1.94)	2.98 (-1.46, 7.43)	15.0 (-48.3, 78.3)	-0.01 (-4.22, 4.20)	-0.16 (-1.12, 0.80)
Livestock-associated microbial					
scn-negative *S. aureus* (Yes/No)	**1.52 (0.14, 2.90)**	3.43 (-0.98, 7.84)	47.0 (-14.6, 109)	-3.40 (-7.46, 0.67)	0.46 (-0.49, 1.41)
Pig-2-bac qPCR	-0.30 (-1.76, 1.16)	-1.14 (-5.67, 3.39)	-14.3 (-77.6, 49.1)	0.09 (-4.12, 4.30)	-0.69 (-1.62, 0.24)
Percent pig contributions[b]	0.02 (-0.09, 0.14)	-0.06 (-0.41, 0.28)	-0.36 (-5.24, 4.52)	--	-0.04 (-0.11,0.03)
Index score of livestock-	0.34 (-0.17, 0.86)	0.98 (-0.62, 2.58)	14.4 (-7.90, 36.7)	**1.79 (0.39, 3.19)**	-0.22 (-0.57,0.14)

Note. IHO = industrial hog operation. CI = confidence interval.

All beta coefficients were estimated using conditional fixed effects linear regression models.

[a]0 = always (80% or greater), 1 = Sometimes (10-79%); 2 = Never (less than 10%)

[b]% pig contributions defined as the percentage of OTUs contributed to the worker from the pig's total microbiome (nares and perineum) estimated via

[c]Index score between 0 and 3 was calculated based on presence of one or more of livestock-associated microbial nasal carriage exposure markers: 1) *scn*-negative *S. aureus* (yes/no); 2) Pig-2-bac qPCR (presence/absence); 3) Total percent pig contributions (presence/absence).

[d]Morisita-horn index, defined as a a dissimilarity index ranging between 0 and 1; Index was calculated based on the similarity in the nasal microbiome bacterial community structure of the current timepoint to the previous timepoint's bacterial community structure.

Table 5. Relation of accumulating occupational exposures and protective activities and *S. aureus* nasal carriage at each timepoint with alpha diversity measure and bacterial contributions from the IHO pig to the IHO workers nasal microbiome over time, 2013-2014, North Carolina, USA.

	IHO worker				
	Shannon diversity	**Phylogenetic distance**	**Observed OTUs**	**% pig contributions[b]**	**Morisita-horn[c]**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Accumulating occupation exposures					
Time since last IHO work shift (8-hour shift)	-0.01 (-0.24, 0.23)	-0.19 (-1.59, 1.22)	-1.88 (-25.87, 22.1)	-0.01 (-4.65, 4.64)	**0.15 (0.04, 0.26)**
Hours at IHO per week (per 8-hours)	0.00 (-0.01, 0.00)	-0.01 (-0.05, 0.03)	-0.15 (-0.78, 0.48)	-0.08 (-0.20, 0.04)	**0.005 (0.003, 0.01)**
Hours of IHO pig direct contact per week (8-hour shift)	0.00 (-0.02, 0.01)	-0.02 (-0.10, 0.06)	-0.26 (-1.62, 1.10)	-0.18 (-0.44, 0.08)	**0.01 (0.004, 0.02)**
Proportion of time at IHO spent in direct contact with pigs	-0.15 (-0.54, 0.24)	-0.47 (-2.81, 1.86)	-8.71 (-48.7, 31.3)	-1.61 (-9.35, 6.13)	**0.48 (0.35, 0.60)**
Accumulating Index score of livestock-associated microbial exposure markers[a]	-0.10 (-0.34, 0.13)	-0.07 (-1.50, 1.35)	-2.19 (-26.5, 22.1)	2.12 (-2.54, 6.78)	**0.24 (0.15, 0.33)**

Note. IHO = industrial hog operation. CI = confidence interval.

All beta coefficients were estimated using conditional fixed effects linear regression models.

[a]Accumulating index score between 0 and 8 was calculated based on the repeated presence of one or more of: 1) *scn*-negative *S. aureus* (yes/no); 2) Pig-2-Bac qPCR (presence/absence); 3) Total percent pig contributions (presence/absence) across three timepoints.

[b]% pig contributions (%) defined as the percentage of OTUs contributed to the worker from the pig's total microbiome (nares and perineum) estimated via SourceTracker tool in QIIME -- SourceTracker is designed to predict the source (IHO pig) of microbial communities in a set of sink samples (IHO worker)

[c]Morisita-horn index, defined as a a dissimilarity index ranging between 0 and 1; Index was calculated based on the similarity in the nasal microbiome bacterial community structure of the current timepoint to the previous timepoint's bacterial community structure.

Table 6. Relation of accumulating occupational exposures and protective activities and *S. aureus* nasal carriage at each timepoint with alpha diversity measure and IHO pig bacterial contributions to children living in IHO worker's households nasal microbiome over time, 2013-2014, North Carolina, USA.

	IHO child				
	Shannon diversity	**Phylogenetic distance**	**Observed OTUs**	**% pig contributions[b]**	**Morisita-horn[c]**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Accumulation of occupation exposures across time					
Time since last IHO work shift (8-hour shift)	-0.12 (-1.71, 1.48)	25.3 (-43.23, 93.85)	0.42 (-4.52, 5.36)	0.53 (-4.04, 5.11)	**1.08 (-0.07, 2.22)**
Hours at IHO per week (8-hour shift)	0.00 (-0.01, 0.00)	0.1 (-0.14, 0.27)	0.00 (-0.01, 0.02)	0.00 (-0.01, 0.02)	**0.005 (0.001, 0.01)**
Hours of IHO pig direct contact per week (8-hour shift)	0.00 (-0.01, 0.01)	0.1 (-0.29, 0.58)	0.01 (-0.02, 0.04)	0.00 (-0.02, 0.03)	**0.01 (0.003, 0.02)**
Proportion of time at IHO spent in direct contact with pigs	-0.11 (-0.61, 0.39)	7.0 (-14.50, 28.56)	0.45 (-1.10, 1.99)	0.25 (-1.19, 1.68)	**0.45 (0.14, 0.77)**
Accumulating Index score of livestock-associated microbial exposure markers[a]	0.20 (-0.31, 0.71)	6.7 (-15.62, 29.05)	0.52 (-1.08, 2.12)	-0.28 (-1.77, 1.20)	**0.64 (0.27, 1.01)**

Note. IHO = industrial hog operation. CI = confidence interval.
All beta coefficients were estimated using conditional fixed effects linear regression models.
[a]Accumulating index score between 0 and 8 was calculated based on the repeated presence of one or more of: 1) *scn*-negative *S. aureus* (yes/no); 2) Pig-2-bac qPCR (presence/absence); 3) Total percent pig contributions (presence/absence) across three timepoints.
[b]% pig contributions (%) defined as the percentage of OTUs contributed to the worker from the pig's total microbiome (nares and perineum) estimated via SourceTracker tool in QIIME -- SourceTracker is designed to predict the source (IHO pig) of microbial communities in a set of sink samples (IHO worker)
[c]Morisita-horn index, defined as a a dissimilarity index ranging between 0 and 1; Index was calculated based on the similarity in the nasal microbiome bacterial community structure of the current timepoint to the previous timepoint's bacterial community structure.

Table 7. Bacterial OTUs contributed from the IHO pig to the nasal microbiome to the IHO workers and children living in their households, 2013-2014, North Carolina, USA.

Source	Sink (n_{OTUs})	Bacterial OTUs
IHO pig	**IHO workers (n=43)**	
		Acinetobacter_lwoffii:Prolinoborus_fasciculus
		Aerococcus_suis
	❖	*Aerococcus_urinaeequi:Aerococcus_viridans*
		Anaerovibrio_lipolyticus
		Clostridium_baratii:Clostridium_sardiniense
		Clostridium_lactatifermentans
		Corynebacterium_freneyi:Corynebacterium_xerosis
		Corynebacterium_kutscheri
		Corynebacterium_stationis
		Corynebacterium_testudinoris
		Dolosicoccus_paucivorans
		Facklamia_tabacinasalis
		Globicatella_sulfidifaciens
	❖	*Lactobacillus_acidophilus:Lactobacillus_amylovorus*
	❖	*Lactobacillus_antri:Lactobacillus_frumenti:Lactobacillus_oris:Lactobacillus_panis:Lactobacillus_reuteri:Lactobacillus_vaginalis*
		Lactobacillus_delbrueckii:Lactobacillus_equicursoris:Lactobacillus_leichmannii
		Lactobacillus_gasseri:Lactobacillus_hominis:Lactobacillus_johnsonii:Lactobacillus_taiwanensis
		Lactobacillus_reuteri
		Methanobrevibacter_millerae
		Micrococcus_lylae
	❖	*Moraxella_pluranimalium*
	❖	*Moraxella_porci*
		otu10338:Bacteroides_oleiciplenus:Bacteroides_stercorirosoris
		otu1271:Prevotella_baroniae:Prevotella_oris
	❖	*otu13543:Parabacteroides_distasonis*
		otu1396:Dorea_longicatena
		otu15839:Barnesiella_intestinihominis
		otu16326:Alloiococcus_otitis:Facklamia_tabacinasalis
		otu3209:Alloprevotella_rava
		otu3213:Eubacterium_infirmum
		otu3992:Alloprevotella_rava
		otu4722:Prevotella_shahii:Prevotella_stercorea
		otu7724:Alloprevotella_rava
		otu8971:Bulleidia_extructa
		otu9103:Prevotella_bryantii
		Rothia_mucilaginosa:Rothia_nasimurium
		Rothia_nasimurium
		Selenomonas_bovis
		Staphylococcus_carnosus:Staphylococcus_condimenti:Staphylococcus_haemolyticus:Staphylococcus_piscifermentans:Staphylococcus_simulans
	❖	*Staphylococcus_pettenkoferi*
		Staphylococcus_sciuri
		Streptococcus_equinus
		Streptococcus_hyointestinalis
		Treponema_porcinum
	IHO children (n=14)	
	❖	*Aerococcus_urinaeequi:Aerococcus_viridans*
		Anaerococcus_prevotii:Anaerococcus_tetradius
		Corynebacterium_amycolatum:Corynebacterium_lactis
		Corynebacterium_freiburgense:Corynebacterium_variabile
	❖	*Lactobacillus_acidophilus:Lactobacillus_amylovorus*
	❖	*Lactobacillus_antri:Lactobacillus_frumenti:Lactobacillus_oris:Lactobacillus_panis:Lactobacillus_reuteri:Lactobacillus_vaginalis*
	❖	*Moraxella_pluranimalium*
		Moraxella_porci
	❖	*otu13543:Parabacteroides_distasonis*
		otu13948:Leptotrichia_goodfellowii
		Parvimonas_micra
		Peptoniphilus_coxii
	❖	*Staphylococcus_pettenkoferi*
		Weissella_paramesenteroides

OTUs shown are those that contribute 1% relative abundance to IHO workers or IHO children at each timepoint.
OTUs are sorted alphabetically within each *S.aureus* nasal carriage outcome strata (*S. aureus*, MDRSA and *scn*-negative *S. aureus)*
Symbols indicate the overlap in carriage between *S. aureus* nasal carriage outcome strata (S. aureus, MDRSA and scn-negative S. aureus)
❖ OTUs contributed by the IHO pig to IHO workers and IHO children

Table 8. Bacterial OTUs exclusivity among IHO workers, by S. aureus nasal carriage outcomes, 2013-2014, North Carolina, USA.

IHO workers	Differential OTUs	Exclusively carried by
	S. aureus	
◆★■	*Corynebacterium_kutscheri*	carrier
◆★■	*Lactobacillus_acidophilus:Lactobacillus_amylovorus*	carrier
◆★	*otu4722:Prevotella_shahii:Prevotella_stercorea*	carrier
◆★	*Pseudomonas_deceptionensis:Pseudomonas_fragi:Pseudomonas_lundensis:Pseudomonas_psychrophila*	carrier
◆★	*Psychrobacter_faecalis:Psychrobacter_pulmonis*	carrier
◆★	*Erwinia_mallotivora:Erwinia_papayae:Pantoea_agglomerans:Pantoea_ananatis:Pantoea_brenneri:Pantoea_eucalypti:Pantoea_vagans*	non-carrier
	otu11544:Gluconacetobacter_liquefaciens	non-carrier
	MDRSA	
✚■	*Clostridium_baratii:Clostridium_sardiniense*	carrier
◆★+■	*Corynebacterium_kutscheri*	carrier
	Globicatella_sulfidifaciens	carrier
◆★+	*Lactobacillus_acidophilus:Lactobacillus_amylovorus*	carrier
■	*Lactobacillus_antri:Lactobacillus_frumenti:Lactobacillus_oris:Lactobacillus_panis:Lactobacillus_reuteri:Lactobacillus_vaginalis*	carrier
■	*wanensis*	carrier
◆★+■	*otu4722:Prevotella_shahii:Prevotella_stercorea*	carrier
◆★+	*Pseudomonas_deceptionensis:Pseudomonas_fragi:Pseudomonas_lundensis:Pseudom*	carrier
◆★+	*Psychrobacter_faecalis:Psychrobacter_pulmonis*	carrier
✚■	*Staphylococcus_sciuri*	carrier
■	*Corynebacterium_freneyi:Corynebacterium_xerosis*	carriers
◆★+■	*Erwinia_mallotivora:Erwinia_papayae:Pantoea_agglomerans:Pantoea_ananatis:Pan*	non-carrier
	scn*-negative *S. aureus	
	Bacillus_aestuarii:Bacillus_arbutinivorans:Bacillus_bataviensis:Bacillus_djibelorensis:Bacillus_drentensis:Bacillus_fucosivorans:Bacillus_fumarioli:Bacillus_niacini:Bacillus_novalis:Bacillus_pocheonensis:Bacillus_pseudomegaterium:Bacillus_senegalensis:Bacillus_soli:Bacillus_vireti:Sporosarcina_koreensis	carrier
◆+	*Erwinia_mallotivora:Erwinia_papayae:Pantoea_agglomerans:Pantoea_ananatis:Pantoea_brenneri:Pantoea_eucalypti:Pantoea_vagans*	carrier
	Methylobacterium_adhaesivum	carrier
	otu11544:Gluconacetobacter_liquefaciens	carrier
	otu13996:Chthoniobacter_flavus	carrier
	otu16072:Rhodopila_globiformis	carrier
◆+■	*otu4722:Prevotella_shahii:Prevotella_stercorea*	carrier
◆+	*Pseudomonas_deceptionensis:Pseudomonas_fragi:Pseudomonas_lundensis:Pseudomonas_psychrophila*	carrier
✚	*Clostridium_baratii:Clostridium_sardiniense*	non-carrier
◆+■	*Corynebacterium_kutscheri*	non-carrier
◆+■	*Lactobacillus_acidophilus:Lactobacillus_amylovorus*	non-carrier
	otu16847:Barnesiella_intestinihominis:Barnesiella_viscericola	non-carrier
◆+	*Psychrobacter_faecalis:Psychrobacter_pulmonis*	non-carrier
	Psychrobacter_sanguinis	non-carrier
■	*Staphylococcus_carnosus:Staphylococcus_condimenti:Staphylococcus_haemolyticus:Staphylococcus_piscifermentans:Staphylococcus_simulans*	non-carrier
✚■	*Staphylococcus_sciuri*	non-carrier

OTUs are sorted alphabetically within each *S.aureus* nasal carriage outcome strata (*S. aureus*, MDRSA and *scn*-negative *S. aureus)*

Symbols indicate the overlap in carriage between *S. aureus* nasal carriage outcome strata (S. aureus, MDRSA and scn-negative S. aureus)

◆ All three *S. aureus* outcomes

★ *S. aureus and MDRSA*

✚ MDRSA and scn negative *S.aureus*

■ IHO pig SourceTracker contributions in IHO workers.

Table 9. Bacterial OTUs exclusivity among children living in IHO worker's households, by *S. aureus* nasal carriage outcomes, 2013-2014, North Carolina, USA.

IHO children	Differential OTUs	Exclusively carried by
	S. aureus	
◆★	*Lysinibacillus_boronitolerans:Lysinibacillus_fusiformis:Lysinibacillus_macroides:Lysinibacillus_odysseyi:Lysinibacillus_p*	carrier
◆	*gasensis:Pseudomonas_flavescens:Pseudomonas_fulva:Pseudomonas_monteilii:Pseudomonas_oryzihabitans:Pseudom* *onas_parafulva:Pseudomonas_plecoglossicida:Pseudomonas_punonensis:Pseudomonas_putida:Pseudomonas_selenii*	carrier
	Pseudomonas_oryzihabitans:Pseudomonas_putida:Pseudomonas_taeanensis	carrier
★	*Clostridium_bolteae:Clostridium_clostridioforme*	non-carrier
	MDRSA	
	Corynebacterium_kroppenstedtii	carrier
	otu6867:Bacteroides_caccae:Bacteroides_cellulosilyticus:Bacteroides_intestinalis:Paludibacter_propionicigenes	carrier
★	*Clostridium_bolteae:Clostridium_clostridioforme*	non-carrier
◆★+	*Lysinibacillus_boronitolerans:Lysinibacillus_fusiformis:Lysinibacillus_macroides:Lysinibacillus_odysseyi:Lysinibacillus_p*	non-carrier
	Pseudomonas_argentinensis:Pseudomonas_benzenivorans:Pseudomonas_cremoricolorata:Pseudomonas_cuatrociene *gasensis:Pseudomonas_flavescens:Pseudomonas_fulva:Pseudomonas_monteilii:Pseudomonas_oryzihabitans:Pseudom* *onas_parafulva:Pseudomonas_plecoglossicida:Pseudomonas_punonensis:Pseudomonas_putida:Pseudomonas_selenii*	
◆★+	*praecipitans:Pseudomonas_spY1410:Pseudomonas_straminea:Pseudomonas_taiwanensis*	non-carrier
	scn*-negative *S. aureus	
	Bacillus_aestuarii:Bacillus_arbutinivorans:Bacillus_bataviensis:Bacillus_djibelorensis:Bacillus_drentensis:Bacillus_fucos *ivorans:Bacillus_fumarioli:Bacillus_niacini:Bacillus_novalis:Bacillus_pocheonensis:Bacillus_pseudomegaterium:Bacillus* *_senegalensis:Bacillus_soli:Bacillus_vireti:Sporosarcina_koreensis*	carrier
	Tumebacillus_ginsengisoli	carrier
◆+	*Lysinibacillus_boronitolerans:Lysinibacillus_fusiformis:Lysinibacillus_macroides:Lysinibacillus_odysseyi:Lysinibacillus_p* *akistanensis:Lysinibacillus_sphaericus:Lysinibacillus_xylanilyticus*	non-carrier
	Pseudomonas_argentinensis:Pseudomonas_benzenivorans:Pseudomonas_cremoricolorata:Pseudomonas_cuatrociene *gasensis:Pseudomonas_flavescens:Pseudomonas_fulva:Pseudomonas_monteilii:Pseudomonas_oryzihabitans:Pseudom* *onas_parafulva:Pseudomonas_plecoglossicida:Pseudomonas_punonensis:Pseudomonas_putida:Pseudomonas_selenii*	
◆+	*praecipitans:Pseudomonas_spY1410:Pseudomonas_straminea:Pseudomonas_taiwanensis*	non-carrier

OTUs are sorted alphabetically within each *S.aureus* nasal carriage outcome strata (*S. aureus*, MDRSA and *scn*-negative *S. aureus)*

Symbols indicate the overlap in carriage between *S. aureus* nasal carriage outcome strata (S. aureus, MDRSA and scn-negative S. aureus)

◆ All three *S. aureus* outcomes

★ *S. aureus and MDRSA*

✚ MDRSA and scn negative *S.aureus*

■ IHO pig SourceTracker contributions in IHO children.

