Fall 2016

The human nasal and oropharyngeal microbiomes and Staphylococcus aureus colonization

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THE HUMAN NASAL AND OROPHARYNGEAL MICROBIOMES AND *STAPHYLOCOCCUS AUREUS* COLONIZATION

by

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A thesis submitted in partial fulfillment
of the requirements for the Doctor of
Philosophy degree in Epidemiology
in the Graduate College of
The University of Iowa

December 2016

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CERTIFICATE OF APPROVAL

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PH.D. THESIS

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"Wit beyond measure is a man's greatest treasure."

J. K. Rowling
Harry Potter and the Order of the Phoenix

ACKNOWLEDGEMENTS

I would like to thank Dr. James Torner, Dr. Tara Smith, Dr. Kelli Ryckman, Dr. Patrick Breheny, and Dr. John Kirby for their support and advice while completing my Ph.D. thesis. I would also like to thank Dr. James Torner, Dr. Margaret Chorazy, and Dr. Tara Smith for their support and countless hours of mentorship provided throughout my doctoral work as well as Dr. Mark Dalman and Dipendra Thapaliya for all of their assistance. This project was funded by the Great Plains Center for Agriculture Health, a research center funded by National Institute of Occupational Safety and Health (NIOSH), grant number 5U54OH007548. Additional funds were provided by Dr. Tara Smith. Fellowship support was also provided by The University of Iowa Graduate College Post-Comprehensive Summer Research Fellowship Award. Lastly, I would like to thank my parents, siblings, extended family, and friends for their invaluable support and encouragement throughout my graduate education.

ABSTRACT

Staphylococcus aureus has been extensively studied, yet it remains unclear why certain individuals continually carry the bacteria while others do not. Livestock workers are known to be at an increased risk of *S. aureus* colonization, but have not been as studied as other high risk groups, including hospitalized patients, have been. Culture based studies have shown other bacteria may decrease the likelihood of *S. aureus* colonization. Here, we utilize 16s rRNA sequencing to better characterize the ecologic relationships between *S. aureus* and the other microbes in the nares and oropharynx in a population of livestock workers.

A cross-sectional, epidemiological study was conducted enrolling 59 participants (26 of which had livestock contact) in Iowa. Participants were enrolled in one of four ways: from an existing prospective cohort study (n=38), from the Iowa Department of Natural Resources Animal Feeding Operations database (n=17), through Iowa county fairs (n=3), and through snowball sampling (n=1). We collected two sets of swabs from the nares and oropharynx of each participant. The first set of swabs was used to assess the microbiome via 16s rRNA sequencing and the second was used to culture *S. aureus*.

We observed livestock workers to have greater diversity in their microbiomes compared to those with no livestock contact. In the nares, there were 26 operational taxonomic units found to be different between livestock workers and non-livestock workers with the greatest difference seen with *Streptococcus* and *Proteobacteria.* In the oropharynx, livestock workers with swine exposure were more likely to carry several pathogenic organisms. We also observed colonized livestock workers to be more likely to

carry *P. gingivalis* which may act as a bridge allowing *S. aureus* to adhere to *Streptococcus* in the oral cavity. While we observed no significant differences when comparing colonized persons to non-colonized persons in either the nares or oropharynx, *Corynebacterium* was more abundant in the colonized persons. Colonized individuals also had greater diversity in their nasal microbiome compared to non-colonized individuals. However, when comparing persistently colonized persons to intermittently colonized persons, we found *Corynebacterium argentorantense* to be more abundant in the persistently colonized individuals.

We hypothesized the genera present in the nares and oropharynx of *S. aureus* carriers would be different from that of non-carriers and there would be differences in the nasal and oropharyngeal microbiomes based on livestock contact and carrier state (persistent, intermittent, and non-carrier). While there were no significant differences between carriers and non-carriers, we were able to identify several operational taxonomic units that were different between livestock worker carrier and non-carriers as well as differences by carrier state. The results of this study are the first to characterize the livestock worker nasal and oropharyngeal microbiomes. Additionally, the results shed light onto several organisms that may be influential in *S. aureus* carriage. However, further studies are needed to better understand these relationships and determine causality.

PUBLIC ABSTRACT

Staphylococcus aureus is an important cause of infections in the United States and globally and is able to be carried in the nose and throat of healthy people increasing their risk of infection. Livestock workers are at an increased risk of carrying the bacteria. Here we have studied the microbiomes – all of the bacteria present on a body site – of the nose and throat of livestock workers in Iowa. We enrolled 59 participants, 26 of which had contact with livestock. We have identified several bacterial differences in the microbiomes of livestock workers compared to those without livestock contact as well as several bacterial differences in the nose and throat of people colonized with *S. aureus* compared to those not colonized with *S. aureus* including *Corynebacterium.* This study is the first we are aware of to characterize the microbiome of livestock workers which will help us begin to understand if and how the microbiome plays a role in disease. This study also adds to our understanding of what bacteria may impact whether a person carries *S. aureus* in their nose, throat, or not at all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

Staphylococcus aureus (*S. aureus*) is an important pathogen globally and is traditionally associated with hospital-, community-, and most recently, livestock-associated infections. *S. aureus* is one of the most frequent causes of bacterial infections and has traditionally been the leading cause of skin and soft tissue infections (SSTIs) [1], bacteremia [1], and infective endocarditis [1], but also causes a wide range of other infections from osteomyelitis [2] to pneumonia [3].

Based upon epidemiological studies, it has been estimated roughly one third of the general population carry *S. aureus* [4] and 1.5% - 3% carry methicillin-resistant *S. aureus* (MRSA) [5], though rates are higher in high risk groups, such as hospitalized patients [5, 6]. Individuals who carry any type of *S. aureus* are considered asymptomatic carriers and may act as a reservoir for the pathogen in the community and in healthcare settings. These carriers have been shown to be able to transmit the bacterium to others either through direct contact or through contact with fomites (objects which may become contaminated with the infectious organism and serve as a mode of transmission for the organism) [7]. While carriage itself is not necessarily harmful to the individual, it is a known risk factor for developing infections [8]. Over the past several decades, *S. aureus* infections have become increasingly difficult to treat with the rise in antibiotic resistance to multiple classes of drugs.

Antibiotic-resistant organisms, particularly MRSA, are responsible for a great deal of excess mortality and excess cost globally. Economic computational modeling has been used to estimate the costs associated with community-associated MRSA (CA-

MRSA) infections in the US estimating the cost per infection to be between $7,000 and $20,500 [9]. This is two to four times higher than the average influenza case ($3,000-$4,000) [10]. It has also been estimated that CA-MRSA infections result in over $2.7 billion in productivity loss in the US [9]. Patients with MRSA skin and soft tissue infections (SSIs) have been shown to have longer length of stays and higher hospital costs than those SSI patients infected with other organisms. In a study in the Northeastern US, it was estimated that patients with MRSA SSIs were hospitalized an average of one day longer and had hospitalization costs an average of $1000 more than those patients infected with other organisms (both antimicrobial resistant and susceptible organisms) [11]. In 2007, it was estimated MRSA caused an excess of roughly €44,000 to the European Union with methicillin-susceptible *S. aureus* (MSSA) causing an estimated €128,500. MRSA was also responsible for approximately 5,000 deaths due to blood stream infections as well as roughly 250 excess bed days with MSSA being responsible for 700 excess bed days in the EU [12].

Colonization and infection with *S. aureus* has been extensively studied [13-15], predominantly in the hospital [6, 16, 17] and community [18, 19] settings; however, it remains unclear why some individuals persistently carry the bacteria while others do not, and why some individuals suffer repeated infections with *S. aureus* despite rounds of antibiotic treatments. Previous studies have shown other bacteria present on the body may decrease the likelihood of carrying *S. aureus [20-23]*. However, previous studies have been primarily based on traditional culture methods which underestimate the complexity of the microbiome - the collection of all the organisms on a body site. It has

been estimated microbes outnumber human cells ten to one. These microbes play a crucial role in human health such as aiding in digestion, regulating the immune system, and keeping potentially pathogenic bacteria, such as *S. aureus*, at bay.

While the importance of the human microbiome has been recognized for many years, there is still very little known about it – especially in unique populations, such as livestock workers who are more likely to carry *S. aureus*. In order to understand what bacteria may be important in preventing *S. aureus* colonization and infection, it is necessary to characterize what bacteria are present in the niches *S. aureus* inhabits. In this study we aim to characterize the microbiome of the nose (anterior nares) and throat (oropharynx) of livestock workers, as they are an understudied population with increased risk of *S. aureus* colonization. We hypothesize the interaction – both competitive and cooperative - between *S. aureus* and the other organisms constituting the microbiota will influence carrier status.

Aim 1: To characterize the nasal and oropharyngeal microbiomes of livestock workers to the nasal and oropharyngeal microbiomes of persons with no livestock contact in Iowa.

Aim 2: To determine if there are differences between the microbiomes of those carrying *S. aureus* and those not carrying *S. aureus* in either or both the anterior nares and oropharynx.

Aim 2.1: To determine if there are microbiota differences in *S. aureus* carriers with livestock exposure compared to *S. aureus* carriers without livestock exposure and if there are microbiota differences in *S. aureus* non-carriers with

livestock exposure compared to *S. aureus* non-carriers without livestock exposure.

Aim 2.2: To determine if the diversity of bacteria in the nares and oropharynx of participants differs by the individual's prior colonization status.

In order to address these aims, we conducted an epidemiological, cross-sectional study of 59 individuals living in eastern Iowa between March 2015 and April 2016. The population included both individuals with and without contact with livestock. Microbial DNA was collected from the nares and oropharynx of all participants. Additionally, participants completed a series of questionnaires to collect data on demographics, health status, known risk factors, and livestock contact.

The research presented in this thesis aims to determine the composition of the microbiome of the nares and oropharynx of livestock workers, as well as determine associations between microbiome characteristics and *S. aureus* colonization. The results of this study may contribute to ongoing efforts to eliminate colonization of *S. aureus*. In the future it may be possible to treat bacterial infections caused by the growth of harmful bacteria, such as *S. aureus*, by transplanting beneficial or neutral bacteria. These neutral bacteria take the place of the pathogen and promote healthy microbiota.

CHAPTER 2: BACKGROUND

2.1 Identification of *Staphylococcus aureus*

S. aureus are gram-positive cocci and grows in clusters aerobically and rapidly at 37°C. When grown on solid media, *S. aureus* produces raised, round, smooth colonies [24]. A wide range biochemical and molecular tests are available to identify *S. aureus* and MRSA from culture. Those relevant to the work presented here are described below.

2.1.1 Biochemical identification

S. aureus is catalase-positive and can be distinguished from *Streptococci* by the catalase test with the catalase enzyme catalyzing hydrogen peroxide into water and oxygen [25]. *S. aureus* also produces coagulase, an enzyme that converts fibrinogen to fibrin which is deposited on the surface of the cell [24]. Coagulase has been hypothesized to be a virulence factor or an indicator of pathogenicity, though this is contentious [24, 25]. Additionally, some *S. aureus* isolates lack coagulase [25]. Lastly, rapid latex agglutination tests, such as the Pastorex Staph-Plus kit by Bio-Rad (Hercules, CA, USA), may be used to identify *S. aureus.* This test contains latex particles coated with IgG binding protein A and fibrinogen which in turn binds to the clumping factor on the bacteria's surface. Because some strains of *S. aureus* lack clumping factor, the Pastorex test also incorporates capsular polysaccharides of *S. aureus* to help reduce false-negative results [25, 26].

2.1.2 *mecA*

The *mecA* gene is carried on the staphylococcal chromosome cassette (SCC*mec*) which is present only in MRSA isolates [27] along with the regulatory genes *mecI* and *mecR* [28]. The *mecA* gene encodes for penicillin-binding protein (PBP), PBP2a. PBP2a

is a 78-kDa protein involved in the formation of the cell-wall and is acquired in MRSA from a non-*S. aureus* origin [29]. PBPs are essential for bacterial survival and as such are the target β-lactam antibiotics. β-lactam's have a structural backbone closely related to PBPs and are able to inhibit them. However, PBP2a has a unique transpeptidase that is not inhibited by β-lactam antibiotics and it remains able to form the cell wall structure even though the other PBPs are inhibited [30]. The source of the *mecA* gene in MRSA is unknown, though homologues have been found in other staphylococcal species [31, 32].

2.1.3 PVL

Panton-Valentine leukocidin (PVL) is a two-component leukocidin and pore-forming protein encoded by the *lukS-PV* and *lukF-PV* genes [33]. In 1932, Sir Phillip Noel Panton and Francis Valentine observed an association with the production of PVL and the formation of SSTIs, particularly abscesses [34]. In 2003, Vandenesch et al., observed an association between CA-MRSA isolates and the PVL genes that was consistently observed across isolates from North America, Europe, and Australia. The authors suggested this may indicate that PVL may confer selective advantages for CA-MRSA strains. And even if it is not a true virulence factor, PVL may serve as a genetic marker for community-associated isolates [35]. A large portion of CA-MRSA strains contain PVL, while it is absent from a majority of HA-MRSA strains and found at a much lower rate in CA-MSSA strains [33].

2.1.4 *spa*

The *spa* gene is specific to *S. aureus* and encodes for protein A which is expressed on the surface of the bacterium. Protein A plays a role in preventing opsonization and

phagocytosis by weakly binding the Fc region of IgG [36]. The *spa* gene contains a polymorphic X-region with a variable number of tandem repeats which can be used to distinguish between strains [37]. *spa* typing works by assigning each repeat region of the gene an alpha-numeric code. The *spa* type then corresponds to the order of those codes [38]. Ridom StaphType (Ridom, GmbH) software can then be used to construct consensus sequences and detect *spa* repeats in order to assign *spa* types from a large, global, continually updating database [38].

2.2 Colonization with *S. aureus*

Colonization or carriage – the growth and survival of the bacteria within the host – with *S. aureus* is known to occur at multiple body sites. The nose has been classically considered the most frequent site of colonization [8]. However, this may be due to sampling bias as the number of studies examining the nose as a colonization site far exceeds any other body site. Other body sites known to carry *S. aureus* are: skin [39], perineum [40], oropharynx [39, 41-44] gastrointestinal tract [45-47], axilla [48], and vagina [39, 48] (Figure 2-1). In a longitudinal study of 1,243 students and factory workers in Mexico by Hamden-Partida et al., 743 (59.8%) participants were colonized with *S. aureus* in either the nose, throat, or both [49]. Of the 743 colonized individuals, 282 (38%) were colonized in the throat only and 296 (39.8%) were colonized in the nose and throat providing 578 (77.8%) throat carriers. One hundred and sixty-five of the 743 carriers were colonized only in the nares (22.2%) providing an overall nasal colonization rate of 62% (461/743). In this population, the throat was the more important site of colonization [49].

Carriage status has traditionally been categorized into three states: non-carriers, intermittent carriers, and persistent carriers. Non-carriers are typically considered those individuals who never carry *S. aureus* or who carry it less than 10% of the time. Intermittent carriers are those individuals who pick up and drop *S. aureus* colonization. Individuals in this category are typically classified as those colonized between 10% and 80% of the time, although these classifications differ between studies. Persistent carriers are those individuals who always colonize with *S. aureus*. Persistent carriage is generally categorized as those colonized greater than 80% of the time. It has been estimated 50% of the general population are non-carriers, 30% are intermittent carriers, and 20% are persistent carriers of *S. aureus* [50].

Hanson et al., unpublished data, conducted a prospective study of Iowans in Johnson and Keokuk counties aimed at better understanding *S. aureus* colonization of the nares and oropharynx, transmission, and infection. This study is the most comprehensive study of *S. aureus* colonization to date in healthy persons. In this study, 79 (44.1%) adults were colonized with *S. aureus* at baseline in either the nose or throat [42]. Thirty-three of the 79 were colonized only in their throat and 35 in both their nose and throat. Sensitivity of nasal and oropharyngeal swabs was calculated for both Johnson and Keokuk counties. In Johnson County, the throat was the more sensitive swabbing site at 93.5% compared to the nares (45.6%). In Keokuk county, the sensitivity for both nasal and throat swabs was the same, 75.8%. Overall, the throat was the more sensitive site at 86.1% compared to 58.2% in the nares [42]. In the longitudinal study, 12 of the 129 adults were oropharynx only colonizers and 22 were colonized in both the nose and throat. In the longitudinal

portion of this study, the throat was again found to be more sensitive than the nose in identifying *S. aureus* carriers (77.3% vs. 72.7% respectively) [41, 42]. In this study, 72 participants (47.4%) were categorized as non-carriers, 49 (32.2%) were categorized as intermittent carriers, and 31 (20.4%) were categorized as persistent carriers [42].

2.2.1 Significance of the anatomical sites

The anterior nares have the most contact with the outside environment and act as a protective barrier [51]. Lined with keratinized, stratified squamous epithelium cells, sweat glands, sebaceous (oil/wax producing) glands, and hair, the anterior nares is more similar to the skin than to the oropharynx [52]. The anterior nares acts as the first line of defense against potential pathogens, blocking them from entering the respiratory tract by acting as a filter to trap larger particles introduced through inhalation. Inhaled air also contains a wide variety of microbes in low numbers in most environments. The anterior nares may also be challenged with microbes dripping from the sinus cavity and anatomical sites higher in the nasal passage [53].

Though connected through the upper respiratory tract, the anterior nares and oropharynx are distinct anatomical sites. The oropharynx is located behind the mouth between the nasopharynx and laryngopharynx. Unlike the anterior nares, the oropharynx is lined with non-keratinized stratified squamous epithelial cells. Like the nasopharynx, the oropharynx also acts as a barrier to the respiratory system and is challenged with microbes from inhalation, ingestion, saliva, and mucus [53].

2.3 Risk factors for colonization with *S. aureus*

There are many known risk factors for colonization with *S. aureus* in the nares and oropharynx. Age is a known risk factor, with children being more likely to be persistently colonized, and the likelihood of persistent carriage decreasing with increasing age. However, as age increases the risk of being an intermittent carrier increases [8]. Carriage rates are also observed to differ by ethnicity and gender, with white [54] males [55, 56] being at the greatest risk for persistent colonization. Immunosuppressive conditions such as diabetes mellitus, HIV, cancers, hemodialysis, renal disease, and liver disease are all at an increased risk of carriage as well. However, many of the studies done to date have primarily considered colonization in the nares, though recent studies have found the oropharynx to be equally as important as the nares and to be a distinct colonization site [43, 44, 57]. Additionally, in the Hanson et al. prospective cohort study, it was found that adults persistently colonized with *S. aureus* preferentially colonized the oropharynx. This study found several risk factors that increased the risk of oropharyngeal colonization including, environmental contamination, family size, and race [41, 42]. However, further research is still needed to determine what may predispose an individual to colonize either the nares or oropharynx. Several recent studies have hypothesized microbiome differences may impact colonization with *S. aureus*.

2.4 Epidemiology of healthcare-associated *S. aureus*

S. aureus has been a known cause of human infections since the late 1800's when it was first identified from the wound of a surgical infection [58]. Treatment of *S. aureus* infections in the 19th century and early half of the 20th century was limited until penicillin

was first used as treatment in the early 1940's. Antibiotic resistance in *S. aureus* first emerged in the 1940s to penicillin only one year after the antibiotic was first introduced [59]. Infections were almost exclusively in hospitalized patients [60] though *S. aureus* was not recognized as a major problem in hospitals until the mid-1960's. By this time, resistance to penicillin had already emerged [61]. By 1960, roughly 80% of hospital associated *S. aureus* infections were resistant to penicillin [61]. In 1967, the first major outbreak of MRSA in hospitals occurred [62] which was followed by the steady rise in MRSA in healthcare facilities. Over the following decades, resistance has emerged to almost all classes of antibiotics, which has resulted in a decrease in treatment options [63]. In 2006, MRSA was the most frequent cause of SSTIs presenting to emergency departments in the United States [64]. Between 2009 and 2010, *S. aureus* caused 15.6% of all hospital-associated infections, which was more than any other reported infectious agent [65].

2.5 Epidemiology of community-associated *S. aureus*

Until the 1990's, MRSA was considered a problem only for those with healthcare contact, particularly those with hospital ICU stays [66]. In the mid-1990's MRSA infections – predominately SSTIs – began to occur in the community in individuals without the traditional hospital associated risk factors [67]. CA-MRSA is typically defined as MRSA acquired by an individual with no healthcare contact. CA-MRSA is often also associated with the presence of the Panton-Valentine leukocidin (PVL) gene [66], which is not present in hospital-associated MRSA (HA-MRSA) strains. However, recent evidence suggests *S. aureus* carrying the PVL gene is emerging in the hospital

setting [68]. Recently, CA-MRSA strains have been causing outbreaks within hospital settings and the distinction between HA- and CA-MRSA has become rather blurred [69, 70]. In some hospitals, CA-MRSA is beginning to replace the traditional HA-MRSA strains that have been endemic for years [71].

2.6 Epidemiology of livestock-associated *S. aureus*

Individuals working with livestock are also known to be at an increased risk of *S. aureus* colonization and infection [72-74]. *S. aureus* colonization and infection has been associated with swine, cattle, and poultry. Voss et al. were the first to report MRSA transmission from swine in 2004. The authors reported a prevalence of MRSA pig farmers that was >760 times higher than among patients admitted to Dutch hospitals. Molecular typing demonstrated that MRSA was transmitted from pigs to pig farmers, among pig farmers and their family members, and from a patient to a nurse [75].

The bacterium has been isolated from horses [76-78], cattle [79, 80], dogs [81] and cats [82], in addition to swine [83-86]. These strains are considered livestock-associated MRSA (LA-MRSA). Frequently animals, particularly swine, do not have as severe as disease as humans, but necrosis, leg weakness and lameness, mastitis, and septicemia have been observed [87-89]. Since LA-MRSA strains were first identified in in the Netherlands, these strains have appeared as the cause of both colonization and infection in Europe [90-93], Asia [94, 95], North America [96, 97], and South America [98].

These infections can be dangerous not only for the person who works directly with the animals, but potentially for their families and entire community. van Cleef et al.

found working in stables with MRSA positive animals, having direct contact with sows, and living with a MRSA-positive swine worker all significantly increased the risk of nasal LA-MRSA carriage [99]. Livestock exposure has also been shown to increase the risk of infections from *S. aureus* in otherwise healthy workers [100].

Frana et al. conducted a study of 29 veterinary students from Iowa State University who were working with swine to assess MRSA acquisition and duration of colonization in the nares. Each of the students was shown to be MRSA-free over the two weeks prior to their work with swine. Samples were collected from veterinary students, swine, and the environment (treatment carts, fences, and gates). Of the students visiting swine MRSA-positive farms, 22.2% were positive for MRSA in the nose. No students visiting MRSA-negative swine farms were ever found to be MRSA-positive. A majority (76.9%) of the MRSA isolates identified among the students was t002, which was also the type most frequently found in swine (75% of isolates) and on the environmental samples (83.8% of all samples) [101].

2.6.1 LA-MRSA associated strains

A range of *S. aureus* strains have been associated with LA-MRSA, including clonal complexes (CC) 9, 97, 130, 522, and multilocus sequence type (ST) 5. However, those strains belonging to ST398 (CC398) are the most widely disseminated strains. Several *spa* types are associated with ST398 with t034 [96], t1451 [96], and t571 [102] being commonly seen in the USA. Globally, t034 and t011 are the most frequently observed LA-associated *spa* types [96].

Whole genome sequencing has shown CC398 likely originated in humans as an MSSA strain which subsequently was passed to livestock where it acquired the SCC*mec* cassette encoding resistance to methicillin and tetracycline, likely due to agricultural antibiotic usage. Tetracycline resistance is nearly universal in LA-CC398 isolates and rarely seen in human isolates [96]. This has also resulted in the CC398 livestock-associated strains appearing to be less transmissible, with colonization and infection likely related to the proximity and density of the animal contact [103, 104]. However, there remains some contention over the carriage duration of CC398 strains. While studies have reported colonization is transient and disappears quickly after the loss of animal contact [103], others have noted persistence carriage without direct animal contact [105, 106].

Infections ranging from SSTIs [72, 102] to wound infections [107, 108], otitis [92], bacteremia [74, 98, 109], and pneumonia [72, 110] have been reported with CC398. While a range of infections have been documented, it has been suggested CC398 isolates are associated with less morbidity compared to human-associated strains. Several studies in Europe have shown CC398 isolates are responsible for only a small portion of invasive disease, with one study finding ST398 was responsible for only 0.4% of invasive infections [111]. However, a French study found ST398 was the dominant form of MSSA causing osteomyelitis in patients with diabetic foot ulcers, accounting for 38% of the cases [93].

2.7 Treatment and Decolonization

2.7.1 Treatment of common MRSA infections

Treating *S. aureus* infections is increasingly difficult with the rise of antimicrobial resistance. In order to help slow the rise of antimicrobial resistance and to provide consistent, evidence-based recommendations on treatment the Infectious Disease Society of America (IDSA) produced clinical practice guidelines for the treatment of MRSA infections [112]. These guidelines provide evidence-based recommendations for adult and pediatric MRSA infections by infection type and provide information on what antimicrobials should and should not be used as well as dosing information [112]. For SSTIs, drainage is the recommended treatment for simple infections with antibiotics only being administered for severe, extensive, and/or aggressive disease such as cellulitis. Clindamycin, trimethoprim-sulfamethoxazole (TMP-SMX), tetracycline, and linezolid are recommended for five to 10 days and should not be used in conjunction with rifampin. For invasive or complicated SSTIs, intravenous (IV) vancomycin, linezolid, daptomycin, or clindamycin may be used for seven to 14 days [112]. In children, simple SSTIs – such as impetigo – may be treated with 2% topical mupirocin ointment. Tetracyclines should also not be used in children under eight [112]. Treatment for more complicated and invasive infections, such as bacteremia, are typically treated more aggressively. IV vancomycin or daptomycin should be administered for at least two weeks unless there is evidence of complicated disease (implants, metastatic infection sites, etc.). For complicated bacteremia, treatment should be administered for 4-6 weeks and six weeks or longer for infective endocarditis. Combination treatments with rifampin

or gentamicin is not recommended. Rifampin and vancomycin combination therapy is recommended for infective endocarditis of a prosthetic valve [112]. The guidelines provide recommendations for osteomyelitis, pneumonia, septic arthritis, infections of the central nervous system, as well as recommendations for vancomycin treatment failure [112].

2.7.2 Decolonization

Many healthcare facilities now screen for MRSA with some facilities also attempting to decolonize carriers in an attempt to prevent infections from ever occurring. Many surveillance and eradication programs have focused on the anterior nares, as it has been traditionally considered the most important site for colonization; however, successful decolonization programs screen for MRSA at more than one site [113]. Decolonization regimens often include at least one of the following treatments: an antimicrobial nasal ointment, antimicrobial mouth rinse, antimicrobial whole body wash, and/or systemic antibiotics plus rifampin. Combination therapies are considered the most effective, though combinations including oral antibiotics have been associated with increases drug resistance. As such, germicides and topical antibiotics are the preferred decolonization agents [114, 115].

Decolonization of *S. aureus* is most commonly achieved by using chlorhexidine and mupirocin. Mupirocin nasal ointment has been shown to be effective in at least temporarily eradicating MRSA from the nares. However, carriage often returns and it has been estimated only 48% of patients remain decolonized after 6 months [116]. Several studies have also shown that while mupirocin may be effective at decolonizing patients, it

still may not reduce the number of infections or hospital length of stay [117, 118]. Recent studies have also shown that while decolonization may decrease the amount of MRSA circulating in hospitals, resistance to antiseptics emerges. Cho et al., found that while implementing active MRSA surveillance and decolonization with both chlorohexidine baths and intranasal mupirocin decreased the incidence of MRSA (thought the trend was not significant, likely due to the small sample size), a significant increase in low-level mupirocin resistance was observed over the 40 month study period increasing from 0% to 19.4% [119]. Low-level mupirocin resistant *S. aureus* infection isolates have been associated with treatment failure in patients [120].

Baratz et al. conducted a study of patients undergoing total joint arthroplasty. Patients were decolonized with a five-day regimen of intranasal mupirocin ointment twice daily as well as daily baths with 4% chlorohexidine soap. All patients, regardless of MRSA carriage status, underwent antibiotic prophylaxis with one dose of a first-generation cephalosporin prior to surgery and two does postoperatively. MRSA patients were also given one does of vancomycin during surgery in addition to decolonization and prophylaxis. Twenty-two percent of patients never decolonized. Furthermore, while decolonization resulted in a decreased amount of nasal *S. aureus* carriers, there was no reduction in the number of SSIs caused by either MSSA or MRSA. *S. aureus* carriers were at an increased risk of SSI with a relative risk of 1.77 compared to non-carriers, though the trend was not significant ($p=0.20$) [121].

Other topical antiseptics, such as polyhexanide, have been shown to be effective at eliminating *S. aureus* in vivo [122]. However, Landelle et al. conducted a double-

blind, randomized controlled trial of polyhexanide and found no significant differences in decolonization rates between the polyhexanide group and placebo [122]. Currently, mupirocin is the most effective topical antimicrobial agent at even temporarily eliminating *S. aureus* carriage, so strong antibiotic stewardship guidelines and practices are necessary when considering universal decolonization policies [123, 124].

Systemic antimicrobial agents in combination with rifampin have also been used to decolonize MRSA patients. Rifampin is often used in combination due to its effectiveness at penetrating tissues and secretions [115]; however drug resistance develops quickly when it is used alone [125]. Walsh et al. conducted a randomized double-blinded multicenter trial of rifampin with either novobiocin or trimethoprim-sulfamethoxazole (tmp/sulfa) on MRSA decolonization. The authors found novobiocin plus rifampin to be more effective at decolonizing patients compared to tmp/sulfa plus rifampin (67% compared to 53%) though the difference was not significant ($p=0.18$). Though resistance developed significantly more frequently in the tmp/sulfa group (14% developed resistance) compared to the novobiocin plus rifampin group (2%), $p=0.04$. The authors also found decolonization was age dependent with older patients being significantly more likely to decolonize successfully ($p<0.01$) as well as site dependent with wounds being significantly less likely to decolonize compared to all other sites (nares, rectum, sputum), $p=0.02$. Only 48% of subjects successfully decolonized in their wounds with no difference between treatment regimens [125].

As stated above, while mupirocin is the gold standard in short term eradication, it alone is not effective at long term *S. aureus* eradication. Because of this, combination

therapies have been attempted. Simor et al. conducted a randomized, controlled trial of chlorhexidine gluconate washes, intranasal mupirocin ointment, oral rifampin and doxycycline for seven days vs no treatment to assess long term (8 months) decolonization. After 3 months, 74% of those undergoing decolonization remained decolonized and only 54% of those remained decolonized after 8 months. Emergence of mupirocin resistance was low at 5% [113].

Current therapies are only temporarily or partially effective at decolonization with resistance on the rise and new antiseptic agents proving ineffective in human studies. It has also been hypothesized that topical decolonization agents may negatively impact the microbiome and increase the risk of infections from other organisms. A meta-analysis of nine randomized controlled trials (3396 patients total) found while mupirocin significantly reduce the number of nosocomial *S. aureus* infections, it did not reduce *S. aureus* SSIs. The authors also found patients treated with mupirocin were at a significantly increased risk of infections caused by other micro-organisms (RR 1.38, 95% CI: 1.12-1.72) including *Enterobacter cloacae*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae* [126]. While this is an area requiring a great deal of further study, information gained from research into the microbiome may eventually lead to novel interventions to suppress *S. aureus* carriage while minimizing the risk of colonization and infection with other pathogenic organisms.

2.8 The human microbiome

In 2001, Joshua Lederberg first coined the term the human microbiome and defined it as signifying “the ecologic community of commensal, symbiotic, and

pathogenic microorganism that literally share our body space" [127]. More traditionally, the microbiome simply means a "small biome – or ecosystem – compromising all of the microorganisms of a particular environment whose genes interact with the environment" [128]. Until recently, microbiology and infectious disease research has been based on culture methodologies. However, culture methodologies are limited in that one can only identify what we currently understand and are able to grow; only a small proportion of the microbial diversity that exists in nature [129]. It has been estimated that microbial cells outnumber human cells ten to one [130]. Advances in DNA based technologies have allowed us to expand our understanding of the microbial community. These advances have lead researchers to analyze the genomes of the bacterial communities without having to culture them first. These technologic advances allow researchers to study what microbes may be present at any particular anatomical site with the goal of better understanding how our microbial communities interact with their host and affect human disease [130].

2.8.1 The nasal microbiome

The human microbiome project (HMP) laid the foundation for our understanding of the microbiome in healthy persons. The HMP analyzed the nasal microbiome by swabbing both nostrils at the anterior nares and pooling the specimens before sequencing [131, 132]. In the HMP, the nasal microbiome varied quite a bit between subjects; however, organisms from the *Corynebacterium* (mean 31%, s.d 21%), *Propionibacterium* (24% sd. 17%), and *Staphylococcus* (17% sd. 18%) genera dominated the samples. With regard to pathogenic organisms, there were very limited class A-C pathogens as defined

by the National Institute of Allergy and Infectious Disease (NIAID) [133]. The only class A-C pathogenic organisms consistently found in the nares of HMP subjects above 0.1% abundance was *S. aureus*. A wide range of opportunistic pathogens were identified (defined as opportunistic by PATRIC [134]) including *S. epidermidis, S. aureus, Propioniobacterium acnes, K. pneumonia, E. coli,* and *S. pneumoniae. P. acnes* and *S. epidermidis* were the most abundantly identified opportunistically pathogenic species at 42.5% and 12.7% across all samples respectively. *S. epidermidis* was identified to some degree in 93% of samples [131].

2.8.2 The oropharyngeal microbiome

The HMP also characterized the oropharyngeal microbiome in healthy persons. The oral microbiome was observed to have the greatest alpha diversity (diversity within samples) of any of the anatomical sites tested by the HMP. *Streptococcus* was the most dominant genera identified in the oral cavity, particularly *S. mitis* [131]. The HMP also showed there were a decreased level of organisms belonging to the Firmicutes phyla on the oropharynx compared to other anatomical sites along the digestive tract; however, there were more organisms from the Bacteroidetes, Fusobacteria, Actinobacteria, and TM7 phyla compared to the other anatomical sites [135]. The genera most frequently identified were *Streptococcus, Veillonella, Prevotella, Neisseria, Fusobacterium, Actinomyces,* and *Leptotrichia*. Several pathogens were identified as colonizing the oropharynx including *S. pneumoniae, S. pyogenes, Neisseria meningitides,* and *Haemophilus influenza*. *Moraxella catarrhalis* (a known sinus pathogen) was detected at a relatively low abundance (mean >0.5%, sd. 4.7%). Despite having a differences in

tissue type, many anatomical sites in the oral cavity have similar microbiomes, likely due to the path of swallowed saliva [135].

2.8.3 Characteristics of frequently identified bacterial genera in the nasal and oropharyngeal microbiomes

Corynebacterium have been frequently identified in the nasal or oropharyngeal microbiomes. Organisms belonging to the *Corynebacterium* genus are gram-positive, aerobic (or facultative anaerobic), catalase positive organisms. They are frequently found in the mucosa and skin of humans and animals. *C. diphtheriae* is the most notable pathogen from this genera [136].

Streptococcus is another frequently identified genus considered normal flora in the nares and oropharynx. Organisms belonging to this genus are coccus shaped, gram-positive, and grow in a distinctive chain. A majority of these organisms are catalase negative, oxidase negative, facultative anaerobes. Organisms from this genus are classified into two groups, alpha-hemolytic and beta-hemolytic. Alpha- hemolysis, or partial hemolysis, is caused by hydrogen peroxide oxidizing hemoglobin and is characterized by green pigmentation on blood agar. Many viridans group streptococci are alpha-hemolytic as well as *S. pneumoniae*. Beta-hemolysis, or complete hemolysis, is the complete lysis of red blood cells caused by the exotoxin, streptolysin. *S. pyogenes* (group A *Streptococcus*) and *S. agalactiae* (group B *Streptococcus*) are the most notable pathogenic beta-hemolytic species [136].

Members of the genus *Staphylococcus* are also frequently identified in the nasal and oropharyngeal microbiomes. This genus is characterized by gram-positive, spherical

organisms that grow in a grape-like structure. They are aerobes or sometimes facultative anaerobes that are catalase positive and grow well in the presence of salts. *S. aureus* (described in detail above) is the most notable pathogen in the genus. Other pathogens include *S. epidermidis*, *S. lugdunensis*, and *S. saprophyticus* and are considered opportunistic. The pathogenic organisms in the genus are often beta-hemolytic, coagulate positive, toxin producers. Staphylococci rapidly develop resistance to antimicrobials [136].

Klebsiella are also found in the nares and oropharynx. This genus is characterized by gram-negative, oxidase negative, rod-shaped bacteria with a polysaccharide-based capsule. The *Klebsiella* genus is small, only containing six species, most notably *K. pneumoniae*. They are facultative anaerobes that are commonly found in nature and grow easily in culture. *K. pneumoniae* infections, while not frequent causes of pneumonia, produce severe disease and is a frequent causes of hospital associated infections. It is also a cause of urinary tract infections in immunocompromised patients. *K. pneumoniae* subspecies *rhinoscleromatis* is a cause of rhinosclermona of the nose and pharynx [136].

Morexella is another small genus containing only six species. These are gram-negative, catalase positive organisms with *M. catarrhalis* being the most clinically relevant species. They are common flora of the respiratory tract and occasionally cause bacteremia, endocarditis, meningitis, and conjunctivitis. *M. atarrhalis* frequently produces β-lactamase [137].

Propionibacterium are frequent colonizers of the skin (in the sweat glands and sebaceous glands), oral cavity, intestines, conjunctiva, and ear canal. Their most notable

feature is their ability to synthesize propionic acid – a foul smelling carboxylic acid that prohibits the growth of molds and some bacteria. *P. acnes* is an opportunistic pathogen associated with several inflammatory conditions, most notably acne vulgaris. *P. acnes* is a frequent contaminant of blood and cerebrospinal fluid samples, but also causes SSIs (particularly device associated SSIs) as well as central nervous system shunt infections, osteomyelitis, and endocarditis. Because of this, it can be difficult to determine if *P. acnes* is a contaminant or the infectious agent [136].

Lastly, members of the large genus *Actinomyces* are frequently found in the nasal and oropharyngeal microbiomes as well as in livestock. These bacteria are gram-positive, facultative anaerobes (with the exception of *A. meyeri* which is an obligate anaerobe) and grow well on most culture media. *A. israelii* and *A. gerencseriae* are causes of actinomycosis, a chronic disease of painful abscesses in the mouth, lungs, and gastrointestinal tract [138].

2.9 *S. aureus* and the human microbiome

Several studies have identified several organisms that may interfere with *S. aureus* colonization of the nares. Recently, Bessesen et al. conducted a matched case-control study of hospitalized inpatients at the Department of Veterans Affairs Eastern Colorado Health Care System to assess differences in the nasal microbiota between MRSA carriers (n=26) and non-carriers (n=26). The authors found a negative correlation between MRSA colonization and *Streptococcus* spp., particularly *S. mitis*, as well as with *Lactobacillus gasseri* and *Klebsiella palustris*. Patients persistently colonized with MRSA had lower alpha-diversities than the non-colonized controls. The authors found

that in vivo, *S. mitis* inhibited the growth of MRSA due to an unknown mechanism involving hydrogen peroxide [139].

Yan et al. sought to determine the bacterial diversity of the nares of 12 individuals over four time points using pyrosequencing and traditional culture methods [51]. It was discovered that the anterior nares was composed primarily of *Actinobacteria* (48 – 52.5% of all sequences). Yan et al. also sought to determine what the differences were in the nasal microbiota between *S. aureus* carriers (n=6) and non-carriers (n=6). A total of 28 operational taxonomic units (OTUs) were identified with statistically different abundances between *S. aureus* carriers and non-carriers. Of those 28 OTUs, OTUs from the *Corynebacterium* genus (7 OTUs) were the most represented and varied the most between carriers and non-carriers. *C. pseudodidiptheridicum* was found to possibly inhibit *S. aureus* carriage through competitive inhibition – suppression of one or several bacterial species due to the presence of other bacteria – as it was found more often in non-carriers than carriers. After culturing the species pairs on agar, the theory of competitive inhibition was supported. *S. aureus* was unable to grow near *C. pseudodidiptheridicum* and vice versa. Proteobacteria were more common and in greater proportions in noncarriers compared to carriers. Interestingly, *S. epidermidis* – which has been previously implicated as a possible source of competitive inhibition for *S. aureus* – and *Acrinobacteria* did not differ significantly between carriers and non-carriers. Overall, non-carriers had less bacterial diversity than persistent carriers did [51].

C. accolens has also been shown to possibly inhibit *S. aureus* growth. In a study of 103 patients by Alverez et al. at a French hospital, *C. accolens* was found more often

in those patients who were not colonized with *S. aureus*, which is supportive of competitive inhibition. *Staphylococcus haemolyticus* was found exclusively in *S. aureus* non-carriers [140]. However, Yan et al. found C. *accolens* to not only be more common in those colonized with *S. aureus* – a directly opposing finding of Alverez et al. – but also found *C. accolens* growth to be supportive of *S. aureus* and vice versa [51].

Using a 16s rRNA gene microarray (PhyloChip), Lemon et al. examined both the nasal and oropharyngeal microbiomes to determine differences between the anatomical sites in seven healthy volunteers [53]. The authors found the oropharynx had increased species richness compared to the anterior nares; however, the nares had more interpersonal variation than the oropharynx. The researchers found bacteria belonging to the Firmicutes (containing the Staphylococcaceae family) and Actinobacteria (containing the Cornybacteriaceae family) phyla were most common in the nares and Firmicutes, Proteobacteria, and Bacteroidetes were the most abundant bacterial phyla in the oropharynx. While bacteria belonging to the Firmicutes phylum was found at the nares and oropharynx, the families within the Firmicutes phylum differed by site. An inverse correlation was also found between the Firmicutes and Actinobacteria in the nares and the Firmicutes and Proteobacteria in the oropharynx. More specifically, Lemon et al. found an inverse correlation between members of the Staphylococcaceae family and Actinobacteria in the nares [53]. It appears *Corynebacteria* may play an important role in the determination of *S. aureus* carriage in the human nares and possibly oropharynx.