Supplementary Material Table S1. Read statistics for participant types (IHO workers and their household IHO children, 2014, North Carolina, USA.

Raw reads	1,700,289
Successfully merged read-pairs	1,472,777
Average reads/sample pre-processing	13,602
Average successful paired reads/sample	11,782
Average high quality reads/sample	11,779
Average number of chimeras/sample	335
Average final clean reads/sample	11,310
Average read length	253

Read quality parameter are outlined within the methods section.

Chimera identification procedures are outlined within the methods section.

Supplementary Materials Table S2. Relation of hand washing with *S. aureus* and Livestock-associated microbial exposure markers nasal carriage, 2013-2014, North Carolina, USA.

	Beta (95% CI)
***S. aureus* nasal carriage outcomes**	
S. aureus	0.01 (-0.20-0.22)
MDRSA	-0.12 (-0.27-0.03)
Livestock-associated microbial nasal carriage exposure markers[a]	
scn–negative *S. aureus*	0.10 (-0.10-0.28)
Pig-2-Bac	-0.22 (-0.46-0.02)
Percent pig contributions[b]	-0.01 (-0.29-0.26)

[a]Livestock-associated microbial nasal carriage exposure markers defined as: 1) *scn*-negative *S. aureus* (yes/no); 2) Pig-2-bac qPCR (presence/absence); or 3) Total percent pig contributions (presence/absence).

[b] Pig contributions (%) defined as the percentage of OTUs contributed to the worker from the pig's total microbiome (nares and perineum) estimated via SourceTracker tool in QIIME.

Chapter Six: Miscellaneous results

Read statistics for all samples that underwent microbiome sequencing:

Supplementary Material Table S1: Read statistics for participant types (IHO pigs, IHO workers, AFHO pigs, AFHO workers, IHO children, CR adults and CR children), 2014, North Carolina, USA.	
Raw reads	4,303,017
Successfully merged read-pairs	3,714,638
Average reads/sample pre-processing	13,836
Average successful paired reads/sample:	11,944
Average high quality reads/sample:	11,944
Average number of chimeras/sample	661
Average final clean reads/sample:	11,095
Average read length:	253

Research question: Does species assignment correlate with their qPCR targets that should have high correlations? The results presented here are the statistically significant correlations

Staphylococcus is highly collinear with its molecular confirmatory marker (*fem* a). *Staphylococcus* is highly collinear with Pig-2-Bac (pig fecal marker) and CC398 *S. aureus*.

Validation table of genus level classification of Resphera taxonomic database in relation to qPCR molecular targets

	Pig-2-bac	*fem A*	*cc398*	*mec A*	*moc A*	*Strep suis*	*Strep hains*	*Strep pneumoniae*	*influenza A*
Pig-2-bac	---	0.75***	0.81***	---	---	---	0.4656*	---	---
fem A	0.75***	---	0.94***	---	---	---	---	---	---
cc398	0.81***	0.94***	---	---	---	---	---	---	---
Strep hains	0.47*	---	---	---	---	---	---	---	---
Staphylococcus	0.75***	0.92***	0.92***	---	---	---	---	---	---
Corynebacterium	---	---	---	---	---	---	---	---	0.83***
Moraxella	---	---	---	---	---	0.51*	0.50*	---	---
Ruminococcus	---	---	---	-0.43*	0.55*	---	---	---	---
Enterobacteriaceae_unassigned	---	---	---	---	0.59***	---	0.64***	---	---
Firmicutes_unassigned	---	---	---	---	0.82***	---	---	---	---
Actinomyces	---	---	---	---	---	0.66***	---	---	---
Rothia	---	---	---	---	---	---	0.76***	---	0.47*
Lachnospiraceae_unassigned	---	---	---	---	---	---	---	0.60**	---
Porphyromonadaceae_unassigned	0.63***	0.72***	0.80***	---	---	---	---	---	---
Intrasporangiaceae_unassigned	---	---	---	---	0.76***	---	---	---	---
Veillonella	---	---	---	0.47*	---	0.48*	---	---	---
Paenibacillus	---	---	---	0.66***	---	---	---	---	---
Brevundimonas	0.65***	0.73***	0.80***	---	---	---	---	---	---
Alistipes	---	---	---	---	---	---	---	---	1.00***
Abiotrophia	---	---	---	---	---	---	---	0.99***	---
Neisseriaceae_unassigned	---	---	---	0.70***	---	---	---	---	---
Papillibacter	---	---	---	---	---	---	---	0.62***	---

** indicates a correlation coefficient with an alpha significance value of less than or equal to 0.05*

*: $p < 0.05$, **: $p < 0.01$; ***: $p < 0.001$

Manuscript 1

Research question: Does the epidemiological study origin of the sample (4 month epidemiological study and Cross-sectional/Thrasher study) bias alpha diversity comparisons? [performed via Student's t-test]

Differences in alpha diversity between 4 month IHO worker vs IHO pig , North Carolina: 2013-2015.

	4 mos IHO worker	IHO pig	
	Mean (SD)	Mean (SD)	P-value
Alpha Diversity Measures			
Shannon	5.76 (0.96)	6.44 (0.99)	0.079
Inverse Simpson	1.06 (0.13)	1.07 (0.02)	0.768
Observed OTUs	164 (148)	628 (27)	0.000
Phylogenetic distance	14.3 (7.98)	40.2 (1.5)	0.000
Species richness	302 (198)	1296 (72)	0.000
Species eveness	0.46 (0.13)	0.18 (0.03)	0.000

Note. IHO, industrial hog operation. CR, Community Referent.

P-values estimated from t-test comparing alpha diversity measures between Thrasher IHO workers and CR adults

There are significant differences in alpha diversity between the 4 month (4 mos) IHO worker and the IHO pig (except for Shannon diversity and inverse simpson).

Differences in alpha diversity between thrasher IHO worker vs IHO pig , North Carolina: 2013-2015.

	Thrasher IHO worker	IHO pig	
	Mean (SD)	Mean (SD)	P-value
Alpha Diversity Measures			
Shannon	6.76 (1.17)	6.44 (0.99)	0.463
Inverse Simpson	1.04 (0.06)	1.07 (0.02)	0.154
Observed OTUs	261 (190)	628 (27)	0.000
Phylogenetic distance	21.2 (11.4)	40.2 (1.5)	0.000
Species richness	507 (453)	1296 (72)	0.000
Species eveness	0.59 (0.20)	0.18 (0.03)	0.000

Note. IHO, industrial hog operation. CR, Community Referent.

P-values estimated from t-test comparing alpha diversity measures between Thrasher IHO workers and CR adults

There are significant differences in alpha diversity between the Thrasher IHO worker and the IHO pig (except for Shannon diversity and inverse simpson).

Differences in alpha diversity between 4 month IHO worker vs. AFHO worker , North Carolina: 2013-2015.

	4 mos IHO worker	AFHO worker	
	Mean (SD)	Mean (SD)	P-value
Alpha Diversity Measures			
Shannon	5.76 (0.96)	8.61 (0.14)	0.000
Inverse Simpson	1.06 (0.13)	1.01 (0.002)	0.311
Observed OTUs	164 (148)	626 (75)	0.000
Phylogenetic distance	14.3 (7.98)	42.4 (3.3)	0.000
Species richness	302 (198)	1464 (117)	0.000
Species eveness	0.46 (0.13)	0.67 (0.07)	0.002

Note. IHO, industrial hog operation. CR, Community Referent.
P-values estimated from t-test comparing alpha diversity measures between Thrasher IHO workers and CR adults

There are significant differences in alpha diversity between the 4 month (4 mos) IHO worker and the AFHO worker (inverse simpson).

Differences in alpha diversity between Thrasher IHO worker vs. AFHO worker , North Carolina: 2013-2015.

	Thrasher IHO worker	AFHO worker	
	Mean (SD)	Mean (SD)	P-value
Alpha Diversity Measures			
Shannon	6.76 (1.17)	8.61 (0.14)	0.000
Inverse Simpson	1.04 (0.06)	1.01 (0.002)	0.251
Observed OTUs	261 (190)	626 (75)	0.000
Phylogenetic distance	21.2 (11.4)	42.4 (3.3)	0.000
Species richness	507 (453)	1464 (117)	0.000
Species eveness	0.59 (0.20)	0.67 (0.07)	0.344

Note. IHO, industrial hog operation. CR, Community Referent.
P-values estimated from t-test comparing alpha diversity measures between Thrasher IHO workers and CR adults

There are significant differences in alpha diversity between the Thrasher IHO worker and the AFHO worker (inverse simpson and species evenness).

Differences in alpha diversity between 4 month IHO worker vs thrasher IHO worker , North Carolina: 2013-2015.

	4 mos IHO worker	Thrasher IHO worker	
	Mean (SD)	Mean (SD)	P-value
Alpha Diversity Measures			
Shannon	5.76 (0.96)	6.76 (1.17)	0.005
Inverse Simpson	1.06 (0.13)	1.04 (0.06)	0.493
Observed OTUs	163.62 (147.72)	261.11 (189.64)	0.076
Phylogenetic distance	14.33 (7.98)	21.15 (11.38)	0.033
Species richness	302.30 (197.64)	506.84 (453.18)	0.068
Species eveness	0.46 (0.13)	0.59 (0.20)	0.013

Note. IHO, industrial hog operation.
P-values estimated from t-test comparing alpha diversity measures between 4 month vs. Thrasher IHO worker

There are significant differences in alpha diversity between the 4 month (4 mos) IHO worker and the thrasher IHO worker (except inverse simpson, observed OTUs, and species richness).

Research question: Does *S. aureus* nasal carriage differ by carriage status (yes or no) in IHO pigs?
Note: All AFHO pig isolates were *S. aureus* negative. No results presented here by carriage status.; IHO worker differences presented in manuscript 2. [performed via Student's t-test]

Comparison of IHO pig microbiome, by *S. aureus* outcome (*S. aureus,* MDRSA and *scn*-negative *S. aureus)*. North Carolina (2013-2015).

	IHO pig		
	Shannon diversity	**Phylogenetic distance**	**Observed OTUs**
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)
S. aureus			
Carrier	7.50 (1.03, 13.98)	43.74 (20.58, 66.90)	609.27 (432.21, 786,32
Non-carrier	6.17 (5.44, 6.91)	39.32 (35.18, 43.45)	634.29 (571.06, 697.52
MDRSA			
Carrier	7.50 (1.03, 13.98)	43.74 (20.58, 66.90)	609.27 (432.21, 786,32
Non-carrier	6.17 (5.44, 6.91)	39.32 (35.18, 43.45)	634.29 (571.06, 697.52
***Scn*-negative**			
Carrier	7.50 (1.03, 13.98)	43.74 (20.58, 66.90)	609.27 (432.21, 786,32
Non-carrier	6.17 (5.44, 6.91)	39.32 (35.18, 43.45)	634.29 (571.06, 697.52

Note. IHO, industrial hog operation. AFHO, antibiotic-free hog operation.
IHO pig *S. aureus* positive isolates were all MDRSA and *scn*-negative *S. aureus.*
P-value estimated from the Student's t-test.

IHO pig *S. aureus* positive isolates were all MDRSA and *scn*-negative *S. aureus*. There are no significant differences in alpha diversity of the IHO pig nasal microbiome by *S. aureus* nasal carriage outcomes (carriage vs. not).

Research question: Does *S. aureus* nasal carriage differ by carriage status (yes or no) in AFHO worker? [performed via Student's t-test]

Comparison of AFHO worker microbiome, by *S. aureus* outcome (*S. aureus,* MDRSA and *scn*-negative *S. aureus)*. North Carolina (2013-2015).

	AFHO worker		
	Shannon diversity	**Phylogenetic distance**	**Observed OTUs**
S. aureus			
Carrier	8.63 (8.07-9.19)	38.6 (31.5-56.5)	548 (33.0-1062)
Non-carrier	8.56 (6.58-10.53)	44.0 (3.59-73.7)	657 (370-944)
MDRSA			
Carrier	8.47 (7.47-9.50)	40.3 (9.21.2-74.6)	572 (208-1313)
Non-carrier	8.66 (8.09-9.21)	47.9 (28.3-52.2)	761 (309-836)
***scn* negative**			
Carrier	--	--	--
Non-carrier	--	--	--

Note. IHO, industrial hog operation. AFHO, antibiotic-free hog operation.
P-value estimated from Student's t-test.

There are no significant differences in alpha diversity of the AFHO worker nasal microbiome by *S. aureus* nasal carriage outcomes (carriage vs. not).

Research question: Are there significant differences in the microbiome of IHO pigs and AFHO pigs (by anatomical site and combined), and between pigs and their respective workers (by anatomical site and combined)? [performed via anosim comparisons of the R-statistic]

Differences in beta diversity between pigs (by anatomical site) and worker, by mode of hog production (IHO vs. AFHO), North Carolina: 2013-2015.

Comparison category	Beta diversity metric	*R* statistic	*P-value*	n
IHO pig nares vs. AFHO pig nares	Unweighted UniFrac	0.638	0.001	20
	Weighted UniFrac	0.685	0.001	
	Bray-Curtis	0.789	0.001	
	Binary Jaccard	0.819	0.001	
IHO pig perineum vs. AFHO pig perineum	Unweighted UniFrac	0.396	0.002	18
	Weighted UniFrac	0.415	0.002	
	Bray-Curtis	0.477	0.001	
	Binary Jaccard	0.474	0.001	
IHO pig vs. AFHO pig (combined)	Unweighted UniFrac	0.485	0.001	38
	Weighted UniFrac	0.526	0.001	
	Bray-Curtis	0.603	0.001	
	Binary Jaccard	0.629	0.001	
IHO pig nares vs. IHO worker nares	Unweighted UniFrac	0.083	0.216	50
	Weighted UniFrac	0.260	0.002	
	Bray-Curtis	0.128	0.083	

	Binary Jaccard	0.142	0.068	
IHO pig perineum vs. IHO worker nares	Unweighted UniFrac	0.116	0.144	49
	Weighted UniFrac	0.393	0.001	
	Bray-Curtis	0.190	0.026	
	Binary Jaccard	0.185	0.047	
IHO pig (combined) vs. IHO worker				17
	Unweighted UniFrac	0.14302	0.012	
	Weighted UniFrac	0.39215	0.001	
	Bray-Curtis	0.2578	0.001	
	Binary Jaccard	0.264	0.001	
AFHO pig nares vs. AFHO worker nares	Unweighted UniFrac	0.502	0.001	
	Weighted UniFrac	0.581	0.001	
	Bray-Curtis	0.618	0.002	
	Binary Jaccard	0.618	0.001	
AFHO pig perineum vs. AFHO worker nares	Unweighted UniFrac	0.308	0.009	16
	Weighted UniFrac	0.272	0.021	
	Bray-Curtis	0.331	0.006	
	Binary Jaccard	0.336	0.003	
AFHO pig (combined) vs. AFHO worker	Unweighted UniFrac	0.214	0.023	
	Weighted UniFrac	0.281	0.009	
	Bray-Curtis	0.3359	0.004	
	Binary Jaccard	0.3627	0.002	

IHO worker nares vs. AFHO worker nares	Unweighted UniFrac	-0.214	0.962	47
	Weighted UniFrac	0.002	0.482	
	Bray-Curtis	-0.113	0.839	
	Binary Jaccard	-0.033	0.601	

Note. IHO, industrial hog operation. AFHO, antibiotic-free hog operation.
p-values estimated using the R-statistic.

There are significant differences in the beta diversity of the IHO pig and AFHO pig by anatomical site and combined. When taking into account community membership and composition, the IHO pigs nares are close to significance (except weighted UniFrac $p < 0.05$) compared to the IHO workers nasal microbiome. When taking into account community membership and composition, the IHO pigs perineum are significantly different compared to the IHO workers nasal microbiome.

Research question: Are there significant differences in the microbiome of IHO pigs, IHO workers and AFHO workers by *S. aureus* carriage outcomes? [performed via anosim comparisons of the R-statistic]

Differences in beta diversity of pigs and workers microbiomes, by *S. aureus* nasal carriage (IHO vs. AFHO), North Carolina: 2013-2015.

Comparison category	Beta diversity metric	*R* statistic	*P-value*
IHO pig S. aureus carrier vs. non-carrier	Unweighted UniFrac	-0.048	0.595
	Weighted UniFrac	-0.001	0.500
	Bray-Curtis	0.001	0.445
	Binary Jaccard	-0.025	0.533
IHO worker S. aureus carrier vs. non-carrier	Unweighted UniFrac	0.074	0.105
	Weighted UniFrac	0.075	0.035
	Bray-Curtis	-0.018	0.700
	Binary Jaccard	0.015	0.271
AFHO worker S. aureus carrier vs. non-carrier	Unweighted UniFrac	-0.236	0.808
	Weighted UniFrac	0.091	0.382
	Bray-Curtis	0.036	0.394
	Binary Jaccard	0.073	0.362
IHO pig MDRSA carrier vs. non-carrier	Unweighted UniFrac	-0.048	0.609
	Weighted UniFrac	-0.007	0.488
	Bray-Curtis	0.010	0.439
	Binary Jaccard	-0.025	0.541
IHO worker MDRSA carrier vs. non-carrier	Unweighted UniFrac	0.135	0.103

	Weighted UniFrac	0.290	0.001	*
	Bray-Curtis	0.035	0.306	
	Binary Jaccard	0.036	0.321	
AFHO worker MDRSA carrier vs. non-carrier				
	Unweighted UniFrac	-0.127	0.611	
	Weighted UniFrac	0.000	0.440	
	Bray-Curtis	-0.145	0.626	
	Binary Jaccard	-0.255	0.901	
IHO pig *scn* negative-*S. aureus* carrier vs. non-carrier	Unweighted UniFrac	-0.048	0.623	
	Weighted UniFrac	-0.007	0.469	
	Bray-Curtis	0.010	0.469	
	Binary Jaccard	-0.025	0.536	
IHO worker *scn* negative-*S. aureus* carrier vs. non-carrier	Unweighted UniFrac	0.045	0.283	
	Weighted UniFrac	0.168	0.022	*
	Bray-Curtis	-0.043	0.680	
	Binary Jaccard	-0.015	0.528	
AFHO worker *scn* negative-*S. aureus* carrier vs. non-carrier	Unweighted UniFrac	---	---	
	Weighted UniFrac	---	---	
	Bray-Curtis	---	---	
	Binary Jaccard	---	---	

Note. IHO, industrial hog operation. AFHO, antibiotic-free hog operation.
p-values estimated using the R-statistic.

Significant differences in beta diversity (Weighted UniFrac only) are observed only in the IHO workers across all *S. aureus* nasal carriage outcomes measures (carriers vs. not).

Research question: Are there significant differences in the microbiome of IHO workers by study origin? [performed via anosim comparisons of the R-statistic]

Differences in beta diversity between a pigs and worker microbiomes, by study (4 month IHO worker, Thrasher IHO worker), North Carolina: 2013-2015.