As stated above, *S. epidermidis* has previously been thought to negatively interact with *S. aureus*. A longitudinal study of the anterior nares of five healthy adults and a

cross-section of 42 hospitalized subjects found *S. epidermidis* to be negatively correlated with *S. aureus* ($p=0.004$) in inpatients [141]. *S. epidermidis* was also the most frequent species identified in those that were classified as inpatient *S. aureus* non-carriers. *P. acnes* was also negatively associated with *S. aureus* colonization in the nares. This study also found that hospitalized patients had significantly less diverse nasal microbiomes than hospitalized patients. Healthy subjects had twice as many OTUs as hospitalized subjects ($p<0.001$). Within the healthy individuals, a longitudinal study was done with four time points ($n=5$) showing that although the microbial make-up of the individuals was different, in all five subjects, it remained stable [141]. Several other bacteria commonly colonizing the nares have been implicated in inhibiting *S. aureus* colonization, most commonly *S. pneumoniae* [141].

Several studies considering *S. aureus* colonization in the oropharynx do exist. Roos et al. aimed to determine if orally and nasally administering several strains of lactobacilli that demonstrated inference capabilities *in vitro* against both PVL positive and negative MRSA would reduce MRSA colonization in seven patients colonized with MRSA for over one year. All three of the patients colonized in the nose and throat cleared *S. aureus* colonization in the nares and oropharynx, though clearance occurred more quickly in the nares. Two patients were unable to clear nasal colonization – these patients were never throat carriers – and two patients did not remain in follow up long enough to determine clearance [142]. Uehara et al. also considered oral *S. aureus* colonization and aimed to determine if MRSA colonization in the nares and throat of neonates was related to colonization with viridans streptococci [143] (a pathogen causing

sepsis, pneumonia, and meningitis in neonates). It was observed that MRSA colonization at both anatomical sites was lower in the group that was colonized with viridans streptococci ($p<0.001$) with no significant differences in clinical characteristics [143].

L. fermentum has also been shown to inhibit *S. aureus* growth in animal models [21] as has *S. epidermidis* [144]. Studies assessing bacterial inhibition of *S. aureus* in the nares are limited and studies of *S. aureus* in the oropharynx are even more so. Additionally, most studies have small samples sizes and provide conflicting results. Because of the limitations of the literature, there is a need to investigate bacterial communities and their impact on *S. aureus* carriage in the nares and oropharynx further.

2.10 Analytic methods

2.10.1 Taxonomic classification

As with all organisms, bacteria are grouped into subspecies, species, genus, family, order, class, phylum, and kingdom based on biochemical, morphological, and/or genetic similarity with less similarity in the higher orders [145]. Historically, the species level has been the most important level of classification; however, there is not one clear definition of a bacterial species [145]. In 1944, Ernst Mayr proposed a species be defined as a group of organisms remaining phenotypically similar because of recombination between them [146]. More simply put, a species is the largest group of organisms able to produce fertile offspring. While this definition is well suited for the domain Eukarya, it poses an issue for Archaea and Bacteria whose members primarily reproduce through asexual reproduction [147]. Furthermore, genes can be transferred to organisms of another species through horizontal gene transfer[147]. Due to these issues, the species

definition for Archaea and Bacteria is typically based on biochemical, morphological, and genetic characteristics, though many definitions exist across fields [147]. The issues surrounding the lack of consensus around the definition of a species is known as the species problem.

When molecularly classifying bacteria, the 16s ribosomal subunit gene – or the 16s rRNA gene – is most frequently used as it contains both highly conserved and hypervariable regions allowing it to act as a "genetic fingerprint". Additionally, the 16s rRNA gene is relatively inexpensive and simple to sequence making it an ideal candidate for a genomic marker [148]. While the 16s rRNA gene provides a way of identifying bacteria, it does not solve the species problem. Defining the species based entirely on the 16s rRNA gene is problematic for three main reasons: the hypervariable regions of the 16s rRNA gene allow for small numbers of base-pair changes occurring frequently over evolutionary time [148], bacteria are frequently only sequenced once in a study allowing for a small amount of sequencing error [148, 149], and named species genomes may not be available in reference databases for novel sequences [148]. Because of these issues, sequences belonging to bacteria that would be considered of the same species by other classification methods, may not share sequences that are 100% similar [148]. To circumvent these issues, operational taxonomic units (OTUs) can be used to replace the species designation for community analysis. OTUs are groupings of bacteria with sequences with some divergence, but are assumed to be otherwise identical genomes. OTUs are typically clustered together based on sequences that are 95%, 97% or 99% similar to each other [148].

Once sequences are clustered into OTUs, a representative sequence of each OTU is chosen and traditional taxonomy is assigned to it using a reference database such as SILVA, Greengenes, or RDP. However, the species problem still prevents OTUs from being classified down to the species level. Instead, most reference databases can only assign taxonomy down to the genus level. Once classification is assigned to the representative sequence, the classification is given to all sequences belonging to that OTU [150, 151]. The OTUs are used in downstream analysis to assess the composition of the community.

2.10.2 Alpha diversity

Alpha diversity is defined as the richness – or the number of OTUs [152] – of the sample. Each sample is given its own value of alpha diversity. Multiple measures exist to estimate the alpha diversity of a sample [153]. All measures take richness into account with several also considering the sample's evenness – the measure of the relative abundance of different OTUs making up the richness. A list of the commonly used alpha diversity metrics is provided below.

2.10.2.1 Chao1 diversity index

The Chao1 diversity index is a non-parametric estimator based on mark-release-recapture (NMR) statistics for estimating the size of a population. NMR statistics are based on the proportion of OTUs that have been observed (recaptured) previously to those seen only once in the sample. Chao1 estimates the total OTU richness by

$$S_{Chao1} = S_{obs} + \frac{n_1^2}{2n_2}$$

where

S_{obs} = the number of observed OTUs

n_1 = the number of singletons (OTUs observed once)

n_2 = the number of doubletons (OTUs observed twice)

The Chao1 measure is particularly useful for low-abundance datasets; however, it strongly correlates with the number of sequences assessed. The maximum value of S_{Chao1} is ($S2_{obs}$+1)/2 until S_{obs} reaches the square root of twice the total richness.

2.10.2.2 Abundance-based coverage estimator (ACE)

Similar to Chao1, the abundance-based coverage estimator (ACE) is a nonparametric measure based on NMR statistics. ACE incorporates data from all OTUs with less than 10 sequences as opposed to only singletons and doubletons as is done by Chao1. Similar to Chao1, ACE underestimates the true richness of the sample with low sample sizes (number of sequences).

$$S_{ACE} = S_{abund} + {}^{S_{rare}}/_{C_{ACE}} + \left({}^{F_i}/_{C_{ACE}}\right)\gamma_{ACE}^2$$

where

S_{abund} = the number of OTUs with > 10 observations

S_{rare} = the number of OTUs with ≤ 10 observations

C_{ACE} = estimates the sample coverage by:

$$C_{ACE} = 1 - {}^{F_1}/_{N_{rare}}$$

F_i = the number of OTUs with i individuals

$N_{rare} = \sum_{i=1}^{10} iF_i$

γ_{ACE}^2 = the coefficient of variation of the F_i's by:

$$\gamma_{ACE}^2 = max\left[\frac{S_{rare}\sum_{i=1}^{10} i(i-1)F_i}{C_{ACE}(N_{rare})(N_{rare}-1)} - 1{,}0\right]$$

2.10.2.3 Shannon diversity index

The Shannon diversity index – also known as the Shannon-Wiener index, Shannon-Weaver index, and Shannon entropy – is a measure of the amount of entropy (information) in a particular system. It is positively correlated with OTU richness and evenness and like the Chao1 index, gives more weight to the rare OTUs [154]. It is calculated by:

$$H' = -\sum_{i=1}^{n} p_i log_b p_i$$

where

p_i = the proportional abundance of OTU *i*

b = the base of the logarithm (the natural logarithm or base=2 are both commonly used)

The Shannon diversity index is one of the most frequently used indices [152, 154]; however, the main issue with this index is the difficulty in interpreting its meaning [154, 155] as it is a measure of entropy and not diversity. *H'* is a measure of uncertainty in the OTU identification within a sample, not the number of OTUs in the sample [156]. The *H'* is a measure with no meaning and does not predict the probability of identifying the next OTU [154]. The interpretation is as richness and evenness increase, as does the index [154]. All samples with an *H'* of 3.0 will have the same entropy, and therefore have equivalent diversities [156]. The Shannon index is an underestimation of the true alpha diversity due to incomplete coverage with the coverage being dependent on the richness

and evenness in each sample; therefore, error rates will be different for each sample included in the study. This error also increases for samples with fewer sequences [154].

2.10.2.4 The Simpson's index and Inverse Simpson's diversity index

The Simpson's index (also known as the Gini-Simpson's index) is the probability two sequences belonging to the same OTU will be randomly chosen [157]. The values are constrained between 0 and 1 with the index increasing as the sample's diversity decreases so that samples with an index of 1.0 have all OTUs at equal abundance [157]. When compared to the other indices, it places more weight on the dominant OTUs [158]. It is calculated by:

$$D = \sum p_i^2 \text{ or } D = \left[\sum_{i=1}^{S} n_i(n_i - 1) \Big/ N(N-1)\right]$$

where

p_i = the proportional abundance of OTU i

S = the number of OTUs

n_i = the number of sequences within the i^{th} OTU

N = the total number of sequences in the sample

The Simpson's diversity index is simple to understand; however, it is not necessarily intuitive as diversity increases with the decrease in D. Because of this, the inverse – or $1/D$ – is frequently used (sometimes referred to as the Simpson's reciprocal index or Simpson's dominance) so that the increase in the index value reflects an increase in diversity [159]. For the inverse Simpson's diversity index, the values begin at 1 and increase with increasing diversity with the maximum value being the greatest number of

OTUs present. A value of 1 indicates the sample contained 1 OTU and a value of 7 indicates the sample contained 7 OTUs.

2.10.3 Beta diversity

Similar to alpha diversity, beta diversity is the measure of distance or dissimilarity between sample pairs – or the comparison of groupings of samples based on metadata – and acts as a dissimilarity score between the samples [147]. Beta diversity provides information on what taxa are shared between the samples and to what degree allowing researchers to answer questions on how an environment may change over time or if it is influenced by a disease state or condition [147]. Two distinct types of beta diversity exist: measures based on presence-absence data and measures based on abundance data. Presence-absence metrics only allow for the distinctions of what OTUs are different between groups while abundance-based metrics allow for differences based on how much of an OTU is present, i.e. the groups may share the same OTUs but several of the OTUs may be rare in group 1 while they are dominant in group 2 [160]. Incomplete sampling and rare OTUs have less of an influence on abundance-based metrics compared to presence-absence metrics [160]. Unlike measures of alpha diversity which are scalar and thus can be given one number to describe them, measures of beta diversity have magnitude and direction and tend to require more advanced methods to describe them [161]. A description of commonly used beta diversity metrics is provided below.

2.10.3.1 Sørensen index

The Sørensen index, also known as the Sørensen-Dice index or Sørensen similarity index, is a presence-absence measure of beta-diversity [161]. It measures the

similarity in OTU composition between two samples. It is calculated by the equation [162]:

$$\beta = 1 - \frac{2ab}{a + b\prime}$$

where

a = the number of OTUs in site 1

b = the number of OTUs in site 2

ab = the number of OTUs shared between the two sites

When more than two samples are being compared, the overall similarity between samples is calculated by averaging overall the sample pairs [162]. Because the index is based on presence-absence data, it is better suited for heterogeneous data.

2.10.3.2 Jaccard index

The Jaccard index is very similar to the Sørensen index and also compares the similarity between samples using presence-absence data. The Jaccard index compares the unique OTUs of both samples or populations to the shared OTUs in those samples or populations. This is done using the following equation [163].

$$\beta = 1 - \frac{a}{a + b + c}$$

where

a = the number of OTUs shared between groups

b = the number of unique OTUs in group 2 not present in group 1

c = the number of OTUs in group 1 not present in group 2

2.10.3.3 UniFrac and Weighted UniFrac distances

UniFrac is a presence-absence measure based on phylogenetic information allowing it to account for shared ancestry between communities [164]. It is based on the idea that communities differing more should have more divergent phylogenetic trees due to the adaption to their specific community environment which may be detrimental to the comparison community [164]. It assumes if two communities are similar, few adaptions would be required for an organism to move between them [164]. UniFrac measures phylogenetic distances between sets of taxa by dividing branch lengths that are not shared between the groups being compared with a distance of 0 meaning the groups are identical and a distance of 1 meaning the groups share no OTUs [165]. UniFrac requires a constant sequencing depth to be accurate so data must be normalized to a set depth prior to analysis [165]. It is calculated by:

$$Unweighted_{AB} = \sum b_A \Delta b_b \Big/ \sum b_A \cup b_B$$

where

b = the set of branch lengths in the phylogenetic tree

A and B = the communities being compared

Δ = the symmetric difference between the groups

$\cup$ = the union between the groups

The weighted UniFrac brings abundance data into account by weighting each branch of the phylogenetic tree by the difference in proportional abundance of the OTUs between groups [165]. This allows low abundance OTUs to have less of an impact on the total distance correcting the issue where low abundance taxa skew unweighted UniFrac

measurements, especially when those OTUs are on a long branch of the tree [165]. It is calculated by:

$$Weighted_{AB} = \sum_i^n \left(b_i + \left| \frac{A_i}{A_r} - \frac{B_i}{B_r} \right| \right) \Big/ \sum_i^n b_i$$

where

A = sample 1

B = sample 2

B_i = length of branch i

$\frac{A_i}{A_r}$ = proportional abundances of associated with B_i

$\frac{B_i}{B_r}$ = proportional abundances of associated with B_i

2.10.3.4 Bray-Curtis dissimilarity

The Bray-Curtis index creates a dissimilarity matrix and quantifies the degree of dissimilarity between samples based on count data at each site (OTU). The index ranges from zero to one with a zero value indicating the samples have the same OTU composition and one indicating no OTUs are shared between them. The Bray-Curtis measure ignores variables with zero counts at both locations (joint absences). The method assumes the samples were taken from an areas of equal size and that raw count data is used. It is calculated by summing the differences between counts for each site and dividing them by the total abundances of the samples being compared using the following equation [148].

$$\beta = 1 - \left[2C_{ij} \Big/ S_i + S_j \right]$$

where

S_j = the number of OTUs at site *j*

S_i = the number of OTUs at site *i*

C_{ij} = the total number of OTUs at the location with the fewest OTUs

2.10.4 Ordination

Because beta diversity metrics are not scalar and instead calculate a distance between samples, they create a matrix of distances between all samples in the dataset instead of singular values [161]. The distance matrix created can be visualized in multiple ways including, but not limited to, phylogenetic trees and ordination. Frequently reported ordination methods include canonical correspondence analysis (CCA), nonmetric multidimensional scaling (NMDS), detrended correspondence analysis (DCA), Principal components analysis (PCA) and Principal coordinates analysis (PCoA). Here, the focus will be on PCoA.

2.10.4.1 PCoA

As stated above, PCoA is a method of visualizing information from large matrices and works by transforming correlated data into a set of uncorrelated variables called Principal coordinates (PC) [166]. The points (or samples) on plot are plotted multidimensionally so they are as close as possible to the distance matrix [167]. The direction accounting for the greatest amount of space between the points is the first PC, the direction separating the second most is the second coordinate and so on. These PCs are assigned a percentage value based on how much of the variability in the distance matrix they explain. Typically the first two PCs are plotted – or the first three PCs on a 3-dimensional plot – so it is possible to visualize the distances [167]. Samples, or points,

clustering together are more similar and have more similar microbiomes. Points can be color-coded and/or represented as different shapes to incorporate metadata to determine if clustering occurs based on information such as sample type or environmental characteristics [167].

2.10.5 Significance testing

Permutational multivariate analysis of variance (PERMANOVA), also called nonparametric MANOVA, is a nonparametric way of assessing variation between groups based off of a dissimilarity matrix [168]. PERMANOVA measures the spread around the special mean or centroid [169]. PERMANOVA is preferred over MANOVA and other parametric tests which assume a normal distribution. In community analysis, data is heavily right-skewed due to the large number of zero counts from rare OTUs. Additionally, community analyses typically have more OTUs than there are degrees of freedom making traditional MANOVA inappropriate [170]. The null hypothesis of the test is there are no differences in the position or spread (in multivariate space) between the groups being compared. In PERMANOVA, the sum of squared differences between individual points and their group centroid is equal to the sum of squared inter-point distances divided by the total number of points [169]. This allows for the calculation of the sum of squares from the distance/dissimilarity matrix using any distance measure. PERMANOVA uses a pseudo-F statistic to calculate the p-value through permutation or shuffling the sampling unit across the variable of interest recalculating the test statistic each time in order to create the distribution which allows for PERMANOVA to avoid the assumption of normality [170].

2.10.6 Based-upon repeat pattern analysis

The based-upon repeat pattern (BURP) algorithm is designed to identify clonal relationships between *S. aureus* spa based on evolutionary relatedness [171]. This is done by assessing the genetic cost- or the number of mutations differentiating two isolates – between the samples. The algorithm creates a genetic cost distance matrix between all samples in the dataset to determine the number of genetic mutations required to connect any two strains [171]. Isolates are then grouped together into the same clonal complex (CCs) if they have a genetic set below a pre-specified value which the developed recommend be set to a cost of four (or a four mutation difference) [171]. The main limitation to the BURP analysis is all isolates must be *spa*-typable to assess genetic relatedness, though the number of non-typable isolates is low (estimated at 0.1%) [171].

2.11 Research significance

The studies carried out for this dissertation aim to add to our current knowledge of *S. aureus* carriage in healthy livestock workers and community members. Additionally, very little is known regarding the microbiome of livestock workers. This study (Aim 1) is the first we are aware of to characterize the healthy microbiomes of the nares and oropharynx of livestock workers, a necessary first step to understanding how the microbes interact with the host as well as impact disease. Similarly, there is little research surround *S. aureus* carriage, particularly with regard to the differences in the microbiome by carrier state. This study (Aim 2) hopes to add to the current knowledge of what may constitute a microbiome protective against *S. aureus* colonization or what may constitute a microbiome at risk of *S. aureus* colonization. We also aim to begin to understand if any

bacterial components of the microbiome may have an impact on whether a person is a persistent carrier, intermittent carrier, or a non-carrier.

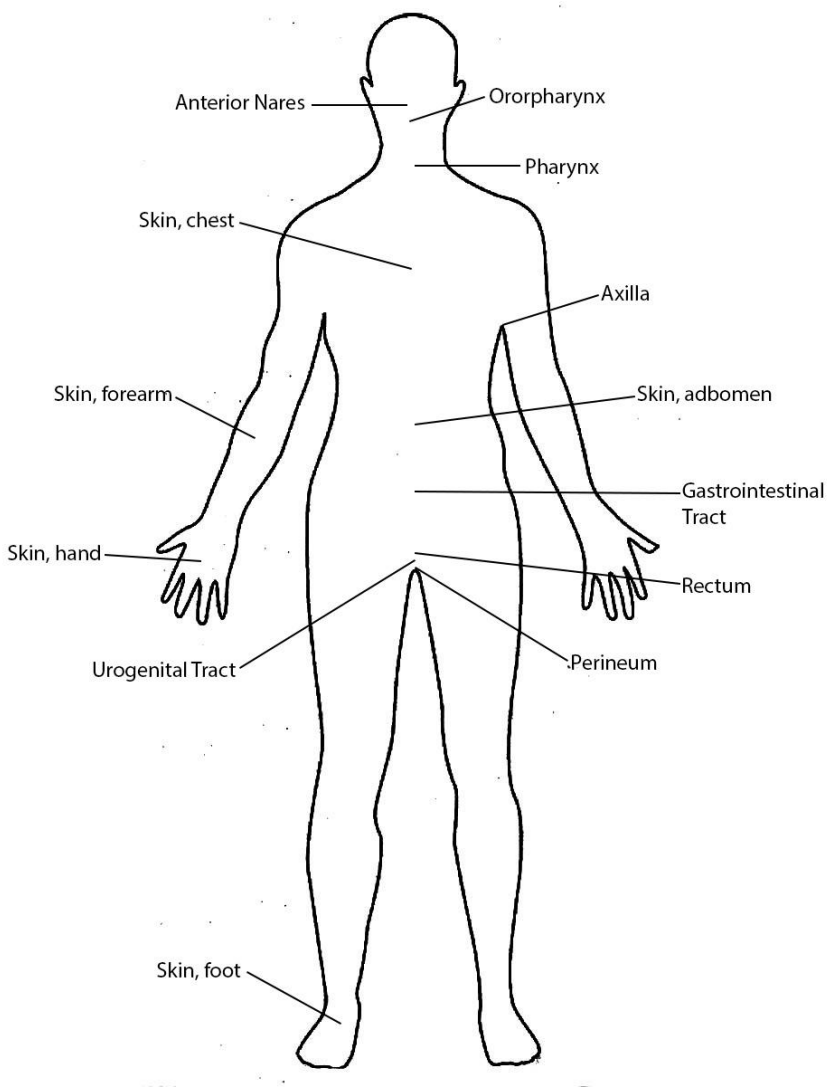

Figure 2-1: Anatomical sites known to harbor *S. aureus* in adults

CHAPTER 3: RESEARCH DESING AND METHODOLOGY

3.1 Study design

We conducted an epidemiological, cross-sectional study of 59 individuals – 30 participants with no livestock exposure (unexposed group) and 29 participants with livestock exposure (exposed group) living in Eastern Iowa between April 2015 and March 2016.

3.1.1 Enrollment

Participants were enrolled in one of four ways. The first enrollment method used was from a pre-existing cohort study of urban and rural Iowans. This cohort consisted of 95 family units (177 individuals over 18 years of age). One individual from each family was contacted by letter and then by phone call to schedule enrollment. If the original contact person for each family was either not interested or ineligible for participation, a letter was sent to the other members of the family unit until all eligible adults in the cohort were contacted. Only one individual from each family unit was eligible for participation, so if one adult from the family unit was enrolled, then all other adults in that family were considered ineligible and not contacted. Participants enrolled from the pre-existing cohort were both livestock workers and non-livestock workers. A detailed description of the pre-existing cohort – including inclusion and exclusion criteria – is provided in the appendix.

In addition to being enrolled from the pre-existing cohort, livestock workers were enrolled in three additional ways: snowball sampling, from Iowa County Fairs, and from the Iowa Department of Natural Resources (DNR) Animal Feeding Operations (AFO) Database.

3.1.2 Snowball sampling

In order to obtain additional livestock workers, we asked the already enrolled livestock workers if they would be interested in helping us reach out to other livestock workers they knew (who did not live in their household and did not work on the same operation). A letter along with a $5 Walmart gift card was sent to all enrolled livestock workers asking if they would be interested in passing information along to others with livestock contact. If the enrolled livestock worker responded yes, they were sent three packets including a study brochure, introduction letter, and response form. The enrolled workers did not have to inform the study team how many packets were handed out or to whom. Interested potential participants then called the study team to set up enrollment.

3.1.3 Iowa County Fair enrollment

A booth was set up at two Iowa County Fairs (Jones County and Johnson County). A study team member passed out information on the study to livestock workers attending the fair. Participants could both take an information packet and contact the study team at a later date, or could answer several eligibility questions and schedule an enrollment date while at the fair. We asked all interested persons to provide a contact name and phone number for a follow-up or reminder call.

3.1.4 Iowa DNR AFO database enrollment

The Iowa DNR AFO database is a list of all 8,000 regulated livestock and poultry facilities in the State of Iowa. There are three ways facilities can be included into the AFO database. The first is because they are a large operation required to obtain a permit from the state and/or a manure management plan. Secondly, operations can volunteer to

provide the state with information on their facility to be included in the database. The last way to be included is if the facility has had compliance issues reported to the state.

Three types of feeding operations are included in the database. The first are confinement feeding operations. Animals in these facilities are housed in buildings that are totally roofed. All operations in the state considered to be confinement facilities must retain all manure and file a manure management plan, thus all confinement operations are included in the AFO database. The second type of facility included are open feedlots. An open feedlot is an open roofed (or only partially roofed) facility with no ground cover or vegetation in the confined area. The third type of facility is a combined operation were some animals are housed in confinement and other in an open feedlot. All types of AFOs included are confined to a lot, yard, corral, or building for at least 45 days of the year. Livestock on pastures are not included in the database. The AFO database includes the contact information for each facility (name, phone number, and address) as well as the type and number of animals housed at the facility.

Operations were chosen from the DNR AFO database based on county. Individuals from Johnson, Linn, Keokuk, Washington, and Louisa Counties were contacted. Only one individual from each AFO system was eligible for enrollment. The contact person provided by the DNR was mailed an invitation letter and called to set up enrollment.

3.1.5 Inclusion and exclusion criteria

In order to be considered eligible to participate, participants must have been 18 years of age, able and willing to provide consent, and willing to fill out the necessary

questionnaires and provide nasal, oropharyngeal, and skin swabs (A separate arm of this study analyzed three skin sites. This data is not part of this thesis). Lastly, participants in the exposure group must have contact with livestock and participants in the unexposed group cannot have contact with livestock.

Participants were considered ineligible if they had taken antibiotics or inhaled corticosteroids in the past three months, have received the nasal influenza vaccine (FluMist) in the past month, had an active infection of any sampling site or the upper respiratory tract at the time of enrollment, were HIV positive, and/or had been hospitalized for longer than 24 hours in the last three months. We also requested participants not eat or drink within 60 minutes of sample collection.

In order to minimize excluding potential participants, any participant who had taken antibiotics or steroids, had the nasal influenza vaccine, had an active infection, or been hospitalized for more than 24 hours, was asked if they were willing to schedule the sampling visit at a later time when they would be eligible. In order to ensure participants did not consume food or beverages prior to sampling, we asked them not to eat or drink when scheduling their home visits and scheduled visits outside of meal times. Once we arrived at the participant's home, we waited to collect oropharyngeal samples until the end of the visit to help ensure one hour has passed since last eating or drinking.

Eligibility was determined on the initial phone call. All interested participants completed a brief survey assessing the eligibility criteria. All eligible participants were then asked if they would like to enroll in the study. If the participant was interested, the enrollment visit was scheduled at this time.

All participants were compensated $40.00 for participation. This study and all protocols were approved by the University of Iowa Institutional Review Board prior to enrollment.

3.2 Data collection and management

Study enrollment occurred in person at the participant's home. After the participant was consented into the study, he or she was asked to fill out a questionnaire containing questions regarding demographic characteristics, known and hypothesized risk factors for *S. aureus* colonization, history of *S. aureus* infections, and questions regarding their livestock contact (Table 3-1). If the participant worked with livestock, an additional questionnaire was filled out asking questions regarding what animals the participant had contact with, what type of work they performed at the plant/farm, how long they have worked with animals, the number of animals they have contact with on an average day, when their last contact was, the average number of hours they work with animals a week, hand hygiene, and personal protective equipment. Similarly, if a participant was a healthcare worker, he or she was asked to fill out a questionnaire on the type of healthcare facility they work in, type of work done on the job, patient contact, and hand hygiene. The enrollment questionnaire (Appendix B1), the livestock exposure questionnaire (Appendix B2), and the healthcare exposure questionnaire (Appendix B3) are provided.

Questionnaires were all filled out by the participant at time of enrollment. Following filling out the questionnaires, each participant provided two sets of swabs from their nares and oropharynx. All samples were collected by a trained researcher. All

samples were transported back the University of Iowa Center for Emerging Infectious Diseases (CEID) at the University Research Park for processing.

3.3 Laboratory methodologies

3.3.1 Sample collection

After the participant was consented and filled out the required questionnaires, the swabs were collected. All participants who consented to the study, had all samples collected. Sterile, dry, nylon-flocked swabs (Copan Diagnostics, Murrieta, CA) were used to collect both nasal and oropharyngeal swabs. Two sets of swabs were collected from each participant with the first set being used for 16s rRNA sequencing and the second for culture. For the anterior nares samples, one swab was inserted 2cm into the one nostril and rotated around the anterior nares mucosa four times. The swab was then removed and inserted into the other nostril and the process repeated and placed back into the tube and labeled with the participant's ID number and anatomical site. This was then repeated with the second swab. For the oropharyngeal swabs, the swab was inserted into the mouth – avoiding the lips, teeth, hard palate, and tongue – and rubbed against the oropharynx above the tonsils for 5 seconds (2.5 seconds on each side of the mouth). The swab was then removed, again avoiding all other anatomical structures of the mouth, and placed back into the tube and labeled with the participant's ID number and anatomical site (Figure 3-1). This process was then repeated using the second swab. Swabs were transported in a cooler back to the CEID where they were processed immediately.

3.3.2 Sample processing: samples collected for 16s rRNA sequencing

Upon arrival at the CEID, samples designated for 16s rRNA sequencing were processed immediately. Bacterial DNA was extracted using the MO BIO PowerSoil DNA isolation kit (MO BIO Laboratories Inc, Carlsbad, CA) adapted for swabs. Swab heads were left in the bead beating tube during the bead beating process. Negative controls (only kit reagents) were used in each batch of extractions. DNA extractions were stored at -80°C until sequencing. Extracted DNA was then quantified using a fluorescent nucleic acid assay on a Qubit (Life Technologies, Grand Island, NY). DNA with concentrations greater than 100ng/µL was diluted to 100ng/µL before being sent for sequencing. DNA with concentrations below the detectable range on the Qubit was concentrated using an ethanol precipitation. This was done by adding 5M NaCl, linear acrylamide, and 2.5 volumes 100% ethanol to the DNA. Samples were then centrifuged for 30 minutes at top speed. The ethanol was carefully aspirated off and the pellet was washed with 70% ethanol and centrifuged again for 3 minutes at top speed. The ethanol was aspirated off and the pellet was allowed to dry before being re-suspended in 30 µL Tris buffer. Samples that were either diluted or concentrated were then quantified a second time on the Qubit. Thirty microliters of sample was sent to the University of Minnesota Genomics Center for 16s rRNA sequencing (including library preparation). Sequencing of the V1-V3 region was done using 300 nt paired-end reads on the Illumina MiSeq.

DNA was normalized to 5ng/µL for amplicon polymerase chain reaction (PCR). This was followed by a PCR clean-up step using AMPure XP beads to remove and free primers and primer dimers in order to prepare for indexing. Index PCR was then done to

attach the dual indices and sequencing adapters using the Nextera XT Index kit followed by another PCR clean-up step and library validation. Fluorometry was used for library quantification followed by normalization and pooling. The library was diluted to 4 nM and 5 µl of diluted DNA was used for pooling. The library was then denatured (using NaOH and heat) and diluted to prepare for sequencing on the MiSeq using the v3 chemistry. Primer sequences (Table C-1) and cycling conditions can be found in Appendix C.

3.3.3 Sample processing: samples collected for *S. aureus* culture

Samples collected for *S. aureus* culture were brought back to the CEID and inoculated into Baird-Parker broth (BPB) and incubated for 24 hours at 37°C. Following incubation, all isolates were plated onto Baird-Parker agar (BPA) and incubated at 37°C for 48 hours. BP agar is a selective and differential medium for the isolation of *S. aureus*. Positive colonies appear dark gray/black, are shiny, and have clear halos surrounding them. These presumptive positive colonies were subcultured to Columbia CNA agar with 5% sheep blood (Becton, Dickinson and Company, Sparks, MD, USA) and incubated for 18-24 hours. The presumptive positive colonies were then confirmed as *S. aureus* using the catalase, coagulase, and Pastorex Staph Plus rapid latex agglutination assay (Bio-Rad, Redmond, WA, USA). Glycerol stocks of all positive isolates were made using a 1:1 TSB and glycerol mixture and stored at -80°C.

3.3.3.1 Antimicrobial Susceptibility testing (AST)

Phenotypic resistance to antimicrobials was assessed using minimum inhibitory concentration (MIC) testing according to the Clinical and Laboratory Standards

Institute's standards [172] on the VITEK 2. Isolates were tested for resistance to the following antimicrobials: benzylpenicillin (penicillin G), cefoxitin, oxacillin, gentamicin, ciprofloxacin, levofloxacin, moxifloxacin, erythromycin, clindamycin, quinupristin/dalfopristin, linezolid, daptomycin, vancomycin, minocycline, tetracycline, tigecycline, nitrofurantoin, rifampicin, and trimethoprim/sulfamethoxazole (Table 3-2). An isolate was classified as multi-drug resistant *S. aureus* (MDR-SA) if it was non-susceptible to ≥1 antimicrobial agent in ≥ 3 antimicrobial categories or was MRSA [173].

3.3.4 Molecular characterization of *S. aureus*

DNA was extracted from all *S. aureus* isolates using the Promega Wizard Genomic DNA purification kit (Promega Corporation, Madison, WI, USA) modified for *S. aureus* through the addition of lysozyme, mutanolysin, and lysostpahin to the cell lysis step. All extracted DNA was stored at -20°C.

All isolates were characterized using polymerase chain reaction (PCR). Strain typing was done by typing the Staphylococcal protein A (*spa*) gene and analyzed using Ridom Staph Type software (Ridom, GmbH, Germany). PCR was also be done to look for the presence of the *mecA* gene and the Panton-Valentine leucocidin (PVL) gene. Each gene is described in further detail below. All molecular procedure utilized positive and negative controls. A flow diagram of the laboratory methods is provided (Figure 3-2).

3.3.4.1 *spa* gene

The *spa* gene was amplified using the methods described by Shopsin et al. [174] using primers that have been validated for use with Ridom StaphType software. Amplified DNA was sequenced using Sanger sequencing at the University of Iowa

Institute Of Human Genetics. Strain typing was done by comparing the genetic sequence of the isolate to a database of known sequences using the Ridom StaphType software. The based upon repeat pattern (BURP) algorithm was used to identify genetic clusters [171].

3.3.4.2 *mecA* gene

Amplification of the *mecA* gene was done following the protocol used by Bosgelmez-Tinaz et al. [175]. The presence of amplified product was determined through the use of agarose gel electrophoresis. While the presence of the *mecA* gene does not mean the gene is functional, any isolate containing the *mecA* gene is designated as MRSA. This is because the gene can be shared with other bacteria regardless of if it is expressed or not.

3.3.4.3 PVL genes

The *lukS-PV* and *lukF-PV* genes were co-amplified using PCR as described by Lina et al.[176]. Agarose gel electrophoresis was used to determine the presence or absence of the gene.

3.4 Data management and analysis

3.4.1 Data management

Upon return to the CEID, all consent forms and questionnaires were stored in a locked file cabinet. All questionnaire data was entered into the REDCap database housed on the University of Iowa secure servers. All subjects were assigned unique identification numbers to ensure confidentiality. All sequencing data was stored on a password-protected hard drive.

3.4.2 Data analysis

Once sequencing is complete, raw data files was downloaded from a secure server (BaseSpace, Illumina). Once data is obtained from the University of Minnesota, data was assessed for quality and poor quality data was filtered out (poor quality sequencing reads are defined as sequences with low base quality scores, short reads less than 200bp, reads with uncalled nucleotide bases, or any reads that could not assemble into paired reads). FastQC (Babraham Institute, Cambridge, UK) was used to asses sequence quality. Reads were assembled using FLASh with the following parameters: minimum overlap = 30, maximum overlap = 150, and mismatch = 0.1 [177]. Adapters were removed from the merged file using Cutadapt [178]. USEARCH version 8.1.1861 and Python version 2.7.12 were used for chimera removal, operational taxonomic unit (OTU) creation, and taxonomy assignment at the genus level. The Ribosomal Database Project (RDP) classifier was used as the reference database. OTUs were grouped together based on 97% similarity. Species level classification was done for all *Staphylococcus*- associated OTUs as well as several of the differentially represented OTUs using BLAST+2.4.0 and the blastn function. Human-associated OTUs were also removed from the dataset using BLAST+2.4.0 and the blastn function. R version 3.3.1 was used for all statistical analyses and plot generation using the following packages: phyloseq [179], vegan [168], DESeq2 [180], and ampvis [181]. PERMANOVA, through the vegan package, was used to assess diversity differences between groups. PERMANOVA was chosen over ANOVA or MANOVA because parametric test assume the counts of abundances of OTUs to conform to a normal distribution which is untrue due to the rare species which skew the

data [182]. PERMANOVA assumes no distribution and can account for the Bray-Curtis distances as described in section 2.10.5. The DESeq2 and ampvis packages were used to assess microbiota differences between groups. Results were considered significant if the p-value was less than 0.05.

3.4.3 Aim 1 statistical analysis

Aim 1 is to characterize the nasal and oropharyngeal microbiomes of livestock workers and compare it to the microbiomes of those without livestock exposure. The main exposure of interest is livestock exposure (dichotomous, yes/no). The main outcome of interest is the microbial composition of the anterior nares and oropharynx of those with and without livestock contact (continuous).

Alpha diversity was calculated to measure the amount of diversity within each sample using the Inverse Simpson diversity index. Beta diversity was calculated to measure the diversity across all samples using the Bray-Curtis method. PCoA was used to visualize beta diversity. PERMANOVA was used to determine if there was a significant difference in the beta diversity between the two groups (livestock workers and non-livestock workers). The DESeq2 and ampvis packages in R were used to determine if any OTUs were differentially abundant between livestock workers and non-livestock workers.

3.4.4 Aim 2 Statistical analysis

The goal of aim 2 is to determine if there are differences between the microbiomes of those individuals between *S. aureus* carriers and non-carriers in either or both the anterior nares and oropharynx. The main exposure of interest is *S. aureus*

colonization of the nares or oropharynx (dichotomous, yes/no). The main outcome of interest is the microbial composition of the anterior nares and oropharynx (continuous).

Again, alpha diversity was calculated to measure the amount of diversity within each sample using the Inverse Simpson diversity index. Beta diversity was calculated to measure the diversity across all samples using the Bray-Curtis method. This was done for the nares samples and then for the oropharynx samples because an individual is more likely to be colonized in both locations if they are already colonized in one location. PCoA was used to visualize beta-diversity. PERMANOVA was used to determine if the beta diversity between those with *S. aureus* and those without *S. aureus* was significantly different.). The DESeq2 and ampvis packages in R were used to determine if any OTUs were differentially abundant between livestock workers and non-livestock workers.

3.4.2.1 Aim 2.1 Statistical analysis

Aim 2.1 takes aim 2 a step further to determine if there are microbiota differences between *S. aureus* carriers with livestock exposure compared to carriers without livestock exposure and if there are microbiota differences between non-carriers with livestock exposure compared to those non-carriers without livestock exposure. *S. aureus* colonization in the nares and oropharynx stratified by livestock exposure status is the main exposure of interest (dichotomous, yes/no). The microbial composition of the anterior nares and oropharynx is the main outcome (continuous).

As with the prior aims, the alpha diversity was calculated to measure the amount of diversity within each sample using the inverse Simpson diversity index. Beta diversity was calculated to measure the diversity across all samples using the Bray-Curtis method.

PCoA was used to visualize diversity measures. PERMANOVA was used to determine if the beta diversity between those carriers with livestock contact and those without livestock contact are significantly different and then again for the non-carriers. The DESeq2 and ampvis packages in R were used to determine if any OTUs were differentially abundant between livestock workers and non-livestock workers.

3.4.2.2 Aim 2.2 Statistical analysis

The last aim is to determine if the diversity of bacteria in the nares and oropharynx of participants differs by the individual's prior colonization status. This aim was done only on the population enrolled from the longitudinal cohort study, as there is one year's worth of weekly colonization status available for these participants. This was used to determine if the participant is historically a non-carrier, intermittent carrier, or persistent carrier. The main outcome of interest is the diversity of the micro-organisms in the nares and oropharynx of these individuals.

Alpha diversity was calculated to measure the amount of diversity within each sample using the Inverse Simpson diversity index. Beta diversity was calculated to measure the diversity across all samples using the Bray-Curtis method. PCoA was used to visualize beta-diversity. PERMAANOVA was used to determine if there are differences in the core microbiomes by prior colonization status. Prior colonization status was broken down to persistent colonizers vs. non- or intermittent colonizers. The DESeq2 and ampvis packages in R were used to determine if any OTUs were differentially abundant between livestock workers and non-livestock workers.

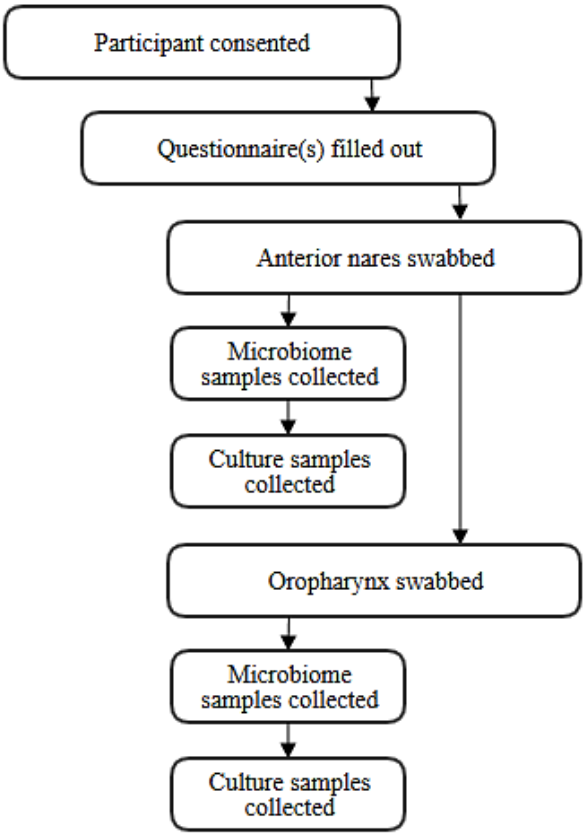

Figure 3-1: Flow chart of enrollment procedures and sample collection

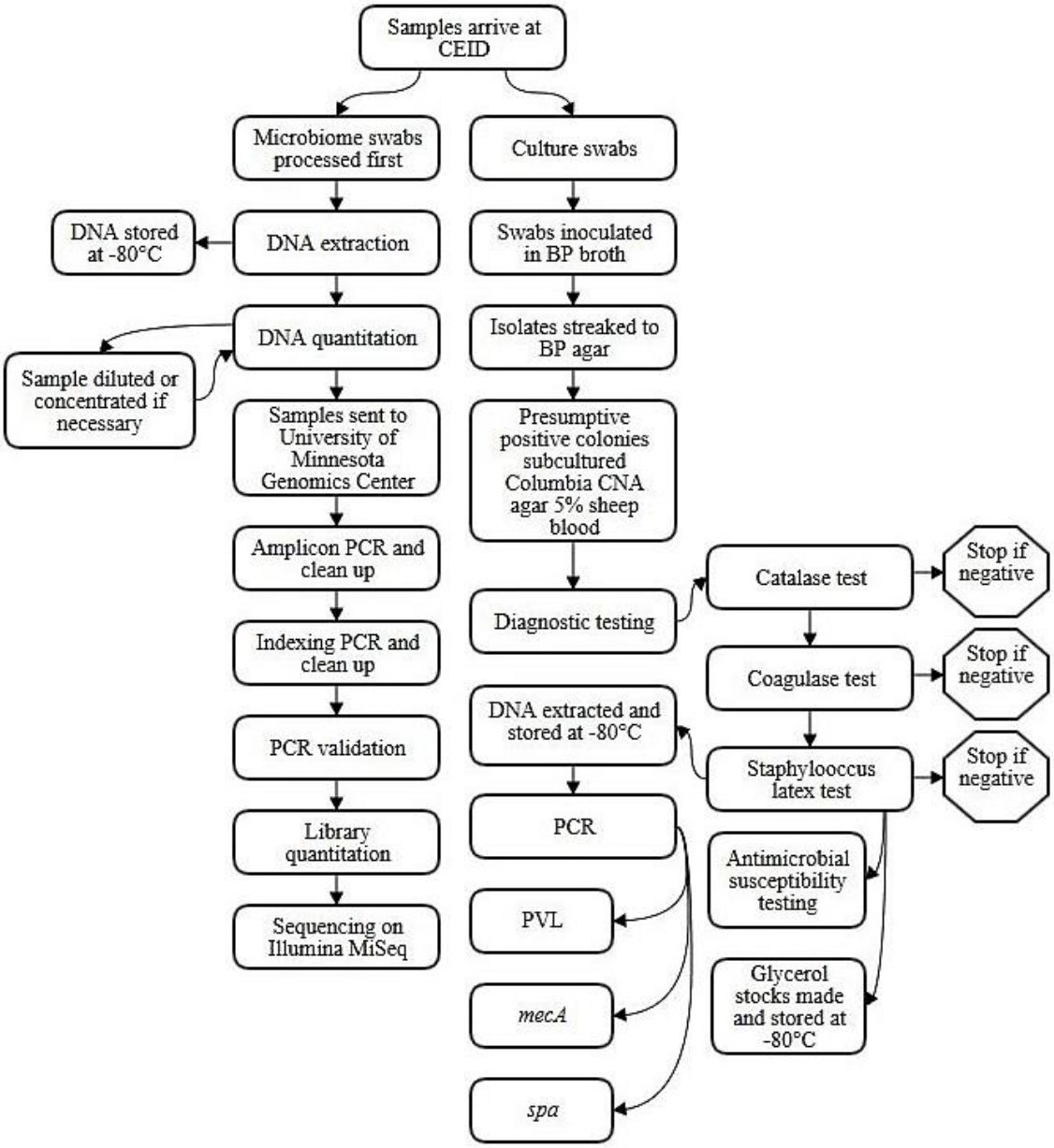

Figure 3-2: Flow diagram of laboratory procedures.

Table 3-1: Variables collected on the participant questionnaires

Covariate	Description	Rationale	Citation
Age	Participant's age. Continuous	Increasing age is associated with a decrease in *S. aureus* colonization.	[8, 183]
Gender	Participant's biologic gender. Dichotomous (male / female)		[8, 183]
Recent hospitalization	Hospitalization in the last 3 months, dichotomous (yes/ no).	Hospitalization is a known risk factor for *S. aureus* and a risk factor for an altered microbiome	[8, 183-186]
Antibiotic usage	Did the participant take antibiotics (oral or intravenous) in the prior 3 months? Dichotomous (yes/ no)	Exclusion criteria. Antibiotic usage is known to temporarily change the relative abundance of bacteria in the microbiome	[187, 188]
Nasal influenza vaccine	Did the participant receive the nasal influenza vaccine in the past month? Dichotomous (yes/no)	Exclusion criteria. The nasal influenza vaccine may temporarily alter the microbiome of the nares	
Current infections	Does the participant have an active infection of the nose, throat, or sinus cavity? Dichotomous (yes/no)	Exclusion criteria. Active infections of these locations may temporarily alter the microbiome of the infection site.	
Use of gym facility	Use of a gym – or fitness center. Dichotomous (yes/ no)	Known risk factor for *S. aureus* colonization.	

Table 3-1: continued

Covariate	Description	Rationale	Citation
Play team sports	Participant is involved in team sports. Dichotomous (yes/no)	Possible risk factor for *S. aureus* colonization.	[189, 190]
Sharing hand and/or bath towels	Do participants share their hand towels or bath towels with other individuals? Dichotomous (yes/no)	Sharing of bath and hand towels is a hypothesized risk factor for *S. aureus* colonization as well as an altered microbiome.	[191, 192]
Working in healthcare	Does the participant work or volunteer in a healthcare facility (dichotomous, yes/no) If yes, what type of facility? (categorical by type of facility - large or small hospital, out patients clinic, dentist office, etc.) If yes, do they have patient contact? (dichotomous, yes/no)	Healthcare settings are known risk factors for *S. aureus* colonization and are hypothesized to impact the microbiome.	
Skin conditions	Does the participant have skin conditions, such as eczema? Dichotomous (yes/no) If yes, what condition?	Skin conditions may put individuals at risk for *S. aureus* infections	[8]

Table 3-1: continued

Covariate	Description	Rationale	Citation
Immunosuppressive conditions	Does the participant have an immunosuppressive condition such as cancer or diabetes? Dichotomous (yes/ no) If yes, what condition?	Known risk for *S. aureus* colonization and altered microbiome	[8]
HIV/AIDS	Does the participant have HIV or AIDS Dichotomous (yes/no)	Exclusionary criteria. HIV/AIDS is likely to heavily alter the composition of the microbiome	[193]
Current tobacco usage	Does the participant smoke tobacco? Dichotomous (yes/ no) Does the participant chew tobacco? Dichotomous (yes/ no)	Smoking is hypothesized to impact *S. aureus* colonization and is known to alter the oral microbiome. Chew tobacco is hypothesized to impact the oral microbiome	[194]
Past tobacco usage	Has the participant ever smoked tobacco? Dichotomous (yes/ no) Has the participant ever used chew tobacco? Dichotomous (yes/ no)	Smoking is hypothesized to impact *S. aureus* colonization and is known to alter the oral microbiome. Chew tobacco is hypothesized to impact the oral microbiome	[194]

Table 3-1: continued

Covariate	Description	Rationale	Citation
Probiotics	Has the participant used probiotics in the past 3 months? Dichotomous (yes/no)	Probiotic usage is hypothesized to help maintain a healthy microbiome, though this is contentious	[195]
Teeth brushing habits	How frequently does the participant brush their teeth? Categorical	The authors hypothesize the frequency of tooth brushing may alter the oral microbiome	[196]
Dentures	Does the participant wear dentures? Dichotomous (yes/no)	Wearing dentures is hypothesized by the authors to possibly influence the oral microbiome	[197]
Type of animal (livestock) contact	What type of animal does participant have contact with? Categorical, (Cattle, swine, poultry, goat, horse, sheep, other)	Animal contact is a known risk factor for *S. aureus* carriage. We hypothesize it may also impact the composition of the microbiome	[76, 79, 198, 199]
Number of animals	The number of animals a participant has contact with in a normal week. Continuous (count)	It is hypothesized working with more animals increases the risk of *S. aureus* carriage and will impact composition of the microbiome.	[200]

Table 3-1: continued

Covariate	Description	Rationale	Citation
Duration of time spent with animals in a week	How much time does the participant spend with livestock during a typical week? Continuous	Longer durations of time spent with animals may increase risk of *S. aureus* colonization.	[201]
Type of work performed with livestock	What type of work does the participant do on the farm? Categorical	It is hypothesized individuals with more contact with animals in their duties or are involved in dirtier tasks may have altered microbiomes	[201]

Table 3-2: Antimicrobial agents included in AST by antimicrobial category

Antimicrobial category	**Antimicrobial agent**
β-lactams (cephamycins)	Cefoxitin
	Benzylpenicillin (penicillin G)
	Oxacillin
Aminoglycosides	Gentamicin
Ansamycins	Rifampicin
Fluoroquinolones	Ciprofloxacin
	Levofloxacin
	Moxifloxacin
Glycopeptides	Vancomycin
Glycylcyclines	Tigecycline
Lincosamides	Clindamycin
Lipopetides	Daptomycin
Macrolids	Erythromycin
Oxazolidinones	Linezolid
Streptogramins	Quinupristin/ dalfopristin
Folate synthesis inhibitors	Trimethoprim/ sulfamethoxazole
Tetracyclines	Tetracycline
	Minocycline
Nitrofurans	Nitrofurantoin

CHAPTER 4: RESULTS

4.1 Study population and demographics

4.1.1 Enrollment

Potential participants were recruited in four ways as described in section 3.1. The first way participants were contacted was through the preexisting cohort. One hundred and six participants were contacted from this cohort and 38 were subsequently enrolled (35.8%). Four participants were contacted through attending the state fairs and three were enrolled (75%). One-hundred and fifty-eight individuals from the Iowa Department of Natural Resources Animal Feeding Operations (DNRs AFO) database were contacted and 17 were enrolled (10.8%). Lastly, we provided two enrolled participants with four packets each to assist with snowball sampling and enrolled one participant through this method (assuming all packets were distributed, 12.5%). Overall, 276 persons were contacted for participation in this study. Ninety participants responded to the enrollment letters providing a response rate of 32.6%. Of the 90 responses, 18 were not interested in participation. Of those not interested, six provided a reason for not wanting to participate. One participant was deceased, three did not have the time to participate, and 2 no longer worked with livestock and as such were not interested in a study investigating livestock exposure. Of the remaining 72 interested persons, 59 were eligible for participation and enrolled into the study providing a participation rate of 21.4% (59/276) of those contacted and 65.6% (59/90) of those who were initially interested. The most common reason for an interested person not being eligible was due to recent antibiotic usage (n=8) followed by tobacco usage (n=3) and cancelling the enrollment visit (n=1). One participant from

the AFO database was also ineligible due to no longer working with livestock. A description of the population can be found in Figure 4-1.

4.1.2 Participant demographics and behaviors

The average age of participants was 54.6 years (range: 28-85 years). Livestock workers were significantly older at 59.1 years compared to non-livestock workers (51.1 years), P=0.027. Forty-one (69.5%) of the fifty-nine participants were males. Livestock workers were predominantly male (n=24, 92.3%) while males only made up 51.5% of the non-livestock workers (n=17) (P = 0.0007) (Tables 4-1). Those without livestock contact were also more likely to brush their teeth daily compared to livestock workers (P <0.001) and more likely to use liquid hand soaps (P <0.001). Livestock workers were more likely to share hand towels within their household compared to non-livestock workers (P < 0.001) (Table 4-2). Lastly, those without livestock contact were more likely to use a gym (0.011) (Table 4-3). There were no other significant differences between those with and without livestock contact.

4.1.3 Livestock exposure

Twenty-six participants had current exposure to livestock. A majority of participants with livestock exposure, worked with swine (n=18). Twelve participants worked with cattle, four with poultry, four with sheep, two with horses, and one with goats (Table 4-4). Several participants currently worked with more than one type of animal with seven participants working with two animal types, two working with three animal types, and one participant working with five animal types (swine, poultry, cattle,

sheep, goats, and horses). The most frequent combination of animal types was swine and cattle (n=4).

4.1.3.1 Contact with swine

As described in Table 4-4, 18 participants had contact with swine. The average number of animals a participant had contact with was 3,024 pigs with a range of 8 to 10,000 pigs. On average, participants worked with swine for 2.5 hours a day (range: 0.5-10 hours), six days a week (range: 2-7 days). The average amount of time participants had been working with swine was 40 years (range: 5-65 years). On average, it had been 30.3 hours since the participant had contact with swine at the time of swabbing (range: 1 hr. – 2 wks., median: 3 hr.).

Swine workers reported a wide range of duties they perform with swine. Seventeen (94.4%) of swine workers reported working on a finishing facility, 13 (72.2%) on a wean-finish facility, 11 (61.1%) working with nursery pigs, one (5.6%) participant working with breeding swine and one (5.6%) with farrowing swine. The most frequently reported activity was cleaning and disinfecting equipment and areas exposed to swine, swine products, or swine waste (n=14, 77.8%) and transporting swine (n=14, 77.8%). The second most frequently reported activities were cleaning swine barns and/or trucks (n=13, 72.2%) and disposing of swine waste (n=13, 72.2%) followed by examining and treating swine (n=12, 66.7%). Eight participants (44.4%) reported being responsible for administrative duties, but still entering areas where swine, raw pork products, and/or swine waste was. Other duties reported were collecting blood or other specimens from

the swine (n=2, 11.1%), packaging cooked (n=3, 16.7%) or raw (n=2, 11.1%) pork products, and slaughtering/butchering swine (n=2, 11.1%).

4.1.3.2 Contact with cattle

Twelve participants reported working with cattle (11 on a beef or dairy farm and one in a beef processing plant). The average number of cattle a participant had contact with was 191 (range: 4-850 cows). On average, cattle farmers spent 1.6 hours a day (range: 0.5-3 hrs.), 6.4 days a week (range: 1-7 days) in contact with cattle (Table 4-4). On average, it had been 24.7 hours since the participant had contact with cattle at the time of swabbing (range: 1 hr. - 7 days, median: 5 hours).

The most frequently reported activity was feeding cattle (n=11, 91.7%) followed by disposing of cattle waste (n=9, 75%) and examining and treating cattle (n=9, 75%). Other frequently reported activities were weaning cattle (n=8, 66.7%) transporting cattle (n=8, 66.7%), cleaning cattle barns and trucks (n=8, 66.7%), working with cattle (vaccinating, castrating, deworming, and dehorning; n=8, 66.7%), cleaning and disinfecting equipment and areas exposed to cattle, cattle products, or cattle waste (n=7, 58.3%) and calving (n=7, 58.3%). Four cattle workers reported breeding cattle (33.3%). Three participants reported collecting blood and other specimens (25%). Other activities reported were packaging cattle products (n=2, 16.7%), slaughtering cattle (n=1, 8.3%), milking cattle (n=1, 8.3%), and administrative duties (n=1, 8.3%).

4.1.3.3 Contact with poultry

Four participants reported working with poultry. The average number of poultry a participant had contact with was 1644 (range: 20-6500 chickens). On average, poultry

farmers spent 1 hour a day (range: 0.25-3 hrs.), seven days a week in contact with poultry (Table 4-4). On average, it had been 1.5 hours since the participant had contact with poultry at the time of swabbing (range: 1 hr. - 2hrs, median: 1.5 hours).

All participants with poultry contact reported cleaning chicken houses, disposing of chicken waste, and cleaning and disinfecting areas exposed to chickens, chicken products, or chicken waste. Two participants (50%) reported gathering eggs, packaging raw and cooked poultry products, examining and treating chickens, and gathering blood and other specimens. One participant (25%) reported slaughtering chickens and one (25%) reported transporting chickens.

4.1.3.4 Other animal contact

Other animals' participants had contact with were sheep (n=4), horses (n=2) and goats (n=1). Those who worked with sheep had contact with an average of 28 sheep (range: 10-50) and worked with them for 1.4 hours a day (range: 0.25-3) for 6.75 days a week (range: 6-7 days). The two participants that had contact with horses spent between 3 and 8 hours with them 6-7 days a week. One of the participants had contact with 12 horses and the other with only one horse. One participants reported having contact with ten goats and spent 2 hours a day, 7 days a week with them.

4.1.4 Healthcare exposure

Eight participants (13.6%) reported either working or volunteering in a human healthcare setting, four of which were in a large hospital, two in a small hospital, one in a public health lab, and one as a school nurse. Four healthcare workers were nurses, one was a physical therapist, one a lab scientist, one worked as a hospital administrator with

no patient contact, and one was a volunteer. One healthcare worker also had livestock exposure. This participant reported working as a nurse with direct patient care who had contact with MRSA positive patients a few times per week. This participant also had contact with swine (n=8), poultry (n=38), and sheep (n=10). The participant had no contact with the live swine and only handled packaging the raw pork products. The participant had regular contact with live chickens as well as live sheep and was responsible for caring for the animals.

On average, healthcare workers had been working in healthcare for the past 18.9 years (range: 5- 45 years) and in their current position for an average of 13 years (range: 1-36 years). Six of the participants (75%) reported having direct patient contact. The average number of patient participants had contact with on a typical day was 18.2 (range: 4-40 patients). It had been an average of 64.7 hours (range: 2 hours – 1 week, median: 36 hours) since the participant's last contact with patients at the time of swabbing. Healthcare workers also reported working with patients an average of 6.4 hours a day (range: 3- 8 hours), 3.5 days per week (range: 3-5 days per week). Five of the healthcare workers with direct patient contact reported directly caring for patients with MRSA. Additionally, one participant reported having contact with MRSA specimens in a laboratory setting six months ago.

4.2 Summary analysis of 16s rRNA sequencing

One thousand six hundred and eighty-two operational taxonomic units (OTUs) were identified in the dataset. The majority of OTUs belonged to the Firmicutes phylum (n=574, 34.1%) followed by Proteobacteria (n=324, 19.3%), Bacteroidetes (n=296,

17.6%), and Actinobacteria (n=293, 17.4%). Twenty-seven OTUs were unclassified bacteria (1.6%). Figure 4-2 depicts the top 98% most abundant phyla. The mean sequencing depth (number of reads) per sample was 30,310 with a range of 1,579 to 64,700 (Figure 4-3).

4.2.1 Negative controls

Negative controls were sequenced to assess if contamination was present in any of the laboratory reagents. The alpha diversity of the samples by sample type (nasal, oropharyngeal, and negative control) is shown in Figure 4-4a. PCoA of the Bray-Curtis distances are shown in Figure 4-4b. Negative controls clustered separately from the nose and throat samples ($P < 0.001$). One hundred and thirty-six OTUs were identified in the negative controls (Figure 4-5). A barplot of the relative abundances can be found in Figure 4-6.

4.3 Analysis of Aim 1: the livestock worker microbiome

The Inverse Simpson diversity index (Figure 4-7) was greater for those with livestock contact compared to those without livestock contact in the nasal samples ($P > 0.001$); however, there was no difference in alpha diversity between those with and without livestock contact in the oropharyngeal samples ($P = 0.542$). The ordination plot of the Bray-Curtis distances for all samples is shown in Figure 4-8. The samples primarily cluster by sample type ($P = 0.001$), but also by livestock exposure ($P = 0.038$, p-value for the interaction between sample type and livestock exposure = 0.035). Because samples cluster by both livestock exposure and sample type, ordination was done to assess the nasal samples (Figure 4-9a) and oropharyngeal samples (Figure 4-9b)

separately. When considering only the nasal samples, there is still a significant difference between those with livestock exposure compared to those without livestock exposure (P = 0.002). However, there is no significant difference for the oropharyngeal samples (P = 0.559). Figure 4-12 depicts the differences in relative abundance of OTUs based between those with livestock contact and those without. As depicted in Figure 4-12, there are a large number of OTUs with greater abundancy in the nasal samples with livestock contact compared to those without livestock contact. Unlike the nasal samples, there is a great deal of similarity between the relative abundances of OTUs in those with and without livestock exposure in the oropharyngeal samples (Figure 4-13).

4.3.1 Differences in animal type

Animal type was grouped into four categories for analysis: cattle, poultry, swine, or contact with more than one type of animal. There was no difference in the Inverse Simpson diversity index by animal type in either the nares (P = 0.762) or the oropharynx (P = 0.941) (Figure 4-10). In the nares, there are no easily discernable clusters in the PCoA plot (Figure 4-11a); however, there is a statistical difference between the animal types (P = 0.009). In the oropharynx, there are no differences between groups (P = 0.297) (Figure 4-11b).

4.3.2 Livestock nasal microbiome

The relative abundances of all phyla present in the nares of participants are depicted in Figure 4-14 by livestock status. Actinobacteria and Firmicutes were the most prevalent phyla in both the livestock workers and non-livestock workers. Bacteroidetes were more abundant in the livestock workers. Figure 4-16 further depicts the most

prevalent OTUs in the nasal microbiome for both the livestock workers and non-livestock workers. The greatest differences were seen with *Streptococcus, Proteobacteria, Peptoniphilus, Streptophyta,* and *Prevotella*. A total of 26 OTUs were differentially represented between the livestock workers and non-livestock workers, 25 of which were significantly more abundant in those with livestock contact. Only two OTUs belonging to the *Streptophyta* genus were more abundant in the non-livestock workers (Figure 4-17). The 2-fold change values and Benjamini-Hochberg adjusted p-values for each significantly differentially abundant OTU can be found in Table D-1.

4.3.2.1 The nasal microbiome and animal type

The 15 most abundant genera in the livestock workers by animal type are shown in Figure 4-18. Unsurprisingly, the abundances of genera for the livestock workers with contact with more than one type of animal tend to overlap with the abundances for the other animal types. *Corynebacterium* and *Staphylococcus* were the most abundant genera with members of the Firmicutes phylum being the most abundant. When comparing swine workers to those with any other animal contact, there were 45 OTUs differentially abundant between those with and without swine contact, nine of which were more abundant in those without contact with swine (Figure 4-19, Table D-2). The majority of OTUs belonged to the Firmicutes phylum with *Clostridium sensu stricto* and *Prevorella* were the most prevalent genera. The most significantly differential OTU observed in those with swine contact belonged to *Clostridium sensu stricto* (2-fold change: 11.94, P = 7.63E-18). For those without swine contact, *Actinomyces* was the most significantly differential (2-fold change: -7.07, P = .00458).

4.3.3 Livestock oropharyngeal microbiome

As was observed in the nasal microbiome, OTUs belonging to the Firmicutes and Proteobacteria phyla were most abundant (Figure 4-15). Unlike the nasal microbiome, there is a great deal of similarity between those with and without livestock contact. There were no OTUs that were significantly differentially abundant between the livestock workers and those without livestock contact in the oropharyngeal microbiome. The top 15 most abundant genera are shown in Figure 4-20. The *Streptococcus* genera was the most prevalent genera observed in the oropharynx followed by *Provetella* and *Heamophilus* genera. Though not significantly different, the genus *Neisseria* was the most different between livestock workers and those without livestock contact.

4.3.3.1 The oropharyngeal microbiome and animal type

The 15 most abundant genera in the livestock workers by animal type are shown in Figure 4-21. As with the nares, the abundances of genera for the livestock workers with contact with more than one type of animal tend to overlap with the abundances for the other animal types. *Streptococcus* and *Prevotella* were the most abundant genera. Unlike the nares, there are a wider variety of phyla present in the oropharynx. There were nine OTUs significantly more abundant in the swine workers compared to those with all other animal types in the oropharynx and two *Lactobacillus* OTUs with increased abundance in those with no swine contact (Figure 4-22, Table D-3).

4.3.4 The livestock worker microbiome and participant behaviors

We assessed if any of the participant behaviors differed between the livestock workers and the non-livestock workers in Table 4-2 (type of soap used, tooth brushing

habits, and shared hand towels) had an impact on the microbiome. There were no significant differences in either the Inverse Simpson diversity index or the Bray-Curtis distances for any of the variables. For the type of soap used (modeled as antibacterial soap, not antibacterial soap, or using both types), the p-value for the Inverse Simpson diversity index was 0.232 in the nares and 0.221 in the oropharynx. The p-value for differences in the Bray-Curtis distances was 0.564 in the nares and 0.833 in the oropharynx. For the frequency of tooth brushing (modeled as daily, every morning, every evening, most days, some days, and never), the p-value for the Inverse Simpson diversity index was 0.80 in the nares and 0.187 in the oropharynx. The p-value for differences in the Bray-Curtis distances was 0.188 in the nares and 0.569 in the oropharynx. For shared hand towels (modeled as shared, not shared, or unsure), the p-value for the Inverse Simpson diversity index was 0.607 in the nares and 0.371 in the oropharynx. The p-value for differences in the Bray-Curtis distances was 0.973 in the nares and 0.410 in the oropharynx.

4.4 Analysis of Aim 2: the microbiome and *S. aureus* carriage

The Inverse Simpson diversity index was not significantly different between the colonized persons and non-colonized persons ($P = 0.144$) (Figure 4-23). There was also no significant difference in alpha diversity between colonized persons and non-colonized persons in either the nares ($P = 0.376$) or the oropharynx ($P = 0.728$). The ordination plot of the Bray-Curtis distances for all samples is shown in Figure 4-24. The samples cluster by both colonization status ($P > 0.001$) and sample type ($P > 0.001$). When considering only the nasal samples, the significant difference between the colonized and non-

colonized persons remained (P = 0.002) (Figure 4-25a); however, there was no difference between the colonized and non-colonized clusters in the oropharyngeal samples (P = 0.899) (Figure 4-25b).

Figure 4-26 shows the relative abundances of all OTUs in the colonized and non-colonized persons in the nares. There is a great deal of similarity between colonized and non-colonized person's microbiota in the nasal samples, though there are several OTUs that are abundant in a greater number of colonized samples. The oropharyngeal microbiomes of colonized persons and non-colonized persons look very similar (Figure 4-27). Figure 4-28 further depicts the nasal microbiome differences between colonized and non-colonized persons. OTUs belonging to the Firmicutes and Actinobacteria phyla dominate the microbiomes of the colonized and non-colonized persons; however, colonized persons have a greater amount of OTUs belonging to the Firmicutes phylum compared to the non-colonized persons. While the oropharyngeal microbiomes are very similar between colonized and non-colonized persons, there is more diversity than seen in the nasal microbiomes. OTUs belonging the Firmicutes phylum are also the most prevalent in the oropharyngeal samples (Figure 4-29).

4.4.1 *S. aureus* carriage and the nasal microbiome

The only significantly different genus between the colonized and non-colonized microbiomes in the nasal microbiome, was *Staphylococcus*. However, this was the *S. aureus* OTU. While the other *Staphylococcus* species were not significantly more abundant in the colonized persons, colonized persons did have more *Staphylococcus* OTUs compared to those not colonized with *S. aureus*. Other *Staphylococcus* species

present were *S. epidermidis*, *S. cohnii*, *S. lentus*, *S. hominis*, *S. lugdunensis*, and *S. massiliensis*. *S. epidermidis* was the most prevalent non-*aureus* S*taphylococcus* species and was present in 100% (n=30) of the *S. aureus* colonized nasal samples compared to 89.6% (n=26) in the non-colonized samples. *S. cohnii* was the second most prevalent at 76.7% (n=23) of *S. aureus* colonized persons and 62.1% (n=18) of non-colonized samples. *S. homini* was prevalent in 60% (n=18) of colonized persons and 55.2% (n=16) of non-colonized persons. The largest difference between colonized and non-colonized persons was with *S. lentus* at 20% (n=6) in the colonized compared to 6.9% (n=2) in the non-colonized. Colonized persons also had slightly higher amounts of Proteobacteria (*Haemophilus* and an unclassified genus), Actinobacteria (*Rothia*), and Bacteroidetes (*Prevotella*). Non-colonized persons had higher amounts of Actinobacteria (*Corynebacterium*) and Cyanobacteria/Chloroplast (*Streptophyta*) compared to the colonized (Figure 4-30a).

4.4.2 *S. aureus* carriage and the oropharyngeal microbiome

There were no OTUs significantly different between the colonized and non-colonized persons in the oropharynx. Many of the most abundant OTUs were similarly abundant in both the colonized and non-colonized persons. *Saccharibacteria genera incertae sedis* was slightly more abundant in the oropharynx of colonized persons. Bacteroidetes (*Porphyomonas)* was slightly more abundant among non-colonized persons (Figure 4-30b). Of the *Staphylococcal* species, *S. epidermidis* was the most prevalent and was in 100% (n=7) in the oropharynx of colonized persons compared to 35.6% (n=21) of

the non-colonized persons. *S. cohnii* was present in the oropharynx of 42.9% (n=3) of the colonized persons and 28.6% (n=2) of the non-colonized persons.

4.4.3 *S. aureus* carriage and participant behaviors

We hypothesized sharing of hand towels would impact the composition of the nasal microbiome by colonization status and the frequency of tooth brushing would impact the microbiome by colonization status in the oropharynx. However, there were no significant differences between colonized and non-colonized persons for either behavior for either the Inverse Simpson diversity index of the Bray-Curtis differences.

4.5 Analysis of Aim 2.1: *S. aureus* carriage and livestock exposure

The Inverse Simpson diversity index was significantly greater for livestock workers in the nares ($P = 0.0015$) though not different in the oropharynx ($P = 0.571$); however, there was no difference in livestock exposure based on colonization status in either the nares ($P = 0.815$) or the oropharynx ($P = 0.484$) (Figure 4-31). The ordination plots of colonization status and livestock exposure in the nares show the samples significantly cluster by colonization status ($P = 0.001$) and livestock exposure ($P = 0.001$) (Figure 4-32a); however, there are no significant clusters in the oropharyngeal samples (Figure 4-32b). When considering only colonized persons, samples associated with livestock exposure clustered separately from those without livestock exposure ($P = 0.004$). Samples associated with livestock exposure cluster together in the non-colonized persons ($P = 0.021$) (Figure 4-33).

The heatmap of the nasal samples shows a greater abundancy of OTUs for the livestock workers compared to non-livestock workers for both the colonized and non-

colonized person with the greatest abundancy in the colonized livestock workers (Figures 4-34 and 4-35). There microbiomes of the oropharyngeal samples were very similar between the livestock workers and non-livestock workers for both the colonized persons and non-colonized persons (Figures 4-36 and 4-37). The predominant phyla in the nasally colonized persons was the Firmicutes followed by Actinobacteria. In the non-colonized persons, Actinobacteria was more prevalent than the Firmicutes (Figures 4-38 and 4-39). In the oropharynx, the Firmicutes was the most abundant phylum (Figures 4-40 and 4-41).

4.5.1 Differences between livestock workers and non-livestock workers' microbiotas by colonization status in the anterior nares.

The most abundant OTUs in the nasally colonized persons belonged to the Firmicutes, Actinobacteria, and Proteobacteria (Figure 4-42a). Twenty-four OTUs were significantly differentially abundant between *S. aureus* colonized livestock workers and non-livestock workers. The majority of the significantly differential OTUs belonged to the Firmicutes phylum (Figure 4-42b). Log 2-fold change values and Benjamini-Hochberg adjusted p-values for each significantly differentially abundant OTU can be found in Table D-4. In the nasally non-colonized persons, Actinobacteria was the most abundant phylum followed by the Firmicutes, and Proteobacteria. Fewer differences were seen in abundance by livestock contact compared to the colonized persons (Figure 4-43a). Nine OTUs were significantly more abundant in the non-colonized livestock workers compared to the non-colonized, non-livestock workers with the majority belonged to the Firmicutes phylum. Two OTUs were more abundant in the non-

colonized, non-livestock workers and both belonged to the Actinobacteria phylum (Figure 4-43b). Log 2-fold change values and Benjamini-Hochberg adjusted p-values for each significantly differentially abundant OTU can be found in Table D-5.

4.5.2 Differences between livestock workers and non-livestock workers' microbiotas by colonization status in the oropharynx.

Livestock workers oropharyngeally colonized with *S. aureus* had less *Strepotoccus, Prevotella, Heamophilus, Campylobacter* and *Veillonella* than oropharyngeally colonized non-livestock workers and had greater abundancies of *Rothia, Fusobacterium, Neisseria,* and *Lachnoanerobaculum* than non-livestock workers (Figure 4-44). *Alloprevotella* was the only significantly differentially abundant OTU and was more abundant in oropharyngeally colonized, non-livestock workers (2-fold change: 5.17, adjusted $P = 0.0006$). Among the oropharyngeally non-colonized persons, the abundancies between livestock workers and those without livestock contact were very similar and there were not significantly differentially abundant OTUs (Figure 4-45).

When looking at livestock exposure, there were no differences in OTUs between the colonized and non-colonized livestock workers with the exception of *S. aureus* in the nares. The same was true for those without livestock exposure in the nares. However, in the oropharynx, colonized livestock workers had significantly more *Porphyromonas* (2-fold change = -8.54, $P = 0.03$) than the non-colonized livestock workers. Additionally, non-colonized livestock workers had significantly more *Atopobium* (2-fold change = 6.25, $P= 0.004$) compared to colonized livestock workers. There were no differences in

the microbiomes of colonized persons with no livestock contact compared to non-colonized persons without livestock contact.

4.6 Analysis of Aim 2.2: microbiome differences in *S. aureus* carriage states

The Inverse Simpson diversity index was significantly different by prior carriage status ($P = 0.021$) and by sample type ($P<0.001$); however, there was not difference in carriage status by sample type ($P = 0.436$) (Figure 4-46). The ordination plot of the Bray-Curtis distances showed no significant differences by carriage status in either the nares ($P = 0.405$) or the oropharynx ($P = 0.23$) (Figure 4-47).

Figures 4-48 and 4-49 show the relative abundances of all OTUs by carriage type. There is a great deal of similarity between the intermittent carriers, persistent carriers, and non-carriers' microbiota in both the nares and oropharynx. The oropharyngeal samples have a greater abundancy of OTUs in the top quadrants of the map compared to the anterior nares and less abundancy in the middle of the map. Figure 4-50 further depicts the similarities in the nasal microbiota between carriage states. OTUs belonging to the Firmicutes and Actinobacteria phyla dominate the microbiomes in persistent, intermittent, and non-carriers. Similarly, the oropharyngeal samples are very similar between carrier states; however, there is more diversity than seen in the nasal samples. OTUs belonging the Firmicutes phylum are also the most prevalent in the oropharyngeal samples (Figure 4-51).

In the nasal samples, there were no OTUs significantly differential represented for any of the carrier states. However, non-carriers had slightly greater abundancies of *Corynebacterium,* and *Finegoldia* compared to persistent and intermittent carriers as well

as more *Streptophyta.* Intermittent carriers had slightly greater abundancies of *Propionibacterium* and unclassified *Actinomycetales* compared to persistent and non-carries as well as increased *Actinomyces* compared to non-carriers. Persistent carries had increased abundancies of *Staphylococcus, Anaerococcus, Peptoniphilus, Haemophilus,* and *Actinetobacter* compared to intermittent and non-carriers (Figure 4-52).

The differences in the most abundant OTUs in the oropharynx by carriage status are shown in Figure 4-53. In the oropharynx, non-carriers had significantly more *Streptococcus* and *Gemella* compared to the intermittent carriers. Log 2-fold changes and p-values for all significantly differential OTUs between the intermittent and non-carriers can be found in Table D-6. Additionally, intermittent carriers had significantly greater abundancies of *Moryella* and *Alloprevotella* compared to persistent carriers while persistent carries had significantly greater abundancies of eight different OTUs compared to intermittent carriers. Log 2-fold changes and p-values for all significantly differential OTUs between intermittent and persistent carriers can be found in Table D-6. There were no OTUs significantly different between persistent carriers and non-carriers. However, when persistent carriers were compared to intermittent and non-carriers combined, three OTUs were significantly different between with persistent carriers having more *Corynebacterium* and *Bacteroides* compared to all others where *Moryella* was significantly more abundant (Figure 4-54, Table D-7).

4.7 *S. aureus* culture

Thirty-three (56.9%) of participants were positive for *S. aureus* in either the nose or throat with two participants (3.4%) being positive in only the throat and 17 participants

(29.3%) being positive in only the nose. Overall, 31 nasal sample were positive for *S. aureus* and 14 oropharyngeal samples were positive for *S. aureus.* Four isolates (from three participants) were positive for the *mecA* gene (8.8%) and two isolates both originating from the same participant were positive for the PVL gene (4.4%).

The most prevalent *spa* type overall was t034 (n=8, 17.8%) followed by t571 (n=4, 8.9%), t012 (n=4, 8.9%), t008 (n=4, 8.9%), and t002 (n=4, 8.9%). In the nares, t034 was still the most prevalent *spa* type (n=7, 22.6%) followed by t002 (n=3, 9.8%). In the oropharynx, t008, t012, t216, and t571 were each found in two samples (14.3% each). Seven isolates were not typable (15.6%): six in the nares (19.4%) and one in the oropharynx (7.1%). Using the BURP clustering algorithm, two clusters were identified in the nares and two in the oropharynx. The seven non-typable isolates were not included in the cluster as there is no way for the algorithm to assess their genetic relatedness to the other strains (Figure 4-55).

4.7.1 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing revealed a high level of antimicrobial resistance to multiple antimicrobial agents. The highest amount of resistance was seen to benzylpenicillin with 82.2% (n=37) of all strains being resistant. Over half of all isolates were resistant to erythromycin at 53.3% (n=24), with an additional 4.4% (n=2) showing intermediate susceptibility. A high amount of resistance was also observed for tetracycline (37.8%, n=17), clindamycin (33.3%, n=15), and minocycline (20.0%, n=9). Oxacillin resistance was observed in 6.7% (n=3) of isolates, slightly lower than what was observed via the *mecA* gene (8.9%, n=4). No resistance was observed for vancomycin,

rifampicin, linezolid, daptomycin, tigecycline, or nitrofurantoin (Figure 4-56). Twenty-two isolates (48.9%) of isolates met the definition for multi-drug resistant *S. aureus* (MDR-SA).

4.8 Comparison of the *S. aureus* culture results and 16s rRNA sequencing

Twenty-three samples were positive for *S. aureus* via both standard culture and 16s rRNA sequencing with 45 positive via culture and 37 positive via sequencing. 16s rRNA identified 14 colonized participants that were not positive via culture; however, 22 samples were positive via culture that were not positive through sequencing. Using culture as the gold standard, sensitivity of 16s rRNA sequencing was 51.1% with a specificity of 79.7%.

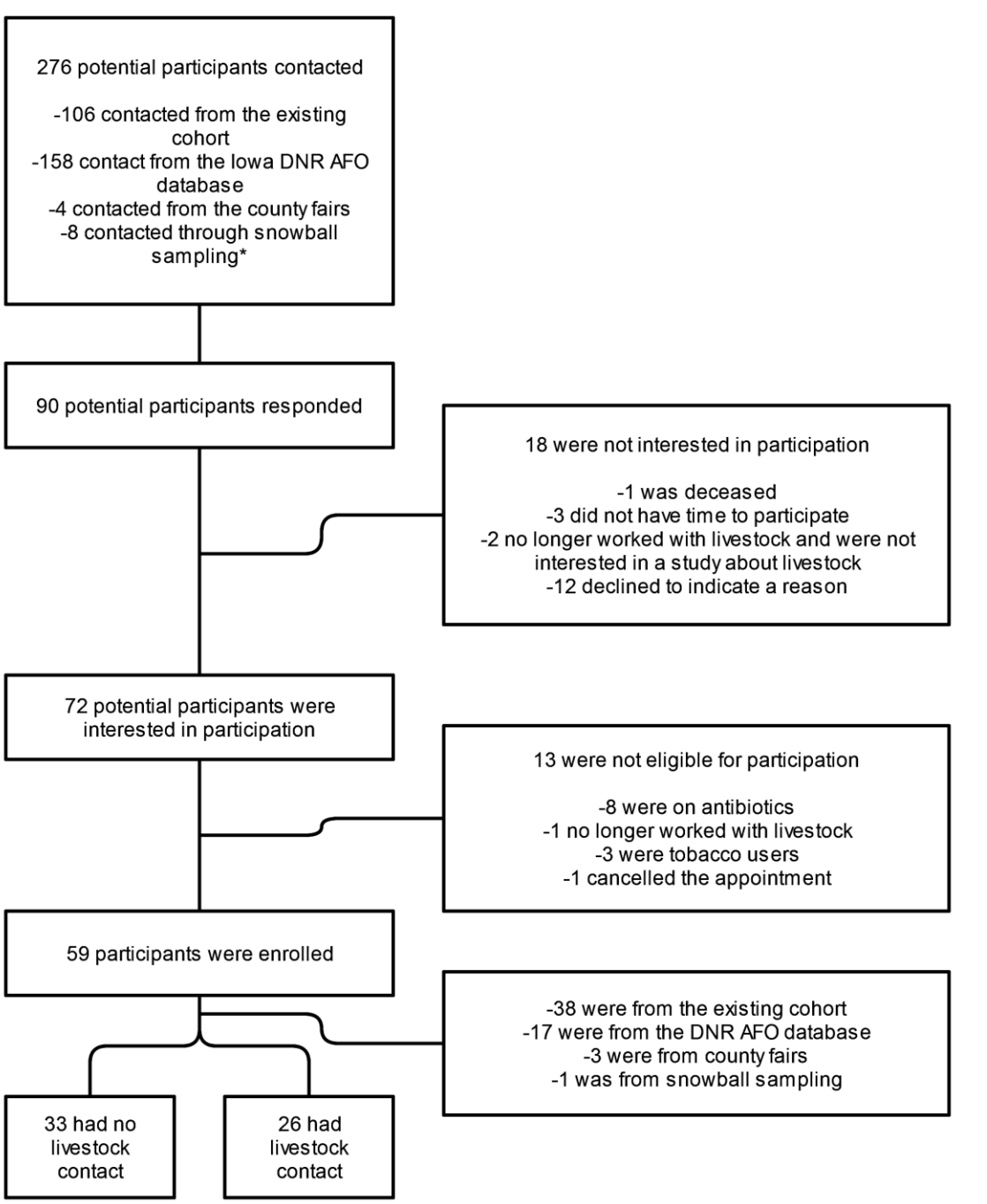

Figure 4-1: Flow diagram of participant enrollment.

*It is not possible to know how many participants were contacted via snowball sampling as we were not able to ask the subjects passing information out for us how many information packets they disseminated. We provided eight packets and assume all were passed out.