Comparison category	Beta diversity metric	*R* statistic	*P-value*
IHO worker 4 months vs. thrasher	Weighted UniFrac	0.086	0.025
	Unweighted UniFrac	0.040	0.083
	Bray-Curtis	0.111	0.004
	Binary Jaccard	0.108	0.004
IHO pig vs. 4 month IHO worker	Weighted UniFrac	0.240	0.001
	Unweighted UniFrac	0.374	0.001
	Bray-Curtis	0.368	0.001
	Binary Jaccard	0.370	0.001
IHO pig vs. thrasher IHO worker	Weighted UniFrac	0.092	0.111
	Unweighted UniFrac	0.056	0.171
	Bray-Curtis	0.103	0.094
	Binary Jaccard	0.058	0.152
4 month IHO worker vs. AFHO worker	Weighted UniFrac	0.136	0.043
	Unweighted UniFrac	0.335	0.008
	Bray-Curtis	0.428	0.003
	Binary Jaccard	0.496	0.001
Thrasher IHO worker vs. AFHO worker	Weighted UniFrac	-0.363	1.00
	Unweighted UniFrac	-0.084	0.586
	Bray-Curtis	-0.084	0.552
	Binary Jaccard	-0.078	0.584

Note. IHO, industrial hog operation. AFHO, antibiotic-free hog operation.
p-values estimated using the R-statistic.

There are significant differences in beta diversity of the microbiome of IHO workers by study (4 mos vs. thrasher), IHO pigs and 4 mos IHO worker, 4 mos IHO workers and AFHO workers. IHO pigs and thrasher IHO workers as well as thrasher IHO workers and AFHO workers have similar beta diversities.

<u>Research question: Is there a study effect on log 2 fold genera abundances of taxa?</u> [performed via generalized linear models (GLMs]

*using one of the log 2 fold differences in OTU

*comparing alpha diversity measures of humans by study; pigs were not considered as they were all collected from the same study

xi: glm shannon jw i,studynew if humanvspig==1 & man1==1, family(gaussian) link(identity) cluster(clusterp2)
outreg2 using studyeffect, word replace
xi: glm inverse simpson jw i.studynew if humanvspig==1 & man1==1, family(gaussian) link(identity) cluster(cluster)
outreg2 using studyeffect, word append
xi: glm chao1 jw i.studynew if humanvspig==1 & man1==1, family(gaussian) link(identity) cluster(cluster)
outreg2 using studyeffect, word append
xi: glm heip e jw i.studynew if humanvspig==1 & man1==1, family(gaussian) link(identity) cluster(cluster)
outreg2 using studyeffect, word append
xi: glm observed otus jw i.studynew if humanvspig==1 & man1==1, family(gaussian) link(identity) cluster(cluster)
outreg2 using studyeffect, word append

	(1)	(2)	(3)	(4)	(5)
	VARIABLESshannon jw	inverse simpson jw	chao1 jw	heip e jw	observed otus jw
	Istudynew 21.000***	-0.0233	204.5*	0.136***	97.49*
	(0.336)	(0.0322)	(110.9)	(0.0527)	(53.38)
	Istudynew 32.843***	-0.0526*	1,162***	0.218**	462.4***
	(0.218)	(0.0287)	(166.3)	(0.100)	(94.15)
	Constant5.765***	1.059***	302.3***	0.457***	163.6***
	(0.206)	(0.0286)	(42.59)	(0.0281)	(31.83)
	Observations47	47	47	47	47

Robust standard errors in parentheses; *** p<0.01, ** p<0.05, * p<0.1

<u>Research question: Sensitivity analysis of log 2 fold LOD half imputation and effect of study;</u> [performed via generalized linear models (GLMs]

NOT SIGNIFICANT EFFECT SIZE CHANGE

xi: glm log2 g moraxella LODhalf i.studynew if humanvspig==1 & man1==1, family(gaussian) link(identity) cluster(cluster) outreg2 using log2fold, word replace
xi: glm log2 g prevotella LODhalf i.studynew if humanvspig==1 & man1==1, family(gaussian) link(identity) cluster(cluster) outreg2 using log2fold, word append
xi: glm log2 g lactobacillus LODhalf i.studynew if humanvspig==1 & man1==1, family(gaussian) link(identity) cluster(cluster) outreg2 using log2fold, word append

	(1)	(2)	(3)	(4)
	VARIABLESlog2 g moraxella LODhalf	log2 g prevotella LODhalf	log2 g prevotella LODhalf	log2 g lactobacillus LODhalf
	Istudynew 2-1.316	0.0344	0.0344	-1.662*
	(0.911)	(0.444)	(0.444)	(0.998)
	Istudynew 30.113	-0.394	-0.394	1.311
	(1.475)	(0.285)	(0.285)	(0.899)
	Constant-9.410***	-4.798***	-4.798***	-8.245***
	(0.682)	(0.247)	(0.247)	(0.820)
	Observations46	46	46	46

Robust standard errors in parentheses
*** p<0.01, ** p<0.05, * p<0.1

Research question: What bacterial OTUs significantly differ by *S. aureus* nasal carriage outcomes (carriers vs. not)?

****

*WHICH OTUS ARE SIGNIFICANT DIFFERENT BY S. AUREUS NASAL COLONIZATION AMONG IHO WORKERS

****

filter samples from otu table.py -i man 2/otu tables/otu table labcontaminants removed filtered xsectional mc2 man2.biom -o man 2/otu tables/otu table labcontaminants removed filtered xsectional mc2 IHOworker.biom -m man 2/AB dissertation xsectional mapping file 1 5 18 truncated quartiles.txt -s 'participant type 2:IHO worker'

group significance.py -i man 2/otu tables/otu table labcontaminants removed filtered xsectional mc2 IHOworker.biom -m man 2/AB dissertation xsectional mapping file 1 5 18 truncated quartiles.txt -o man 2/otu significance man2/IHOworker saureus gtest.txt -s g test -c saureusnew

group significance.py -i man 2/otu tables/otu table labcontaminants removed filtered xsectional mc2 IHOworker.biom -m man 2/AB dissertation xsectional mapping file 1 5 18 truncated quartiles.txt -o man 2/otu significance man2/IHOworker saureus kruskal.txt -s kruskal wallis -c saureusnew

****

*WHICH OTUS ARE SIGNIFICANT DIFFERENT BY MDRSA NASAL COLONIZATION AMONG IHO WORKERS

****

group significance.py -i man 2/otu tables/otu table labcontaminants removed filtered xsectional mc2 IHOworker.biom -m man 2/AB dissertation xsectional mapping file 1 5 18 truncated quartiles.txt -o man 2/otu significance man2/IHOworker mdrsa gtest.txt -s g test -c mdrsa positive new

group significance.py -i man 2/otu tables/otu table labcontaminants removed filtered xsectional mc2 IHOworker.biom -m man 2/AB dissertation xsectional mapping file 1 5 18 truncated quartiles.txt -o man 2/otu significance man2/IHOworker mdrsa kruskal.txt -s kruskal wallis -c mdrsa positive new

****

*WHICH OTUS ARE SIGNIFICANT DIFFERENT BY SCN NEG NASAL COLONIZATION AMONG IHO WORKERS

****

group significance.py -i man 2/otu tables/otu table labcontaminants removed filtered xsectional mc2 IHOworker.biom -m man 2/AB dissertation xsectional mapping file 1 5 18 truncated quartiles.txt -o man 2/otu significance man2/IHOworker scnneg gtest.txt -s g test -c scn negnew

group significance.py -i man 2/otu tables/otu table labcontaminants removed filtered xsectional mc2 IHOworker.biom -m man 2/AB dissertation xsectional mapping file 1 5 18 truncated quartiles.txt -o man 2/otu significance man2/IHOworker scnneg kruskal.txt -s kruskal wallis -c scn negnew

****

*WHICH OTUS ARE SIGNIFICANT DIFFERENT BY SSTI CASES VS. CONTROLS AMONG IHO WORKERS

****

group significance.py -i man 2/otu tables/otu table labcontaminants removed filtered xsectional mc2 IHOworker.biom -m man 2/AB dissertation xsectional mapping file 1 5 18 truncated quartiles.txt -o man 2/otu significance man2/IHOworker ssti gtest.txt -s g test -c anyssti baselinenew
group significance.py -i man 2/otu tables/otu table labcontaminants removed filtered xsectional mc2 IHOworker.biom -m man 2/AB dissertation xsectional mapping file 1 5 18 truncated quartiles.txt -o man 2/otu significance man2/IHOworker ssti kruskal.txt -s kruskal wallis -c anyssti baselinenew

Results: for kruskal (relative abundance); there were not otus significantly diff by any of the s. aureus outcomes in IHO workers
Results: for (g-test) pres/abs: there were marked differences in what s. aureus nasally colonized iho workers carried
*****S. aureus**
*Present only in carrier; Pseudomonas deceptionensis:Pseudomonas fragi:Pseudomonas lundensis:Pseudomonas psychrophila
*Present only in carrier; Staphylococcus equorum:Staphylococcus haemolyticus
*Present only in carrier; Staphylococcus sciuri
*Present only in carrier; Aerococcus urinaeequi:Aerococcus viridans
*Present in non-carrier; Cronobacter malonaticus:Cronobacter sakazakii:Escherichia albertii:Escherichia coli:Escherichia fergusonii:Pantoea dispersa:Shigella boydii:Shigella dysenteriae:Shigella flexneri:Shigella sonnei
*****MDRSA**
*Present only in carrier;
Pseudomonas deceptionensis:Pseudomonas fragi:Pseudomonas lundensis:Pseudomonas psychrophila
*Present only in carrier;Staphylococcus equorum:Staphylococcus haemolyticus
*Present only in carrier;Staphylococcus sciuri
*Present only in carrier;Lactobacillus acidophilus:Lactobacillus amylovorus
*Present only in carrier;Aerococcus urinaeequi:Aerococcus viridans
*Present only in carrier;Cobetia crustatorum
*Present only in carrier;otu11544:Gluconacetobacter liquefaciens
*Present only in carrier;Corynebacterium freneyi:Corynebacterium xerosis
*Present only in carrier;Clostridium baratii:Clostridium sardiniense
*Present only in carrier;Bacillus aestuarii:Bacillus arbutinivorans:Bacillus bataviensis:Bacillus djibelorensis:Bacillus drentensis:Bacillus fucosivorans:Bacillus fumarioli:Bacillus niacini:Bacillus novalis:Bacillus pocheonensis:Bacillus pseudomegaterium:Bacillus senegalensis:Bacillus soli:Bacillus vireti:Sporosarcina koreensis
*Present only in carrier;Halomonas halodenitrificans
*Present only in carrier;Psychrobacter aquimaris:Psychrobacter nivimaris:Psychrobacter proteolyticus
*Present only in carrier;Psychrobacter sanguinis
*Present only in carrier;Staphylococcus carnosus:Staphylococcus condimenti:Staphylococcus haemolyticus:Staphylococcus piscifermentans:Staphylococcus simulans

*Present in non-carrier: Cronobacter malonaticus:Cronobacter sakazakii:Escherichia albertii:Escherichia coli:Escherichia fergusonii:Pantoea dispersa:Shigella boydii:Shigella dysenteriae:Shigella flexneri:Shigella sonnei
*****SCNNEG**
*Present only in carrier;
Pseudomonas deceptionensis:Pseudomonas fragi:Pseudomonas lundensis:Pseudomonas psychrophila
*Present only in carrier;Staphylococcus equorum:Staphylococcus haemolyticus
*Present only in carrier;Cobetia crustatorum
*Present only in carrier;otu11544:Gluconacetobacter liquefaciens
*Present only in carrier;Halomonas halodenitrificans
*Present only in carrier;
Psychrobacter aquimaris:Psychrobacter nivimaris:Psychrobacter proteolyticus
*Present only in carrier;Bacillus aestuarii:Bacillus arbutinivorans:Bacillus bataviensis:Bacillus djibelorensis:Bacillus drentensis:Bacillus fucosivorans:Bacillus fumarioli:Bacillus niacini:Bacillus novalis:Bacillus pocheonensis:Bacillus pseudomegaterium:Bacillus senegalensis:Bacillus soli:Bacillus vireti:Sporosarcina koreensis
*Present in non-carrier:Cronobacter malonaticus:Cronobacter sakazakii:Escherichia albertii:Escherichia coli:Escherichia fergusonii:Pantoea dispersa:Shigella boydii:Shigella dysenteriae:Shigella flexneri:Shigella sonnei
*Present in non-carrier:Staphylococcus sciuri

****
*WHICH OTUS ARE SIGNIFICANT DIFFERENT BY S. AUREUS NASAL COLONIZATION AMONG CR CHILD
****
filter samples from otu table.py -i man 2/otu tables/otu table labcontaminants removed filtered xsectional mc2 man2.biom -o man 2/otu tables/otu table labcontaminants removed filtered xsectional mc2 CRchild.biom -m AB dissertation xsectional mapping file 2 2 182.txt -s 'participant type 2:CR minor'
group significance.py -i man 2/otu tables/otu table labcontaminants removed filtered xsectional mc2 CRchild.biom -m AB dissertation xsectional mapping file 2 2 182.txt -o man 2/otu significance man2/CRchild saureus gtest.txt -s g test -c saureusnew
group significance.py -i man 2/otu tables/otu table labcontaminants removed filtered xsectional mc2 CRchild.biom -m AB dissertation xsectional mapping file 2 2 182.txt -o man 2/otu significance man2/CRchld saureus kruskal.txt -s kruskal wallis -c saureusnew

*Result: No significantly different OTUs by S. aureus status within CR child; this may be due to the weak significance of unweighted unifrac at 0.042
*Also we are using FDR corrected as more robust measure to remove any OTUs differing strictly by chance alone

Research questions: Are the OTUs that are differential between *S. aureus* nasal carriage outcomes (carriers vs. not) are significantly different in relative abundance? (using the G test for independence)

***IHO worker vs. AFHO worker (from MS1)**
****RESULT:staphylococcus, pseduomonas and unassigned genus
****NON FDR corrected p value shows 31 OTUs are sig diff by this measure
****ADDTL. RESULT:above 3 + cronobacter, cloacibacterium, aerococcus, arthrobacter, aeromonas,
****cobetia, clostridum, bacillus, lactobacillus, corynebacterium, facklamia, rhodococcus,
****glunacetobacter, sphingomonas, exiguobacterium, lysinibacillus, psychrobacter, halomonas
****hydrogenophaga, tolumonas, bacteroides, tolumonas
****

***IHO worker with CR adults**
****
*IHO worker only: Pseudomonas deceptionensis:Pseudomonas fragi:Pseudomonas lundensis:Pseudomonas psychrophila
*IHO worker only: Staphylococcus equorum:Staphylococcus haemolyticus
*CR adult only: Prevotella buccalis
****

***IHO minor vs. CR minor**
* No OTUs significantly associated with either groups when looking at FDR P

Research question: what otus are sig diff related to one exposure group?
****

***IHO worker vs. CR adult**
group significance.py -i man 2/otu tables/otu table labcontaminants removed filtered xsectional mc2 IHOworkerCRadult.biom -m AB dissertation xsectional mapping file 2 2 182.txt -o man 2/otu significance man2/IHOworkerCRadult kruskal.txt -s kruskal wallis -c participant type 2

***IHO minor vs. CR minor**
group significance.py -i man 2/otu tables/otu table labcontaminants removed filtered xsectional mc2 IHOminorCRminor.biom -m AB dissertation xsectional mapping file 2 2 182.txt -o man 2/otu significance man2/IHOminorCRminor kruskal.txt -s kruskal wallis -c participant type 2

- RESULT: No differences in OTUS taking into account relative abundance

Significantly different OTUs
RESULTS (looking at FDR correction significance to adjust for multiple comparisons
IHO pigs and AFHO pigs 36 statistically differing taxa

- No significantly different taxa between AFHO pigs and AFHO workers
- IHO pigs and IHO worker carry 300 statistically significant taxa
- IHO worker and AFHO wrokers 63 statistically differing taxa

***top 10 most significantly different taxa**
***IHO pig vs. AFHO pig**

- otu18608:Desulfofrigus fragile:Desulfoluna spongiiphila
- Lactobacillus antri:Lactobacillus frumenti:Lactobacillus oris:Lactobacillus panis:Lactobacillus reuteri:Lactobacillus vaginalis (AFHO pig nares)
- otu6355:Sphingomonas jaspsi (IHO pig nares)
- Moraxella bovoculi (IHO pig perineum)
- Aerococcus urinaeequi:Aerococcus viridans (IHO pig nares)
- Rothia arfidiae:Rothia endophytica (IHO pig perineum)
- Cellulomonas oligotrophica:Cellulomonas terrae (AFHO pig perineum)
- Lactobacillus reuteri (IHO pig nares)
- Terrabacter carboxydivorans:Terrabacter ginsenosidimutans:Terrabacter lapilli:Terrabacter terrae:Terrabacter terrigena:Terrabacter tumescens (AFHO pig nares)
- Acidovorax konjaci (AFHO pig nares)

***IHO pig vs. IHO worker**

- Moraxella bovoculi (IHO pig nares)
- otu13168:Moraxella bovoculi (IHO pig nares)
- Rothia nasimurium (IHO pig nares)
- otu10926:Treponema parvum (IHO pig nares)
- Rothia mucilaginosa:Rothia nasimurium (IHO pig nares)
- Rothia arfidiae:Rothia endophytica (IHO pig nares)
- otu12429:Parvibacter caecicola (IHO pig perineum)
- Micrococcus terreus (IHO pig perineum)
- otu9475:Alloprevotella rava(IHO pig nares)
- Haemophilus parasuis(IHO pig nares)

***IHO worker vs AFHO worker**
***AFHO worker carry all of the 10 most significantly differing OTUs compared to IHO workers**

- otu12248:Flavonifractor plautii:Pseudoflavonifractor capillosus
- Acinetobacter brisouii
- Arthrobacter arilaitensis:Arthrobacter bergerei:Arthrobacter mysorens:Arthrobacter nicotianae:Arthrobacter protophormiae
- Nocardioides hungaricus
- otu6017:Macellibacteroides fermentans:Parabacteroides chartae
- Exiguobacterium antarcticum:Exiguobacterium artemiae:Exiguobacterium oxidotolerans:Exiguobacterium sibiricum:Exiguobacterium undae
- Lactobacillus kitasatonis
- Serinicoccus chungangensis
- Massilia aerilata
- otu9383:Blautia hansenii

Overall: 353 between IHO pigs and IHO workers, 0 between AFHO pigs and AFHO workers, and 64 between IHO workers and AFHO workers using the FDR corrected significance values.

Research question: What OTUs represent the core microibome by participant type (iho)?