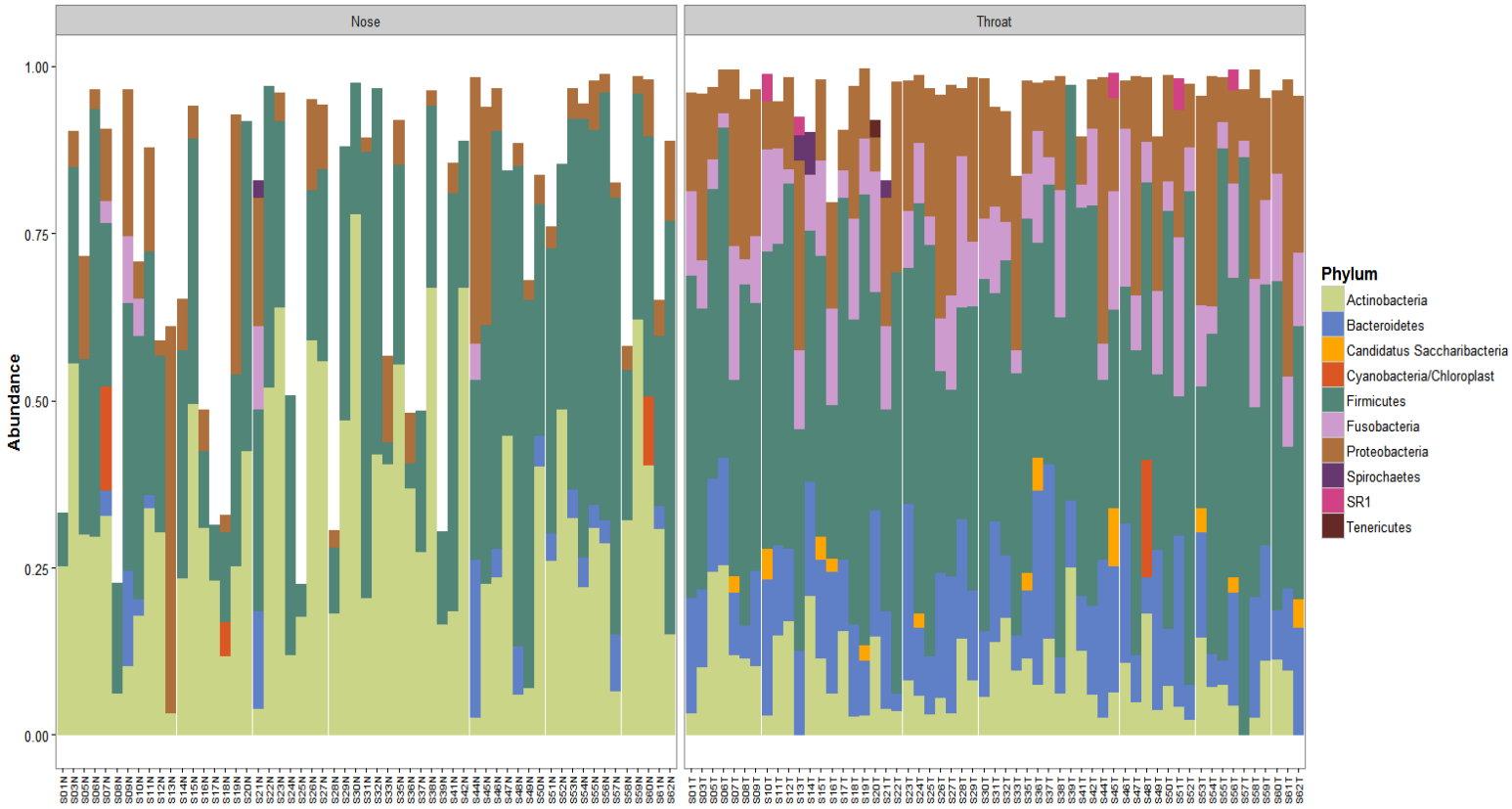

Figure 4-2: Barplot of top 98% of phyla present in all samples by anatomical site.

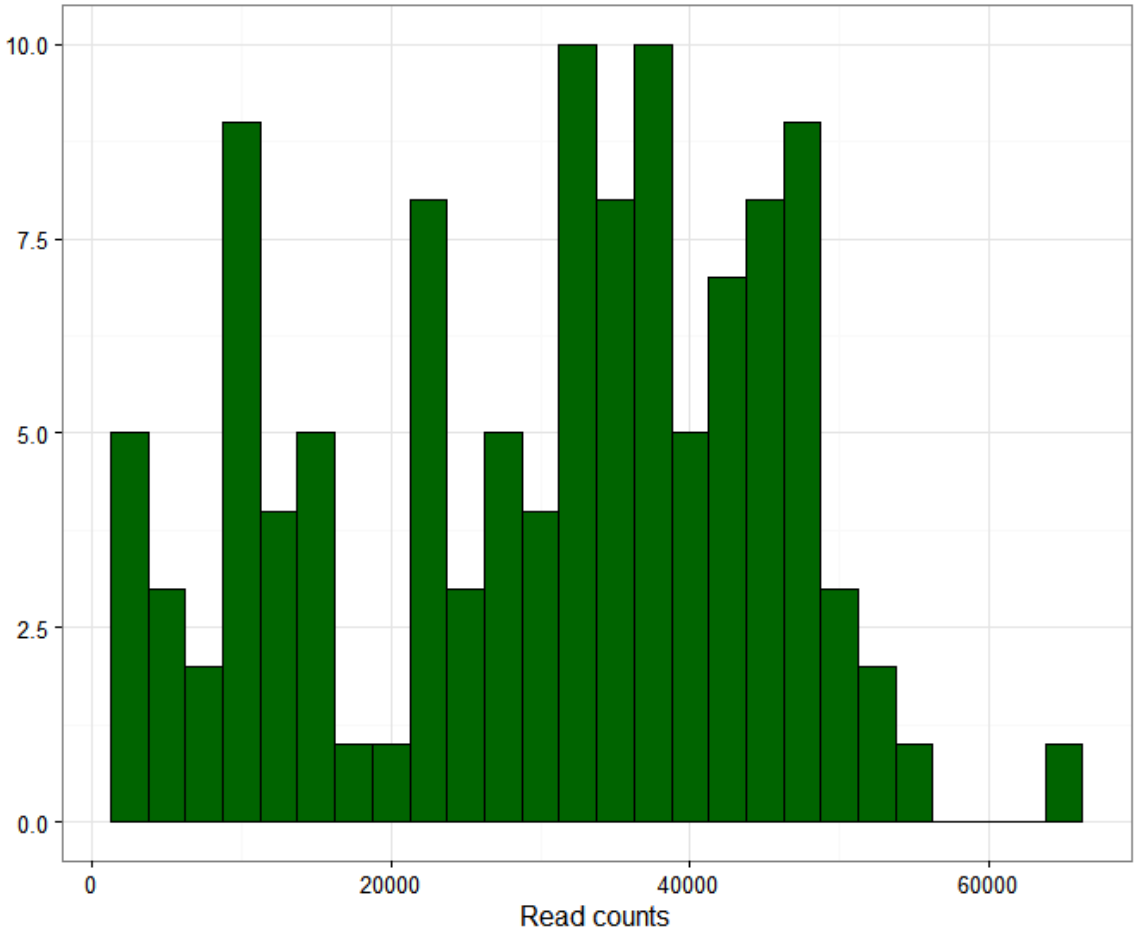

Figure 4-3: Barplot of sequencing depth for all samples.

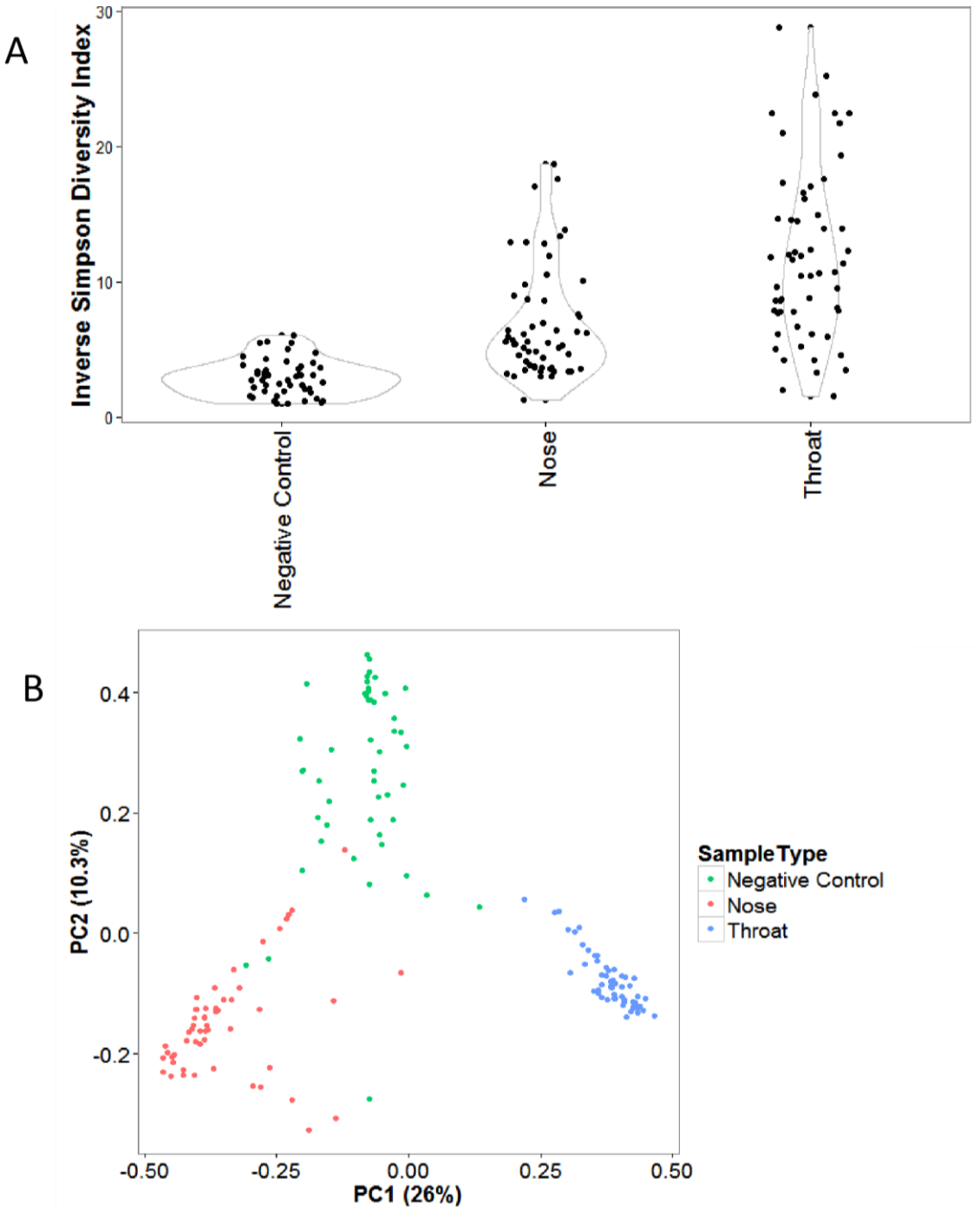

Figure 4-4: Diversity indexes for all samples. (a) Inverse Simpson diversity index violin plot. (b) Ordination plot of Bray-Curtis dissimilarly index of each samples microbiome. PC1 and PC2 = principal coordinates 1 and 2, respectively.

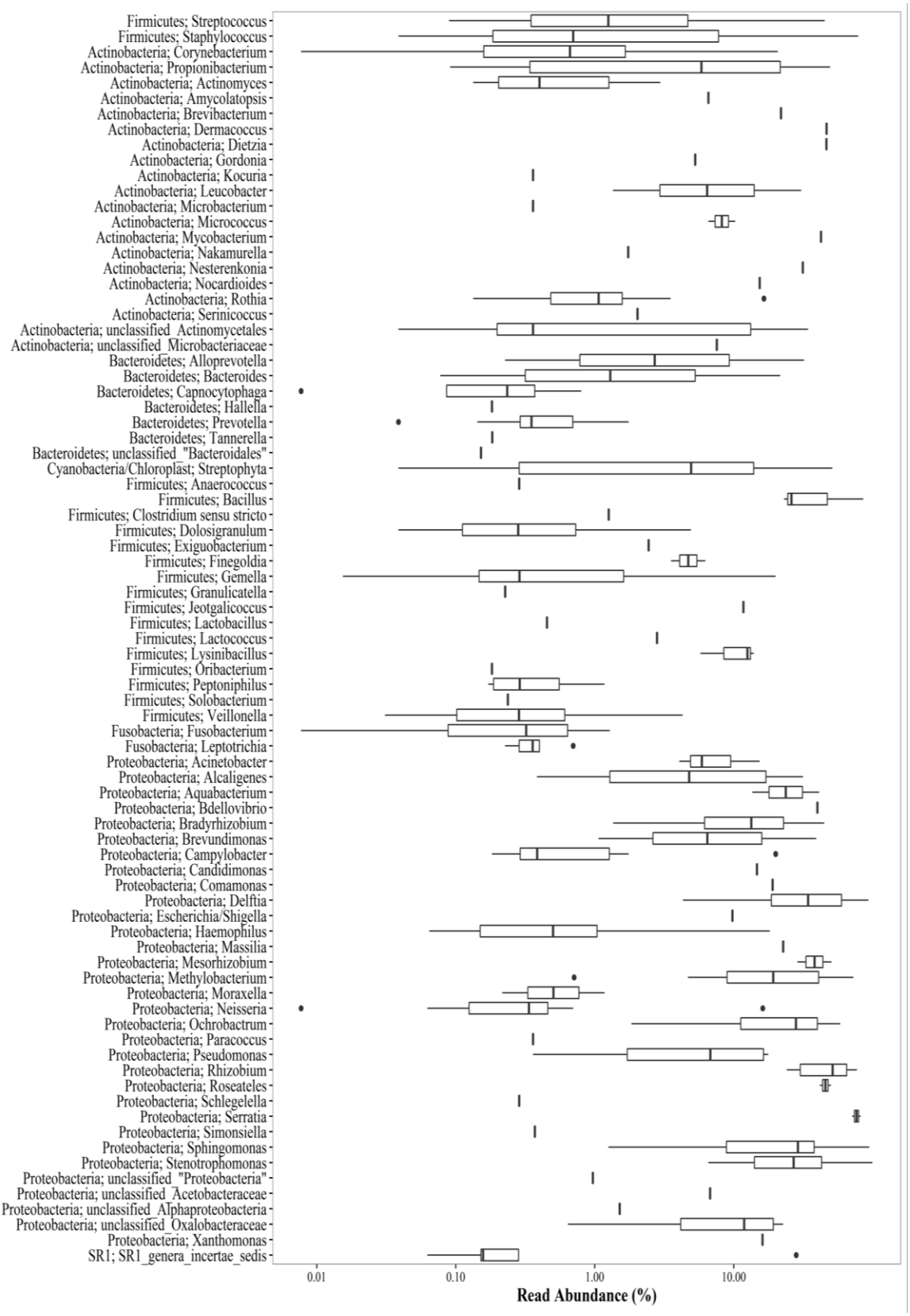

Figure 4-5: Boxplot of OTUs present in negative control samples (log scale)

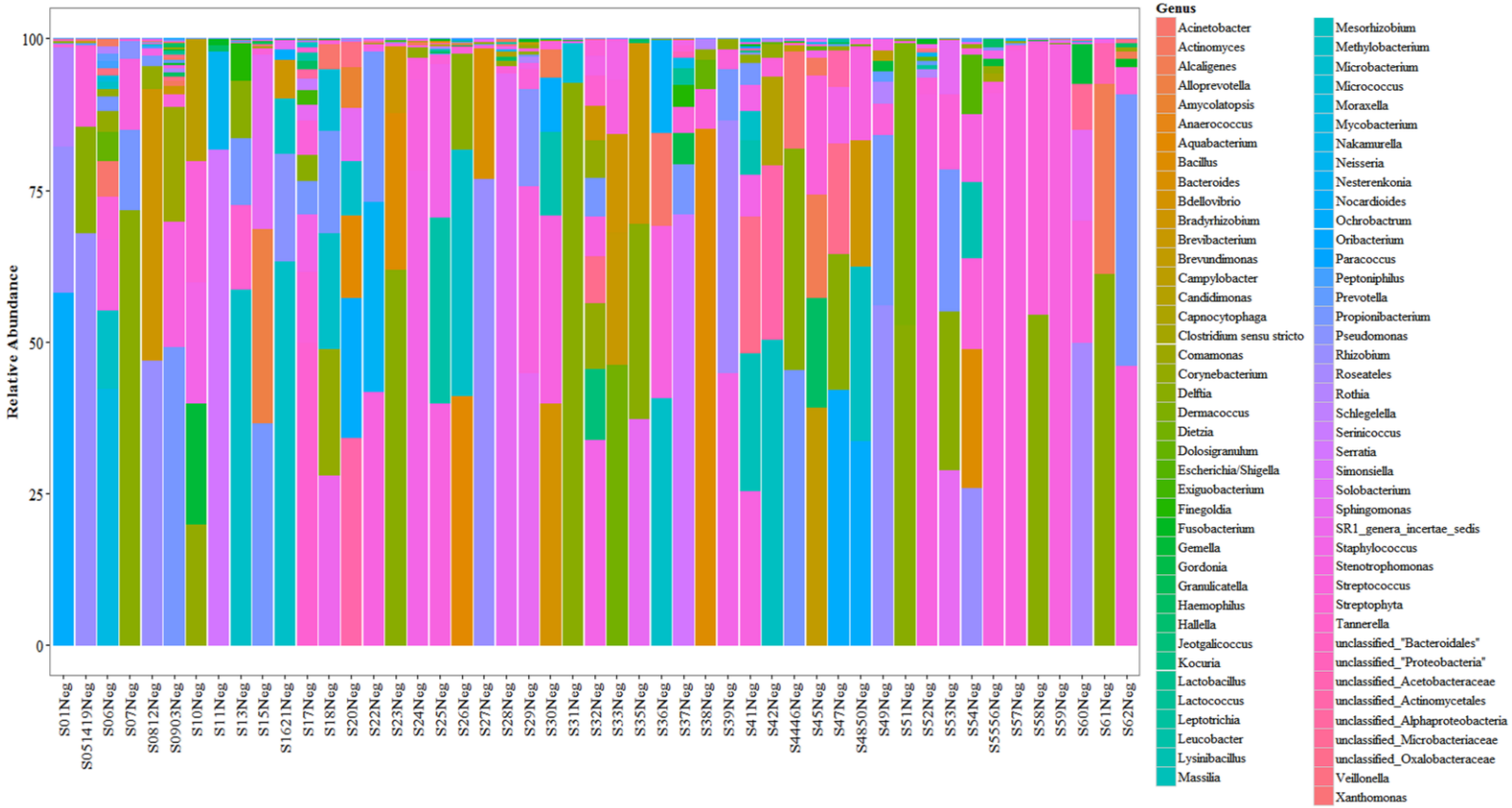

Figure 4-6: Barplot of relative abundances of OTUs present in the negative control samples.

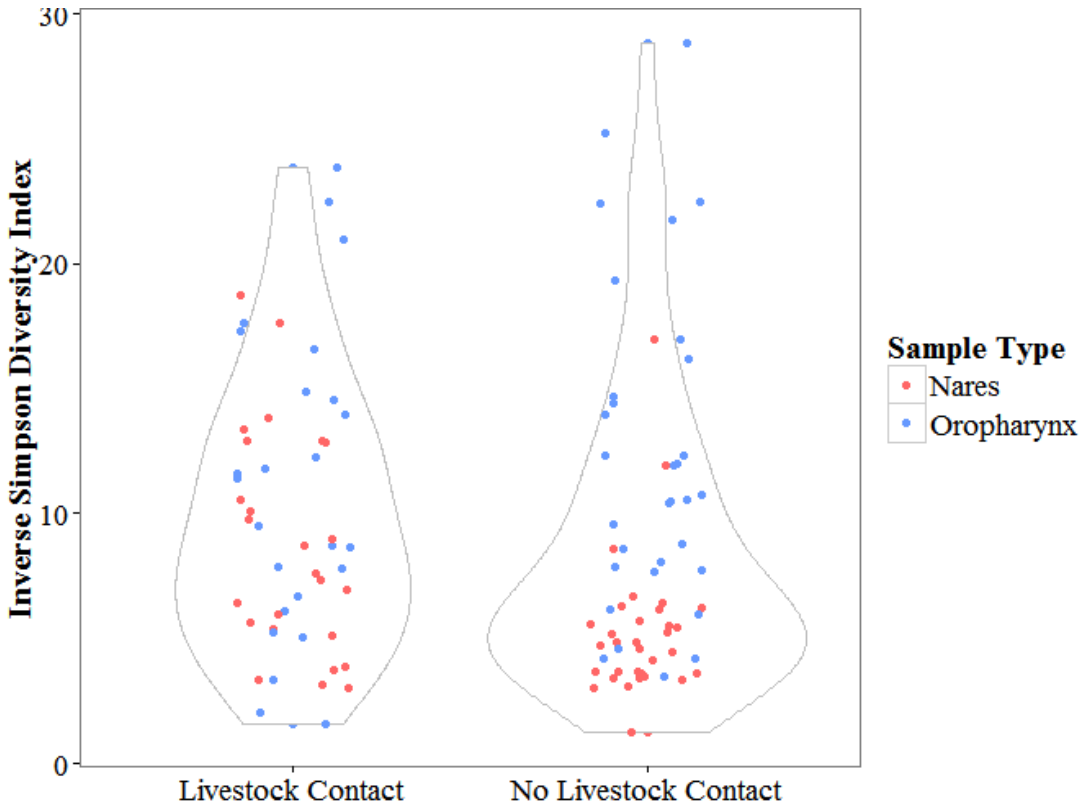

Figure 4-7: Inverse Simpson diversity index comparing alpha diversity of those with and without livestock contact by sample type.

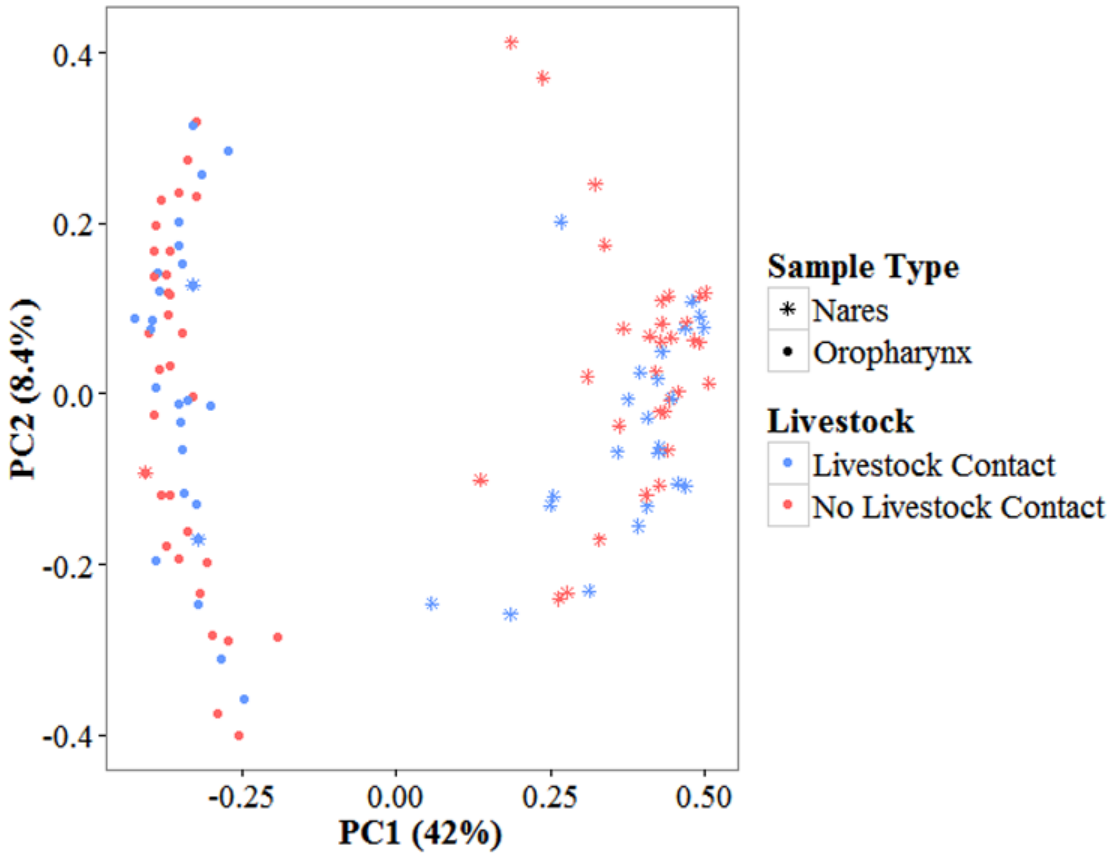

Figure 4-8: Ordination plot of Bray-Curtis dissimilarly index of each samples microbiome. PC1 and PC2 = principal coordinates 1 and 2, respectively.

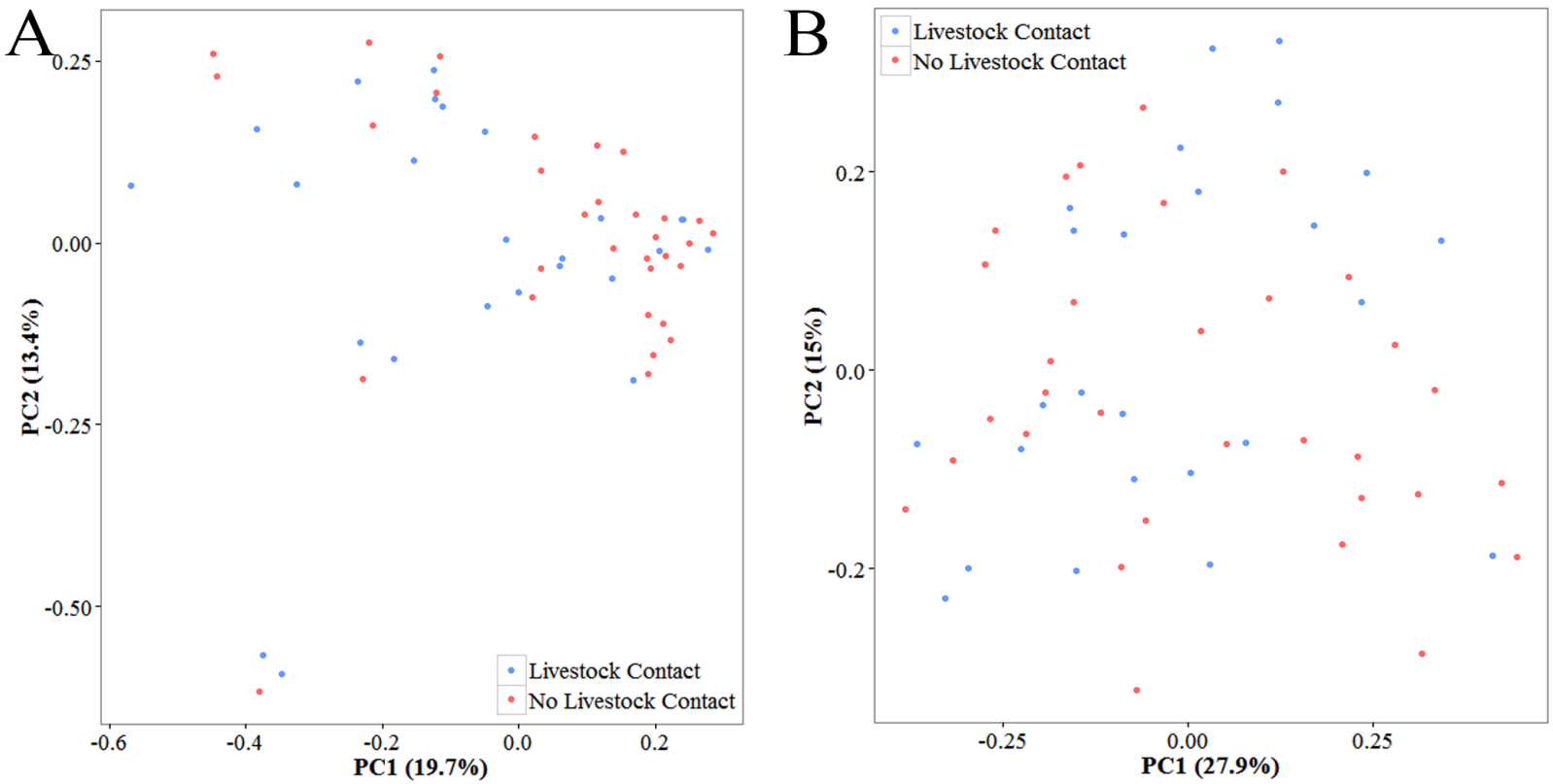

Figure 4-9: Principal coordinates analysis. (a) Ordination of nasal samples based on the Bray-Curtis dissimilarities of the nasal sample microbiomes. (b) Ordination of oropharyngeal samples based on the Bray-Curtis dissimilarities. PC1 and PC2 = principal coordinates 1 and 2, respectively.

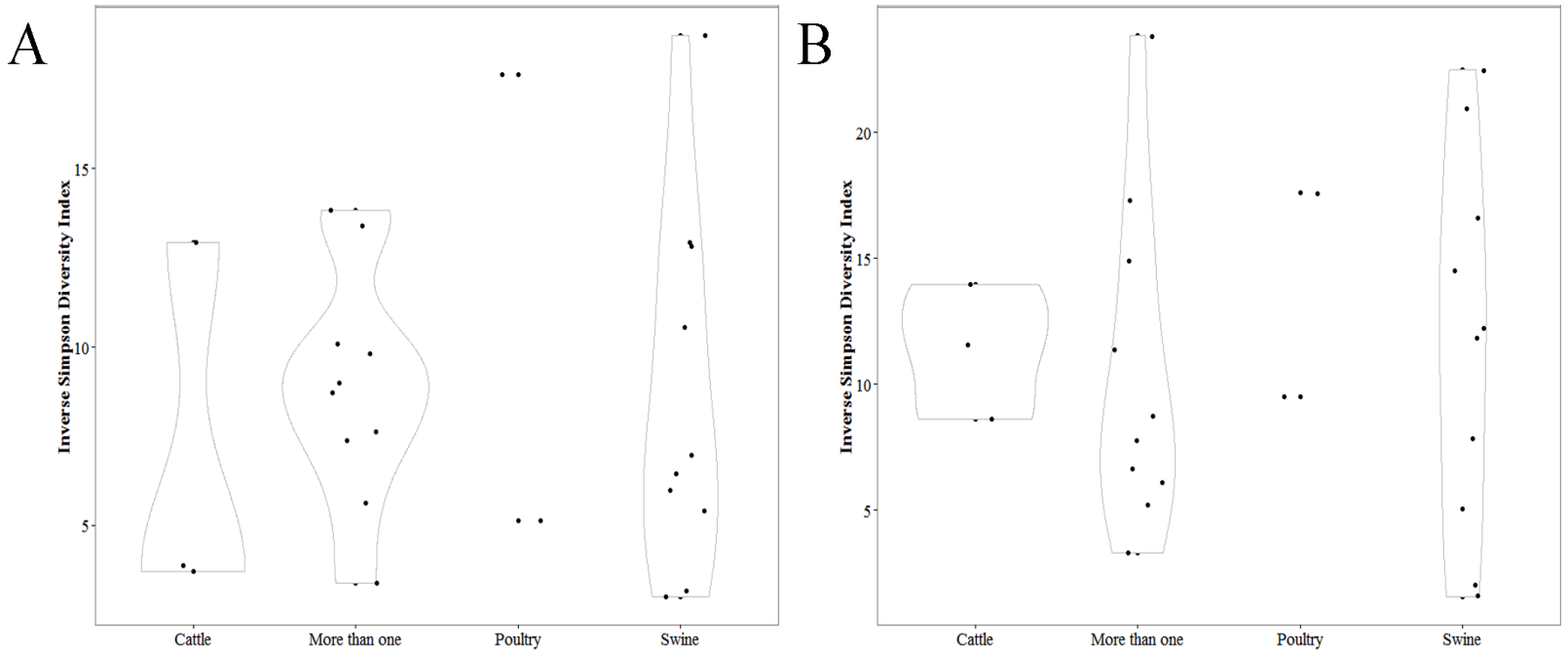

Figure 4-10: Inverse Simpson diversity index comparing alpha diversity of livestock workers by type of animal contact in the (a) nares and (b) oropharynx.

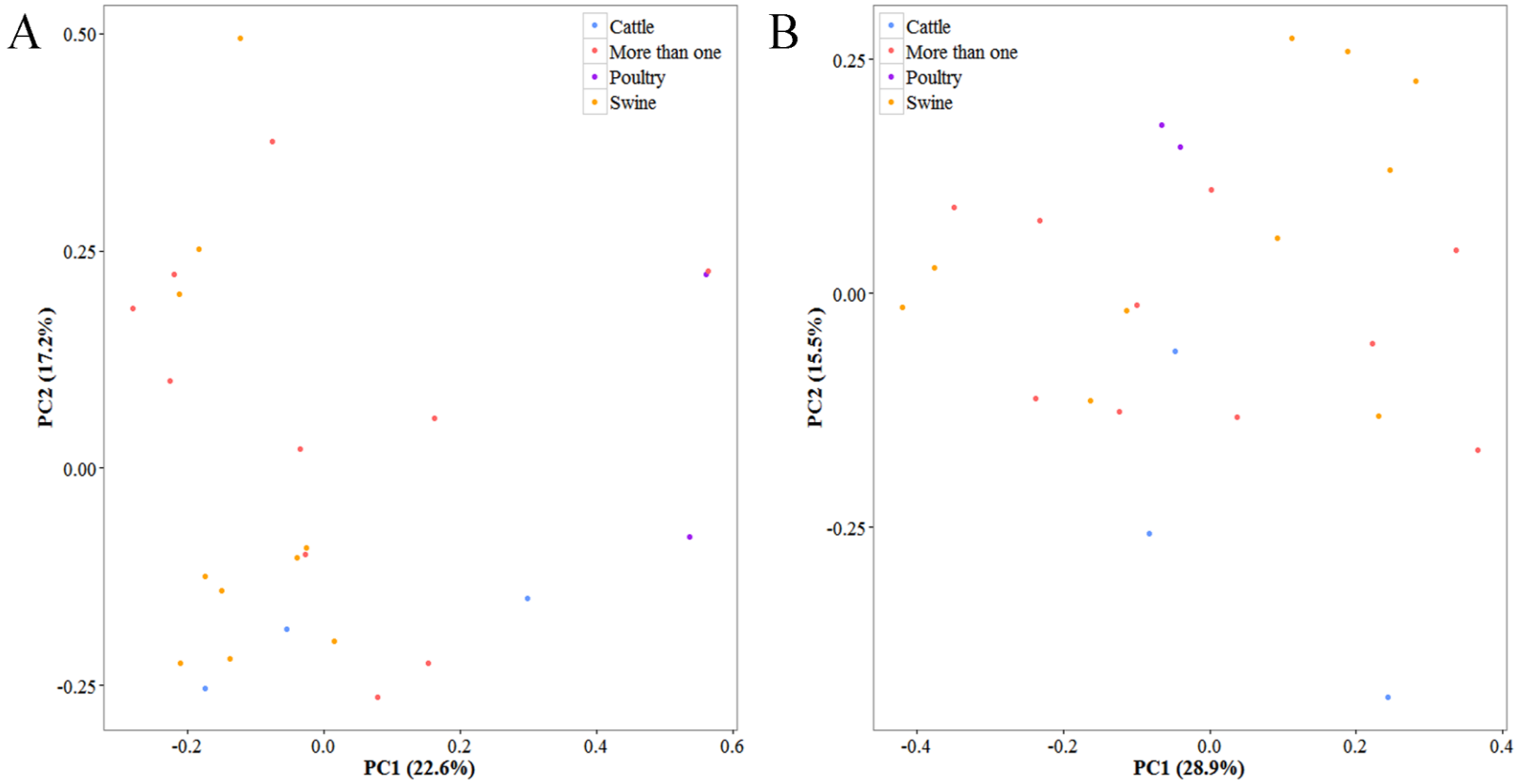

Figure 4-11: Principal coordinates analysis of livestock workers microbiomes by animal contact. (a) Ordination plot of Bray-Curtis dissimilarly index of livestock workers' nares. (b) Ordination of the Bray-Curtis dissimilarities of the livestock workers' oropharynx. PC1 and PC2 = principal coordinates 1 and 2, respectively.

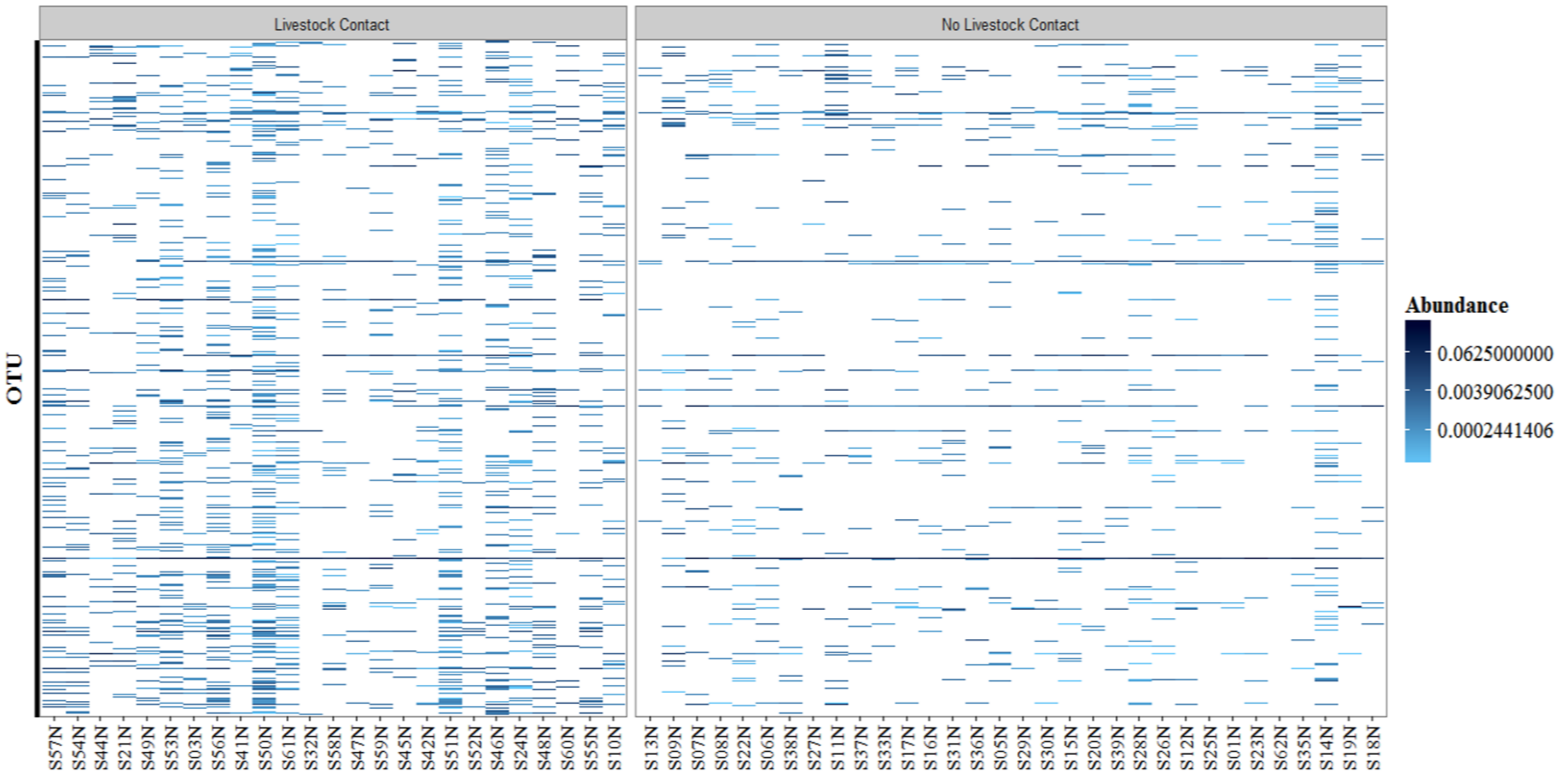

Figure 4-12: Relative abundance of all OTUs by livestock contact for the nasal samples depicted as a heatmap. OTUs are depicted in the same order in both panels.

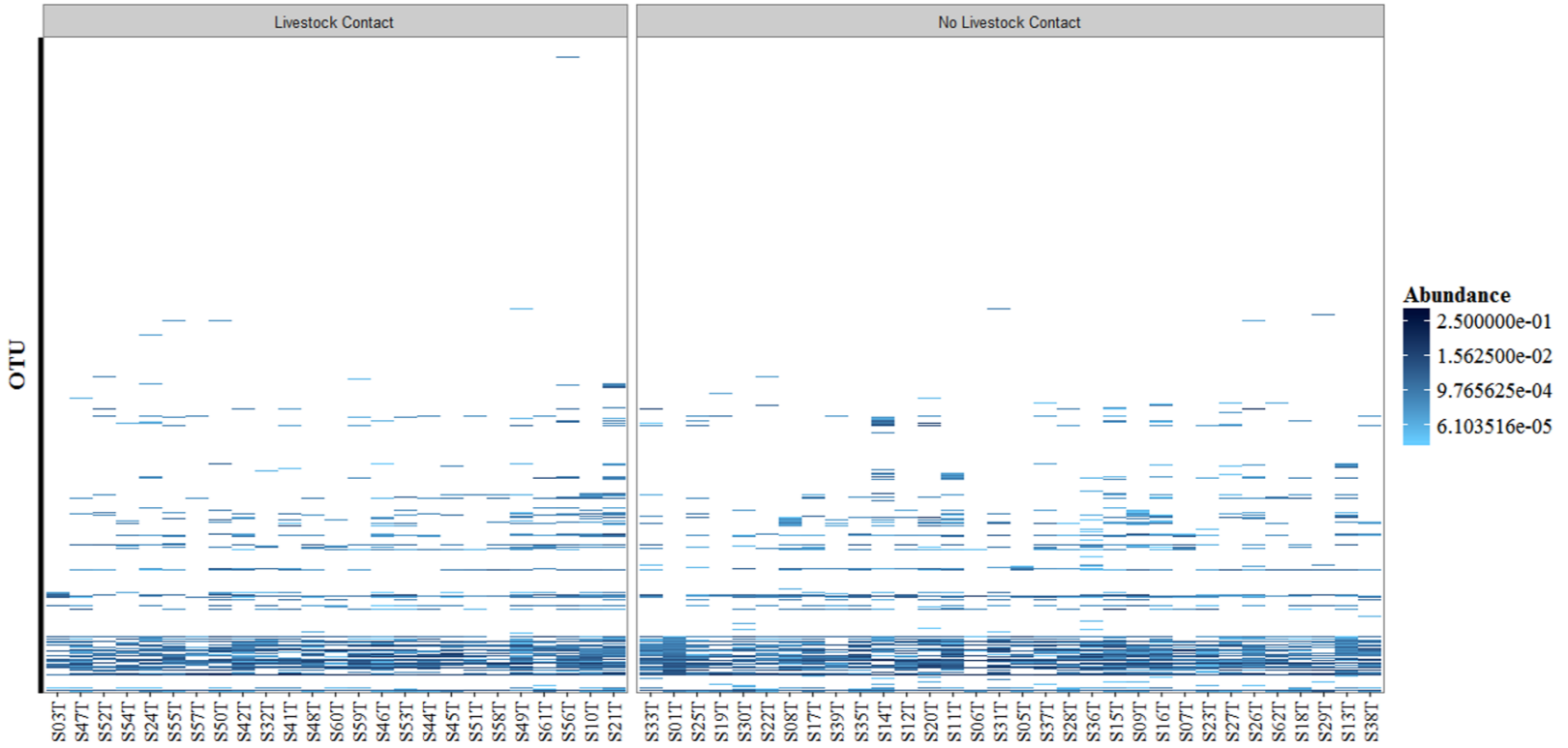

Figure 4-13: Relative abundance of all OTUs by livestock contact for the oropharyngeal samples depicted as a heatmap. OTUs are depicted in the same order in both panels.

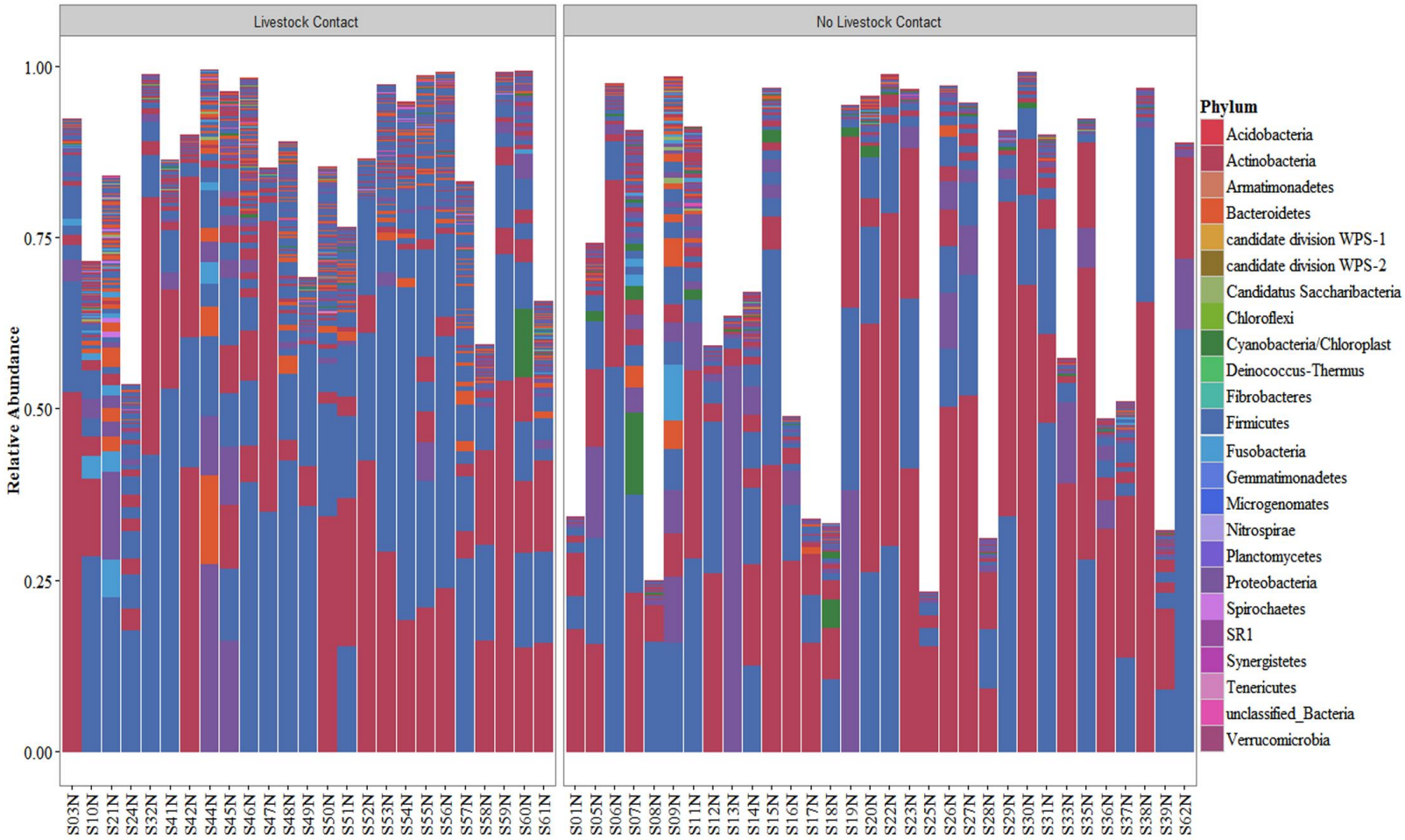

Figure 4-14: Barplot of the relative abundance of all phyla in the nasal samples.

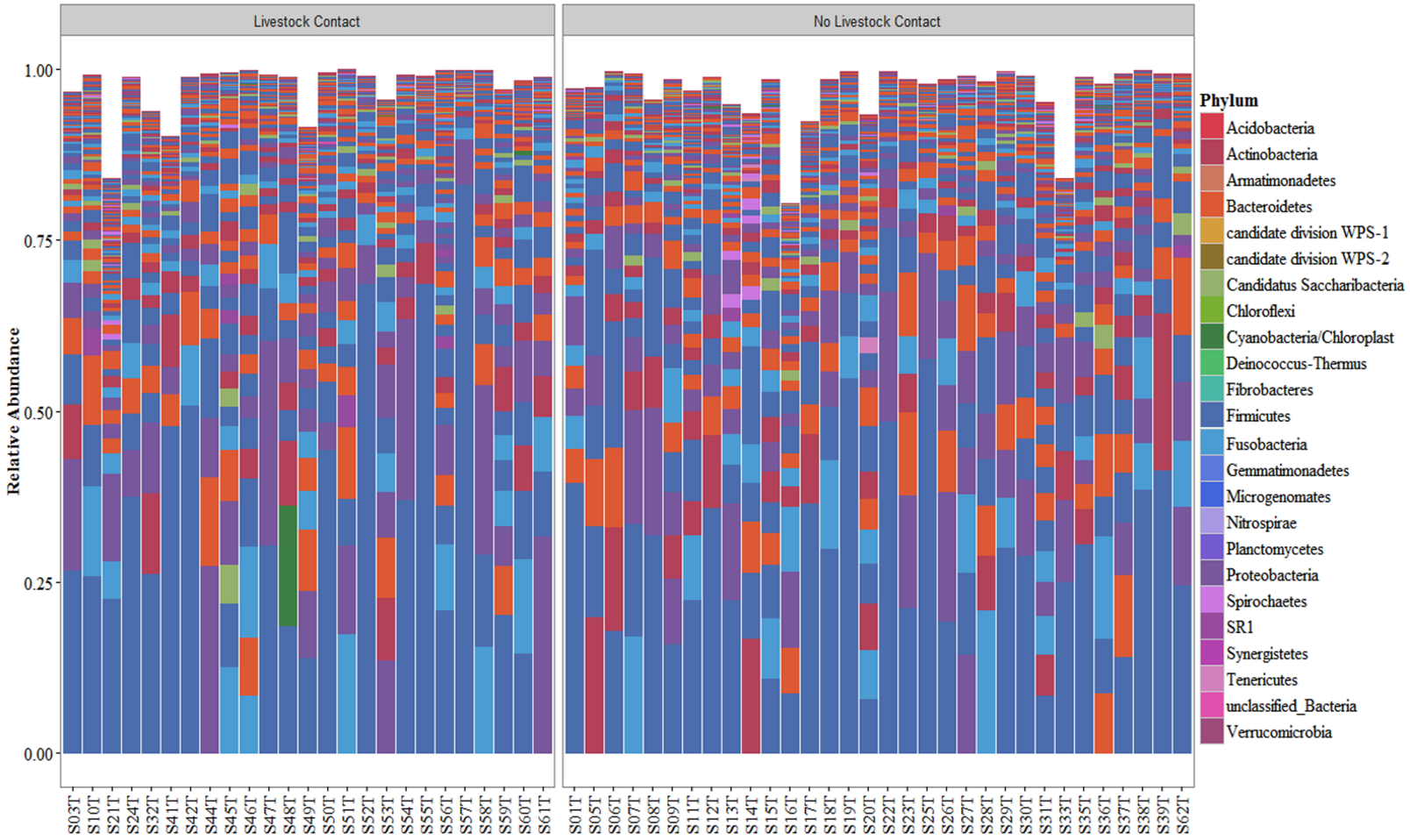

Figure 4-15: Barplot of the relative abundance of all phyla in the oropharyngeal samples.

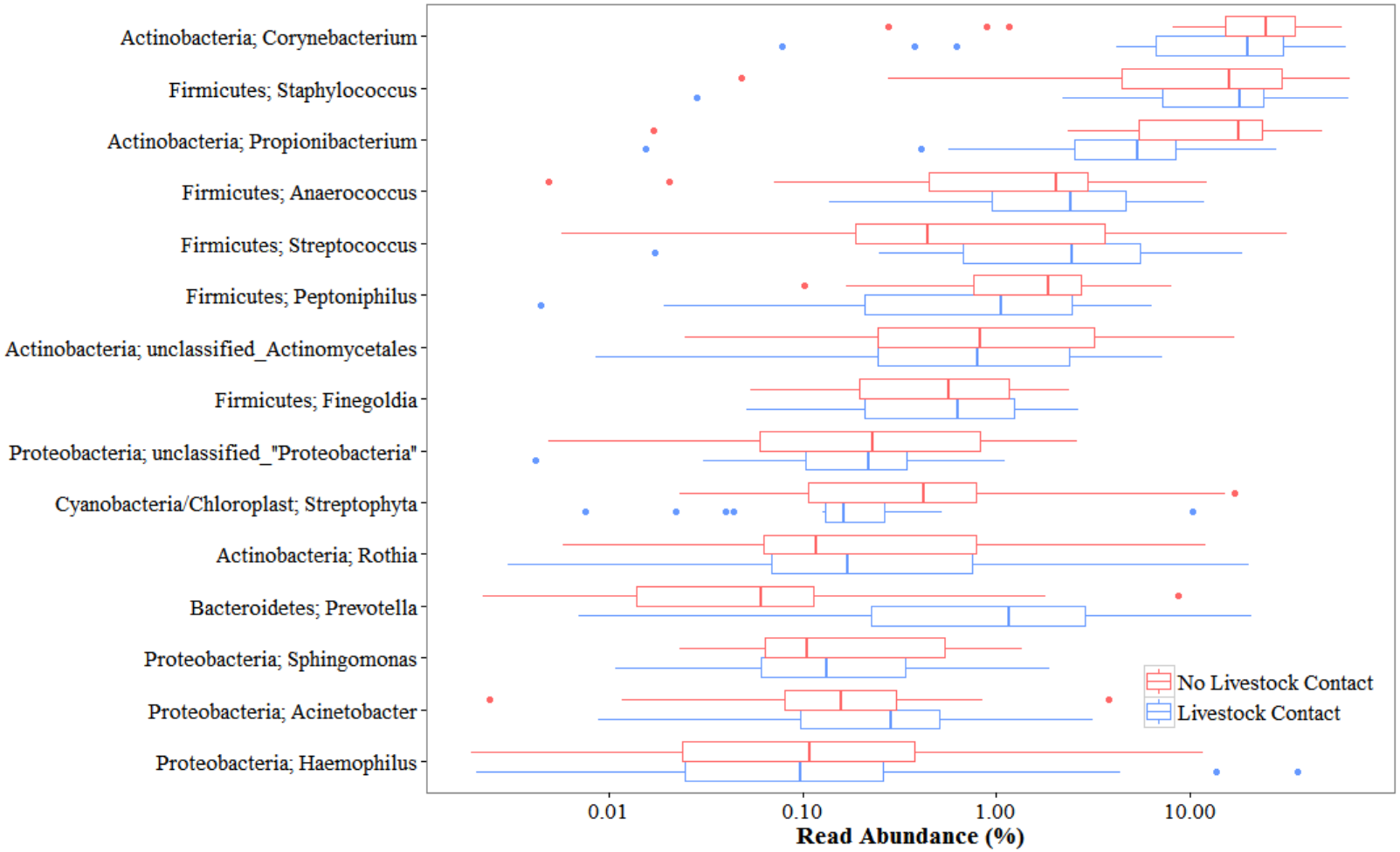

Figure 4-16: Boxplot of the top 15 most abundant OTUs in the nares. Phylum and genus classification are shown. Percent abundances are log transformed.

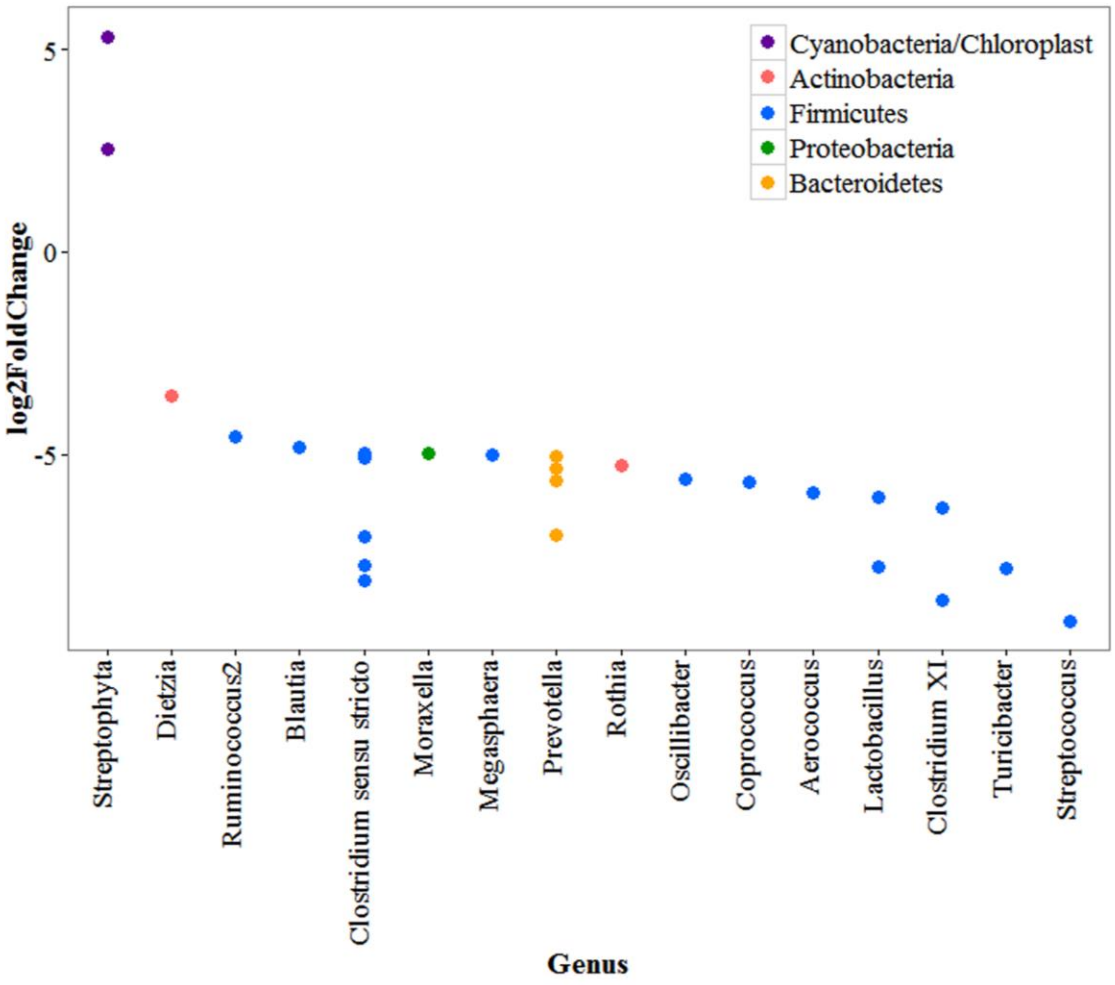

Figure 4-17: Log 2-fold Change of the significantly differentially abundant OTUs in the nares (Benjamini-Hochberg correction applied). Points represent OTUs with phyla represented by color. Negative values represent OTUs significantly more abundant in livestock workers and positive values represent OTUs significantly more abundant in non-livestock workers.

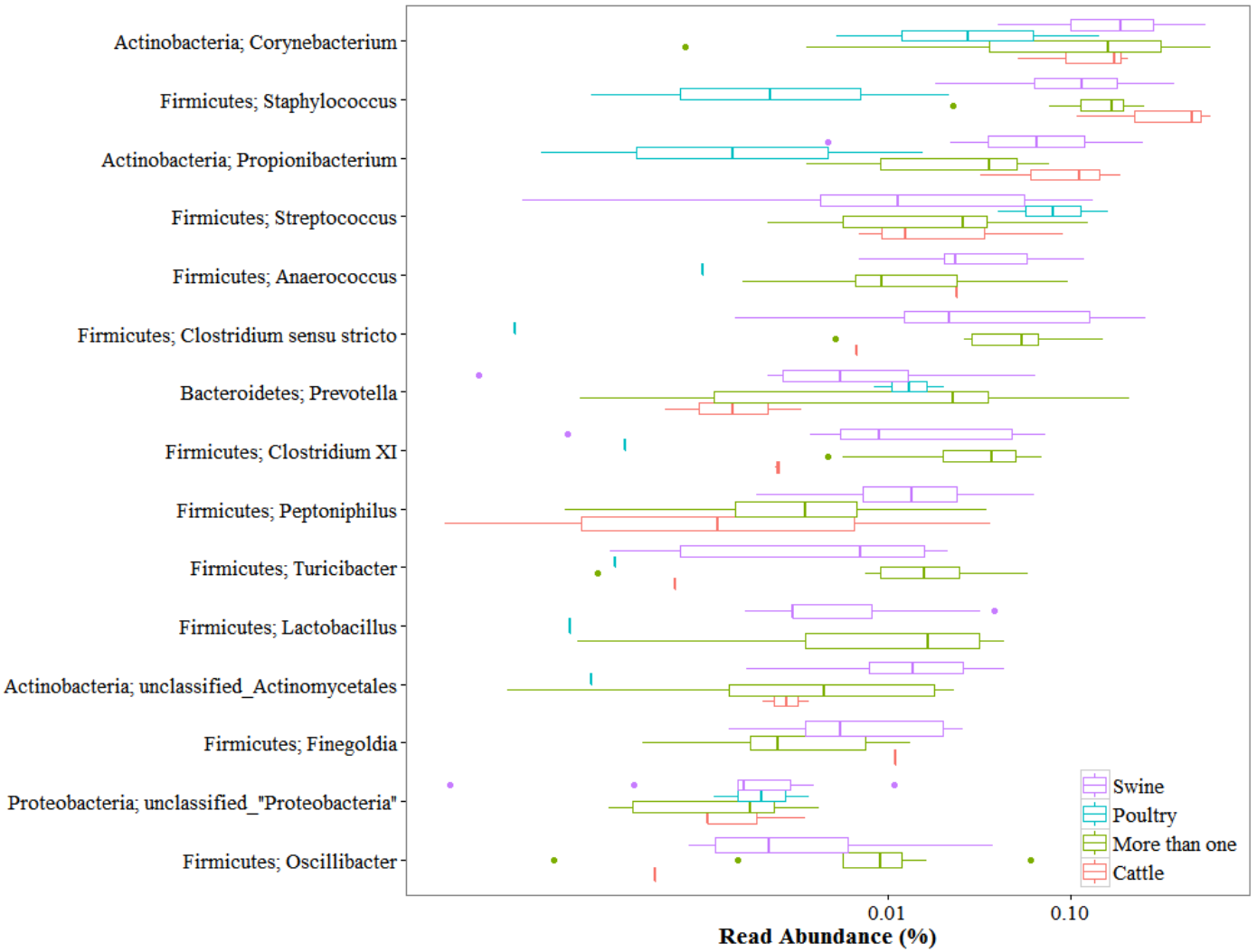

Figure 4-18: Boxplot of the top 15 OTUs in the nares of livestock workers by type of animal contact.

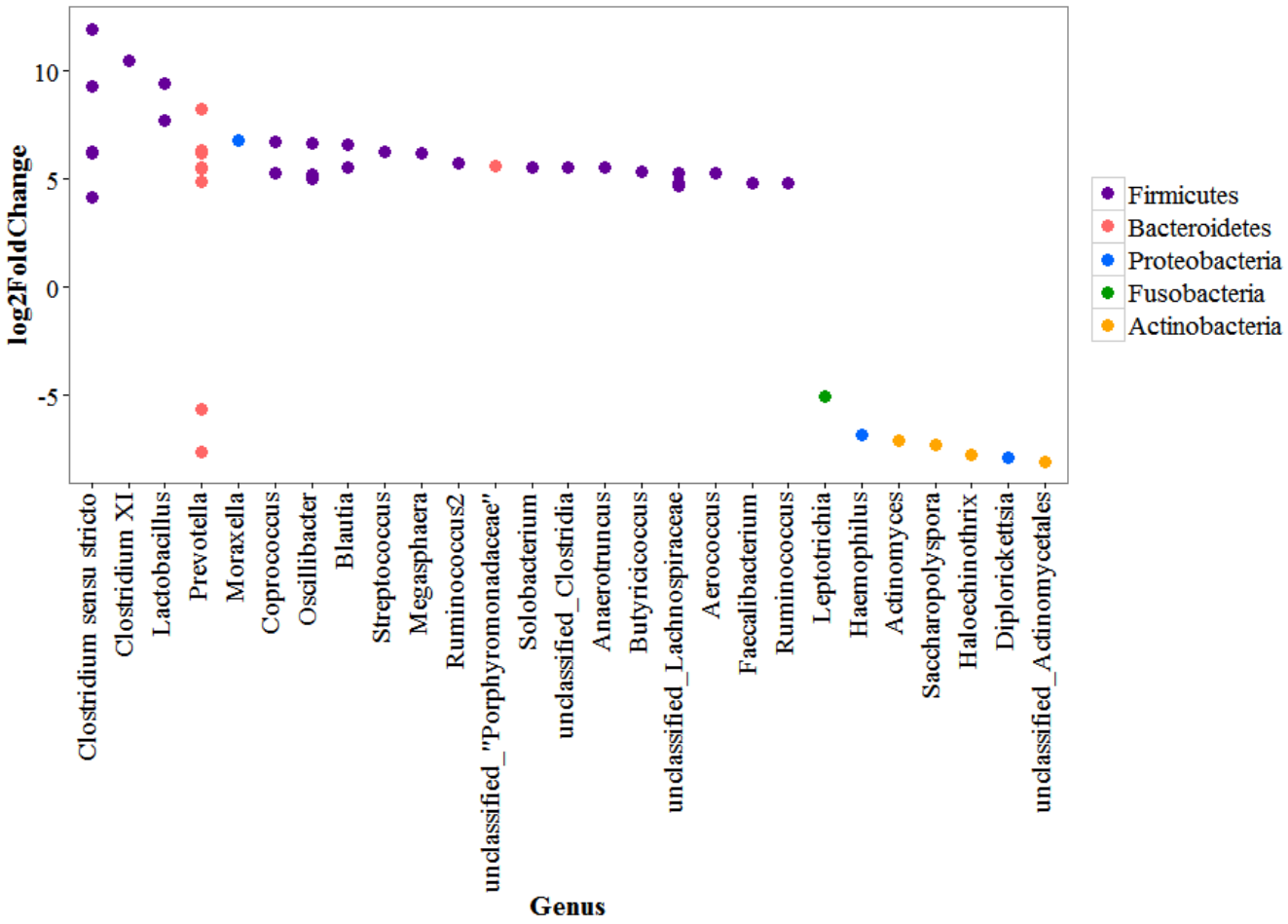

Figure 4-19: Log 2-fold change of the significantly differentially abundant OTUs in the nares by type of animal contact (Benjamini-Hochberg correction applied). Points represent OTUs with phyla represented by color. Negative values represent OTUs significantly more abundant in livestock workers without swine contact and positive values represent OTUs significantly more abundant in livestock workers with swine contact.

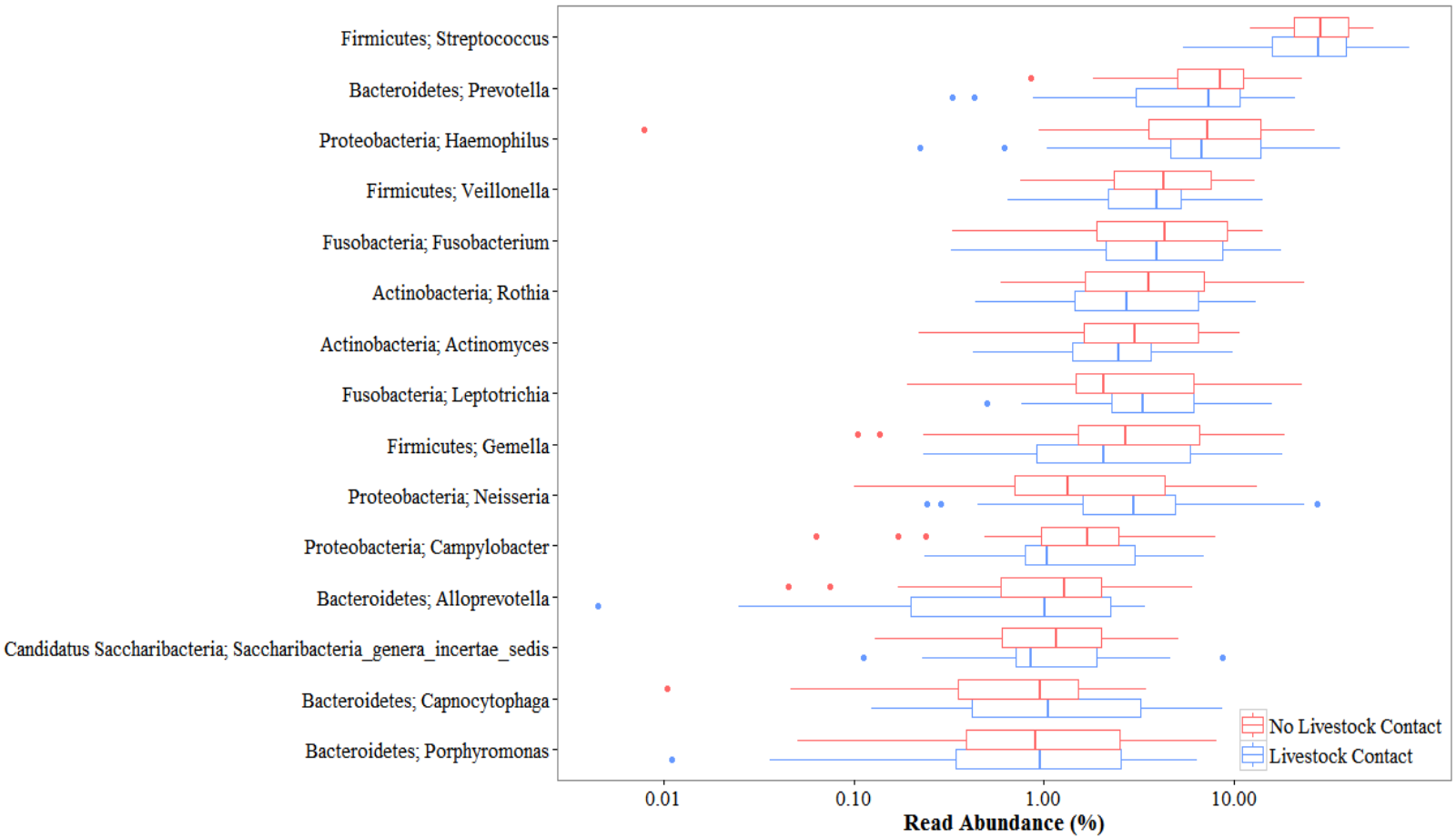

Figure 4-20: Boxplot of the top 15 most abundant OTUs in the oropharynx.

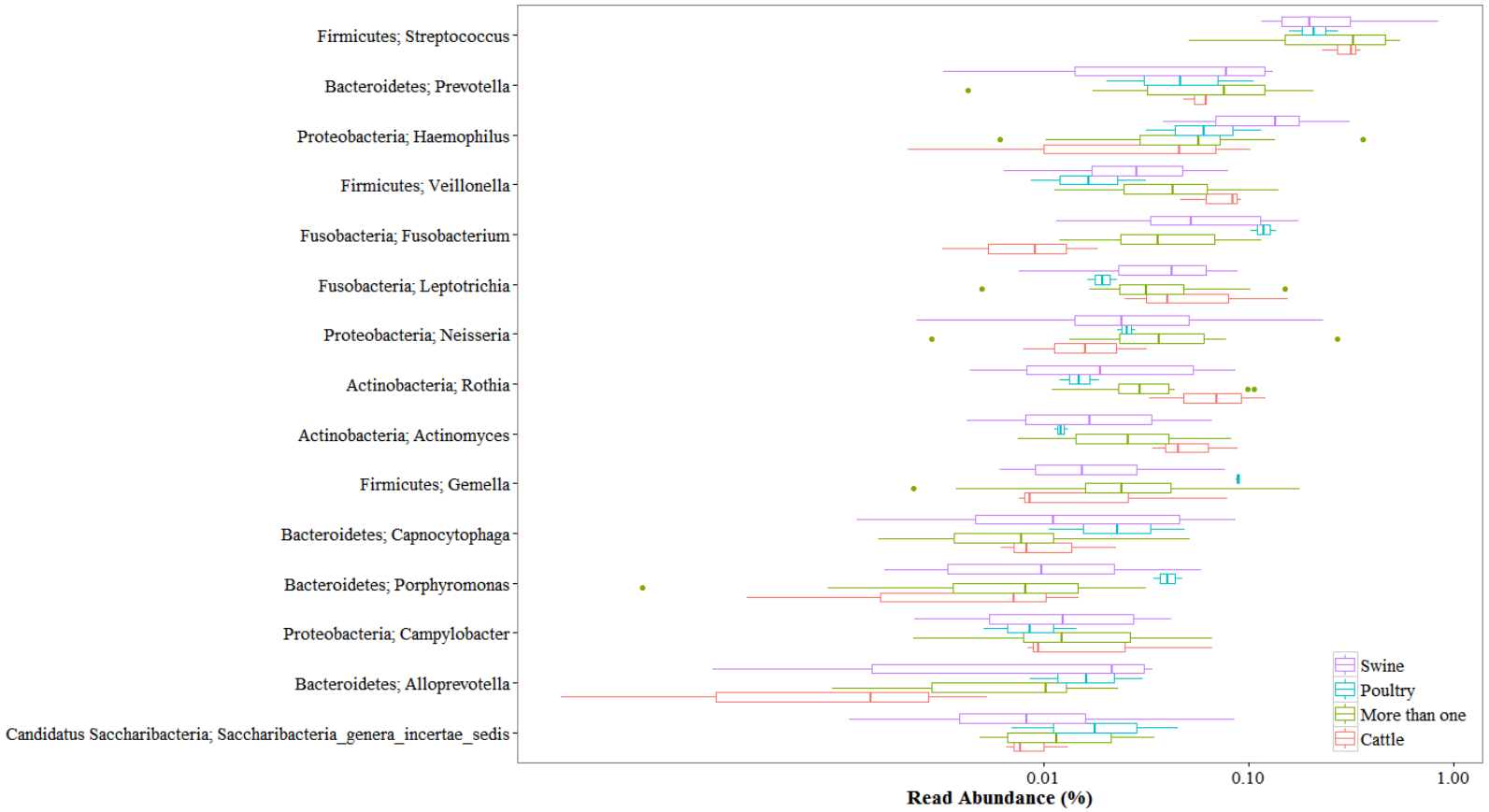

Figure 4-21: Boxplot of the top 15 most abundant OTUs by type of animal contact in the oropharynx. Phylum and genus classification are shown. Percent abundances are log transformed.

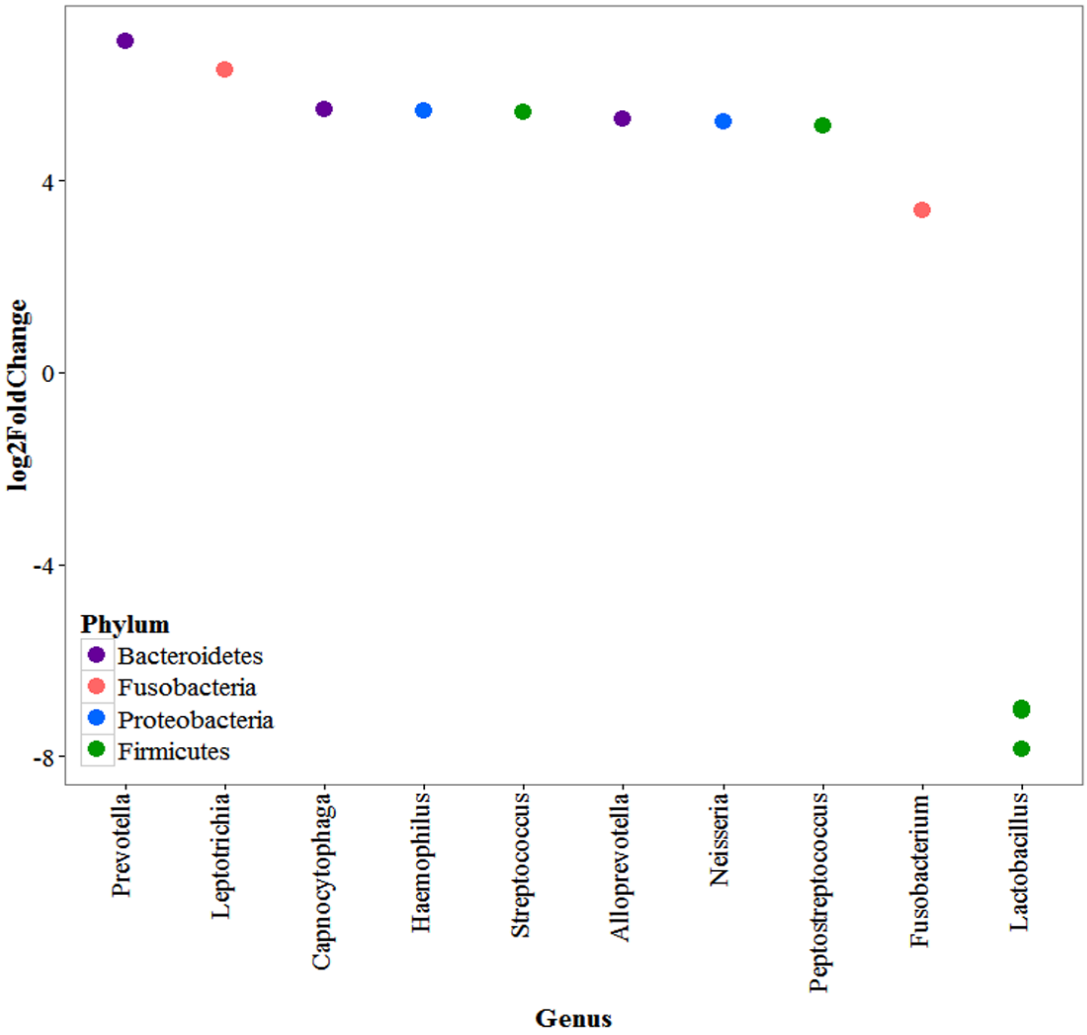

Figure 4-22: Log 2-fold change of the significantly differentially abundant OTUs in the oropharynx by type of animal contact (Benjamini-Hochberg correction applied). Points represent OTUs with phyla represented by color. Negative values represent OTUs significantly more abundant in livestock workers without swine contact and positive values represent OTUs significantly more abundant in livestock workers with swine contact.

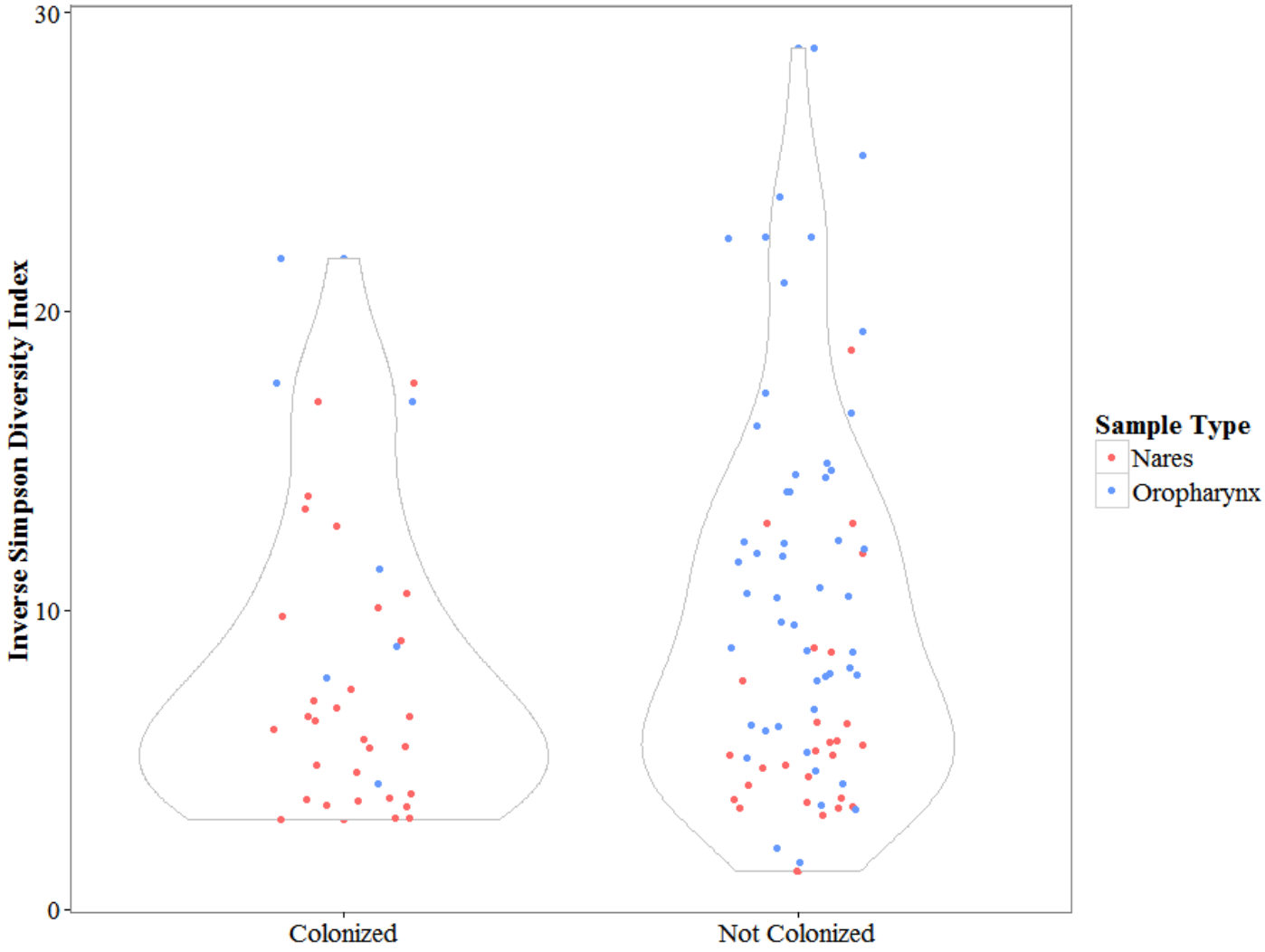

Figure 4-23: Inverse Simpson diversity index comparing alpha diversity by colonization status.

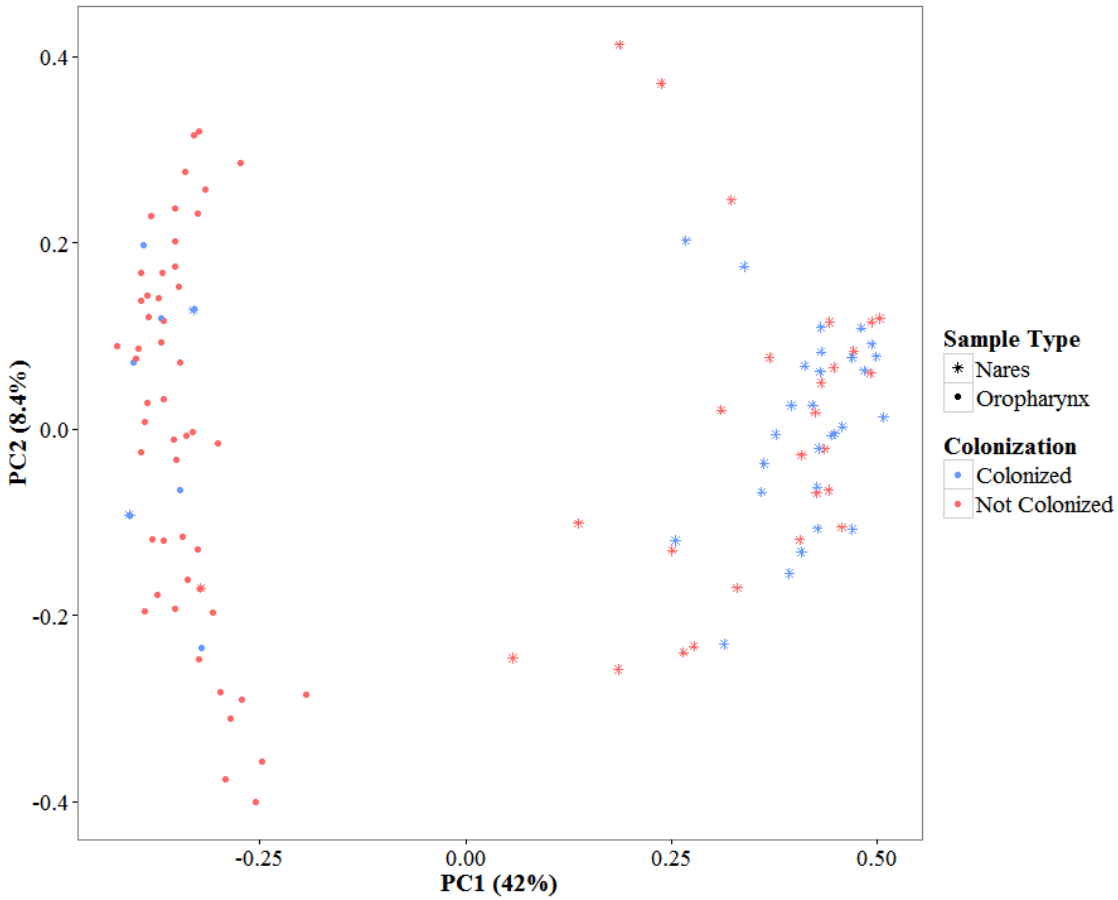

Figure 4-24: Ordination plot of Bray-Curtis dissimilarly index of each samples microbiome. PC1 and PC2 = principal coordinates 1 and 2, respectively.