<u>Core microbiomes were calculated from these classifications to determine the OTUs present in at least 50% of the samples by participant type in QIIME.</u>

compute core microbiome.py -i otu table labcontaminants removed filtered xsectional.biom -o core microbiome man1/IHOpignarescore mbiome man1 --mapping fp mapping file man1.txt --valid states 'participant type 2:IHO pig nares'
compute core microbiome.py -i otu table labcontaminants removed filtered xsectional.biom -o core microbiome man1/IHOpigpericore mbiome man1 --mapping fp mapping file man1.txt --valid states 'participant type 2:IHO pig perineum'
compute core microbiome.py -i otu table labcontaminants removed filtered xsectional.biom -o core microbiome man1/IHOpigcore mbiome man1 --mapping fp mapping file man1.txt --valid states 'participant type 2:IHO pig nares,IHO pig perineum'
compute core microbiome.py -i otu table labcontaminants removed filtered xsectional.biom -o core microbiome man1/IHOworkercore mbiome man1 --mapping fp mapping file man1.txt --valid states 'participant type 2:IHO worker'
*RESULTS:

- IHO pig nares: 100% carry actinobacillus, aerococcus, corynebacterium, lactobacillus, micrococcus, moraxella, and rothia
- IHO pig perineum: 100% carry actinobacillus, lactobacillus, Rothia, Treponema, incertae sedis (genus unassigned), Dorea
- IHO pig: 100% of pig samples carry genus lactobacillus and rothia Rothia arfidiae:Rothia endophytica (nothing; endophytica found in aerosols in german paper from farms using antibiotics, Lactobacillus antri:Lactobacillus frumenti:Lactobacillus oris:Lactobacillus panis:Lactobacillus reuteri:Lactobacillus vaginalis; The use of Lactobacillus as an alternative of antibiotic growth promoters in pigs: A review

- IHO worker: 60% of workers carry genus staphylococcus and streptococcus: Staphylococcus aureus:Staphylococcus capitis:Staphylococcus caprae:Staphylococcus condimenti:Staphylococcus devriesei:Staphylococcus epidermidis:Staphylococcus haemolyticus:Staphylococcus hominis:Staphylococcus lugdunensis:Staphylococcus pasteuri:Staphylococcus petrasii:Staphylococcus simulans:Staphylococcus spC10c:Staphylococcus warneri, streptococcus suis:Streptococcus uberis; zoonotic disease agent rarely affects people and problem forpig health directionality animals to humans; case sichuan province in china reported transmission

Research question: what otus represent the core microbiome by participant type (afho)?

compute core microbiome.py -i otu table labcontaminan
ts removed filtered xsectional.biom -o core microbiome man1/AFHOpignarescore mbiome man1 --mapping fp mapping file man1.txt --valid states 'participant type 2:AFHO pig nares'
compute core microbiome.py -i otu table labcontaminants removed filtered xsectional.biom -o core microbiome man1/AFHOpigpericore mbiome man1 --mapping fp mapping file man1.txt --valid states 'participant type 2:AFHO pig perineum'
compute core microbiome.py -i otu table labcontaminants removed filtered xsectional.biom -o core microbiome man1/AFHOpigcore mbiome man1 --mapping fp mapping file man1.txt --valid states 'participant type 2:AFHO pig nares,AFHO pig perineum'
compute core microbiome.py -i otu table labcontaminants removed filtered xsectional.biom -o core microbiome man1/AFHOworkercore mbiome man1 --mapping fp mapping file man1.txt --valid states 'participant type 2:AFHO worker'

- AFHO pig nares: 100% in AFHO pig nares carried burkholderies(species unassigned), acidovorax, cellulomonas, geobacter, terrabacter, desulfobacteriaceae (genus unassigned), sphingomonas
- AFHO pig perineum: 85% in AFHO pig peri carried acinetobacter, alistipes, firmicutes (genus unassigned), clostridiales (genus unassigned), desulfobacteriaceae (genus unassigned), rhizobiales (genus unassigned), bryobacter, anaerosporobacter, oscillibacter, incertae sedis (genus unassigned)
- AFHO pig: 90% of pig samples carry desulfobacteriaceae (family; unassigned genus); 85% carry acidovorax (genus), cellulomonas (genus), Cellulomonas oligotrophica:Cellulomonas terrae assumed to be sourced by feed based on readings
    - otu18608:Desulfofrigus fragile:Desulfoluna spongiiphila anaerobic bacteria
    - Acidovorax delafieldii:Curvibacter delicatus; associated with infections in humans and found in fish farming
- AFHO worker: 100% of workers carried 4 OTUS intrasporangiaceae (family;unassigned genus), lachnospiraceae (family; unassigned genus),

parabacteroides (genus),bacteroidetes g unassigned (phylum; couldnt not classify any lower)

using information from otu significance test I have deduced species level information for these core otus when possible; file : kruskal pigsbyenvi

- Parabacteroides: otu6067:Parabacteroides distasonis (animal associated) PLOS 0030287
- machnospiraceae:otu15037:Clostridium oroticum:Roseburia faecis:Roseburia inulinivorans (clostridium phylogenetically close to difficle and roseburia known to pig feces
- intrasporangiaceae: Janibacter anophelis:Janibacter corallicola:Janibacter hoylei:Janibacter limosus:Janibacter marinus:Janibacter melonis:Janibacter sanguinis:Janibacter terrae:Knoellia locipacati:Knoellia sinensis:Knoellia subterranea:Tetrasphaera remsis
- janibacter: found in bio filters treating air from livestock facility https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3233082/

Research question: What OTUs are significantly associated with microbial dysbiosis by farm environment (iho & afho)?

***IHO environment core (pig & worker)**
***AFHO environment core (pig & worker)**
compute core microbiome.py -i otu table labcontaminants removed filtered xsectional.biom -o core microbiome man1/IHOcore mbiome man1 --mapping fp mapping file man1.txt --valid states 'participant type 2:IHO worker,IHO pig nares,IHO pig perineum'
compute core microbiome.py -i otu table labcontaminants removed filtered xsectional.biom -o core microbiome man1/AFHOcore mbiome man1 --mapping fp mapping file man1.txt --valid states 'participant type 2:AFHO worker,AFHO pig nares,AFHO pig perineum'
RESULTS:

- IHO envi: 60% of samples carried staphylococcus (genus)
- AFHO envi: 80% of samples acinetobacter (genus), oscillibacter (genus), intrasporangiaceae (family, unassigned genus) /// firmicutes (phylum; unassigned lower classifications), rhizobiales (order; unassigned lowers), desulfobacteraceae (family; unassigned genus)

Research question: Is there a farm-specific at the three AFHO farms visited in 2015? Note: IHO pigs not analyzed due to sampling from only one IHO

Facility-specific impact on the microbiome of pigs and workers

AFHO pig's and AFHO worker's diversity differed between the three AFHO facilities sampled, suggesting a facility-specific influence on the microbiota. Facility-specific influences were not examined for IHO pigs and IHO workers because 1) IHO pigs were sampled from IHO veterinary teaching facility 2) IHO workers, worked at IHO facilities other than the proxy IHO veterinary teaching facility and were not asked about location of employment to conserve confidentiality.[insert code and output for this]

Research question: Are there observed correlations between OTUs in IHO pigs?
We observed great overlap in *Staphylococcus* species presence. We also observed some of inverse associations observed between *Staphylococcus* species and *Corynebacterium* species. [performed via relative abundances of OTUs]
Range= 0 (blue)-1 (red)

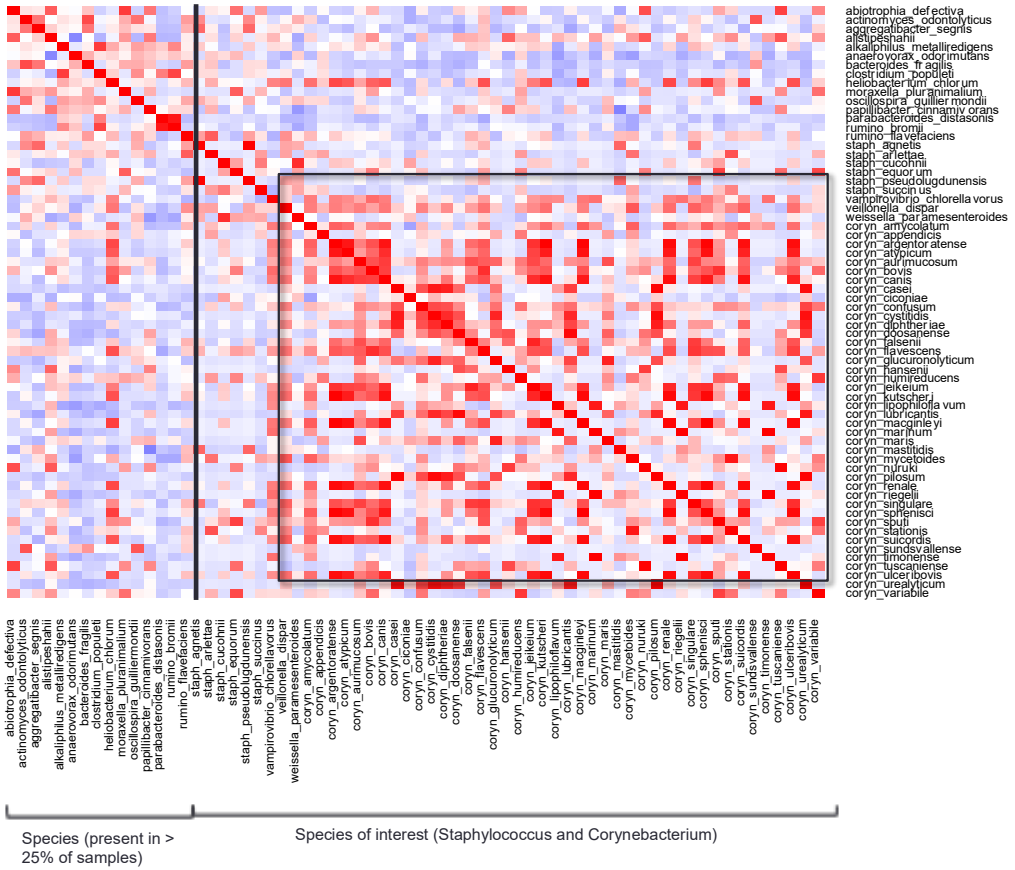
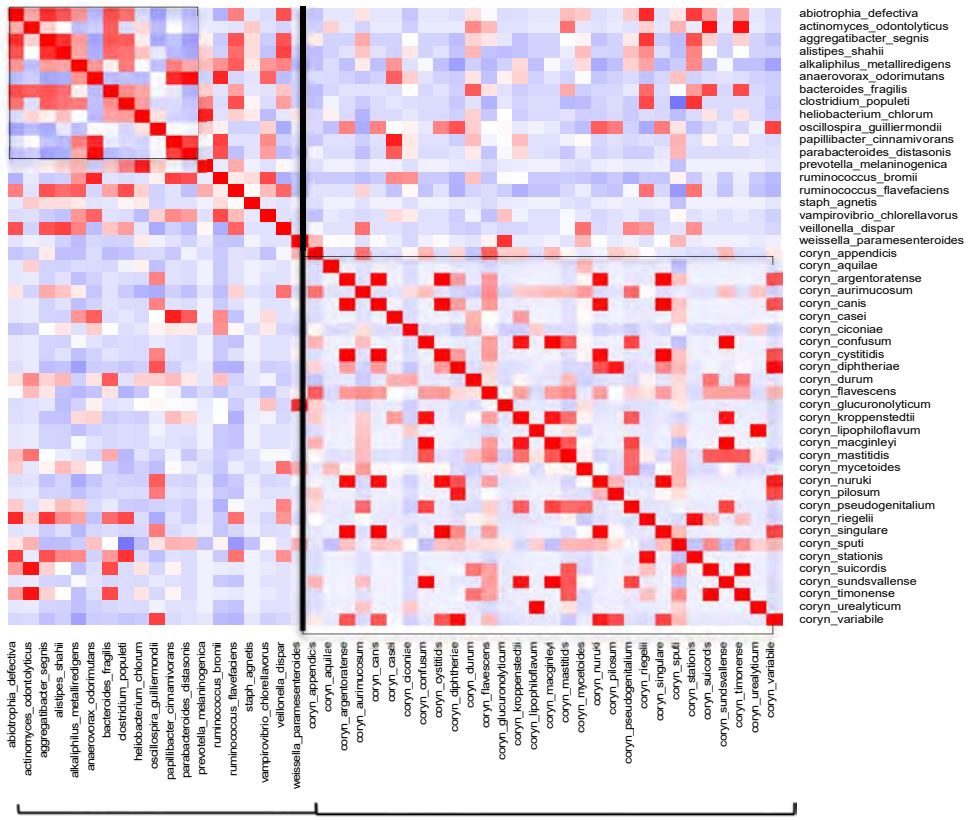


Figure _: Species correlations of the most abundant OTUs at the species level within the IHO pig. In addition to species of interest which include: staphylococcus and corynebacterium. Literature has observed an inverse relationship between staphylococcus species and some corynebacterium species (i.e. and citations). Within IHO pig this directional correlation seems to stand in most cases. Indicated by the blue region.

Research question: Are there observed correlations between OTUs in IHO pigs?
We observed positive correlations observed in AFHO pig within the top most abundant taxa (species level) increase, positive correlations between corynebacterium species decrease and fewer staphylococcus species carried by AFHO pigs compared to IHO pigs. [performed via relative abundances of OTUs]Range= 0 (blue)-1 (red)



Manuscript 2

Research question: Are there significant differences in the beta diversity of IHO workers, children living in the IHO household and CR adults and children living in the CR household? [performed via adonis comparison of R statistic]

Table 4: Differences in beta diversity between IHO workers and children and CR adults and children. North Carolina, 2013-2014.

Comparison category	Beta diversity	R stat*istic*	*p*-value
IHO worker vs. IHO	Unweighted UniFrac	0.054	0.065
	Weighted UniFrac	0.102	0.010
	Bray-Curtis	0.131	0.008
	Binary Jaccard	0.116	0.012
CR adult vs. CR child	Unweighted UniFrac	0.064	0.037
	Weighted UniFrac	0.027	0.145
	Bray-Curtis	0.135	0.006
	Binary Jaccard	0.141	0.002
IHO worker vs. CR	Unweighted UniFrac	0.005	0.374
	Weighted UniFrac	0.004	0.363
	Bray-Curtis	-0.009	0.570
	Binary Jaccard	-0.023	0.732
IHO child vs. CR child	Unweighted UniFrac	0.009	0.279
	Weighted UniFrac	-0.012	0.635
	Bray-Curtis	0.049	0.067
	Binary Jaccard	0.045	0.088

Note. IHO, industrial hog operation. CR, Community resident.

p-values estimated from Student's t-tests comparing beta diversity distance measures between participant types.

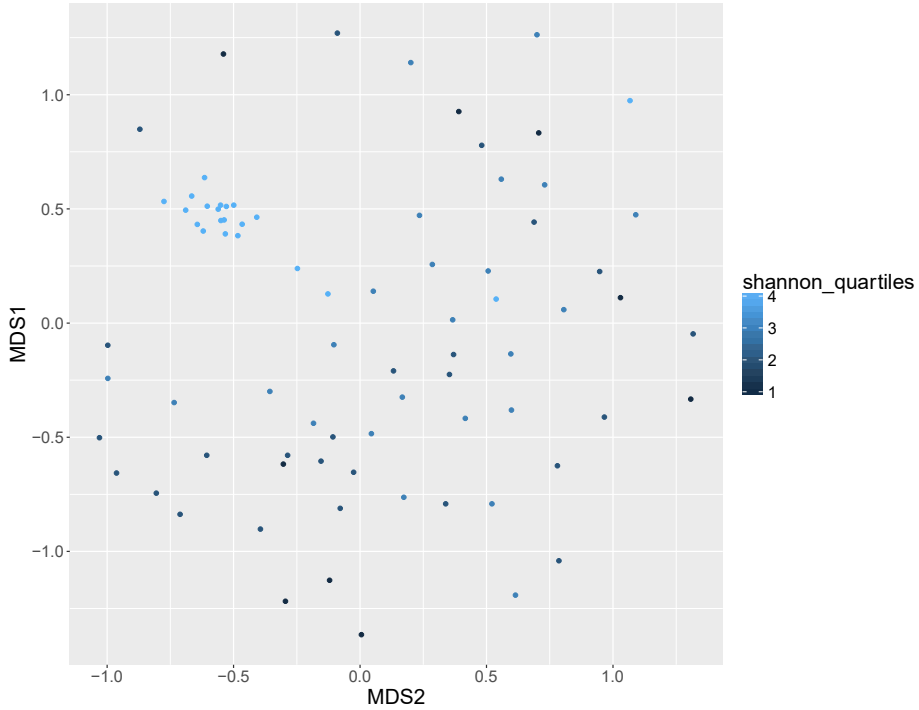
Research question: Are there significant differences in the beta diversity of IHO workers, children living in the IHO household and CR adults and children living in the CR household by *S. aureus* nasal carriage (carriers vs. not)?

Table 5: Differences in beta diversity by S. aureus nasal carriage outcomes (carrier vs. non-carrier), North Carolina: 2013-

		S. aureus		**MDRSA**		***scn* negative *S. aureus***	
Comparison category	**Beta diversity**	***R* statistic**	***p*-**	***R* statistici**	***p*-value**	***R* statistic**	***p*-value**
IHO worker carrier vs. non-	Unweighted	0.046	0.232	0.092	0.247	0.008	0.426
	Weighted UniFrac	0.219	0.014	0.174	0.106	0.216	0.043
	Bray-Curtis	0.184	0.031	0.303	0.015	0.269	0.023
	Binary Jaccard	0.229	0.022	0.406	0.001	0.335	0.005
IHO child carrier vs. non-	Unweighted	-0.077	0.954	-0.143	0.673	---	---
	Weighted UniFrac	-0.051	0.761	-0.079	0.602	---	---
	Bray-Curtis	-0.069	0.848	0.035	0.416	---	---
	Binary Jaccard	-0.039	0.699	-0.061	0.761	---	---
CR adult carrier vs. non-carrier	Unweighted	-0.033	0.670	0.014	0.487	---	---
	Weighted UniFrac	-0.044	0.749	0.298	0.248	---	---
	Bray-Curtis	-0.046	0.748	0.199	0.318	---	---
	Binary Jaccard	-0.052	0.784	0.161	0.369	---	---
CR child carrier vs. non-carrier	Unweighted	0.115	0.042	-0.077	0.469	---	---
	Weighted UniFrac	0.045	0.215	-0.044	0.453	---	---
	Bray-Curtis	0.036	0.250	0.024	0.558	---	---
	Binary Jaccard	0.047	0.203	0.073	0.421	---	---

Note. IHO, industrial hog operation. CR, Community Resident.
p-values estimated from Student's t-tests comparing beta diversity distance measures between participant types by *S. aureus* nasal carriage.

Research question: What <u>portion of spatial variability in bacterial community membership and composition correlated with Shannon diversity index quartiles</u> [performed via METAmds within R adonis by Shannon quartiles constructed from the natural clustering of data; here we do not delineate between participant type to display the influence of alpha diversity on beta diversity]



Panel B, Shannon diversity (overall alpha diversity) correlated with patterns of clustering observed. Ignoring exposure group, nasal microbiomes assigned to the highest quartile of the Shannon diversity index displayed greater similarities in community membership and composition compared to those less diverse communities. Bacterial taxa exclusive to the 4th quartile of the Shannon diversity index are outlined in supplemental Table S5.

Research question: Are there significant differences in the percentage of OTUs contributed to the participants by exposure group (IHO vs. CR)? [performed via GLM]

```
. xi: glm totalpig_perccontribution ihovscr if man2==1, family(gaussian) link(identity) cluster(clusterp2)

Iteration 0:   log pseudolikelihood = -272.79695

Generalized linear models                         No. of obs      =         79
Optimization     : ML                             Residual df     =         77
                                                  Scale parameter =   59.98037
Deviance         =  4618.488581                   (1/df) Deviance =   59.98037
Pearson          =  4618.488581                   (1/df) Pearson  =   59.98037

Variance function: V(u) = 1                       [Gaussian]
Link function    : g(u) = u                       [Identity]

                                                  AIC             =   6.956885
Log pseudolikelihood = -272.7969539               BIC             =   4282.041

                                        (Std. Err. adjusted for 40 clusters in clusterp2)
```

totalpig_perccontribution	Coef.	Robust Std. Err.	z	P>|z|	[95% Conf.	Interval]
ihovscr	6.471098	1.408934	4.59	0.000	3.709639	9.232557
_cons	.009347	.0030557	3.06	0.002	.003358	.0153361

IHO exposure groups (workers and children) on average received 6.5% more bacterial OTUs contributed from the IHO pig compared to the CR exposure groups (adults and children).

Research question: Are there significant differences in the percentage of OTUs contributed to IHO workers vs. CR adults? [performed via GLM]

```
. xi: glm totalpig_perccontribution ihovscr if man2==1 & worker==1, family(gaussian) link(identity) cluste
> r(clusterp2)

Iteration 0:   log pseudolikelihood = -139.36964

Generalized linear models                         No. of obs      =         39
Optimization     : ML                             Residual df     =         37
                                                  Scale parameter =   78.40814
Deviance         =  2901.101296                   (1/df) Deviance =   78.40814
Pearson          =  2901.101296                   (1/df) Pearson  =   78.40814

Variance function: V(u) = 1                       [Gaussian]
Link function    : g(u) = u                       [Identity]

                                                  AIC             =   7.249725
Log pseudolikelihood = -139.3696419               BIC             =    2765.55

                                  (Std. Err. adjusted for 39 clusters in clusterp2)
```

totalpig_perccontribution	Coef.	Robust Std. Err.	z	P>|z|	[95% Conf.	Interval]
ihovscr	13.28512	2.871893	4.63	0.000	7.656308	18.91392
_cons	.0035797	.0034216	1.05	0.295	-.0031265	.0102859

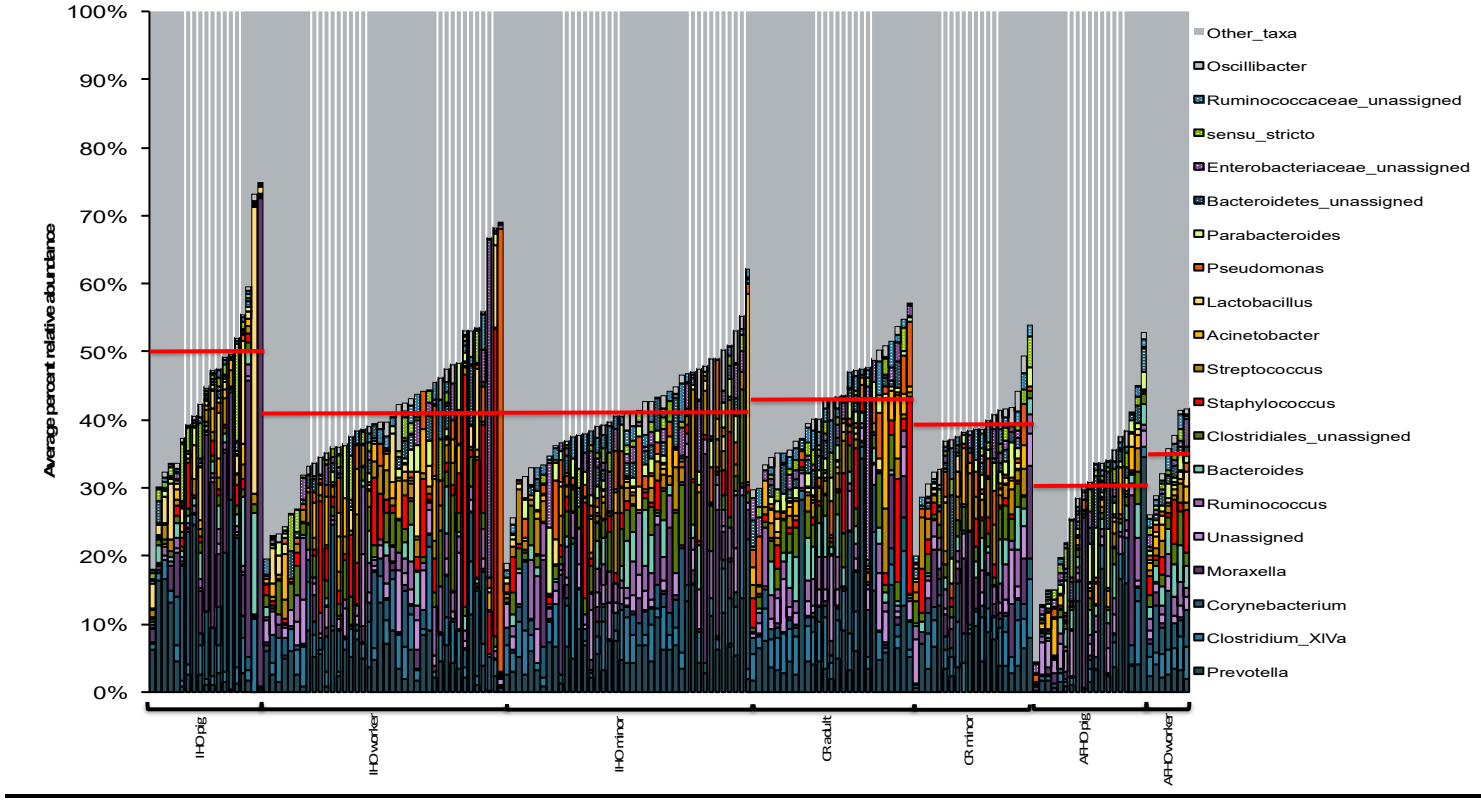
IHO workers on average received 13.2% more bacteria OTUs contributions from the IHO pigs to the IHO worker compared to the CR adult.

Research question: Are there significant differences in the percentage of OTUs contributed to IHO child vs. CR child? [performed via GLM]

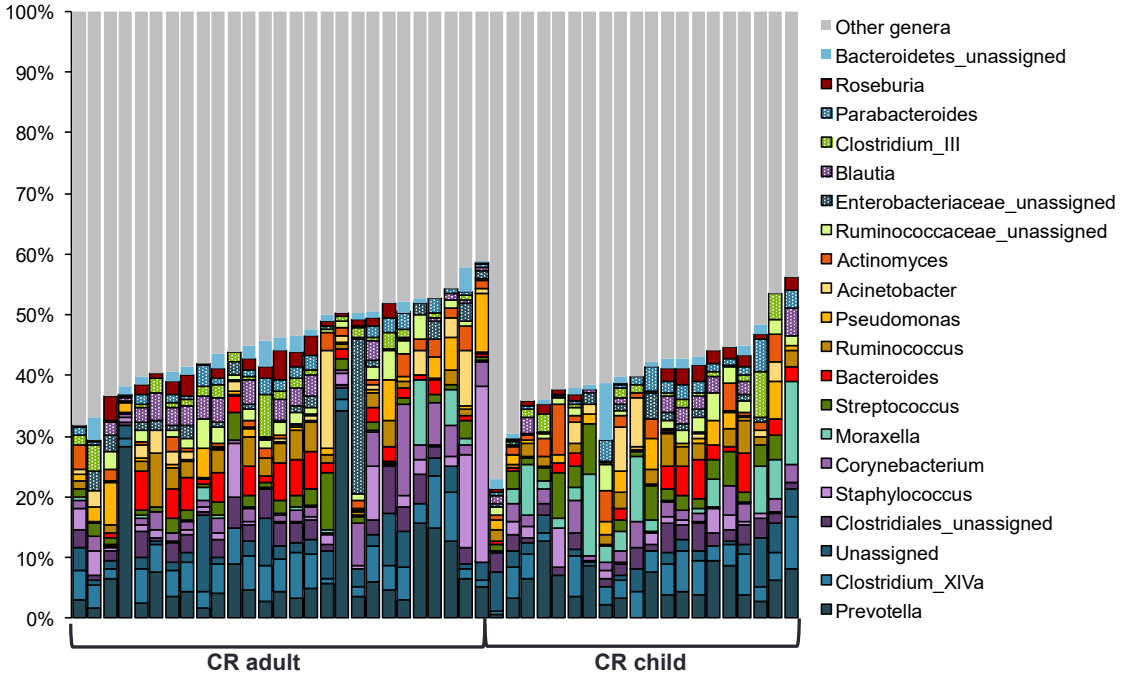
```
. xi: glm totalpig_perccontribution ihovscr if man2==1 & worker==0, family(gaussian) link(identity) cluste
> r(clusterp2)

Iteration 0:   log pseudolikelihood =  84.599774

Generalized linear models                         No. of obs      =         40
Optimization     : ML                             Residual df     =         38
                                                  Scale parameter =  .0008969
Deviance         =  .0340819855                   (1/df) Deviance =  .0008969
Pearson          =  .0340819855                   (1/df) Pearson  =  .0008969

Variance function: V(u) = 1                       [Gaussian]
Link function    : g(u) = u                       [Identity]

                                                  AIC             = -4.129989
Log pseudolikelihood =  84.59977411               BIC             = -140.1433

                                        (Std. Err. adjusted for 40 clusters in clusterp2)
------------------------------------------------------------------------------
             |               Robust
totalpig_perccontribution |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
            ihovscr |  -.0025074   .0093482    -0.27   0.789    -.0208296    .0158148
              _cons |   .0151143   .0055464     2.73   0.006     .0042436    .0259851
------------------------------------------------------------------------------
```

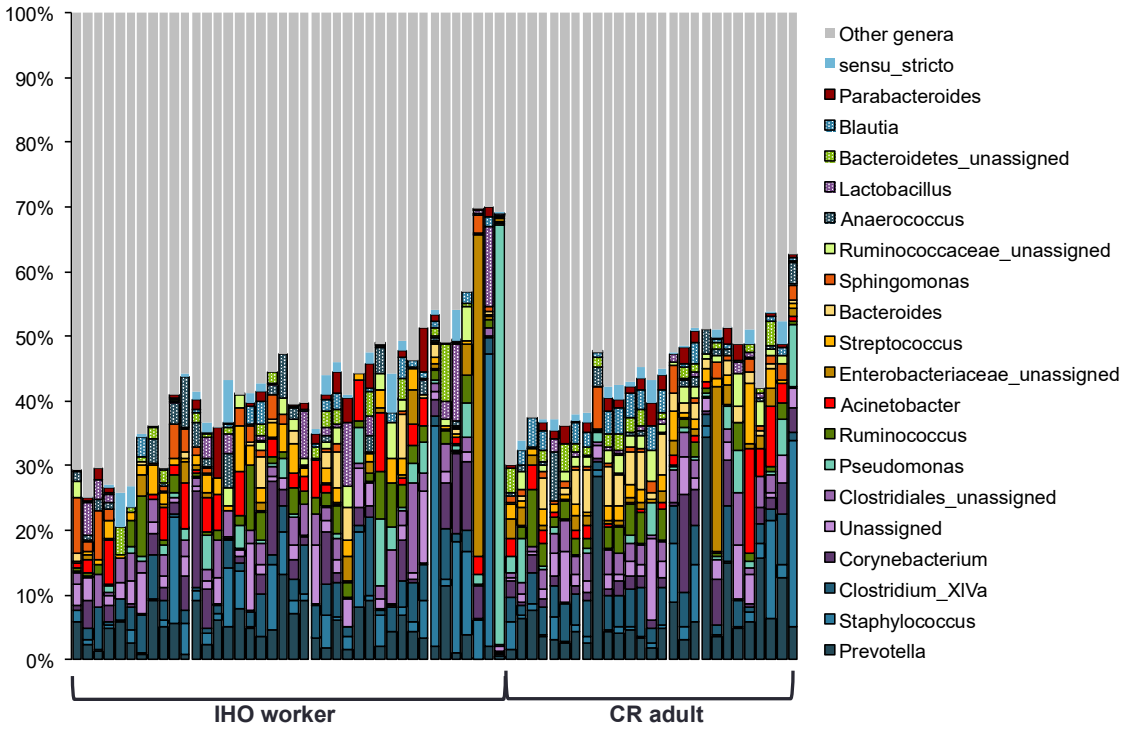
There are no significant differences in the percent bacterial OTUs contributed from the IHO pig to IHO children and the CR children.

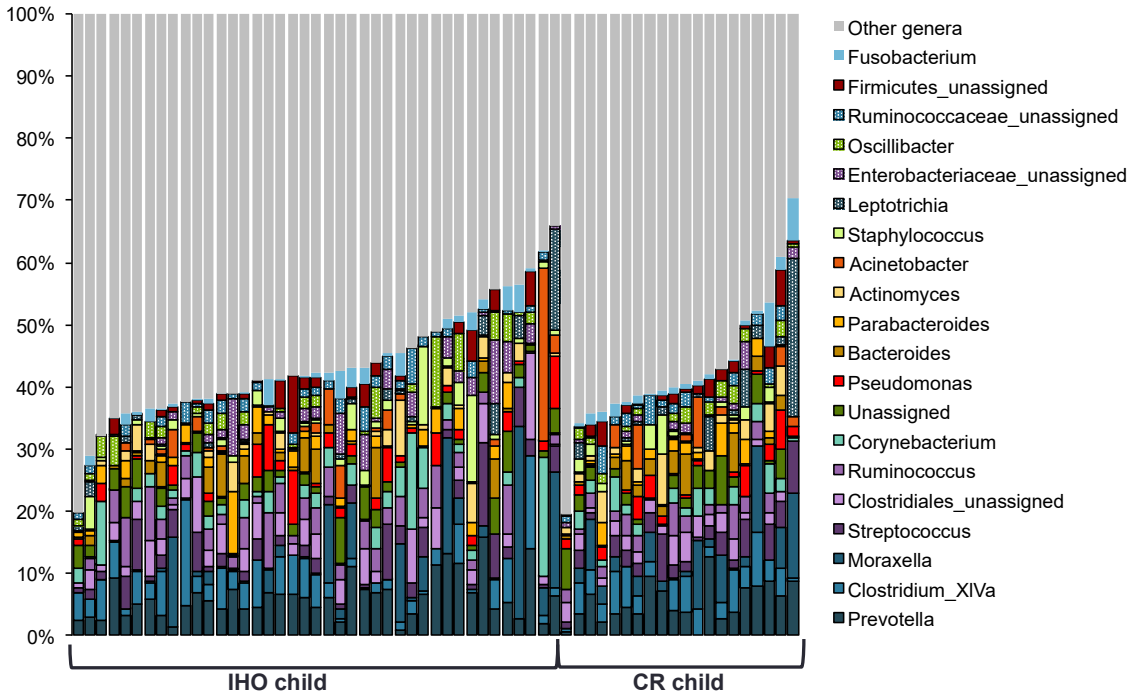
Research question: Are there difference in the relative abundance of the 19 most abundant bacterial OTUs across all participant types: (the red line delineates the average of all "other taxa contributed to that participant type) [using relative abundances of OTUs]



The reduced levels of common genera within the AFHO group supports the notion that AFHO pigs and humans have a divergent microbiome composition compared to the IHO environment. CR adults and minors represent an intermediate between IHO and AFHO populations.


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
CR adult
CR child
Other genera
Bacteroidetes_unassigned
Roseburia
Parabacteroides
Clostridium_III
Blautia
Enterobacteriaceae_unassigned
Ruminococcaceae_unassigned
Actinomyces
Acinetobacter
Pseudomonas
Ruminococcus
Bacteroides
Streptococcus
Moraxella
Corynebacterium
Staphylococcus
Clostridiales_unassigned
Unassigned
Clostridium_XlVa
Prevotella


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
IHO worker
CR adult
Other genera
sensu_stricto
Parabacteroides
Blautia
Bacteroidetes_unassigned
Lactobacillus
Anaerococcus
Ruminococcaceae_unassigned
Sphingomonas
Bacteroides
Streptococcus
Enterobacteriaceae_unassigned
Acinetobacter
Ruminococcus
Pseudomonas
Clostridiales_unassigned
Unassigned
Corynebacterium
Clostridium_XlVa
Staphylococcus
Prevotella


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
IHO child
CR child
Other genera
Fusobacterium
Firmicutes_unassigned
Ruminococcaceae_unassigned
Oscillibacter
Enterobacteriaceae_unassigned
Leptotrichia
Staphylococcus
Acinetobacter
Actinomyces
Parabacteroides
Bacteroides
Pseudomonas
Unassigned
Corynebacterium
Ruminococcus
Clostridiales_unassigned
Streptococcus
Moraxella
Clostridium_XlVa
Prevotella

Manuscript 3

Research question: Are there significant differences in alpha diversity between IHO workers and IHO children on average over time? [performed via Student's t-test]

```
. ttest shannon_jw, by(worker)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |      61    5.515653    .1135744    .8870444     5.28847    5.742835
       1 |      63    5.649946    .1357533    1.077509    5.378579    5.921313
---------+--------------------------------------------------------------------
combined |     124    5.583882    .0886095    .9867139    5.408485     5.75928
---------+--------------------------------------------------------------------
    diff |           -.1342937    .1775512               -.4857741    .2171867
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =  -0.7564
Ho: diff = 0                                     degrees of freedom =      122

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.2254         Pr(|T| > |t|) = 0.4509          Pr(T > t) = 0.7746

. ttest pd_whole_tree_jw, by(worker)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |      61    10.40281    .3530303    2.757255    9.696639    11.10897
       1 |      63    13.11814    .8228266    6.530984    11.47333    14.76294
---------+--------------------------------------------------------------------
combined |     124    11.78237    .4672029    5.202551    10.85757    12.70717
---------+--------------------------------------------------------------------
    diff |           -2.715333    .9055745               -4.508008   -.9226573
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =  -2.9985
Ho: diff = 0                                     degrees of freedom =      122

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0016         Pr(|T| > |t|) = 0.0033          Pr(T > t) = 0.9984

. ttest observed_otus_jw, by(worker)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |      61    91.60656    4.884025    38.14546    81.83705    101.3761
       1 |      63    138.8571    14.16398    112.4231    110.5437    167.1705
---------+--------------------------------------------------------------------
combined |     124    115.6129    7.851032    87.42539    100.0723    131.1535
---------+--------------------------------------------------------------------
    diff |           -47.25059    15.17697               -77.2949   -17.20627
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =  -3.1133
Ho: diff = 0                                     degrees of freedom =      122

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0012         Pr(|T| > |t|) = 0.0023          Pr(T > t) = 0.9988
```

On average over time, there are significant differnces in phylogenetic distance and observed OTUs (not Shannon diversity) within the IHO worker nasal microbiome.

<u>Research question: Are there significant differences in alpha diversity of IHO children *S. aureus* carriers vs. not, on average, over time?</u> [performed via Student's t-test]

```
. ttest shannon_jw if worker==0, by(saureusnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |      30    5.609712    .1240649    .6795317    5.355971    5.863453
       1 |      31    5.424627    .1892187    1.053525    5.038191    5.811063
---------+--------------------------------------------------------------------
combined |      61    5.515653    .1135744    .8870444     5.28847    5.742835
---------+--------------------------------------------------------------------
    diff |            .1850851    .2278258               -.2707932    .6409634
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =   0.8124
Ho: diff = 0                                     degrees of freedom =       59

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.7901         Pr(|T| > |t|) = 0.4198          Pr(T > t) = 0.2099

. ttest pd_whole_tree_jw if worker==0, by(saureusnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |      30    10.49075    .5031445    2.755836    9.461701    11.51979
       1 |      31     10.3177    .5031583    2.801467    9.290114    11.34529
---------+--------------------------------------------------------------------
combined |      61    10.40281    .3530303    2.757255    9.696639    11.10897
---------+--------------------------------------------------------------------
    diff |             .173047    .7117583               -1.251178    1.597272
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =   0.2431
Ho: diff = 0                                     degrees of freedom =       59

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.5956         Pr(|T| > |t|) = 0.8088          Pr(T > t) = 0.4044

. ttest observed_otus_jw if worker==0, by(saureusnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |      30    93.46667     7.49141    41.03214    78.14501    108.7883
       1 |      31    89.80645    6.415274    35.71873    76.70471    102.9082
---------+--------------------------------------------------------------------
combined |      61    91.60656    4.884025    38.14546    81.83705    101.3761
---------+--------------------------------------------------------------------
    diff |            3.660215    9.840276               -16.03013    23.35056
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =   0.3720
Ho: diff = 0                                     degrees of freedom =       59

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.6444         Pr(|T| > |t|) = 0.7113          Pr(T > t) = 0.3556
```

On average over time, there are no significant differences in alpha diversity observed for IHO children by *S. aureus* nasal carriage status.