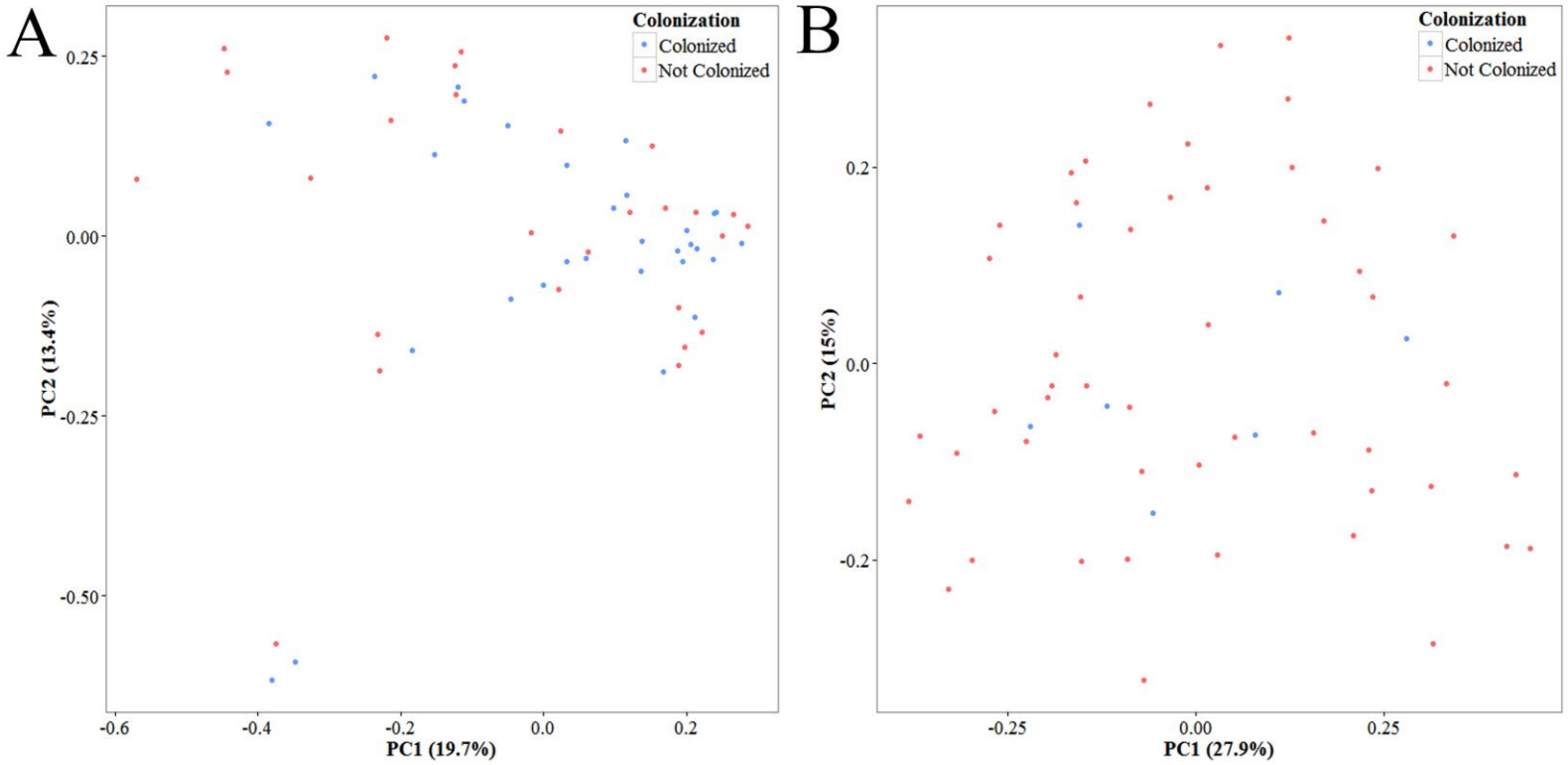

Figure 4-25: Principal coordinates analysis of colonization status. (a) Ordination based on the Bray-Curtis dissimilarities of the nasal sample microbiomes. (c) Ordination based on the Bray-Curtis dissimilarities of the oropharyngeal sample microbiomes. PC1 and PC2 = principal coordinates 1 and 2, respectively.

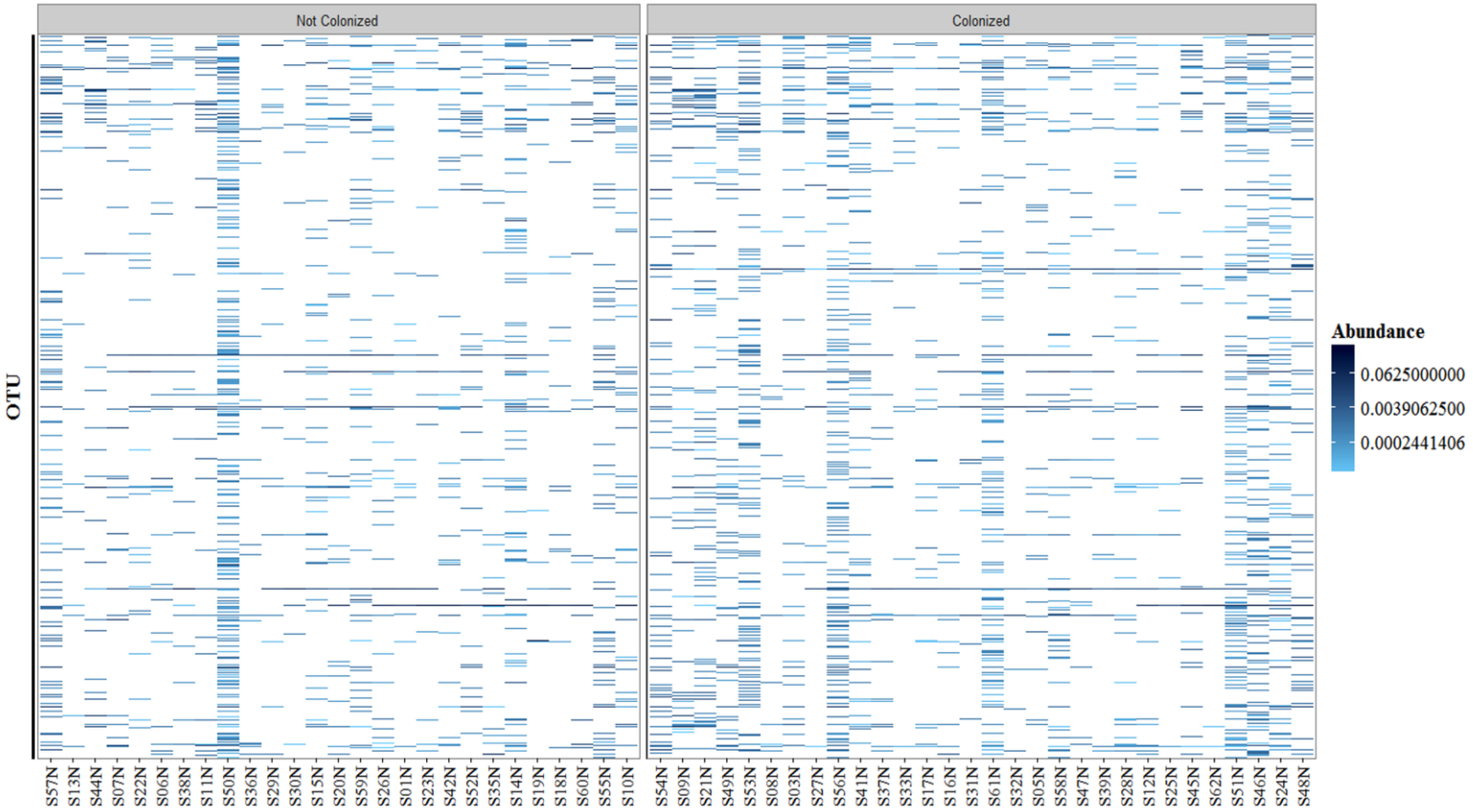

Figure 4-26: Relative abundance of all OTUs by colonization status for the nasal samples depicted as a heatmap. OTUs are in the same order in both panels.

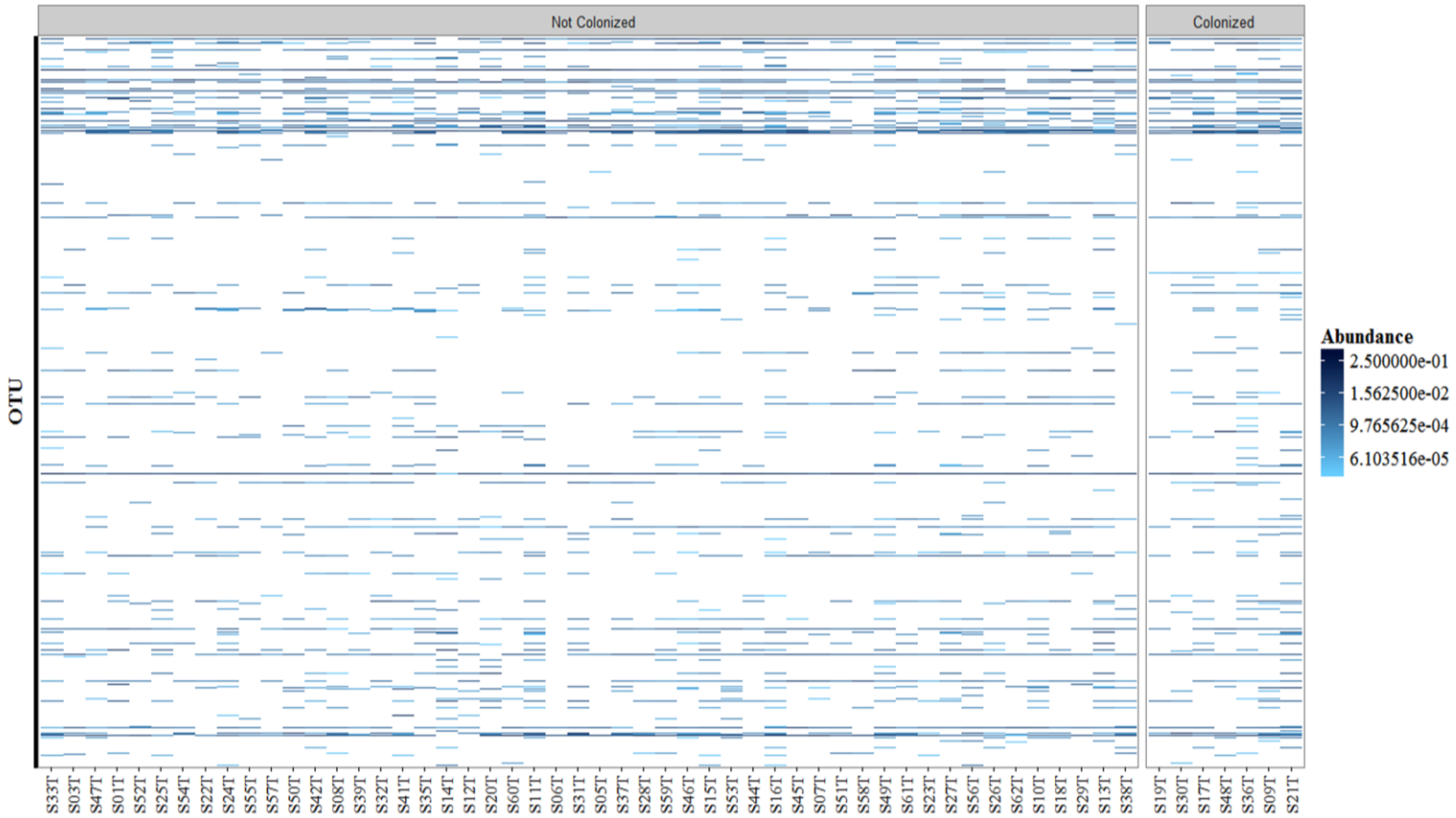

Figure 4-27: Relative abundance of all OTUs by colonization status for the oropharyngeal samples depicted as a heatmap. OTUs are in the same order in both panels.

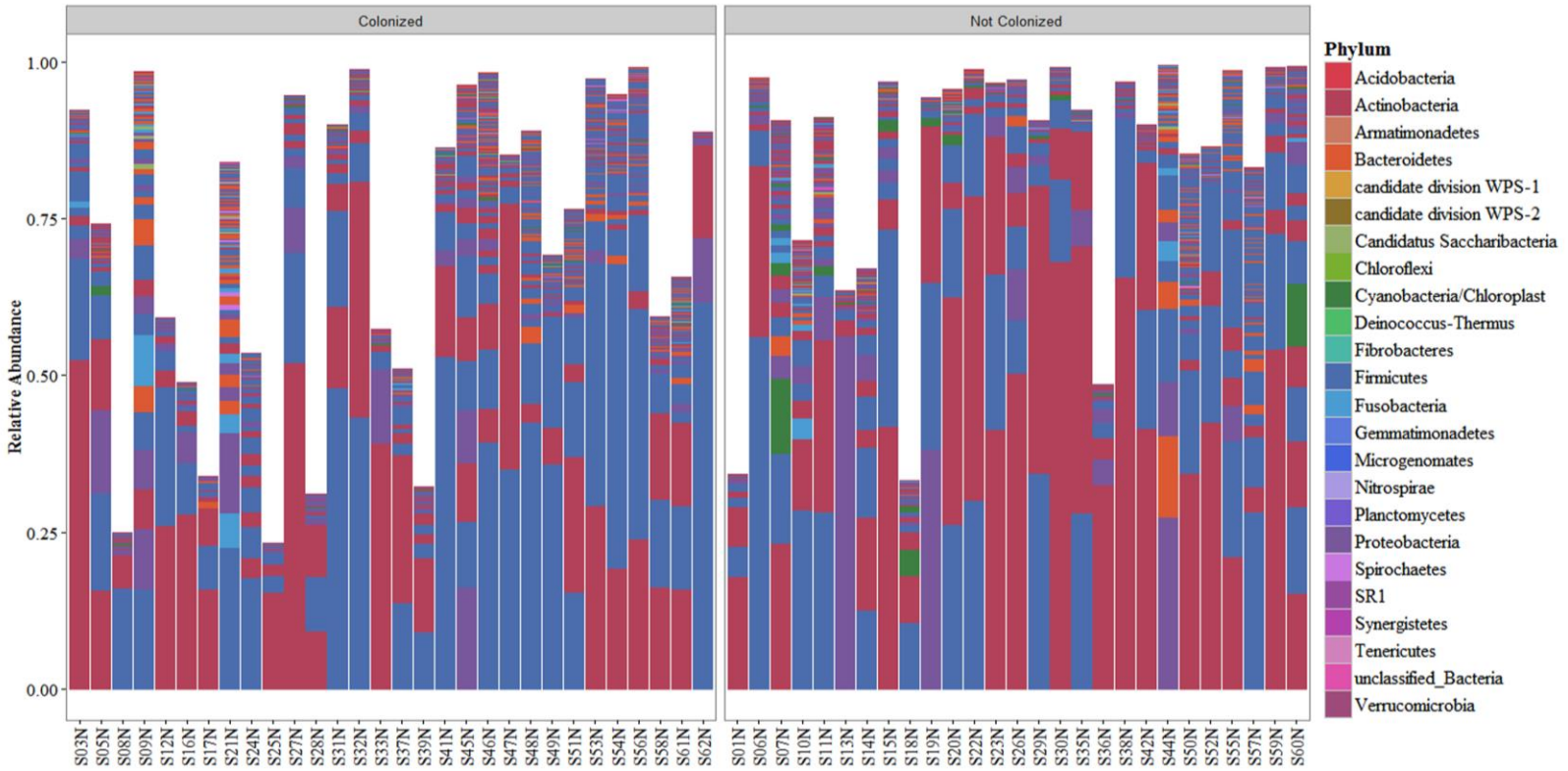

Figure 4-28: Barplot of the relative abundance of all phyla in the nasal samples by colonization status.

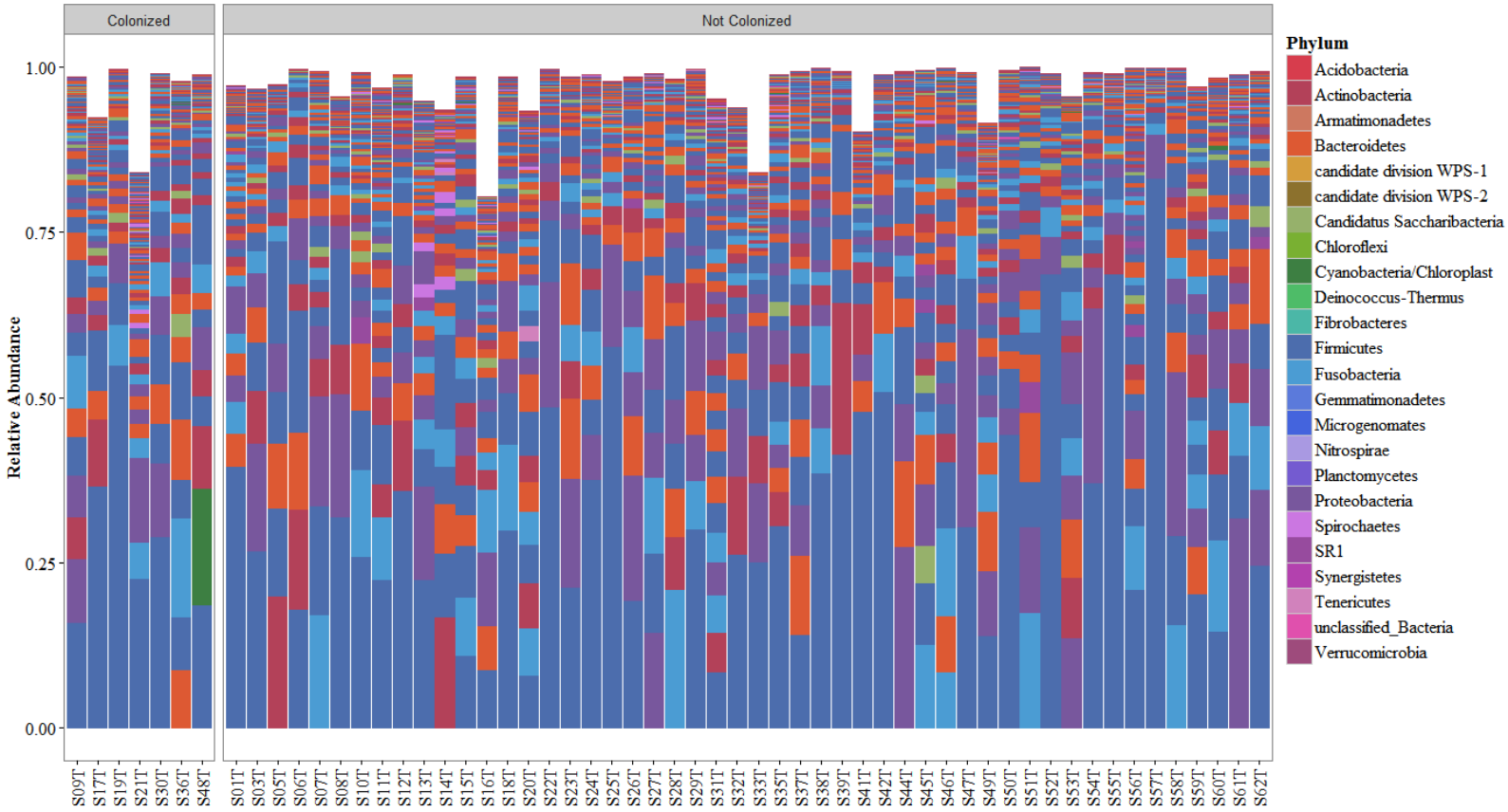

Figure 4-29: Barplot of the relative abundance of all phyla in the oropharyngeal samples by colonization status.

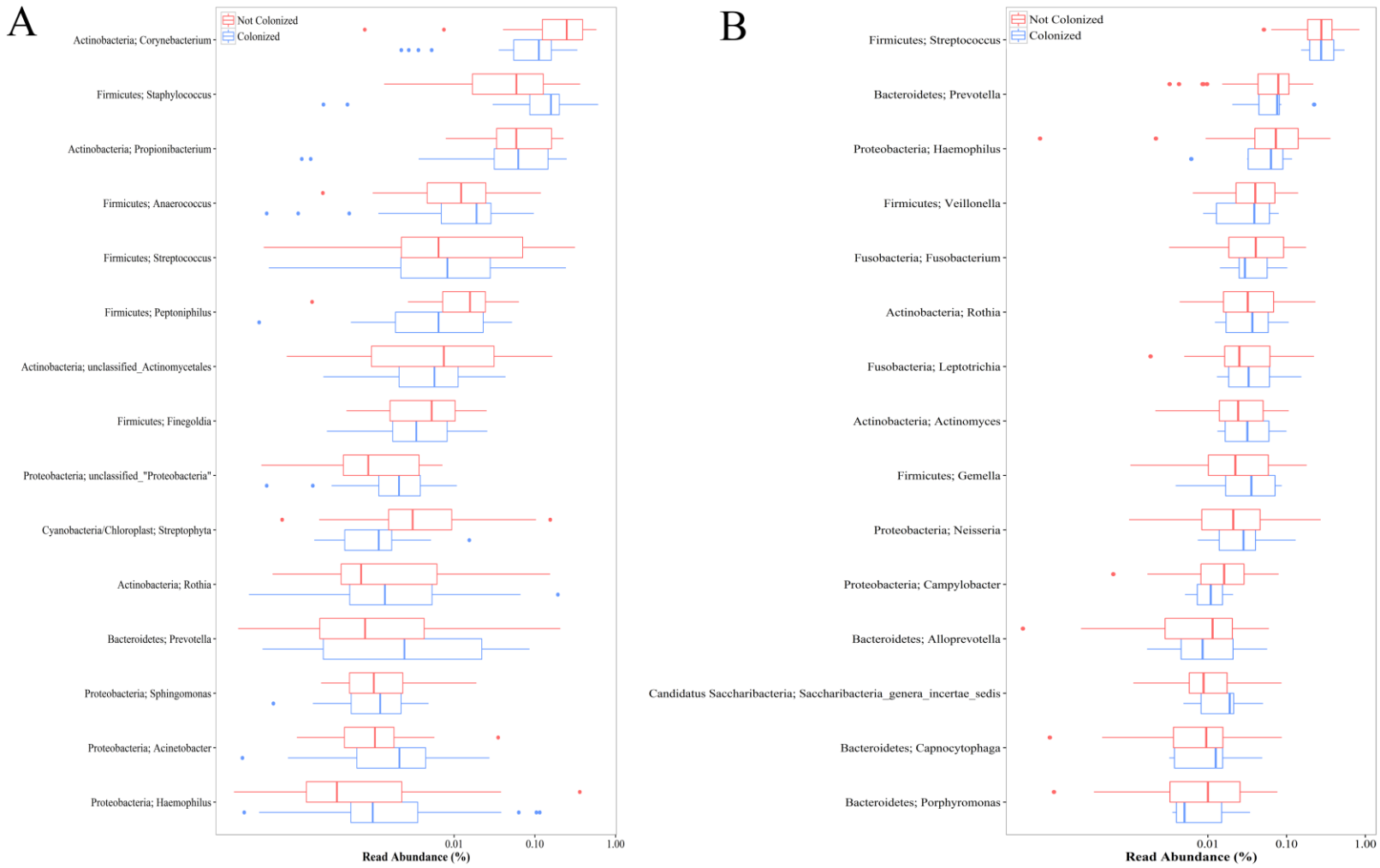

Figure 4-30: Boxplot of the top 15 most abundant OTUs in the nares and oropharynx based on colonization status.

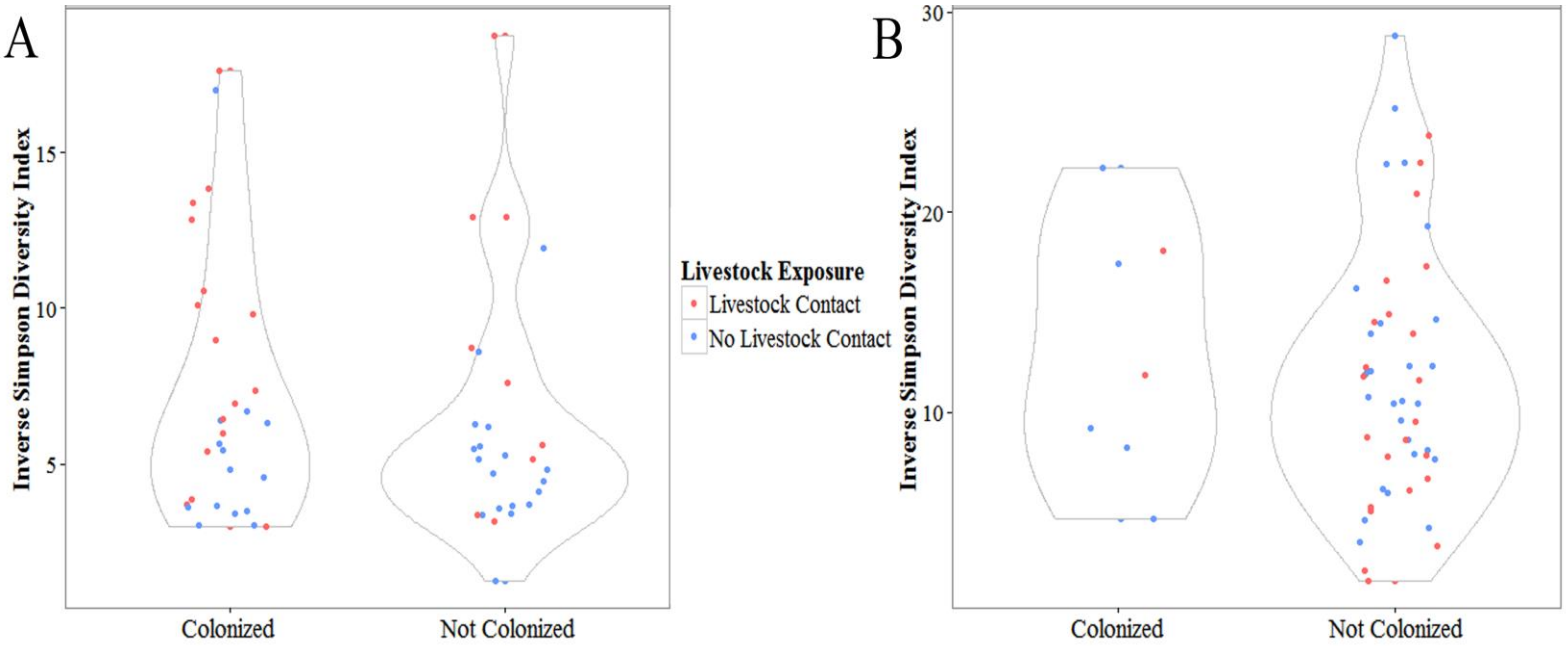

Figure 4-31: Inverse Simpson diversity index comparing alpha diversity by colonization status and livestock exposure. (a) Inverse Simpson diversity index for the nasal samples. (b) Inverse Simpson diversity index for the oropharyngeal samples.

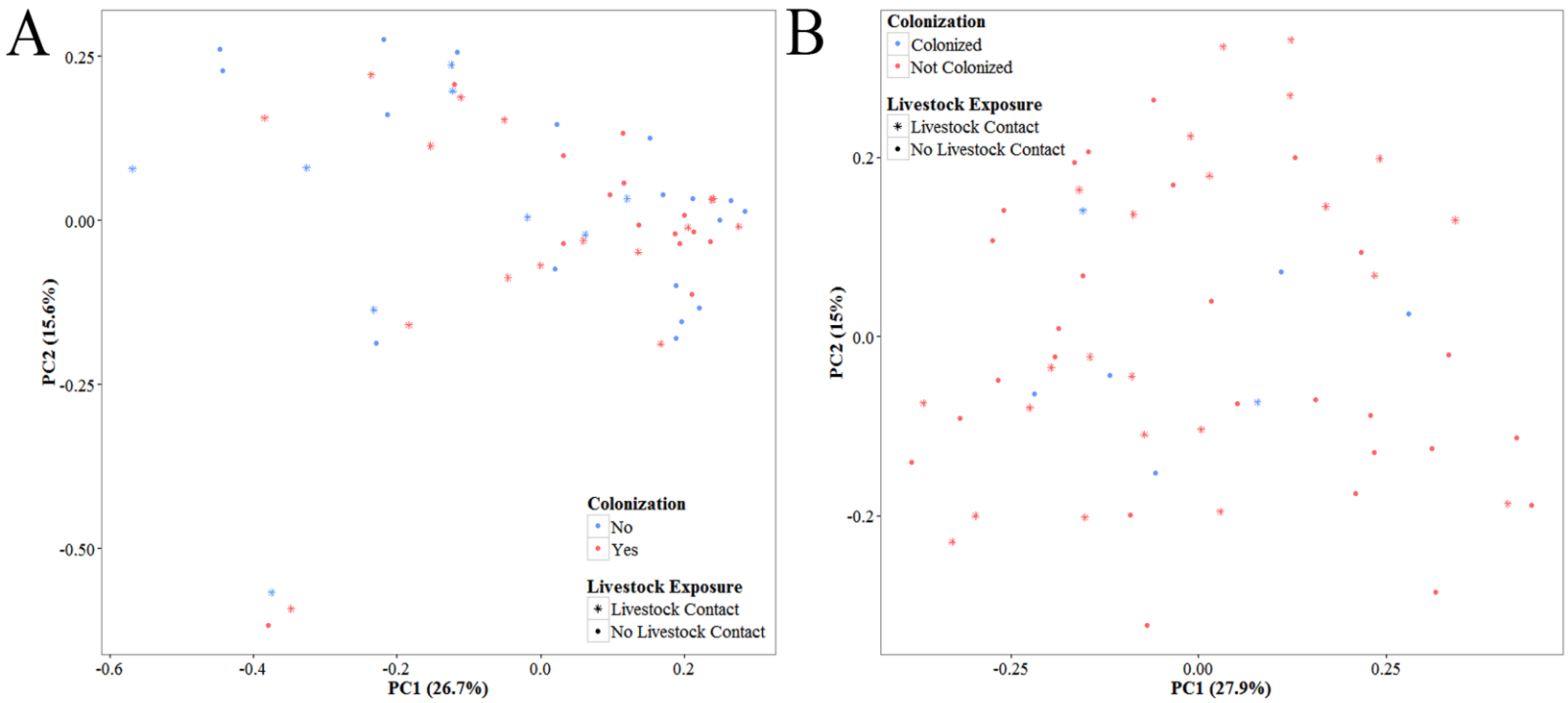

Figure 4-32: Principal coordinates analysis of colonization status and livestock exposure. PC1 and PC2 = principal coordinates 1 and 2, respectively. (a) Ordination plot of Bray-Curtis dissimilarly index of the nares samples. (b) Ordination of the oropharyngeal samples based on the Bray-Curtis dissimilarities index.

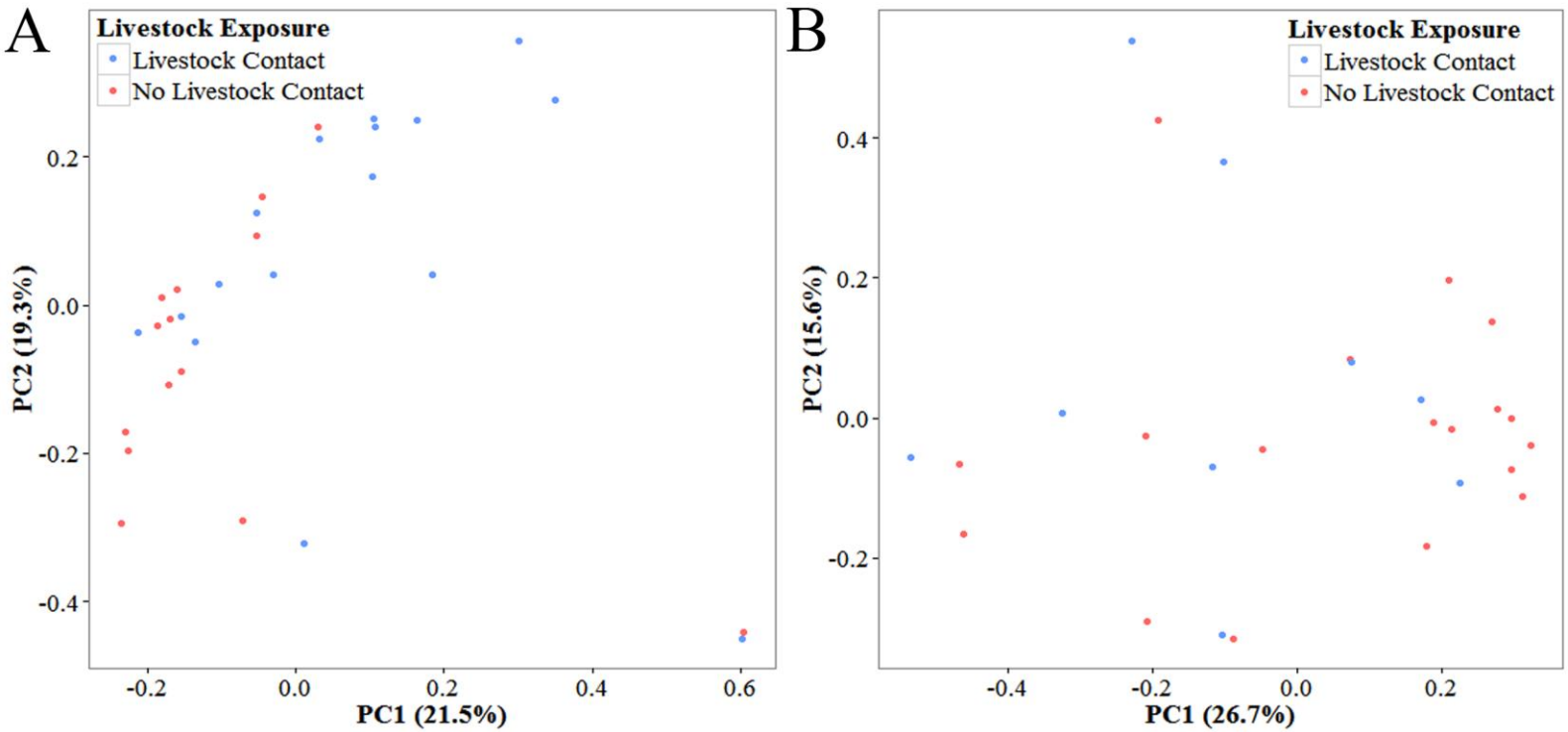

Figure 4-33: (a) Ordination of the colonized samples by livestock exposure status. (b) Ordination of the non-colonized samples by livestock exposure status.

Figure 4-34: Relative abundance of all OTUs by livestock exposure for the nasal samples in the colonized individuals depicted as a heatmap. OTUs are in the same order in both panels.

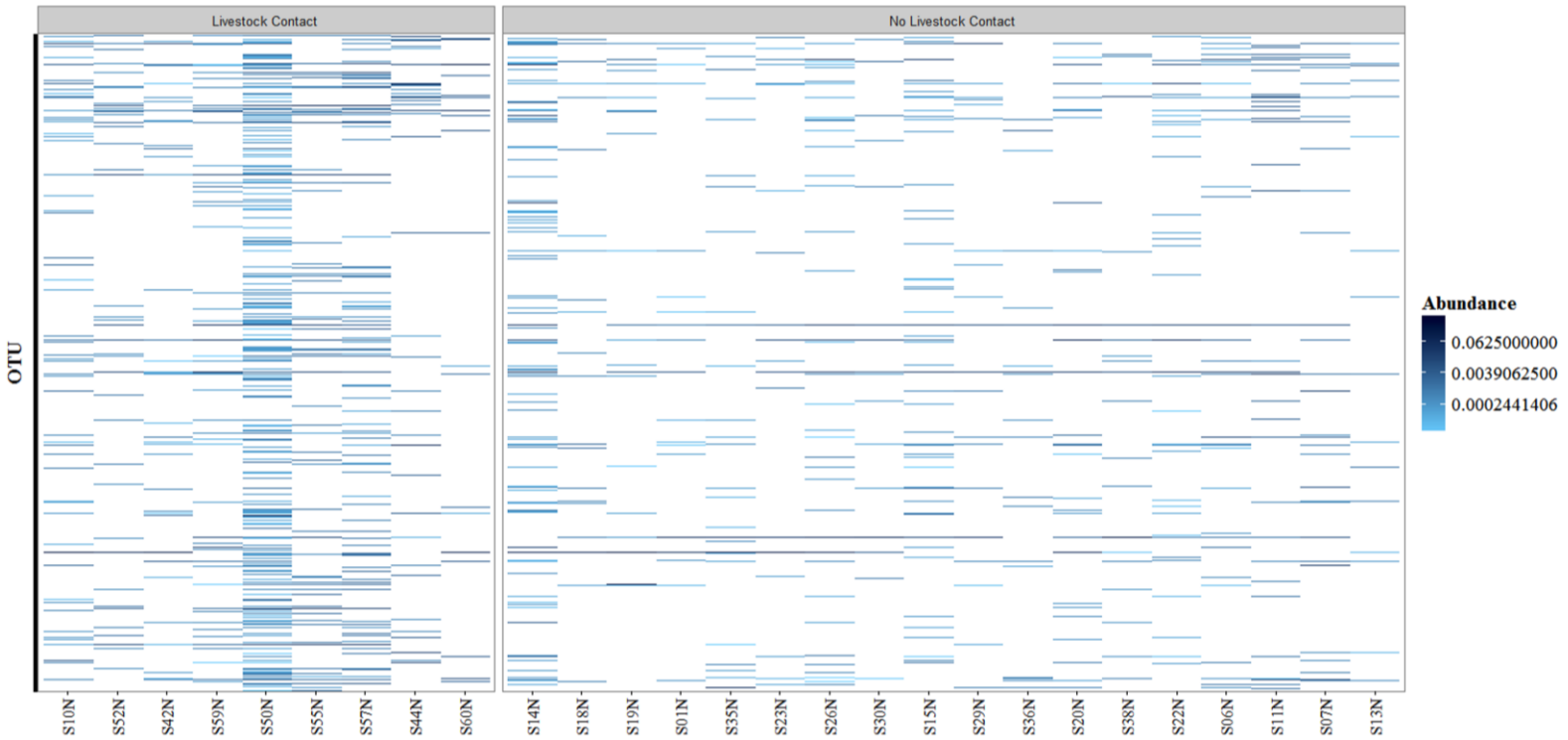

Figure 4-35: Relative abundance of all OTUs by livestock exposure for the nasal samples in the non-colonized individuals depicted as a heatmap. OTUs are in the same order in both panels.

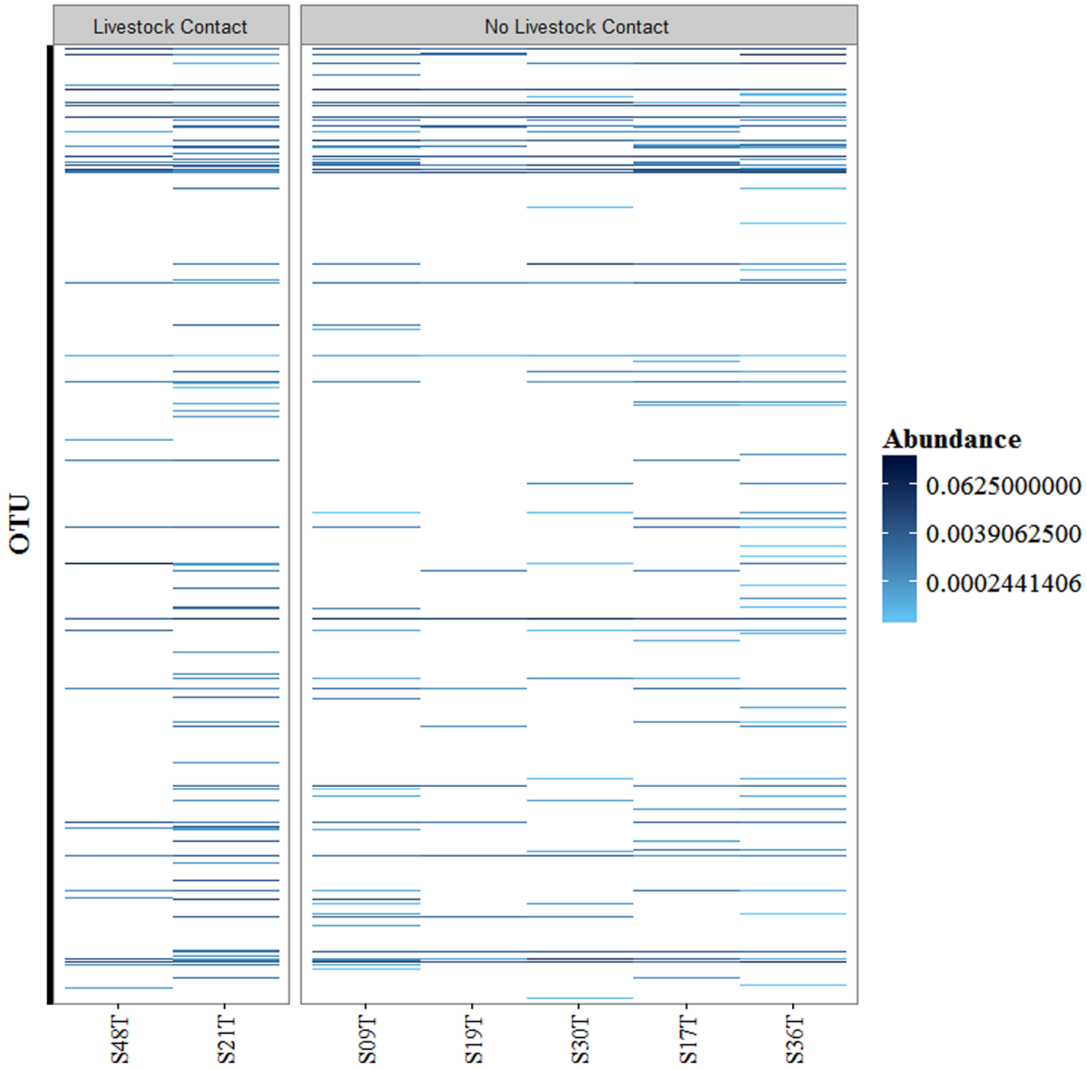

Figure 4-36: Relative abundance of all OTUs by livestock exposure for the oropharyngeal samples in the colonized individuals. OTUs are in the same order in both panels.