<u>Research questions: Are there significant differences in alpha diversity of IHO workers *S. aureus* carriers vs. not, on average, over time?</u> [performed via Student's t-test]

```
. ttest shannon_jw if worker==1, by(saureusnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |      27    5.483536    .1972475    1.024928    5.078087    5.888984
       1 |      36    5.774754    .1855123    1.113074    5.398144    6.151364
---------+--------------------------------------------------------------------
combined |      63    5.649946    .1357533    1.077509    5.378579    5.921313
---------+--------------------------------------------------------------------
    diff |           -.2912187    .2740346               -.8391847    .2567472
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =  -1.0627
Ho: diff = 0                                     degrees of freedom =       61

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.1461         Pr(|T| > |t|) = 0.2921          Pr(T > t) = 0.8539

. ttest pd_whole_tree_jw if worker==1, by(saureusnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |      27    11.88559    1.116857    5.803358    9.589856    14.18132
       1 |      36    14.04255    1.160562     6.96337    11.68648    16.39862
---------+--------------------------------------------------------------------
combined |      63    13.11814    .8228266    6.530984    11.47333    14.76294
---------+--------------------------------------------------------------------
    diff |           -2.156963    1.653374               -5.463089    1.149163
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =  -1.3046
Ho: diff = 0                                     degrees of freedom =       61

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0985         Pr(|T| > |t|) = 0.1969          Pr(T > t) = 0.9015

. ttest observed_otus_jw if worker==1, by(saureusnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |      27     114.037    17.04431    88.56483    79.00196    149.0721
       1 |      36    157.4722    20.90361    125.4217    115.0356    199.9088
---------+--------------------------------------------------------------------
combined |      63    138.8571    14.16398    112.4231    110.5437    167.1705
---------+--------------------------------------------------------------------
    diff |           -43.43519    28.31419               -100.0529    13.18254
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =  -1.5340
Ho: diff = 0                                     degrees of freedom =       61

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0651         Pr(|T| > |t|) = 0.1302          Pr(T > t) = 0.9349
```

On average over time, there are no significant differences in alpha diversity observed for IHO children by *S. aureus* nasal carriage status.

<u>Research question: Are there significant differences in alpha diversity of IHO worker MDRSA carriers vs. not, on average, over time?</u> [performed via Student's t-test]

```
. ttest shannon_jw if worker==1, by(mdrsanew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |      45    5.656248    .1403738    .9416563    5.373343    5.939153
       1 |      18    5.634191    .3282045    1.392454     4.94174    6.326642
---------+--------------------------------------------------------------------
combined |      63    5.649946    .1357533    1.077509    5.378579    5.921313
---------+--------------------------------------------------------------------
    diff |            .0220572    .3029428               -.5837143    .6278286
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =   0.0728
Ho: diff = 0                                     degrees of freedom =       61

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.5289         Pr(|T| > |t|) = 0.9422          Pr(T > t) = 0.4711

. ttest pd_whole_tree_jw if worker==1, by(mdrsanew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |      45    11.97425    .7899292    5.299006    10.38225    13.56625
       1 |      18    15.97786    1.982728    8.412002    11.79467    20.16105
---------+--------------------------------------------------------------------
combined |      63    13.11814    .8228266    6.530984    11.47333    14.76294
---------+--------------------------------------------------------------------
    diff |           -4.003612    1.763273               -7.529494   -.4777305
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =  -2.2706
Ho: diff = 0                                     degrees of freedom =       61

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0134         Pr(|T| > |t|) = 0.0267          Pr(T > t) = 0.9866

. ttest observed_otus_jw if worker==1, by(mdrsanew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |      45    116.7111    12.69238    85.14309    91.13129    142.2909
       1 |      18    194.2222    35.58104    150.9576    119.1528    269.2916
---------+--------------------------------------------------------------------
combined |      63    138.8571    14.16398    112.4231    110.5437    167.1705
---------+--------------------------------------------------------------------
    diff |           -77.51111     30.0109               -137.5216   -17.5006
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =  -2.5828
Ho: diff = 0                                     degrees of freedom =       61

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0061         Pr(|T| > |t|) = 0.0122          Pr(T > t) = 0.9939
```

On average over time, there are significant differences in phylogenetic distance and observed OTUs given the nasal carriage of MDRSA in IHO workers.

<u>Research question: Are there significant differences in alpha diversity of IHO worker *scn*-negative *S. aureus* carriers vs. not, on average, over time?</u> [performed via Student's t-test]

```
. ttest shannon_jw if worker==1, by(scn_negnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |      39    5.551358     .148545    .9276631    5.250644    5.852071
       1 |      24    5.810153    .2633647    1.290218    5.265341    6.354964
---------+--------------------------------------------------------------------
combined |      63    5.649946    .1357533    1.077509    5.378579    5.921313
---------+--------------------------------------------------------------------
    diff |           -.2587952    .2798732               -.8184363     .300846
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =  -0.9247
Ho: diff = 0                                     degrees of freedom =       61

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.1794         Pr(|T| > |t|) = 0.3588          Pr(T > t) = 0.8206

. ttest pd_whole_tree_jw if worker==1, by(scn_negnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |      39    12.09489    1.002573    6.261069    10.06528    14.12449
       1 |      24    14.78092    1.377685    6.749249    11.93096    17.63088
---------+--------------------------------------------------------------------
combined |      63    13.11814    .8228266    6.530984    11.47333    14.76294
---------+--------------------------------------------------------------------
    diff |           -2.686036    1.673235               -6.031876    .6598033
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =  -1.6053
Ho: diff = 0                                     degrees of freedom =       61

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0568         Pr(|T| > |t|) = 0.1136          Pr(T > t) = 0.9432

. ttest observed_otus_jw if worker==1, by(scn_negnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       0 |      39    119.3333    16.67367     104.127    85.57925    153.0874
       1 |      24    170.5833    24.54587    120.2497    119.8063    221.3603
---------+--------------------------------------------------------------------
combined |      63    138.8571    14.16398    112.4231    110.5437    167.1705
---------+--------------------------------------------------------------------
    diff |             -51.25    28.66334               -108.5659    6.065886
------------------------------------------------------------------------------
    diff = mean(0) - mean(1)                                      t =  -1.7880
Ho: diff = 0                                     degrees of freedom =       61

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0394         Pr(|T| > |t|) = 0.0787          Pr(T > t) = 0.9606
```

On average over time, there are no significant differences in alpha diversity observed for IHO children by *scn*- negative *S. aureus* nasal carriage status.

<u>Research question: What is the variability in beta diversity from the previous timepoint in IHO children in *S. aureus* nasal carriage vs. not?</u> [performed via Student's t-test]

```
. ttest morisita_horn_new3 if worker==1 & timepointnew!=0 & saureusnew==1, by(timepointnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |      13     .909281    .0262361    .0945956    .8521175    .9664446
       2 |      11    .8720859    .0752446     .249558    .7044305    1.039741
---------+--------------------------------------------------------------------
combined |      24    .8922333    .0365747    .1791787    .8165727    .9678938
---------+--------------------------------------------------------------------
    diff |            .0371951    .0746344               -.1175871    .1919774
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =   0.4984
Ho: diff = 0                     degrees of freedom =       22

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.6884         Pr(|T| > |t|) = 0.6232          Pr(T > t) = 0.3116

. ttest morisita_horn_new3 if worker==1 & timepointnew!=0 & saureusnew==0, by(timepointnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |       8    .9838054    .0056403    .0159532    .9704682    .9971427
       2 |      10    .9663596    .0094916    .0300151    .9448881    .9878311
---------+--------------------------------------------------------------------
combined |      18    .9741133    .0060613    .0257161     .961325    .9869016
---------+--------------------------------------------------------------------
    diff |            .0174458    .0117929               -.0075541    .0424458
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =   1.4793
Ho: diff = 0                     degrees of freedom =       16

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.9208         Pr(|T| > |t|) = 0.1585          Pr(T > t) = 0.0792
```

There are no significant differences in bacterial community membership and composition (beta diversity) in IHO children in *S. aureus* carriers. There are no significant differences in bacterial community membership and composition (beta diversity) in IHO children in *S. aureus* non-carriers.

<u>Research question: What is the variability in beta diversity from the previous timepoint in IHO children in *S. aureus* nasal carriage vs. not?</u> [performed via Student's t-test]

```
. ttest morisita_horn_new3 if worker==0 & timepointnew!=0 & saureusnew==1, by(timepointnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |      10    .9453623    .0228135    .0721425    .8937547      .99697
       2 |       8    .9493114    .0163218     .046165    .9107166    .9879063
---------+--------------------------------------------------------------------
combined |      18    .9471175    .0142146    .0603074    .9171273    .9771076
---------+--------------------------------------------------------------------
    diff |           -.0039491    .0294702               -.0664231    .0585248
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =  -0.1340
Ho: diff = 0                     degrees of freedom =       16

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.4475         Pr(|T| > |t|) = 0.8951          Pr(T > t) = 0.5525

. ttest morisita_horn_new3 if worker==0 & timepointnew!=0 & saureusnew==0, by(timepointnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |       9    .9476642    .0164339    .0493018    .9097675    .9855609
       2 |      11    .8798539    .0467023     .154894    .7757948    .9839131
---------+--------------------------------------------------------------------
combined |      20    .9103686    .0272478    .1218558    .8533383    .9673988
---------+--------------------------------------------------------------------
    diff |            .0678102    .0539533               -.0455415     .181162
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =   1.2568
Ho: diff = 0                     degrees of freedom =       18

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.8876         Pr(|T| > |t|) = 0.2249          Pr(T > t) = 0.1124
```

There are no significant differences in bacterial community membership and composition (beta diversity) in IHO workers in *S. aureus* carriers. There are no significant differences in bacterial community membership and composition (beta diversity) in IHO workers in *S. aureus* non-carriers.

<u>Research question: What is the variability in beta diversity from the previous timepoint in IHO workers in MDRSA nasal carriage vs. not?</u> [performed via Student's t-test]

```
. ttest morisita_horn_new3 if worker==1 & timepointnew!=0 & mdrsanew==1, by(timepointnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |       7    .9143545    .0405147    .1071917    .8152187     1.01349
       2 |       4    .7603012    .2078821    .4157642    .0987275    1.421875
---------+--------------------------------------------------------------------
combined |      11    .8583351    .0767481    .2545447    .6873297    1.029341
---------+--------------------------------------------------------------------
    diff |            .1540534    .1601428               -.2082148    .5163215
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =   0.9620
Ho: diff = 0                     degrees of freedom =        9

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.8194         Pr(|T| > |t|) = 0.3612          Pr(T > t) = 0.1806

. ttest morisita_horn_new3 if worker==1 & timepointnew!=0 & mdrsanew==0, by(timepointnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |      14    .9493297    .0184596    .0690693    .9094502    .9892091
       2 |      17    .9538433    .0117542    .0484639    .9289254    .9787611
---------+--------------------------------------------------------------------
combined |      31    .9518049    .0103567    .0576639    .9306536    .9729562
---------+--------------------------------------------------------------------
    diff |           -.0045136    .0211503               -.0477709    .0387437
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =  -0.2134
Ho: diff = 0                     degrees of freedom =       29

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.4163         Pr(|T| > |t|) = 0.8325          Pr(T > t) = 0.5837
```

There are no significant differences in bacterial community membership and composition (beta diversity) in IHO workers in MDRSA carriers. There are no significant differences in bacterial community membership and composition (beta diversity) in IHO workers in MDRSA non-carriers.

<u>Research question: What is the variability in beta diversity from the previous timepoint in IHO children in MDRSA *S. aureus* non-carriers; IHO children did not carry MDRSA?</u> [performed via Student's t-test]

```
. ttest morisita_horn_new3 if worker==0 & timepointnew!=0 & mdrsanew==0, by(timepointnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |      18    .9461215    .0147167    .0624376     .915072     .977171
       2 |      19    .9090992    .0284689    .1240932    .8492882    .9689102
---------+--------------------------------------------------------------------
combined |      37    .9271101    .0163512    .0994607    .8939482    .9602719
---------+--------------------------------------------------------------------
    diff |            .0370223     .032583               -.0291246    .1031693
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =   1.1362
Ho: diff = 0                                     degrees of freedom =       35

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.8682         Pr(|T| > |t|) = 0.2636          Pr(T > t) = 0.1318

. ttest morisita_horn_new3 if worker==0 & timepointnew!=0 & mdrsanew==1, by(timepointnew)
1 group found, 2 required
r(420);
```

There are no significant differences in bacterial community membership and composition (beta diversity) in IHO children in MDRSA carriers.

<u>Research question: What is the variability in beta diversity from the previous timepoint in IHO workers in *scn*-negative *S. aureus* nasal carriage vs. not?</u>
[performed via Student's t-test]

```
. ttest morisita_horn_new3 if worker==1 & timepointnew!=0 & scn_negnew==1, by(timepointnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |      10    .9002809    .0332568    .1051673    .8250488     .975513
       2 |       8    .8456891    .1034315    .2925485    .6011124    1.090266
---------+--------------------------------------------------------------------
combined |      18    .8760179    .0482328    .2046345    .7742556    .9777802
---------+--------------------------------------------------------------------
    diff |            .0545918    .0991188               -.1555307    .2647143
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =   0.5508
Ho: diff = 0                                     degrees of freedom =       16

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.7053         Pr(|T| > |t|) = 0.5894          Pr(T > t) = 0.2947

. ttest morisita_horn_new3 if worker==1 & timepointnew!=0 & scn_negnew==0, by(timepointnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |      11    .9716626    .0097399    .0323034    .9499608    .9933643
       2 |      13    .9608483    .0090174    .0325126    .9412011    .9804955
---------+--------------------------------------------------------------------
combined |      24    .9658048    .0065686    .0321794    .9522167     .979393
---------+--------------------------------------------------------------------
    diff |            .0108142    .0132807               -.0167282    .0383567
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =   0.8143
Ho: diff = 0                                     degrees of freedom =       22

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.7879         Pr(|T| > |t|) = 0.4242          Pr(T > t) = 0.2121
```

There are no significant differences in bacterial community membership and composition (beta diversity) in IHO workers in *scn*-negative carriers. There are no significant differences in bacterial community membership and composition (beta diversity) in IHO workers in *scn*-negative non-carriers.

<u>Research question: What is the variability in beta diversity from the previous timepoint in IHO children in *scn*-negative *S. aureus* nasal carriage vs. not?</u>
[performed via Student's t-test]

```
. ttest morisita_horn_new3 if worker==0 & timepointnew!=0 & scn_negnew==0, by(timepointnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |      18    .9533887    .0127646    .0541556    .9264578    .9803197
       2 |      18    .9041643    .0296414    .1257577    .8416264    .9667021
---------+--------------------------------------------------------------------
combined |      36    .9287765    .0164394    .0986365    .8954027    .9621503
---------+--------------------------------------------------------------------
    diff |            .0492245     .032273               -.0163621    .1148111
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =   1.5253
Ho: diff = 0                                     degrees of freedom =       34

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.9318         Pr(|T| > |t|) = 0.1364          Pr(T > t) = 0.0682

. ttest morisita_horn_new3 if worker==0 & timepointnew!=0 & scn_negnew==1, by(timepointnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |       1    .8216035           .           .           .           .
       2 |       1    .9979284           .           .           .           .
---------+--------------------------------------------------------------------
combined |       2     .909766           .           .           .           .
---------+--------------------------------------------------------------------
    diff |           -.1763248           .                       .           .
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =        .
Ho: diff = 0                                     degrees of freedom =        0

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) =      .          Pr(|T| > |t|) =      .          Pr(T > t) =      .
```

There are no significant differences in bacterial community membership and composition (beta diversity) in IHO children in *scn*-negative carriers.

<u>Research question: What is the variability in beta diversity from the previous timepoint in IHO workers in with IHO pig bacterial contributions to the nasal microbiome vs. not?</u> [performed via Student's t-test]

```
. ttest morisita_horn_new3 if worker==1 & timepointnew!=0 & pig_contribution_binary==1, by(timepointnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |      13    .9213712    .0276026    .0995226    .8612303    .9815121
       2 |      13    .8900514    .0643305    .2319467    .7498874    1.030215
---------+--------------------------------------------------------------------
combined |      26    .9057113    .0344367    .1755932    .8347876    .9766349
---------+--------------------------------------------------------------------
    diff |            .0313198    .0700022               -.1131577    .1757973
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =   0.4474
Ho: diff = 0                     degrees of freedom =       24

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.6707         Pr(|T| > |t|) = 0.6586          Pr(T > t) = 0.3293

. ttest morisita_horn_new3 if worker==1 & timepointnew!=0 & pig_contribution_binary==0, by(timepointnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |       8     .964159    .0124947    .0353404    .9346137    .9937043
       2 |       8    .9607341    .0112759    .0318931    .9340708    .9873974
---------+--------------------------------------------------------------------
combined |      16    .9624465    .0081419    .0325676    .9450925    .9798006
---------+--------------------------------------------------------------------
    diff |            .0034249    .0168305               -.0326728    .0395227
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =   0.2035
Ho: diff = 0                     degrees of freedom =       14

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.5792         Pr(|T| > |t|) = 0.8417          Pr(T > t) = 0.4208
```

There are no significant differences in bacterial community membership and composition (beta diversity) in IHO workers given bacterial contributions from the IHO pig. There are no significant differences in bacterial community membership and composition (beta diversity) in IHO workers with no bacterial contributions from the IHO pig.

<u>Research question: What is the variability in beta diversity from the previous timepoint in IHO children in with IHO pig bacterial contributions to the nasal microbiome vs. not?</u> [performed via Student's t-test]

```
. ttest morisita_horn_new3 if worker==0 & timepointnew!=0 & pig_contribution_binary==1, by(timepointnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |      10    .9204767    .0235745     .074549    .8671476    .9738059
       2 |      10    .8536308    .0475076    .1502323    .7461611    .9611005
---------+--------------------------------------------------------------------
combined |      20    .8870538    .0269252    .1204132    .8306987    .9434089
---------+--------------------------------------------------------------------
    diff |            .0668459    .0530352               -.0445768    .1782687
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =   1.2604
Ho: diff = 0                                     degrees of freedom =       18

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.8882         Pr(|T| > |t|) = 0.2236          Pr(T > t) = 0.1118

. ttest morisita_horn_new3 if worker==0 & timepointnew!=0 & pig_contribution_binary==0, by(timepointnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |       9    .9753148    .0053341    .0160024    .9630143    .9876154
       2 |       9    .9707307    .0112442    .0337326    .9448015    .9966599
---------+--------------------------------------------------------------------
combined |      18    .9730228    .0060624    .0257206    .9602322    .9858133
---------+--------------------------------------------------------------------
    diff |            .0045841    .0124453               -.0217987    .0309669
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =   0.3683
Ho: diff = 0                                     degrees of freedom =       16

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.6413         Pr(|T| > |t|) = 0.7174          Pr(T > t) = 0.3587
```

There are no significant differences in bacterial community membership and composition (beta diversity) in IHO children given bacterial contributions from the IHO pig. There are no significant differences in bacterial community membership and composition (beta diversity) in IHO children with no bacterial contributions from the IHO pig.

<u>Research question: What is the variability in beta diversity from the previous timepoint in IHO workers given the nasal carriage (carrier vs. not) of Pig-2-Bac, a known pig fecal marker?</u>
[performed via Student's t-test]

```
. ttest morisita_horn_new3 if worker==1 & timepointnew!=0 & p2bswabnew_binary==1, by(timepointnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |       9    .9238117    .0330015    .0990046      .84771    .9999134
       2 |       6    .8327889    .1393297    .3412866    .4746307    1.190947
---------+--------------------------------------------------------------------
combined |      15    .8874026    .0573471    .2221043    .7644053      1.0104
---------+--------------------------------------------------------------------
    diff |            .0910228     .118826               -.1656851    .3477307
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =   0.7660
Ho: diff = 0                                     degrees of freedom =       13

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.7713         Pr(|T| > |t|) = 0.4573          Pr(T > t) = 0.2287

. ttest morisita_horn_new3 if worker==1 & timepointnew!=0 & p2bswabnew_binary==0, by(timepointnew)

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |      12     .948066    .0204542    .0708556    .9030465    .9930855
       2 |      15    .9506538     .013008    .0503798    .9227544    .9785532
---------+--------------------------------------------------------------------
combined |      27    .9495037    .0113732    .0590971    .9261256    .9728817
---------+--------------------------------------------------------------------
    diff |           -.0025878    .0233357               -.0506487     .045473
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =  -0.1109
Ho: diff = 0                                     degrees of freedom =       25

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.4563         Pr(|T| > |t|) = 0.9126          Pr(T > t) = 0.5437
```

There are no significant differences in bacterial community membership and composition (beta diversity) in IHO workers given the nasal carriage of Pig-2-Bac, a pig fecal marker. There are no significant differences in bacterial community membership and composition (beta diversity) in IHO workers no nasal carriage of Pig-2-Bac, a pig fecal marker.

Research question: Are there significant differences in alpha diversity measures (Shannon diversity, phylogenetic distance and observed OTUs) over time between IHO workers and IHO children? [performed via Student's t-test]

Changes in alpha diveristy with time in IHO workers and children , North Carolina: 2013-2015.

	Timepoint 0		Timepoint 1		Timepoint 2	
	Mean, 95% CI		Mean, 95% CI		Mean, 95% CI	
	IHO Worker	IHO Child	IHO Worker	IHO Child	IHO Worker	IHO Child
Shannon diversity	5.64 (5.20, 6.07)	5.66 (5.45, 5.87)	5.89 (5.44, 6.34)	5.55 (5.06, 6.03)	5.42 (4.85, 6.00)	5.35 (4.85, 5.84)
Phylogenetic distance	13.0 (9.78, 16.3)	9.87 (8.88, 10.9)	14.3 (11.0, 17.6)	11.4 (9.61, 13.1)	12.0 (9.67, 14.4)	10.1 (9.06, 11.1)
Observed OTUs	138 (79.9, 196)	83.4 (71.1, 95.7)	159 (105, 213)	104 (79.0, 129)	120 (78.8, 160)	88.7 (74.2, 103)

S. aureus, MDRSA (multi-drug resistant *S. aureus*), *scn*-negative *S. aureus.*

Each participant contributed a nasal swab for each timepoint (0,1,2).

Student t-test was performed to determine alpha diversity measure and standard error.

Are there significant differences in alpha diversity measures (Shannon diversity, phylogenetic distance and observed OTUs) over time by *S. aureus* nasal carriage status in IHO workers? In IHO children? [performed via Student's t-test]

Changes in alpha diversity over time by *S. aureus* nasal carriage outcomes in 21 Industrial hog operation (IHO) workers and their child household member, North Carolina: 2013-2015.

IHO workers						
	Timepoint 0		**Timepoint 1**		**Timepoint 2**	
	Mean, 95% CI		**Mean, 95% CI**		**Mean, 95% CI**	
S. aureus	**Carriers**	**Non-carriers**	**Carriers**	**Non-carriers**	**Carriers**	**Non-carriers**
Shannon diversity	5.68 (5.02,6.33)	5.59 (4.88,6.29)	6.08 (5.65, 6.52)	5.57 (4.48, 6.66)	5.52 (4.49, 6.54)	5.32 (4.63, 6.01)
Phylogenetic distance	14.2 (9.09, 19.36)	11.47 (7.02,15.92)	14.4 (10.4, 18.4)	14.1 (6.98, 21.3)	13.4 (8.91, 17.9)	10.47 (9.06, 11.89)
Observed OTUs	162 (65.4, 258)	107 (40.9,173)	164 (164, 234)	151 (40.1, 261)	145 (66.1, 224)	91.4 (71.7, 111)
MDRSA	**Carriers**	**Non-carriers**	**Carriers**	**Non-carriers**	**Carriers**	**Non-carriers**
Shannon diversity	5.86 (4.61, 7.11)	5.53 (5.11,5.94)	5.96 (5.43, 6.50)	5.85 (5.18, 6.52)	4.65 (1.09, 8.22)	5.60 (5.12, 6.08)
Phylogenetic distance	17.4 (8.48, 26.22)	10.89 (8.21,13.57)	15.8 (8.11, 23.4)	13.6 (9.65, 17.5)	14.0 (0.65, 27.3)	11.6 (9.33, 13.8)
Observed OTUs	222 (56.64, 387)	96.29 (56.48,136.09)	187 (51.9, 322)	145 (84.0, 206)	159 (-62.6, 381)	110 (70.8, 150)
scn*-negative *S. aureus	**Carriers**	**Non-carriers**	**Carriers**	**Non-carriers**	**Carriers**	**Non-carriers**
Shannon diversity	5.74 (4.28, 7.19)	5.60 (5.16, 6.03)	6.09 (5.61,6.57)	5.71 (4.89, 6.52)	5.51 (4.00, 7.03)	5.37 (4.85, 5.88)
Phylogenetic distance	14.4 (8.06, 20.8)	12.5 (8.24, 16.8)	15.1 (9.91, 20.2)	13.6 (8.60, 18.6)	14.7 (8.42, 21.0)	10.4 (9.23, 11.5)
Observed OTUs	168 (47.54, 288)	126 (51.7, 201)	175 (85.1, 265)	144 (66.7, 222)	167 (55.7, 278)	90.5 (75.1, 106)
IHO children						
S. aureus	**Carriers**	**Non-carriers**	**Carriers**	**Non-carriers**	**Carriers**	**Non-carriers**
Shannon diversity	5.76 (5.43, 6.08)	5.54 (5.24, 5.85)	5.41 (4.54, 6.28)	8.98 (5.12, 6.29)	5.07 (4.14, 6.00)	5.59 (5.03, 6.16)
Phylogenetic distance	10.7 (10.7, 12.3)	5.76 (7.94, 10.0)	11.5 (8.81, 14.1)	11.2 (8.35, 14.1)	8.78 (7.89, 9.67)	11.3 (9.68, 12.8)
Observed OTUs	91 (68.8, 112)	76.0 (62.8, 88.2)	106 (70.6, 140)	102 (57.8, 146)	73.3 (65.1, 81.5)	103 (77.1, 128)

S. aureus, MDRSA (multi-drug resistant *S. aureus*), *scn*-negative *S. aureus.*

Each participant contributed a nasal swab for each timepoint (0, 1, and 2).

Student t-test was performed to determine alpha diversity measure and 95% confidence interval.

Research question: Does an IHO worker's IHO exposure score correlate with differences in alpha diversity, IHO pig SourceTracker contributions and beta diversity (morisita-horn index)? [performed via Student's t-test]

Generatlized linear model of IHO worker's exposure scores to the IHO facility in relation to alpha diversity, IHO pig SourceTracker contributions and beta diversity in workers and chidlren, 2013-2014, North Carolina, USA.

	IHO worker					IHO child				
	Shannon diversity index	Phylogenetic distance	Observed OTUs	Pig contributions (%)[a]	Morisita-horn index	Shannon diversity index	Phylogenetic distance	Observed OTUs	Pig contributions (%)	Morisita-horn index
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	OR (95% CI)
Work activity scores										
Dustiness exposure score										
0	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
1	-0.09 (-0.85, 0.67)	0.38 (-3.67, 4.43)	9.1 (-62.7, 80.9)	-5.1 (-23.2, 13.0)	-0.03 (-0.12, 0.06)	-0.46 (-1.97, 1.05)	-1.1 (-4.17, 2.01)	-9.28 (-35.1, 16.6)	0.04 (-0.03, 0.12)	0.04 (-0.01, 0.09)
2	-0.04 (-0.74, 0.67)	-2.06 (-5.84, 1.71)	-38.7 (-102, 24.6)	-10.3 (-23.9, 3.20)	0.01 (-0.03, 0.04)	-0.01 (-0.66, 0.65)	**-1.4 (-2.37, -0.38)**	-7.51 (-17.0, 1.97)	0.88 (-0.16, 1.92)	-0.25 (-0.53, 0.04)
Cleaning activity score										
0	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
1	0.09 (-0.64, 0.81)	1.99 (-0.41, 4.39)	29.9 (-10.0, 69.8)	2.92 (-3.58, 9.42)	0.02 (-0.01, 0.05)	--	--	--	--	--
2	0.14 (-0.59, 0.87)	2.61 (-0.29, 5.50)	45.6 (-3.6, 94.8)	**14.4 (3.24, 25.6)**	-0.04 (-0.10, 0.03)	-0.53 (-1.29, 0.23)	-0.9 (-2.89, 1.00)	-7.21 (-22.4, 7.95)	-0.78 (-1.80, 0.24)	0.00 (-0.16, 0.17)
Pig contact score										
0	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
1	-0.24 (-0.86, 0.39)	-0.32 (-3.25, 2.61)	-3.3 (-52.7, 46.1)	6.56 (-4.98, 18.1)	0.01 (-0.02, 0.04)	--	--	--	--	--
2	0.23 (-0.33, 0.80)	2.04 (-2.11, 6.19)	38.3 (-33.1, 110)	9.55 (-7.63, 26.7)	-0.05 (-0.14, 0.05)	**0.87 (0.02, 1.73)**	1.1 (-0.86, 3.08)	13.3 (-1.15, 27.8)	0.43 (-0.25, 1.12)	-0.13 (-0.28, 0.01)
Above exposure acitivities score										
0	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
1	0.42 (-0.28, 1.12)	1.12 (-3.20, 5.44)	14.1 (-63.2, 91.4)	5.56 (-6.14, 17.3)	0.01 (-0.03, 0.06)	--	--	--	--	--
2	0.35 (-0.28, 0.98)	-0.41 (-5.45, 4.63)	-15.5 (-105, 74.4)	3.43 (-13.8, 20.7)	-0.02 (-0.12, 0.08)	-0.63 (-1.62, 0.36)	**-2.26 (-3.62, -0.89)**	**-16.0 (-29.5, -2.40)**	0.52 (-0.37, 1.42)	-0.13 (-0.35, 0.08)
PPE use score										
0	Ref	Ref	Ref	Ref	Ref	Ref	**Ref**	**Ref**	Ref	**Ref**
1	-0.15 (-0.77, 0.46)	**-1.05 (-3.70, 1.60)**	-9.96 (-57.5, 37.6)	0.86 (-0.78, 2.50)	-0.02 (-0.04, 0.00)	-0.84 (-1.81, 0.12)	-1.72 (-2.50, -0.93)	**-13.0 (-24.4, -1.62)**	**-1.46 (-1.47, -1.44)**	-0.01 (-0.38, 0.36)
2	0.12 (-0.52, 0.75)	**2.93 (-0.33, 6.20)**	**58.16 (0.4, 115.9)**	**14.3 (4.41, 24.2)**	-0.05 (-0.10, 0.00)	0.10 (-0.43, 0.63)	1.18 (0.54, 1.82)	**7.83 (0.29, 15.4)**	**-1.44 (-1.48, -1.39)**	**0.13 (0.09, 0.17)**

[a]Extreme temperature, extreme malodor, extreme dust, vents off and/or a new herd entering the barns

[b]Used cleaning chemicals and/or pesticides, pressure washed and/or used a torch

[c]Gave pigs shots and/or medicine

[d]Summation of binary (yes/no) of a,b, and c.

[d]Mask, glasses or bodysuit/coveralls

IHO facility exposure scores: IHO facility dustiness score, cleaning activities score, pig contact score, above three exposure activities (dustiness, cleaning and pig contact) score and PPE use score. The IHO facility dustiness score was defined as the sum of the scaled variables including: extreme temperature, extreme malodor, extreme dust, vents off and/or a new herd entering the barns. The cleaning activities score was defined as the sum of binary variable including: used cleaning chemicals and/or pesticides, pressure washed and/or used a torch at the IHO facility. The pig contact score was defined as the sum of binary variable including: gave pigs shots and/or medicine. The above three exposure score was the sum of dustiness, cleaning and pig contact scores. The IHO worker's Personal Protective equipment (PPE) score was defined as the sum of binary variables including: the use of coveralls, glasses and

masks while at work in the IHO facility. All scores were then categorized into 3 bins informed by the structure of the data. These scores offer an interesting perspective on the measurement of frequency and magnitude of exposure to the IHO facilitie

Research question: Does the status of the IHO household members nasal carriage significantly influence differences in the nasal microbiome of the household IHO child over time? [performed via fixed effects model]

Table 5:The influence of an IHO worker's nasal carriage on IHO pig SourceTracker contributions and alpha diversity measures of household IHO children over time. 22013-2014, North Carolina: USA.

	IHO child				
	Shannon diversity index	**Phylogenetic distance**	**Observed OTUs**	**Pig contributions (%)**	**Morisita-Horn index**
IHO worker household member imputed meaures to household IHO children	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
***S. aureus* nasal carriage outcomes**					
S. aureus (Yes/No)	-0.89 (-2.17, 0.39)	-3.22 (-6.30, -0.14)	-36.0 (-75.1, 3.1)	5.03 (1.62, 8.44)	0.00 (-0.79, 0.78)
MDRSA (Yes/No)	-0.10 (-2.02, 1.83)	-2.30 (-7.13, 2.53)	-14.8 (-75.8, 46.3)	-1.06 (-7.16, 5.03)	-0.32 (-1.00, 0.35)
scn negative (Yes/No)	**-1.15 (-2.61, 0.30)**	-2.01 (-5.93, 1.92)	-15.8 (-65.4, 33.7)	0.00 (-5.00, 5.00)	**0.57 (-0.02, 1.15)**
Livestock-associated nasal carriage markers					
Tetracycline resistance (Yes/No)	0.16 (-1.76, 2.09)	0.24 (-4.66, 5.13)	1.55 (-44.2, 47.3)	-0.01 (-0.79, 0.77)	-0.23 (-0.80, 0.33)
S. aureus CC398 qPCR (Presence/Absence)	--	--	--	--	--
Pig-2-bac (Yes/No)	1.42 (-0.36, 3.19)	0.68 (-4.29, 5.64)	12.5 (-48.7, 73.7)	0.02 (-6.10, 6.14)	-0.26 (-0.70, 0.19)
Total percent pig contributions (Yes/No)	-0.02 (-0.19, 0.15)	-0.28 (-0.68, 0.13)	-3.31 (-8.35, 1.72)	--	0.00 (-0.01, 0.01)

[a]0: always (80% or greater), 1: Sometimes (10-79%); 2: Never (less than 10%)

[b]Pig contributions (%) defined as the percentage of OTUs contributed to the worker from the pig's total microbiome (nares and perineum) estimated via SourceTracker tool in QIIME. SourceTracker is designed to predict the source (IHO pig) of microbial communities in a set of sink samples (IHO workers and IHO children)

Research question: Does ever carriage and the maximum average carriage over time of *S. aureus,* livestock-associated *S. aureus,* and IHO pig contributions as well as categorical *S. aureus* nasal carriage (non-carrier, intermittent and persistent carriers) of the IHO household members nasal carriage significantly influence differences in the nasal microbiome of the household IHO child over time? [performed via fixed effects model]

Table 6: Relation of IHO occupational and personal activities with pig contributions to and alpha diversity measures of the worker's nasal microbiome over time . North Carolina: 2013-2014.