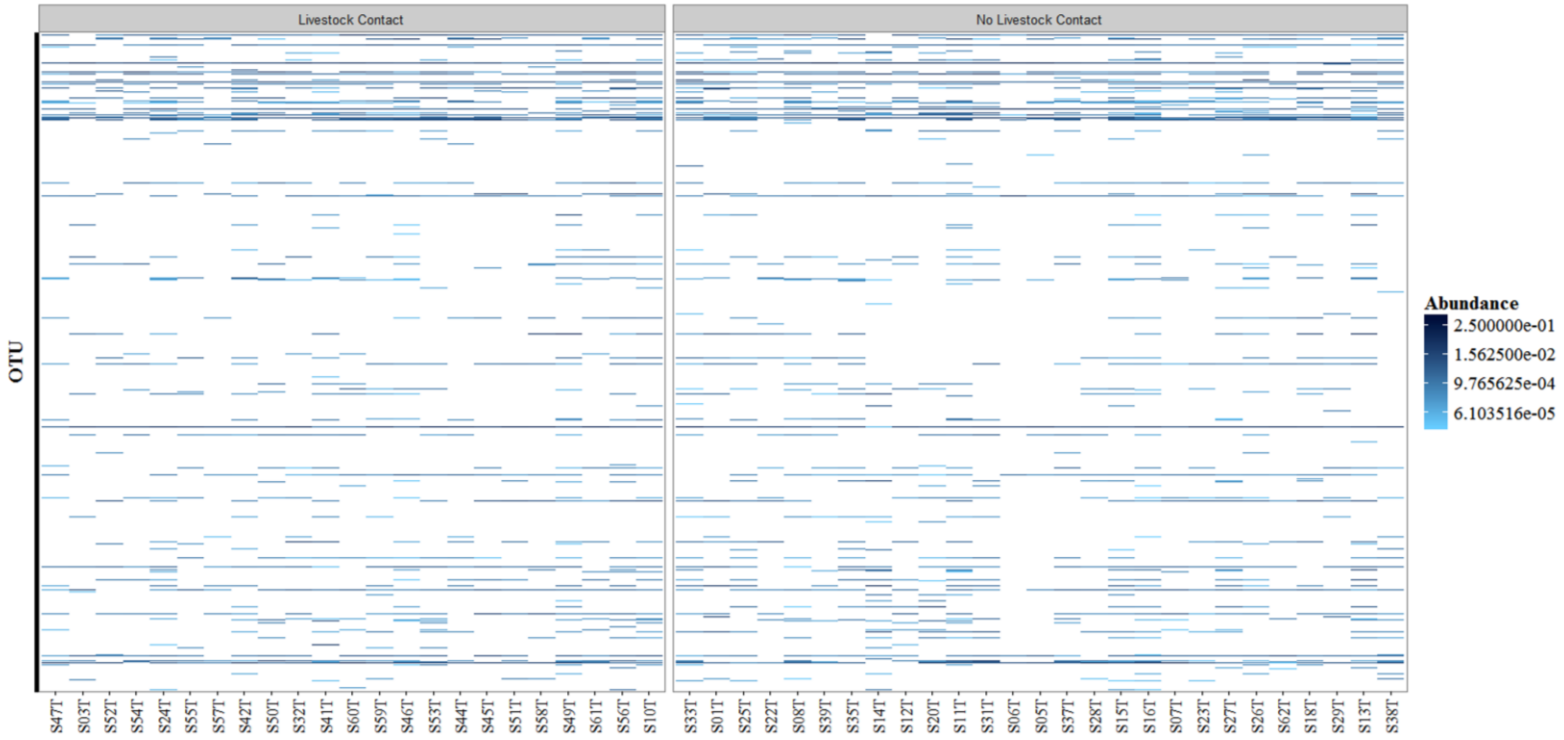

Figure 4-37: Relative abundance of all OTUs by livestock exposure for the oropharyngeal samples in the non-colonized colonized individuals. OTUs are in the same order in both panels.

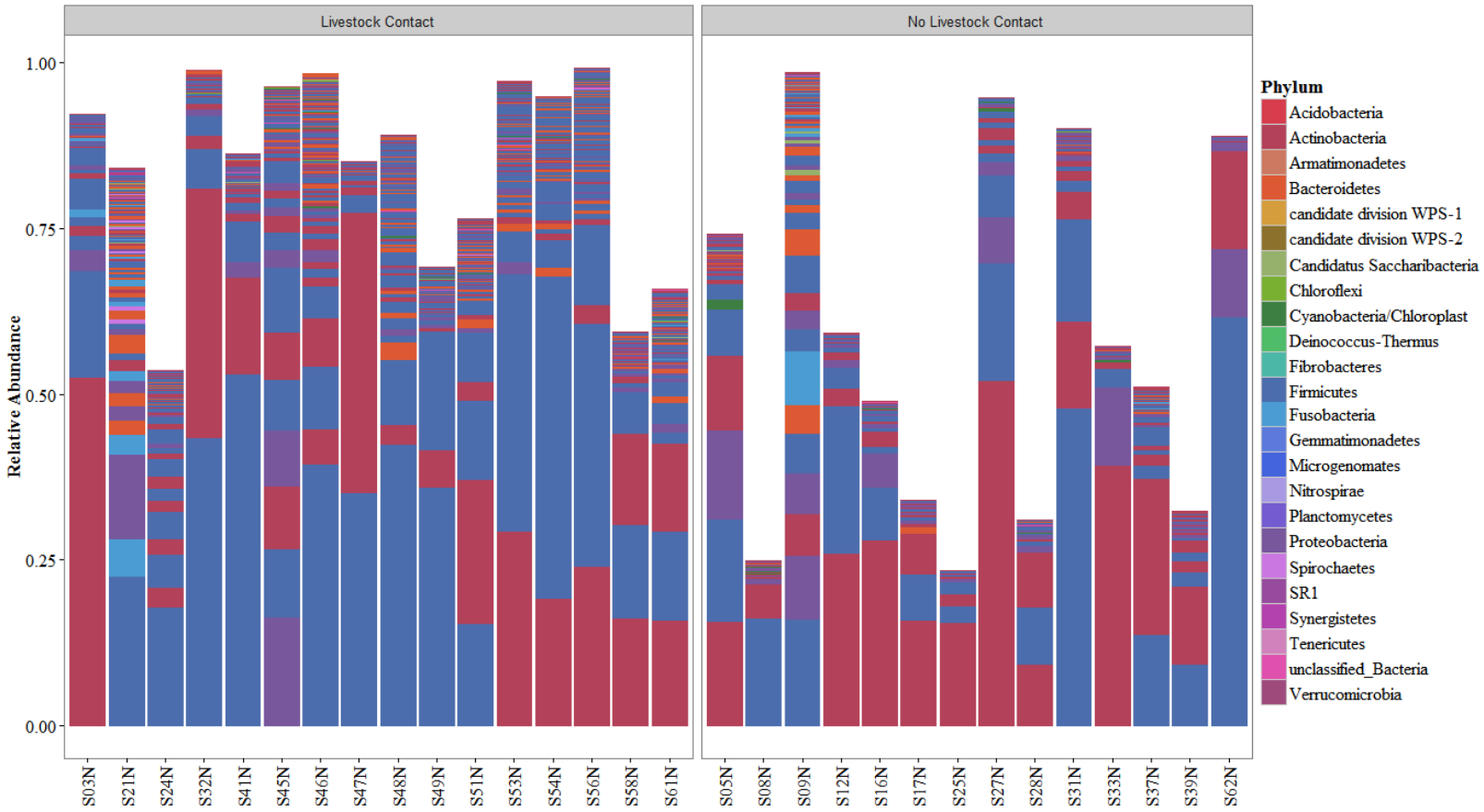

Figure 4-38: Relative abundances of OTUs for colonized nasal samples by livestock contact.

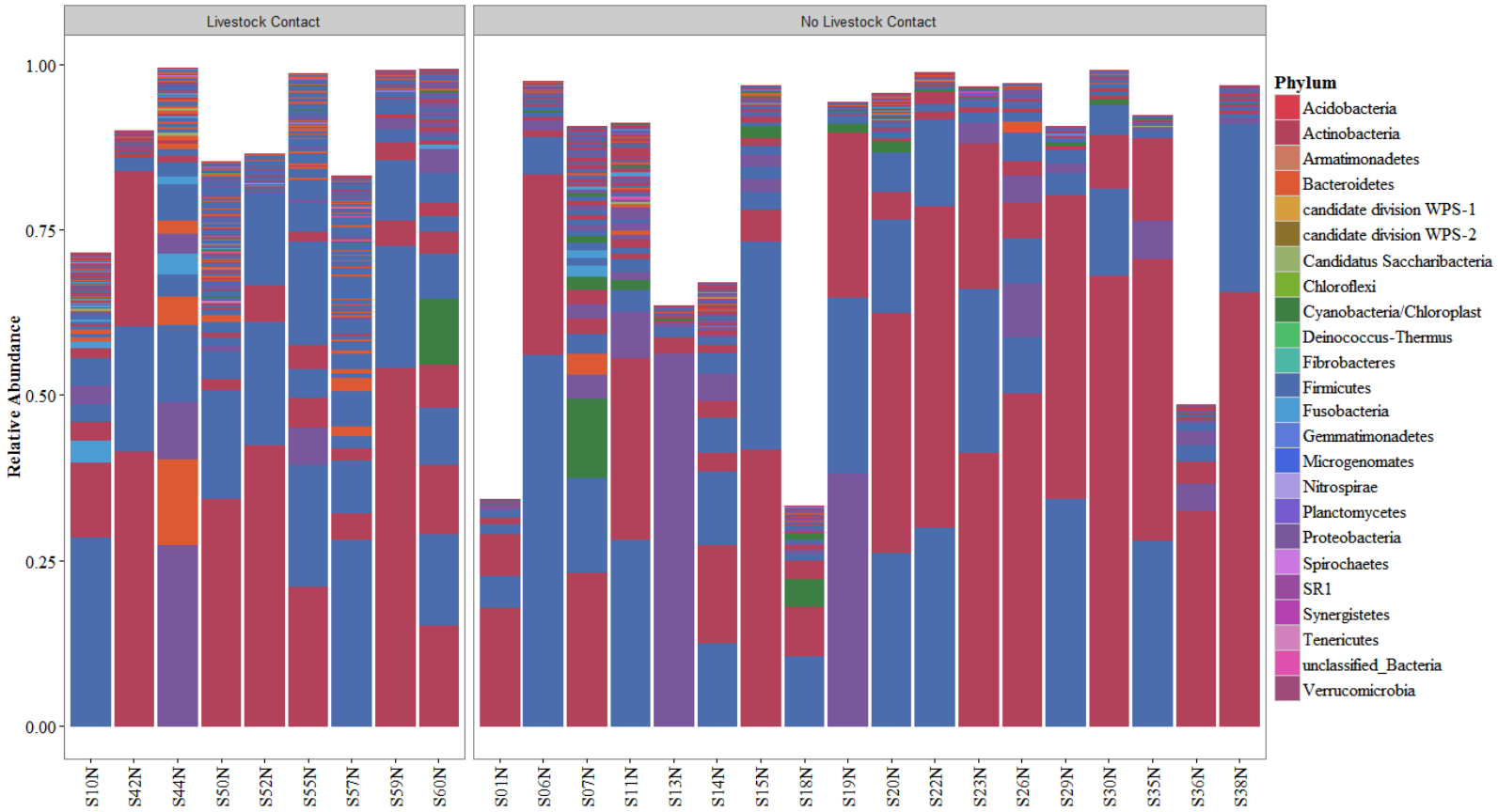

Figure 4-39: Relative abundances of OTUs for non-colonized nasal samples by livestock contact.

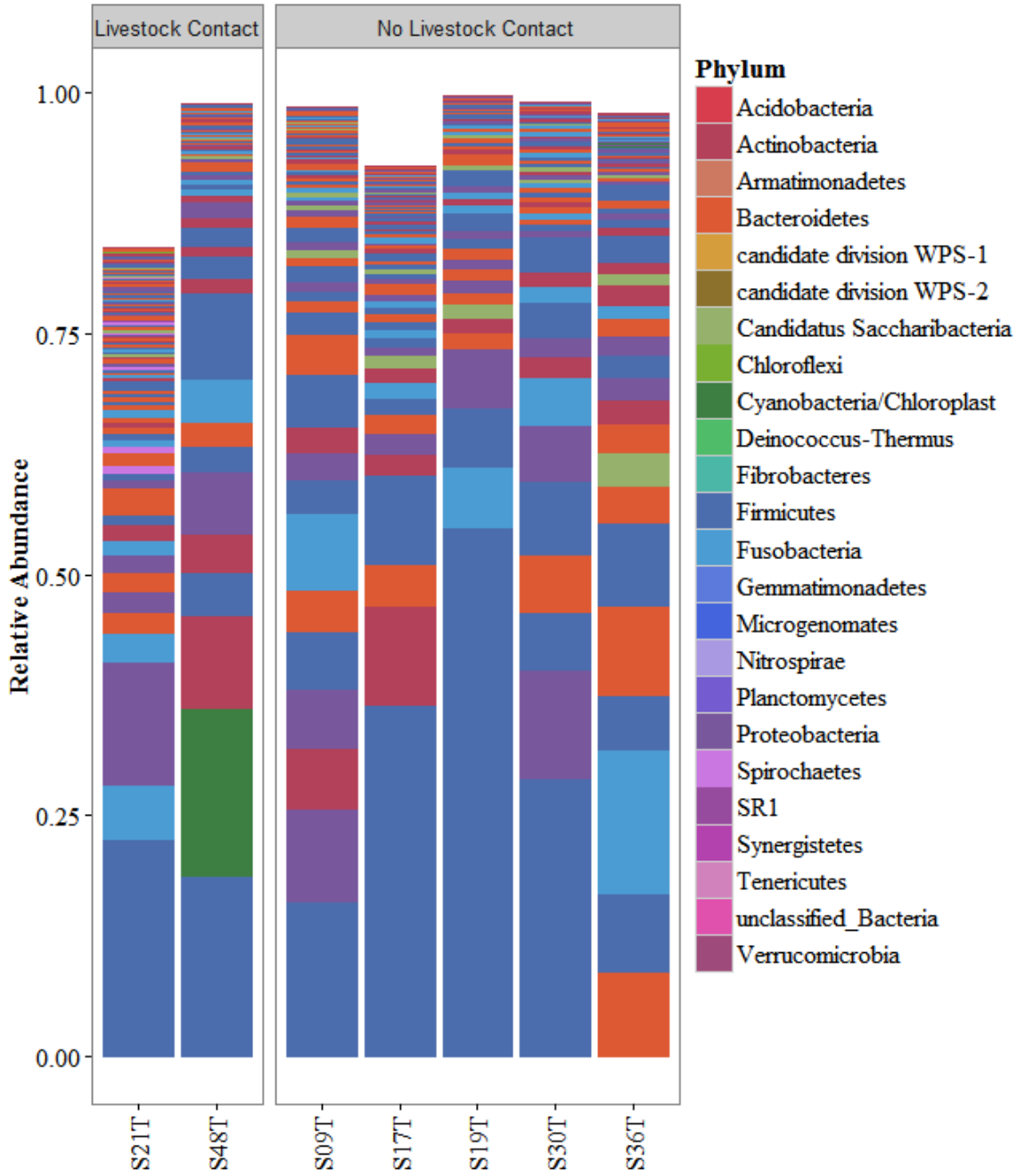

Figure 4-40: Relative abundances of OTUs for colonized oropharyngeal samples by livestock status.

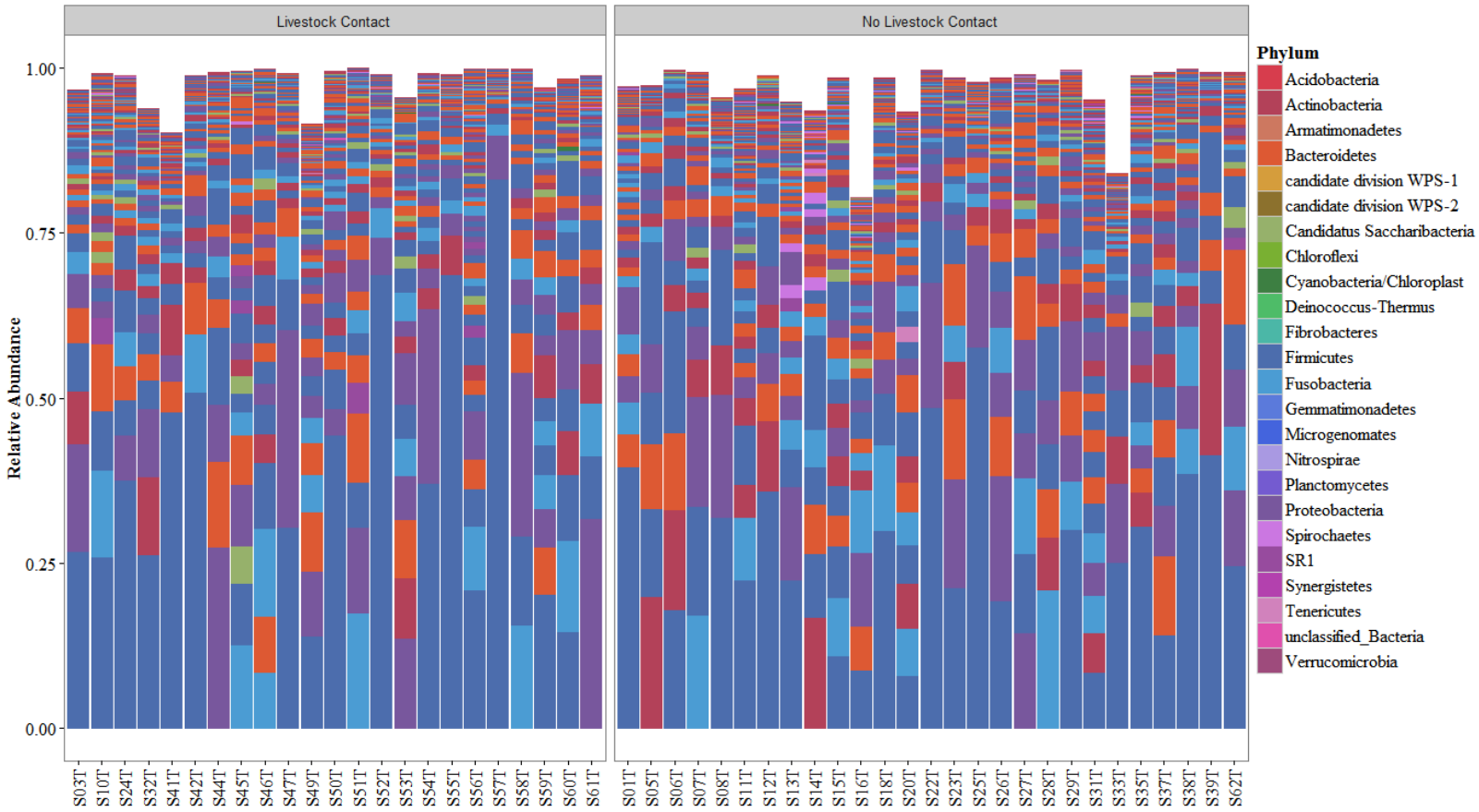

Figure 4-41: Relative abundances of OTUs for non-colonized oropharyngeal samples by livestock status.

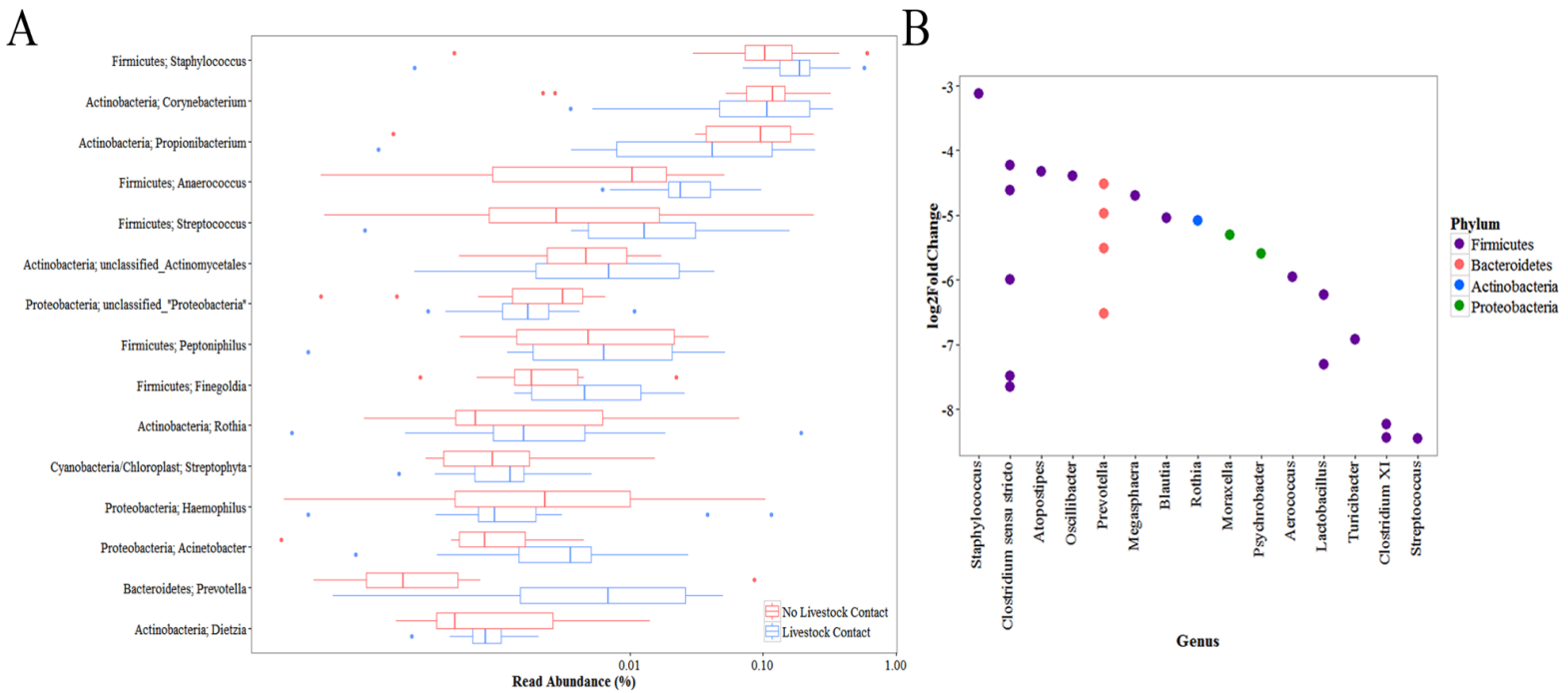

Figure 4-42: (a) Boxplot of the top 15 most abundant OTUs in the nasally colonized persons. Phylum and genus classification are shown. Percent abundances are log transformed. (b) Log 2-fold change of the significantly differentially abundant OTUs between colonized livestock workers and colonized persons with no livestock contact in the nares (Benjamini-Hochberg correction applied). Points represent OTUs with phyla represented by color. Negative values represent OTUs significantly more abundant in colonized livestock workers and positive values represent OTUs significantly more abundant in colonized, non-livestock workers.

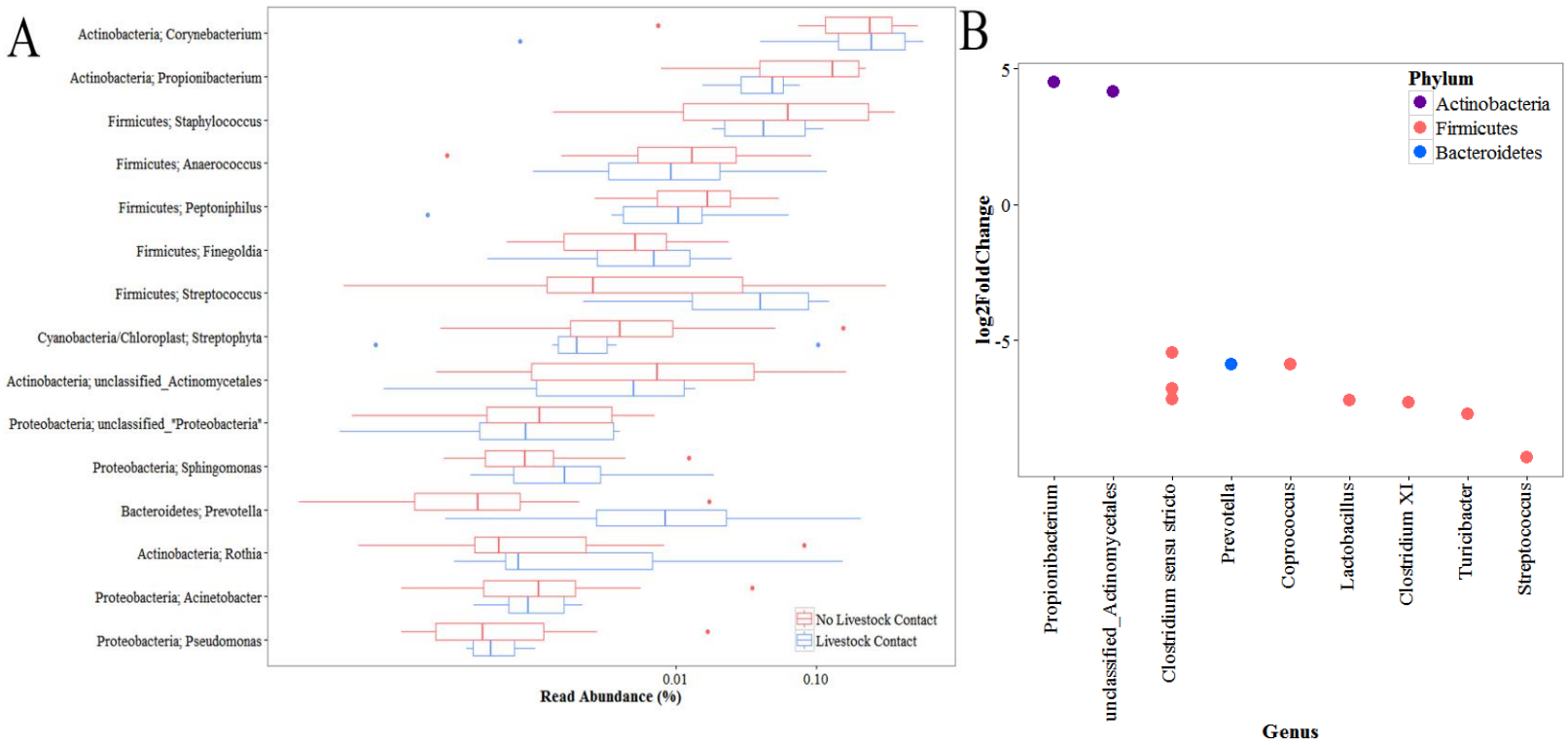

Figure 4-43: (a) Boxplot of the top 15 most abundant OTUs in non-colonized persons. Phylum and genus classification are shown. Percent abundances are log transformed. (b) Log 2-fold change of the significantly differentially abundant OTUs between non-colonized livestock workers and non-colonized persons with no livestock contact (Benjamini-Hochberg correction applied). Points represent OTUs with phyla represented by color. Negative values represent OTUs significantly more abundant in non-colonized livestock workers and positive values represent OTUs significantly more abundant in non-colonized, non-livestock workers.

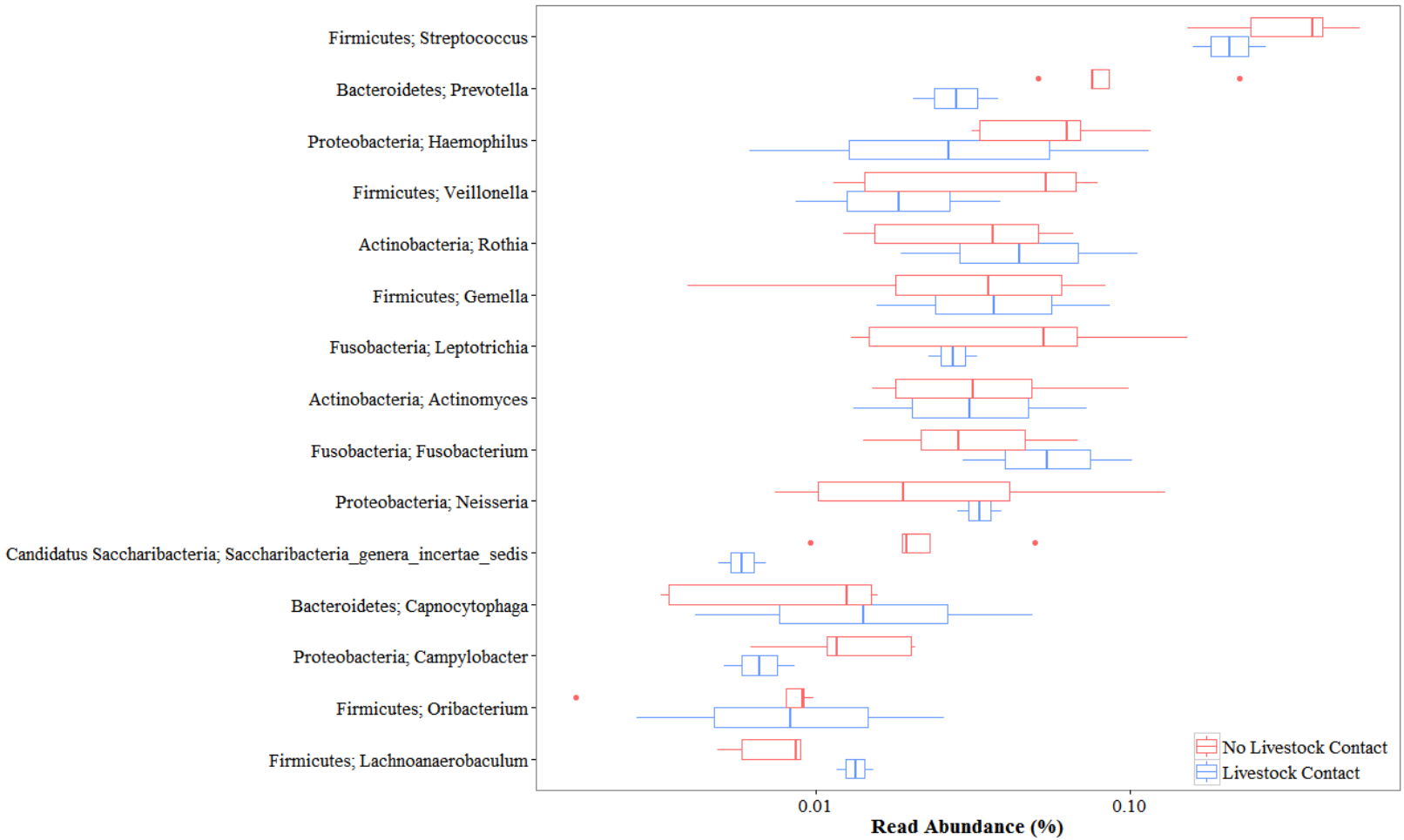

Figure 4-44: Boxplot of the top 15 most abundant OTUs in the oropharynx of colonized persons. Phylum and genus classification are shown. Percent abundances are log transformed.

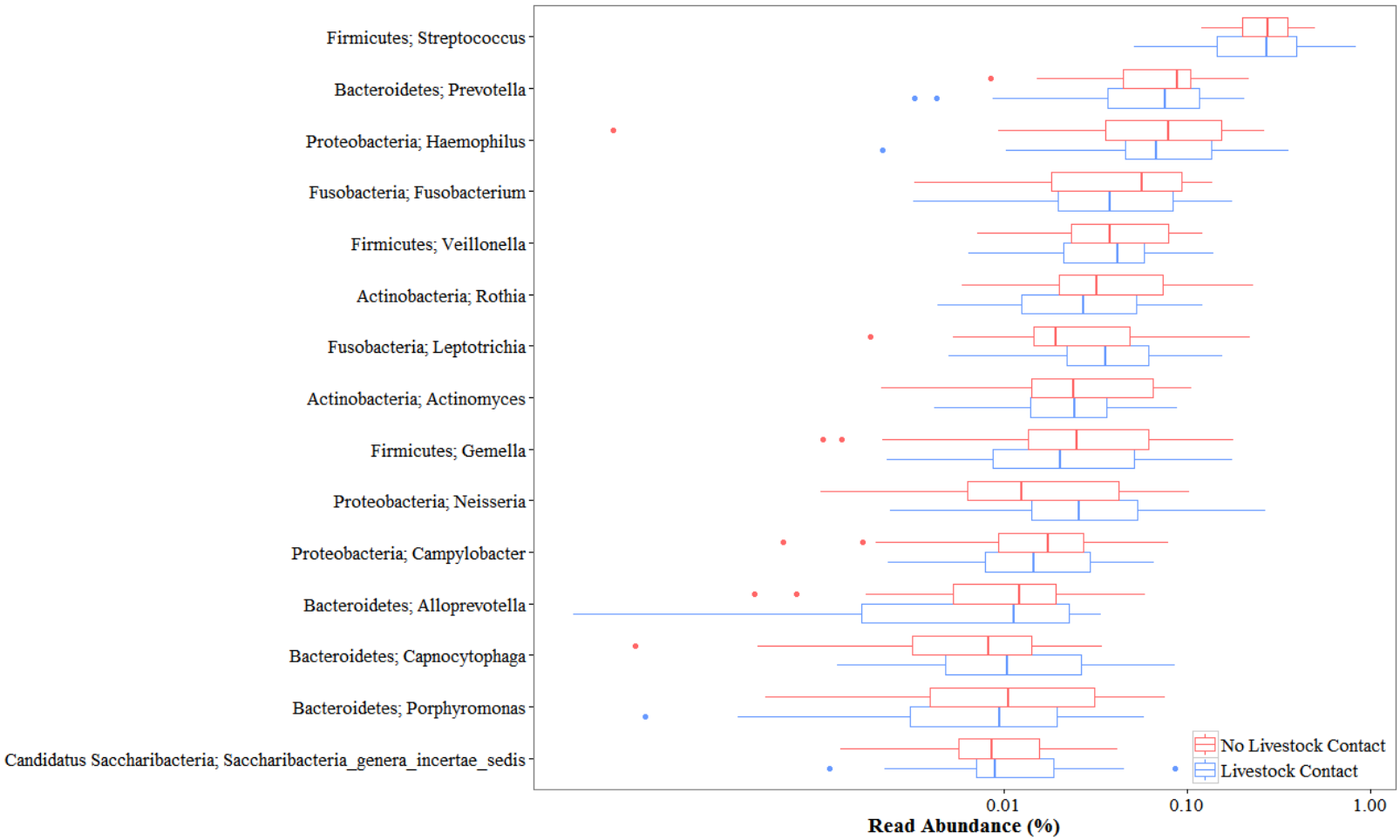

Figure 4-45: Boxplot of the top 15 most abundant OTUs in the oropharynx of non-colonized persons. Phylum and genus classification are shown. Percent abundances are log transformed.

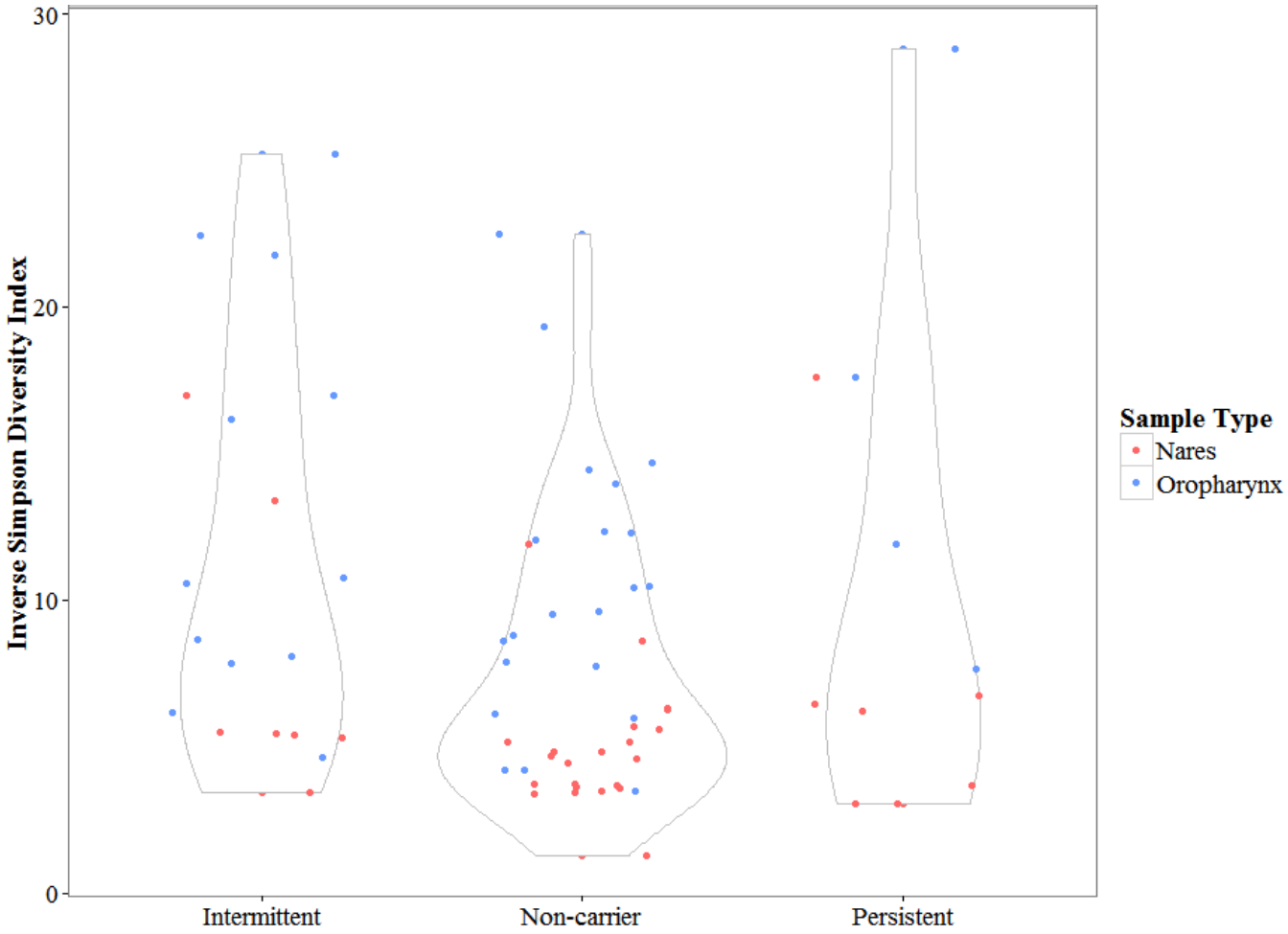

Figure 4-46: Inverse Simpson diversity index comparing alpha diversity by prior *S. aureus* carriage status.

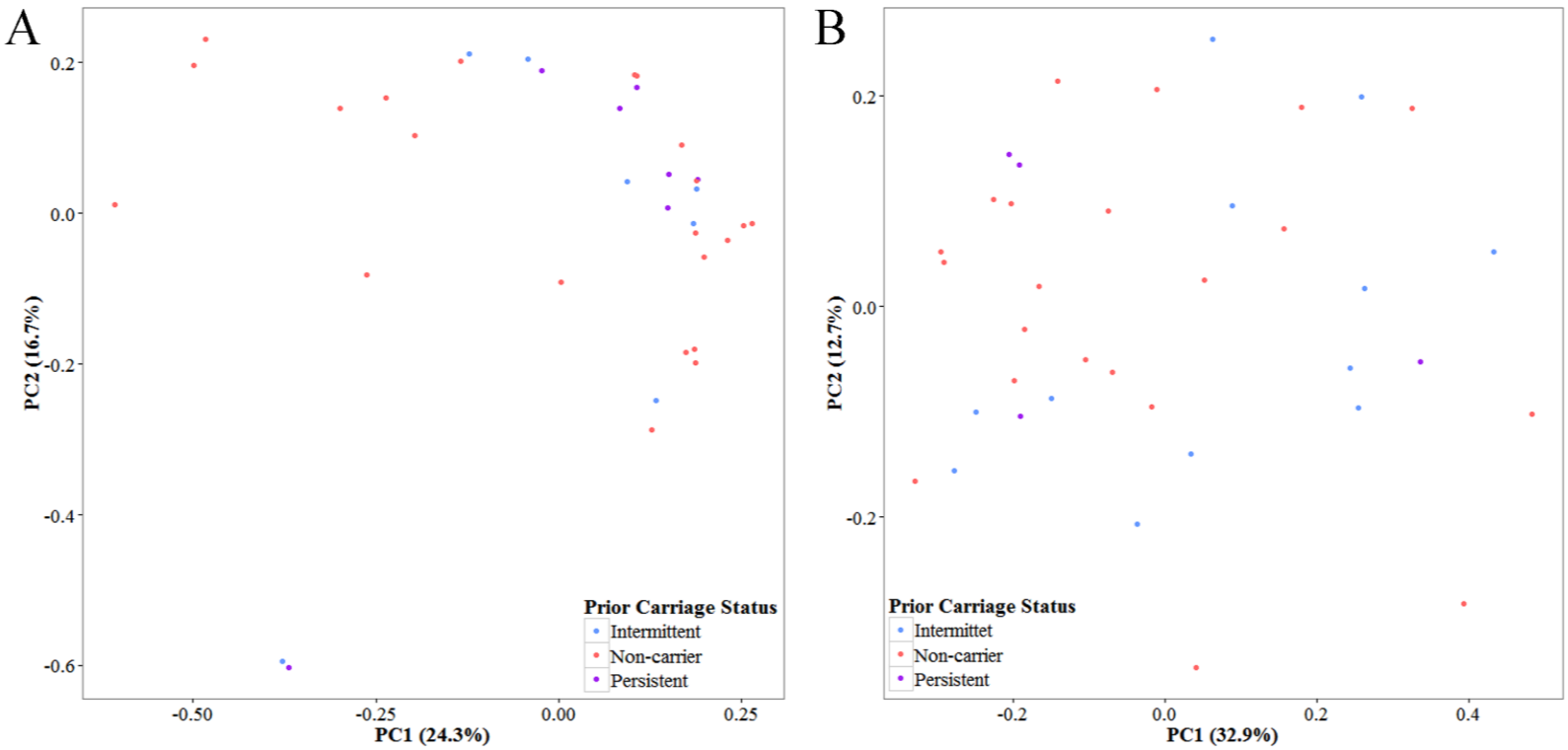

Figure 4-47: Principal coordinates analysis of *S. aureus* prior carriage status. (a) Ordination plot of Bray-Curtis dissimilarly index of prior carriage status and current carrier status. (b) Ordination of the Bray-Curtis dissimilarities index by prior carriage status and sample type. PC1 and PC2 = principal coordinates 1 and 2, respectively.