	IHO worker					IHO child				
	Shannon diversity index	Phylogenetic distance	Observed OTUs	Pig contributions (%)[a]	Morisita-Horn index	Shannon diversity index	Phylogenetic distance	Observed OTUs	Pig contributions (%)	Morisita-Horn index
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Ever *S. aureus* outcome carrier										
S. aureus (Yes/No)	0.25 (-0.31, 0.82)	1.60 (-1.46, 4.66)	32.1 (-18.6, 82.9)	4.22 (-8.21, 16.6)	-0.05 (-0.10, 0.00)	-0.07 (-0.55, 0.40)	-0.17 (-1.86, 1.53)	-0.48 (-23.4, 22.4)	**0.83 (0.08, 1.57)**	-0.05 (-0.11, 0.01)
MDRSA (Yes/No)	-0.02 (-0.55, 0.51)	2.62 (-0.98, 6.22)	48.7 (-14.5, 111.8)	13.4 (-1.44, 28.3)	-0.05 (-0.14, 0.03)	-0.01 (-0.43, 0.42)	0.01 (-1.58, 1.60)	-6.77 (-21.8, 8.2)	0.20 (-0.94, 1.34)	-0.02 (-0.14, 0.10)
scn-negative (Yes/No)	0.26 (-0.24, 0.75)	2.83 (-0.24, 5.90)	54.5 (1.61, 107.4)	9.21 (-4.15, 22.6)	-0.05 (-0.12, 0.01)	-0.17 (-1.37, 1.02)	1.38 (-0.91, 3.66)	13.9 (-23.0, 50.9)	1.84 (-1.40, 5.09)	0.01 (-0.09, 0.10)
Ever Livestock-associated *S. aureus* carrier										
Tetracycline resistance (Yes/No)	-0.40 (-0.99, 0.19)	0.46 (-2.60, 3.52)	9.74 (-46.1, 65.5)	8.38 (-1.96, 18.7)	-0.08 (-0.30, 0.14)	0.96 (-0.40, 1.29)	0.99 (-0.75, 2.73)	10.3 (-5.47, 26.0)	-1.93 (-4.82, 0.96)	-0.08 (-0.41, 0.26)
CC398 (Yes/No)	0.15 (-1.10, 1.40)	3.32 (-2.70, 9.33)	65.1 (-34.6, 165)	11.0 (-14.9, 36.9)	**0.29 (0.08, 0.50)**	0.17 (-0.39, 0.73)	-0.48 (-2.12, 1.17)	-5.65 (-28.4, 17.1)	-0.76 (-1.93, 0.40)	0.05 (-0.03, 0.13)
Pig-2-bac (Yes/No)	-0.20 (-0.98, 0.58)	3.07 (-1.72, 7.86)	55.5 (-24.0, 135)	**21.7 (4.68, 38.7)**	-0.07 (-0.19, 0.05)	0.15 (-0.52, 0.82)	-0.90 (-2.72, 0.92)	-10.9 (-36.4, 14.6)	-1.04 (-2.37, 0.29)	0.02 (-0.06, 0.09)
Maximum *S. aureus* outcome carriage over time/carrier index										
S. aureus	0.11 (-0.09, 0.30)	0.70 (-0.40, 1.81)	14.2 (-4.03, 32.5)	1.46 (-3.10, 6.02)	-0.02 (-0.04, 0.00)	-0.10 (-0.29, 0.10)	-0.41 (-0.98, 0.17)	-4.26 (-11.6, 3.13)	-0.01 (-0.33, 0.31)	-0.02 (-0.05, 0.02)
MDRSA	-0.03 (-0.22, 0.16)	0.80 (-0.77, 2.36)	14.8 (-12.62, 42.2)	4.31 (-2.63, 11.2)	-0.03 (-0.07, 0.01)	-0.01 (-0.43, 0.42)	0.01 (-1.58, 1.60)	-6.77 (-21.8, 8.24)	0.20 (-0.94, 1.34)	-0.02 (-0.14, 0.10)
scn-negative	0.10 (-0.09, 0.28)	1.01 (-0.32, 2.34)	19.3 (-3.62, 42.3)	3.09 (-2.80, 8.99)	-0.03 (-0.06, 0.00)	-0.17 (-1.37, 1.02)	1.38 (-0.91, 3.66)	13.9 (-23.0, 50.9)	-0.03 (-0.06, 5.09)	0.01 (-0.09, 0.10)
Maximum *S. aureus* outcome carriage over time/carrier index										
Tetracycline resistance	0.10 (-0.09, 0.30)	0.87 (-0.91, 2.65)	16.7 (-14.4, 47.8)	4.21 (-4.53, 12.9)	-0.03 (-0.08, 0.02)	**0.70 (0.44, 0.96)**	**2.89 (2.11, 3.66)**	**39.3 (28.8, 49.9)**	**4.13 (3.72, 4.54)**	**-0.06 (-0.10, -0.02)**
S. aureus CC398 qPCR (Presence/Absence)	0.04 (-0.46, 0.54)	2.22 (-1.04, 5.47)	36.7 (-20.0, 93.5)	9.17 (-5.05, 23.4)	-0.03 (-0.11, 0.04)	--	--	--	--	--
Pig-2-bac	-0.08 (-0.34, 0.19)	1.33 (-0.26, 2.92)	23.3 (-3.11, 49.8)	**8.06 (2.54, 13.6)**	-0.02 (-0.06, 0.02)	0.10 (-0.07, 0.27)	-0.47 (-0.98, 0.05)	**-7.86 (-15.3, -0.43)**	-0.35 (-0.75, 0.05)	0.03 (0.00, 0.05)
MaxLA-SA carraige	-0.01 (-0.35, 0.34)	**1.76 (0.22, 3.30)**	**33.9 (5.53, 62.2)**	--	-0.01 (-0.09, 0.08)	0.21 (-0.39, 0.80)	0.35 (-1.09, 1.79)	1.76 (-15.2, 18.7)	--	0.00 (-0.13, 0.14)
Ever pig contributions	-0.09 (-0.59, 0.41)	1.39 (-1.36, 4.13)	24.6 (-24.0, 73.2)	--	-0.04 (-0.09, 0.02)	**0.62 (0.17, 1.07)**	**1.54 (0.21, 2.87)**	**22.6 (5.1, 40.1)**	--	**-0.09 (-0.16, -0.03)**
Maximum pig contriution carriage (Yes/No)	-0.03 (-0.20, 0.14)	0.46 (-0.45, 1.38)	8.20 (-7.98, 24.4)	--	-0.01 (-0.03, 0.01)	**0.21 (0.06, 0.36)**	**0.51 (0.07, 0.96)**	**7.54 (1.7, 13.4)**	--	**-0.03 (-0.05, -0.01)**
Categorical *S. aureus* nasal carriage status (Reference	0.15 (-0.11, 0.42)	1.07 (-0.92, 3.07)	22.3 (-10.8, 55.5)	3.76 (-3.84, 11.4)	-0.04 (-0.08, 0.00)	-0.11 (-0.42, 0.20)	-0.68 (-1.62, 0.27)	-7.22 (-19.2, 4.8)	-0.09 (-0.45, 0.27)	-0.03 (-0.08, 0.03)
Intermittent carriers	-0.59 (-1.22, 0.04)	-1.35 (-4.43, 1.72)	-21.3 (-71.1, 28.4)	5.20 (-8.09, 18.5)	**0.01 (0.00, 0.02)**	0.12 (-0.45, 0.68)	0.33 (-1.73, 2.39)	4.45 (-25.0, 33.9)	**1.49 (0.33, 2.64)**	-0.03 (-0.09, 0.02)
Persistent carriers	0.22 (-0.27, 0.71)	1.88 (-2.01, 5.76)	39.8 (-23.9, 103.5)	7.68 (-7.01, 22.4)	**-0.07 (-0.14, -0.01)**	-0.19 (-0.78, 0.41)	-1.21 (-3.11, 0.68)	-12.8 (-37.1, 11.5)	0.04 (-0.01, 0.10)	-0.06 (-0.16, 0.05)

Ever carriage defined as a participant observed to carry the marker at any timepoint.

Maximum carriage defined as the number of timepoints at which a participant was observed to carry the marker.

Pig contributions (%) defined as the percentage of OTUs contributed to the worker from the pig's total microbiome (nares and perineum) estimated via SourceTracker tool in QIIME.

SourceTracker is designed to predict the source (IHO pig) of microbial communities in a set of sink samples (IHO workers and IHO children)

Categorical *S. aureus* nasal carriage status defined as non-carrier, intermittent (less than7 out of 9 timepoints in full study follow up) and persistent (7 or more timepoints in full study follow up carrying marker) *S. aureus* nasal carriers.

Non-carriers defined as the reference

Ever *S..aureus* nasal carriage outcomes *(*ever *S. aureus,* ever MDRSA, and ever *scn*-negative *S.* aureus), ever LA-SA nasal carriage outcomes (ever tetracycline resistant *S. aureus,* ever CC398, ever pig-2-bac, and ever any of the above three) and ever IHO pig SourceTracker contribution nasal carriage outcome variables were coded as 1 if a given participant carried the marker at any of the three timepoints and zero if never carried. Maximum *S. aureus* nasal carriage outcomes (max *S. aureus,* max MDRSA, and max scn-negative *S.* aureus), LA-SA nasal carriage outcomes (max tetracycline resistant *S. aureus,* max CC398, ever pig-2-bac, and max any of the above three LA-SA markers) and maximum IHO pig SourceTracker contribution nasal carriage outcome variables were coded as 0, 1 or 2 based on the maximum number of timepoints a given participant carried the marker.

S. *aureus* nasal carriage was categorized as non-carriers, intermittent and persistent carriers. Non-carriers were never observed to carry S. *aureus* at all timepoints. Intermittent carriers were observed to carry S. *aureus* in their nares seven out of 9 timepoints during the 4 months total follow-up period (this study focuses on 3 timepoints of these 9). Non-carrier served as the reference for intermittent and persistent S. aureus nasal carriers. Mask usage was categorized as always (reference; 80% or greater mask usage), sometimes (10-79% mask usage) or never (less than 10%) mask usage.

Chapter Seven: Conclusions

Summary of findings

There is epidemiological evidence, using a single pathogen approach, that exposure to IHOs heightens one's risk of *S. aureus* and LA-*S. aureus* nasal carriage in the context of IHO work exposures. However, there has been limited investigation of how IHO work exposures and practices can impact the broader commensal and potentially pathogenic bacterial communities of pigs and humans. This dissertation aimed to characterize and compare the nasal microbiome of pigs, pigs workers and community residents to enhance our understanding of the influence of hog production (with and without antimicrobial drug inputs and confinement) and *S. aureus* nasal carriage outcomes on the nasal microbiome. Secondly, we aimed to enhance our understanding of the influence of hog production and *S. aureus* nasal carriage over time on the temporal variability of microbiome characteristics (alpha diversity, beta diversity and bacterial OTUs contributed to the human nasal microbiome) of IHO workers and children living in their homes.

In Chapter 3, we presented the results of a cross-sectional analysis of IHO pigs, IHO workers, AFHO pigs and AFHO workers. Key microbiome differences were found by mode of production, as we found AFHO pigs and AFHO workers were more diverse than IHO pigs and IHO workers. The mode of pig production had implications on bacterial community structure with clear differences when comparing IHO workers to AFHO workers. Bacterial communities were more similar between AFHO pigs and AFHO workers compared to IHO pigs and IHO workers. We also found significant relationships between personal and occupational exposure activities and the presence of

S. aureus nasal carriage outcomes in relation to changes in alpha diversity and pig microbial contributions to pig workers.

In Chapter 4, data were analyzed from a cross-sectional analysis of IHO workers and IHO workers' children and community resident (CR) adults and CR children. IHO occupational exposure activities and *S. aureus* nasal carriage outcomes (*S. aureus*, MDRSA and *scn*–negative *S. aureus*) were associated with changes in bacterial diversity, community structure and composition, and the percent bacterial contributions from IHO pigs to the nasal microbiome of IHO workers. Our results suggest that IHO workers may be exposed to and colonized by different populations of microbes, including *S. aureus*, compared to individuals who do not have direct IHO occupational exposures (IHO workers' children, CR adults and CR children).

In Chapter 5, we presented the results of a longitudinal analysis of IHO workers and IHO workers' children. Our study found key differences in alpha diversity of the nasal microbiome by timepoint that was influenced by IHO work activities (hours worked per week, number of IHO pigs in direct contact with), MDRSA nasal carriage positivity, and LA-microbial nasal carriage exposure measures (*scn*-negative *S. aureus*, Pig-2-Bac, and percent bacterial contributions from IHO pigs). Accumulating IHO work exposures over time as well as increased time spent in direct contact with IHO pigs appeared to be associated with homogeneous microbial pressures on the nasal microbiomes and community structure of IHO workers and IHO workers' children – meaning the midpoint and endpoint were more similar to their respective adjacent and previous timepoint. OTUs contributed from IHO pigs to IHO workers and IHO workers' children correlated with OTUs exclusively carried by MDRSA and *scn*-negative *S.*

aureus nasal carriers. Increased homogeneity of the nasal microbiome over time among IHO workers and IHO workers' children may be due to pressures on the nasal microbiome created by the IHO environment as well as the OTUs derived from IHO pigs. More consistent and frequent use of a facemask by IHO workers and policy changes to minimize antimicrobial drug use at IHOs may mitigate the direct (IHO worker) and indirect (household child) exposure pressures to pig-associated microbes.

Future Research and Implications

Our research investigating the role of hog production on the nasal microbiome of pigs, pig workers and community residents is a testament to the success we have had in accessing IHO worker populations. Thankfully, due to collaborations with the Rural Empowerment Association for Community Help (REACH), we were able to conduct studies on a unique population of IHO workers, a majority of whom were Hispanic. These workers allowed us to gain insight into the real life experiences of IHO workers and individuals within their communities. Additionally, gaining access to pigs from IHOs and from AFHOs was a great accomplishment in enhancing our knowledge of the influence of IHOs on the pig microbiome and the transfer of bacterial operational taxonomic units (OTUs) to humans both direct and indirectly exposed to IHO pigs.

Due to financial constraints, we had to select samples rather than sequencing all samples. Sample selection may lead to selection biases and small sample size issues. The generalizability of the dissertation cohort is limited due to 89% Hispanic ethnic background and therefore there is a lack of external validity and generalizability to populations outside of IHO workers and possibly other livestock workers who are outside the Hispanic ethnicity. No other livestock-types (i.e. cattle, turkeys, chicken etc.) were

included in analysis. Inclusion of these samples would allow us to characterize reference livestock associated strains from these other livestock types on farms where we sampled pigs and workers. There was also a lack of a true non-exposed referent group within our study population. In the future, the use of a suburban and/or urban unexposed population is advisable to determine differences in the nasal microbiome compared to those directly exposed via their occupation, indirectly exposed via living in the household with IHO workers, or living in close proximity to IHOs.

We had limited longitudinal sample size for each participant by selecting three timepoints out of the 8 total follow-up visits. This dissertation highlights the need for larger sample sizes over tie in order to observe the persistent changes in the nasal microbiomes alpha and beta diversity over time. In future studies, we recommend a sample size of greater than three time points of adjacent follow-up visits to examine persistence of nasal microbiome changes and bacterial taxa carried, without gaps in time.

Our work has highlighted the limited microbiome research in this area and suggests a need for more to improve environmental exposure assessments. There is consistency in the results between two cross-sectional studies supporting that IHO workers' nasal microbiomes are more diverse than other livestock workers. This review of the literature and dissertation highlighted the need for high quality longitudinal epidemiologic studies with bioinformatics analyses of the nasal microbiome of large IHO study populations from various IHOs in the U.S. to enhance knowledge surrounding the generalizability of findings from these IHO workers to the remainder of workers in the U.S. and increase confidence in the results obtained by these studies. Additionally, there is a need for the development of a universal bioinformatics protocol (16s rRNA gene

region to sequence, DNA extraction, primer use, reference databases, quality control parameters and analysis pipelines) in order to have greater confidence in the consistency and repeatability of the results.

Final conclusions

Our study contributes to the literature building around the microbiome of pigs, pig workers and community residents. Our study also has begun to distinguish between the influences of hog production with and without antibiotic use as well as comparing those pig workers with antimicrobial drug exposures to those surrounding community residents in cross-sectional and longitudinal analyses. Additional studies with repeated measures over longer periods of time (more than three timepoints that we investigated here) will help advance the characterization of the nasal microbiome of pigs, pig workers and community residents and its temporal fluctuations.

Our ability to quantify the proportion of bacterial contributions from the IHO pig to the microbiome of IHO worker and the children living in their household as well as among community residents has suggested that the nasal microbiome may serve as an exposure assessment tool to determine the influence of hog production on the microbiome and subsequently human health (e.g., SSTIs, respiratory illnesses). With more research we can have a better understanding of the human health implications of hog production on those directly and indirectly exposed.

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CHAPTER FOUR

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CURRICULUM VITAE

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KEY QUALIFICATIONS

- Environmental Health Epidemiologist with 8 years of quantitative and qualitative research experience in topics including microbiology, bioinformatics, and ecology.
- Knowledge of waterborne-infectious diseases and public health.
- Excellent understanding of epidemiologic principles and surveillance methodologies.
- Strong oral and written communication skills; Written and awarded 5 competitive scholarships, 2 publications, 10 conferences presentations to fellow scientists, as well as the general public.
- Works well in teams to conduct field sample collection and participated in various epidemiologic projects.
- Excellent time management skills and detail-oriented; Met deadlines for grant cycle submissions, reports and manuscripts in timely manner within a self-motivated environment.

SPECIALIZED SKILLS AND TRAINING

- **Research skills:** Study design, implementation and monitoring; data collection and management; epidemiologic and bioinformatics data analysis.
- **Computer skills**: Advanced with STATA, R, QIIME, and Proficient in GIS, Microsoft Word, Excel, Powerpoint and Access.
- **Languages:** Proficient in French and Spanish.

EDUCATION

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
2013-Present

- **PhD in Environmental Health Sciences (degree conferral: August 2018)**
  - Focus within exposure assessment and environmental epidemiology
- **Certificates**
  - Risk Sciences and Public Policy
  - Global Health

University of Maryland, Baltimore County Baltimore MD
2009-2013

- **B.S. in Environmental Science**

PUBLICATIONS

- Davis M, Pisanic N, Rhodes S, *et al.* 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. *Environ Res* 2018: 88–96.

- Enns AA, Vogel LJ, Abdelzaher AM, et al. Spatial and temporal variation in indicator microbe sampling is influential in beach management decisions. Water Res 2012; 46: 2237–46.

PRESENTATIONS

Scientific meetings

Changing Microbiome for Health (CMH) symposium, December *2016.*
Lightning talk: *The role of antimicrobial use in hog production on the nasal microbiome of hogs, hog workers, and community residents. Alexis Brown[1] Christopher Heaney[1] Johns Hopkins Bloomberg School of Public Health.*

NCI/NIH Microbiome in Health and Disease conference, November 2015.
Poster Session *Industrial hog operations (IHO) and their influence on the nasal microbiome of IHO workers and their household contacts. Alexis Brown[1] Christopher Heaney[1] Johns Hopkins Bloomberg School of Public Health.*

Food and Drug Administration (FDA) Summer Research Program, August *2014.*
Poster session *Are Metal Exposure Standards Protecting Us**?** Alexis Brown[1] Ron Brown[2]. [1]Johns Hopkins School of Public Health; [2]U.S. Food and Drug Administration*

Johns Hopkins Seminars and Presentations

Microbiome forum series, December 2017.
The role of antimicrobial use in hog production on the nasal microbiome of hogs, hog workers, and community residents, Alexis Brown[1] Christopher Heaney[1] Johns Hopkins Bloomberg School of Public Health.

Department of Environmental Health and Engineering. 2016.
The role of antimicrobial use in hog production on the nasal microbiome of hogs, hog workers, and community residents.

Department of Environmental Health and Engineering. 2015.
Relation between nasal microbiome and Staphylococcus aureus nasal carriage status among Industrial hog operation workers and their household contacts.

Department of Environmental Health and Engineering. 2014.
Relation between nasal microbiome and Staphylococcus aureus nasal carriage status among Industrial hog operation workers and their household contacts.

RESEARCH EXPERIENCE

Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland 2014-2018
Research scientist

- Managed 2 datasets utilized for microbiome analysis (1 cross-sectional and 1 longitudinal epidemiologic research study) for 3 years.

- Self-trained in bioinformatics skills such as microbiome pipeline generation, data management and analysis.
- Determined the influence of concentrated animal feeding operations (CAFOs) and its use of high antimicrobial drugs on the nasal microbiome of hog and hog workers (specifically pathogenic microorganisms) in comparison to community residents, with no known livestock exposure.
- Ensured the submission of weekly dissertation and project progress reports as well as annual grant reports.
- Assisted oversight of field sampling protocol development for implementation of sample collection.
- Led the coordination, planning and training of 4 principal investigators in the collection of CAFO water, air and surface samples for a month period; samples utilized in legal proceedings to bolster legal team's evidence-based argument supporting the occurrence(s) of environmental injustices in areas surrounding CAFOs in North Carolina.
- Provided technical and administrative assistance in the collection of over 300 samples at 4 swine production operations in the North Carolina area for a pilot study.
    - Published a peer-reviewed manuscript surrounding this research.
    - Collaborated with over 10 principal investigators from 5 renowned research institutions.
- Trained and managed a summer intern in microbiology laboratory bench techniques and experiments.

Food and Drug Administration May-August 2014
Research scientist

- Co-developed a reverse dosimetry method to estimate metal exposure within patients with implanted metal medical devices using urinary metal absorption and excretion data from the national Health and Nutrition Examination Survey (NHANES).
- Contributed and reviewed manuscript(s) (in drafting) informed by research performed Summer 2014.

TEACHING EXPERIENCE

Johns Hopkins Bloomberg School of Public Health
Teaching assistantship(s)

- Fundamentals of Occupational Health
  2016-2017
- Introduction to Environmental and Occupational Health Law
  2014-2017
- Duties/Responsibilities:
    - Led constructive discussions amongst students surrounding course material.
    - Drafted and graded assignments and exams.
    - Maintained clear spreadsheets to track student's grades in class.

- Maintained the course website and online course materials.
- Addressed student's questions surrounding course materials.

SCHOLARSHIPS

- PhRMA Foundation pre-doctoral fellowship nominee 2015-2016
- Environmental Health Sciences Student Award/Diversity Scholarship 2013-2014
- Meyerhoff Scholar 2009-2013
- MARC U*STAR scholar 2011-2013
- Lilly Scholar 2011-2012
- Gamma Theta Upsilon Scholar 2011-2013