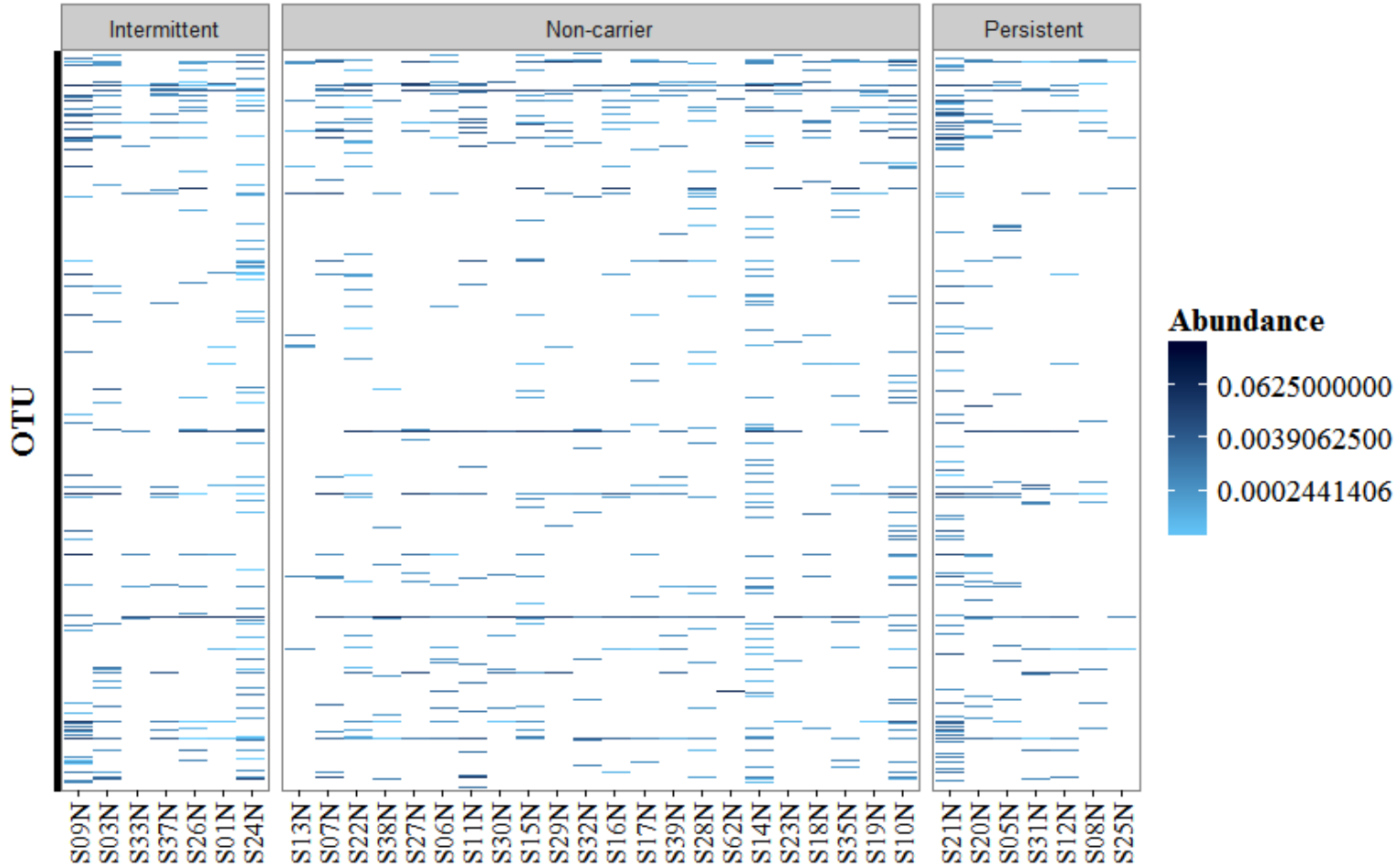

Figure 4-48: Heatmap of relative abundance of all OTUs by *S. aureus* prior carriage status in the nares. OTUs are in the same order in both panels.

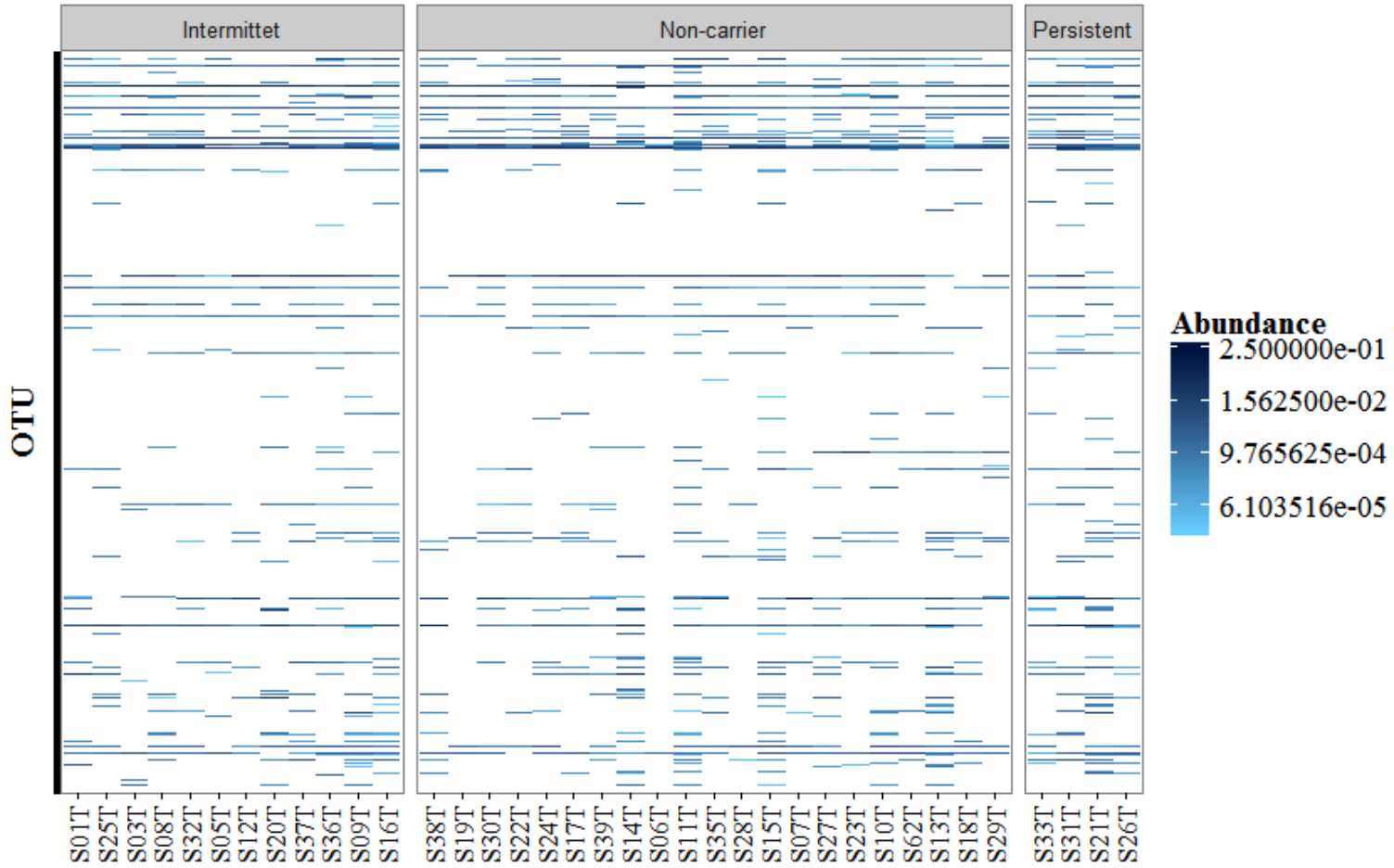

Figure 4-49: Heatmap of relative abundance of all OTUs by *S. aureus* prior carriage status in the oropharynx. OTUs are in the same order in both panels.

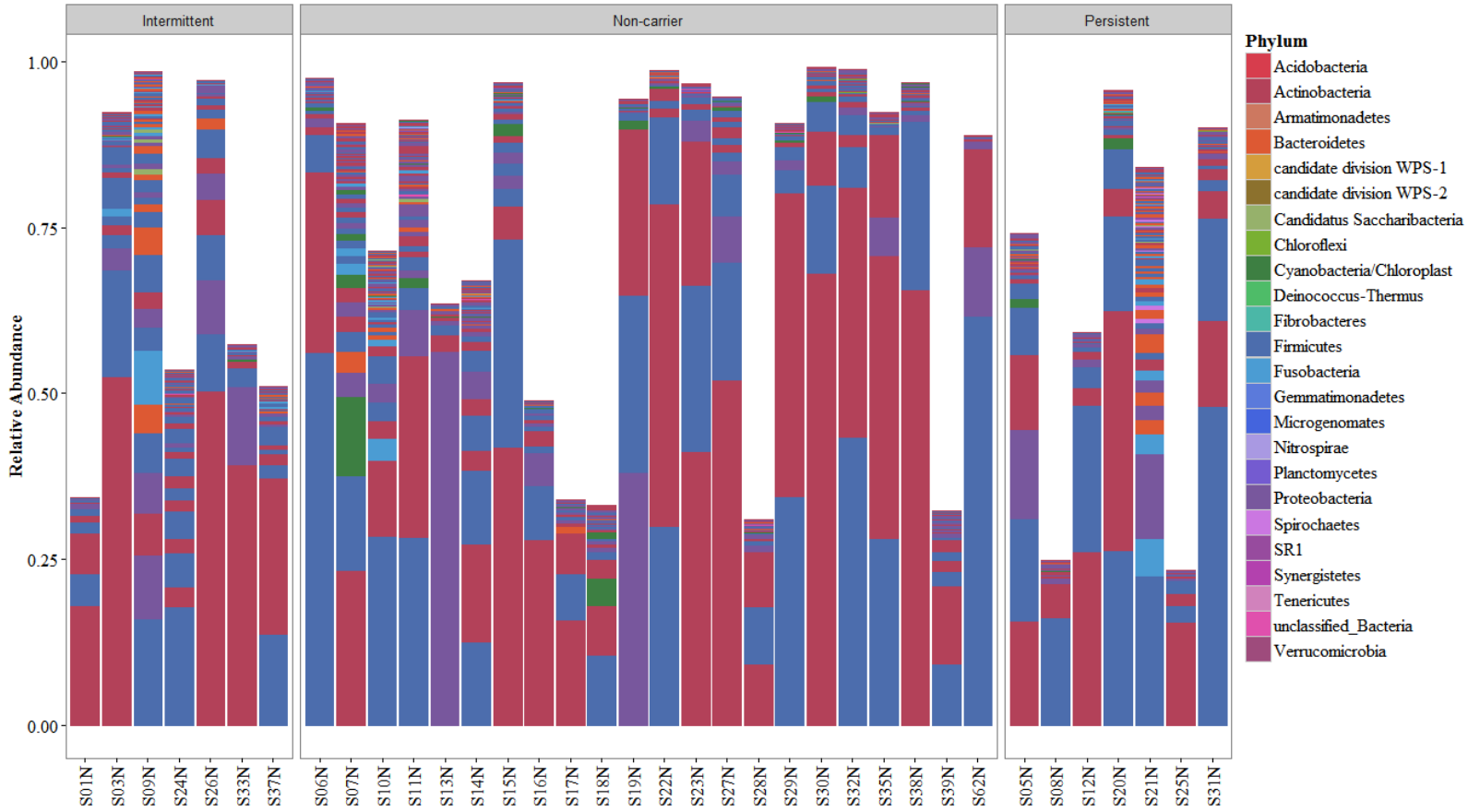

Figure 4-50: Relative abundances of OTUs in the nasal samples by *S. aureus* prior carriage status.

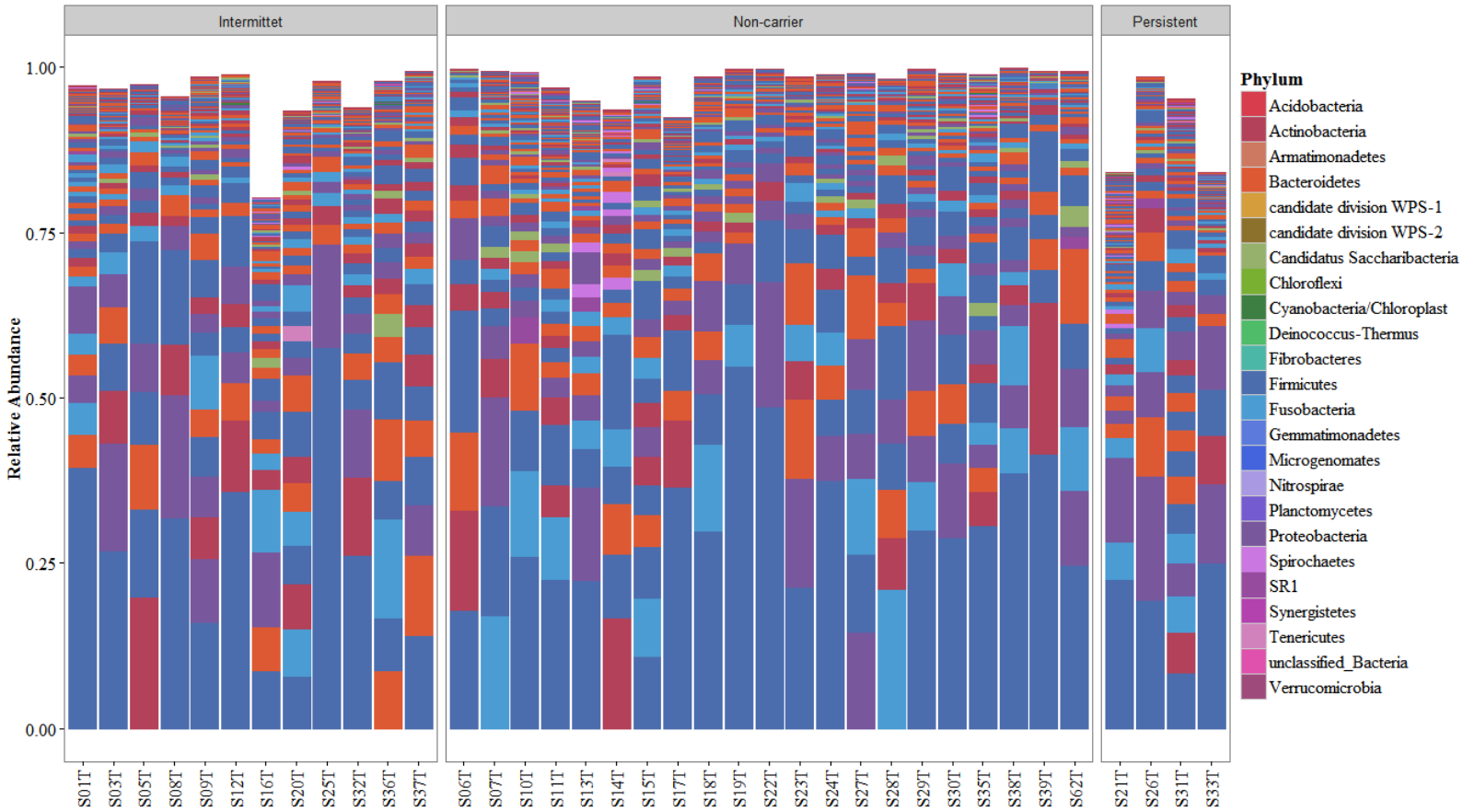

Figure 4-51: Relative abundances of OTUs in oropharyngeal samples by *S. aureus* prior carriage status.

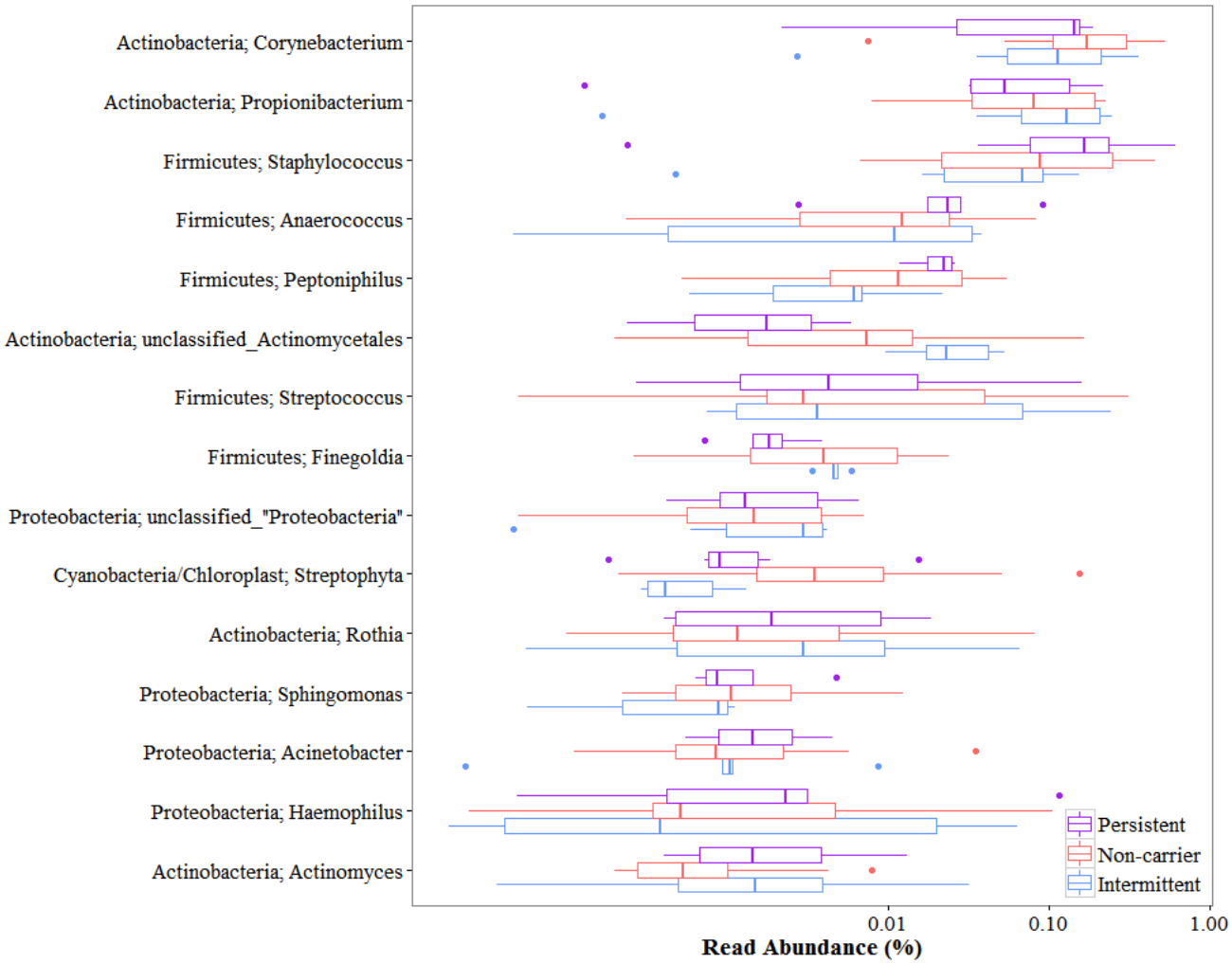

Figure 4-52: Top 15 most OTUs in the nasal microbiome by *S. aureus* prior carriage status.

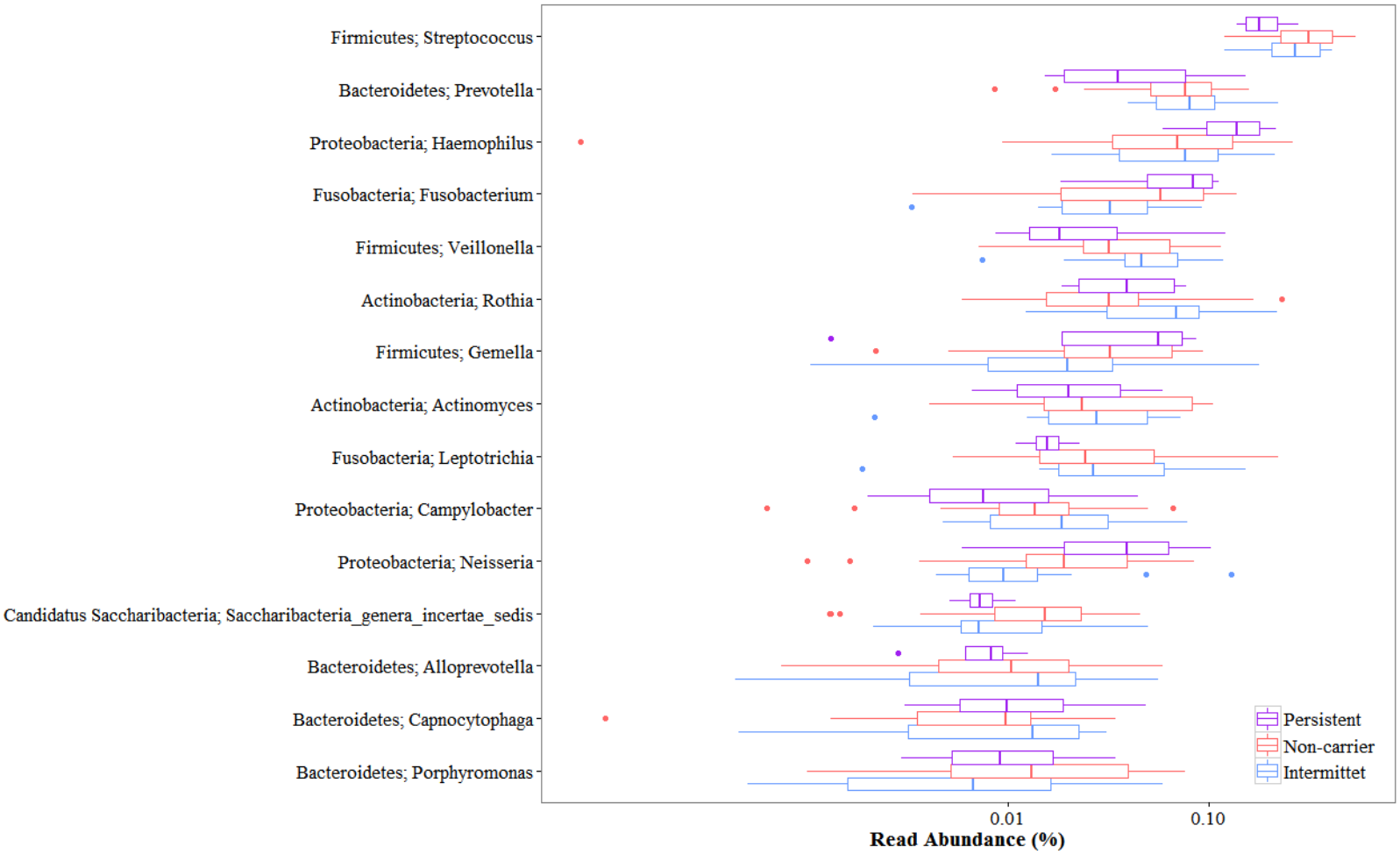

Figure 4-53: Top 15 most OTUs in the oropharyngeal microbiome by *S. aureus* prior carriage status.

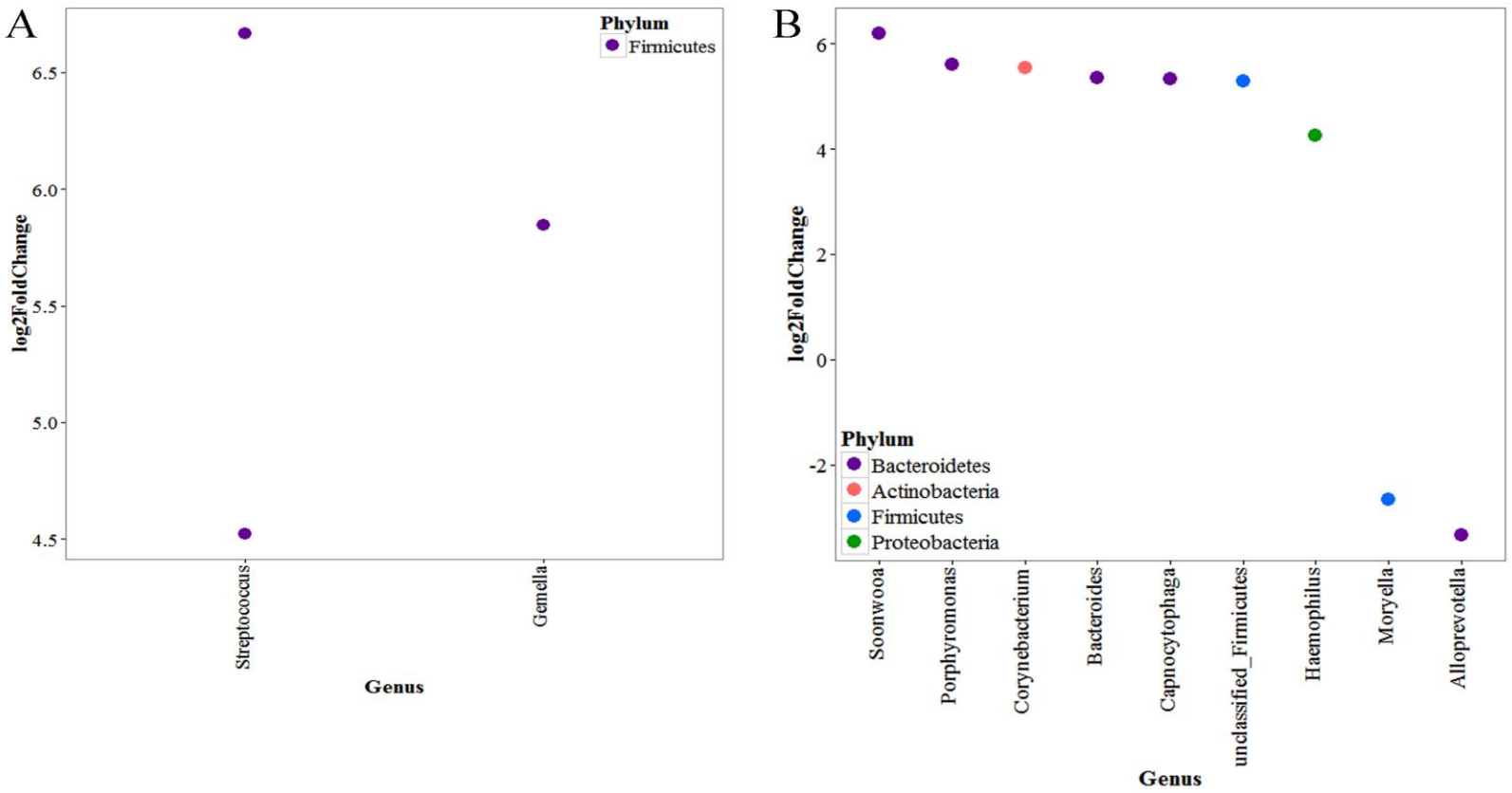

Figure 4-54: Differentially abundant microbiota in the oropharyngeal samples by prior colonization status. (a) Log 2-fold change of the significantly differentially abundant OTUs between intermittently colonized and non-colonized persons (Benjamini-Hochberg correction applied). Positive values represent OTUs significantly more abundant in non-colonized persons and positive values represent OTUs significantly more abundant in intermittently colonized persons (b) Log 2-fold change of the significantly differentially abundant OTUs between intermittently colonized and persistently colonized persons (Benjamini-Hochberg correction applied). Points represent OTUs with phyla represented by color. Positive values represent OTUs significantly more abundant in persistently colonized persons and positive values represent OTUs significantly more abundant in intermittently colonized persons.

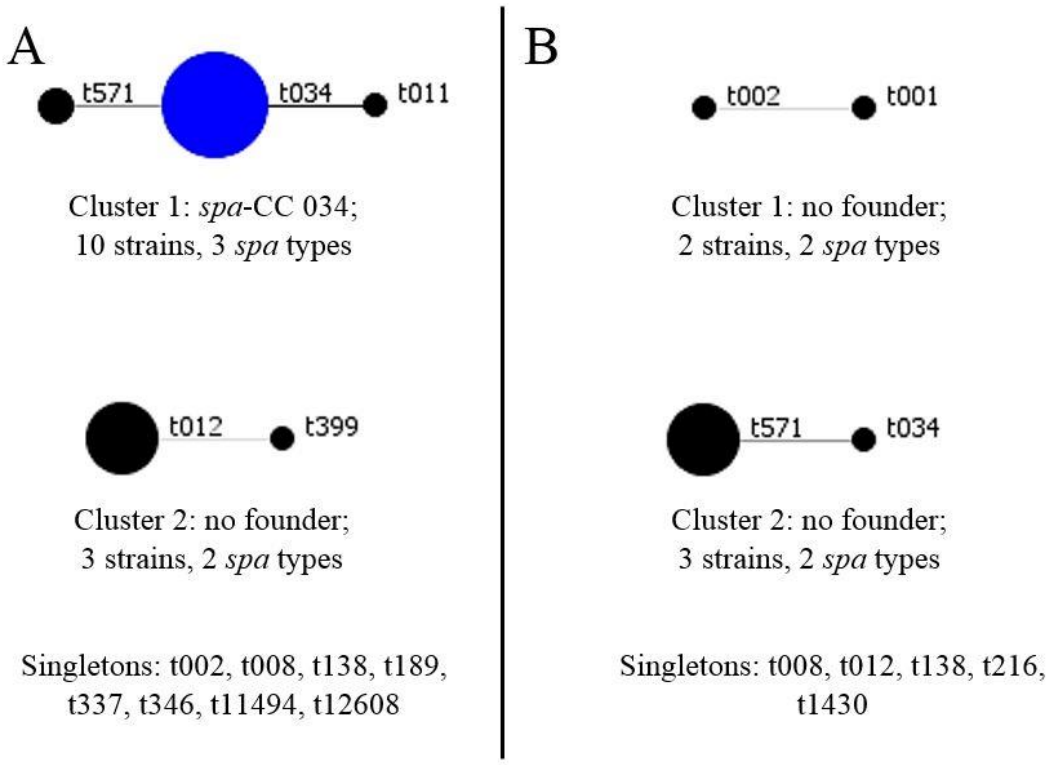

Figure 4-55: BURP analysis of *spa* typing by sample type (n=39). Each circle represents a single spa type. The blue circle indicates the cluster founder. (a) BURP cluster of the nasal samples. Six nasal strains were excluded from the analysis. (n=25) (b) BURP cluster of the oropharyngeal strains. One sample was excluded from the analysis. (n= 13)

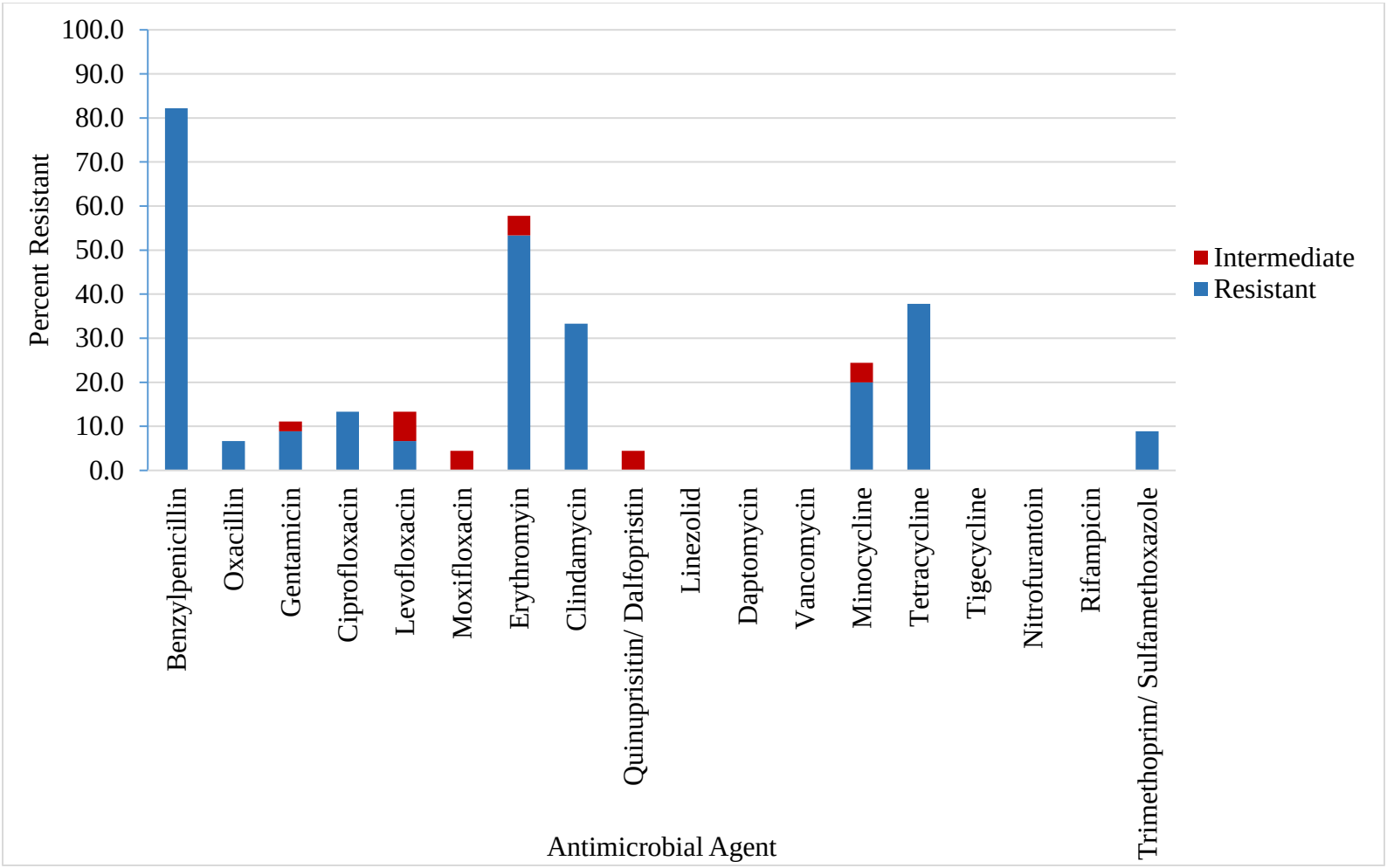

Figure 4-56: Antimicrobial susceptibility to a panel of antimicrobial agents tested using minimum inhibitory concentrations (n=45).

Table 4-1: Participant demographics

	Livestock Contact (n=26)	**No livestock Exposure (n=33)**	**P-value**	**Full Cohort (n=59)**
Age (years)	59.1	51.1	0.027	54.6
BMI	27.4	28.3	0.53	27.9
Sex				
Male	24 (92.3%)	17 (51.5%)		41 (69.5%)
Female	2 (7.7%)	16 (48.5%)	0.0007	18 (30.5%)
Race*				
Caucasian	25 (96.2%)	33 (100.0%)		58 (98.3%)
Other	1 (3.8%)	4 (12.0%)	0.394	5 (8.5%)
Income (net)				
<$20,000	0 (0.0%)	1 (3.0%)		1 (1.7%)
$20,000-$39,999	3 (11.5%)	5 (15.2%)		8 (13.6%)
$40,000-$59,999	3 (11.5%)	6 (18.2%)		9 (15.3%)
$60,000-$79,999	9 (34.6%)	12 (36.4%)		21 (35.6%)
$80,000-$99,999	6 (23.1%)	2 (6.1%)		8 (13.6%)
>$100,000	5 (19.2%)	7 (21.2%)	0.508	12 (20.3%)
Highest level of education				
Less than high school	0 (0.0%)	0 (0.0%)		0 (0.0%)
High school graduate	8 (31.7%)	3 (9.1%)		11 (18.6%)
Some college	3 (11.5%)	5 (15.2%)		8 (13.6%)
College graduate	12 (46.2%)	15 (45.5%)		27 (45.8%)
Graduate level	3 (11.5%)	9 (27.3%)		12 (20.3%)
Professional level	0 (0.0%)	1 (3.0%)	.174	1 (1.7%)
House size				
<1500 sq. ft.	5 (19.2%)	5 (15.2%)		10 (16.9%)
>1500 sq. ft.	19 (73.1%)	27 (81.8%)		46 (77.9%)
Unknown	2 (7.7%)	1 (3.0%)	0.693	3 (5.1%)
Family Size				
1	4 (15.4%)	2 (6.1%)		6 (10.2%)
2	13 (50.0%)	14 (42.4%)		27 (45.8%)
3	3 (11.5%)	5 (15.2%)		8 (13.6%)
4	2 (7.7%)	6 (18.2%)		8 (13.6%)
≥5	4 (15.4%)	6 (18.2%)	0.589	10 (16.9%)

*Race: Participants could identify with more than one racial group. Due to small numbers, race is reported here as Caucasian and other. Other includes: African American (n=1), Asian (n=0), American Indian/ Alaska Native (n=1), and Pacific Islander/ Native Hawaiian (n=2), and other (n=1).

Table 4-2: Health and hygiene characteristics of participants

	Livestock Contact (n=26)	**No livestock Exposure (n=33)**	**P-value**	**Full Cohort (n=59*)**
Asthma				
Yes	0 (0.0%)	3 (9.1%)		3 (5.1%)
No	25 (100.0%)	30 (90.0%)	0.25	55 (93.2%)
COPD				
Yes	1 (4.2%)	1 (3.1%)		2 (3.5%)
No	23 (95.8%)	32 (96.9%)	0.429	55 (96.5%)
Heart Disease				
Yes	2 (8.0%)	6 (18.2%)		8 (13.8%)
No	23 (.92%)	27 (81.8%)	0.445	50 (86.2%)
Diabetes				
Yes	1 (4.0%)	1 (3.0%)		2 (3.4%)
No	24 (96.0%)	32 (97.0%)	1.0	56 (96.6%)
Cancer				
Yes	1 (4.0%)	3 (10.0%)		5 (9.1%)
No	24 (96.0%)	26 (86.7%)		49 (89.1%)
Don't know	0 (0.0%)	1 (3.3%)	0.158	1 (1.8%)
Past Cigarette Use				
Yes	2 (8.0%)	9 (27.3%)		11 (19.0%)
No	23 (92.0%)	24 (72.7%)	0.09	47 (81.0%)
Past Cigar Use				
Yes	0 (0.0%)	4 (12.1%)		4 (6.8%)
No	26 (100.0%)	29 (87.9%)	0.123	55 (93.2%)
Past Chew User				
Yes	2 (7.7%)	3 (9.1%)		5 (8.5%)
No	24 (92.3%)	30 (90.9%)	0.848	54 (91.5%)
Skin Conditions				
Eczema	2 (%)	2 (%)		4 (%)
Psoriasis	2 (%)	0 (0.0%)		2 (%)
Folliculitis	1 (%)	0 (0.0%)		1 (%)
Red bumps/ pimples	2 (%)	0 (0.0%)	0.214	2 (%)
SSTI				
Yes	1 (3.8%)	0 (0.0%)		1 (1.8%)
No	25 (96.2%)	30 (96.8%)		55 (96.5%)
Don't know	0 (0.0%)	1 (3.2%)	0.253	1 (1.8%)
S. aureus infection				
Yes	0 (0.0%)	0 (0.0%)		0 (0.0%)
No	26 (100.0%)	32 (97.0%)		58 (98.3%)
Don't know	0 (0.0%)	1 (3.0%)	0.559	1 (1.7%)

*Several participants opted not to answer several questions

Table 4-2: continued

	Livestock Contact (n=26)	No livestock Exposure (n=33)	P-value	Full Cohort (n=59*)
Dentures				
Yes	0 (0.0%)	2 (6.3%)		2 (3.4%)
No	26 (100.0%)	30 (93.8%)	0.497	56 (96.6%)
Tooth Brushing Frequency				
Every morning	9 (34.6%)	26 (78.8%)		35 (59.3%)
Every evening	17 (65.4%)	15 (45.5%)		32 (54.2%)
Most mornings	2 (7.7%)	1 (3.0%)		3 (5.1%)
Most evenings	4 (15.4%)	4 (12.1%)		8 (13.6%)
Some mornings	6 (23.1%)	1 (3.0%)		7 (11.9%)
Some evenings	2 (7.7%)	1 (3.0%)		3 (5.1%)
No mornings	0 (0.0%)	0 (0.0%)		0 (0.0%)
No evenings	0 (0.0%)	0 (0.0%)	<0.001	0 (0.0%)
Probiotic usage				
Yes	3 (11.5%)	4 (12.1%)		7 (12.1%)
No	23 (88.5%)	29 (87.9%)	1.0	51 (87.9%)
Type of Hand Soap				
Non-antibacterial, bar	11 (42.3%)	12 (36.4%)		23 (40.0%)
Non-antibacterial, liquid	11 (42.3%)	17 (51.5%)		28 (47.5%)
Antibacterial, bar	11 (42.3%)	8 (24.2%)		19 (32.2%)
Antibacterial, liquid	11 (42.3%)	19 (57.6%)		30 (50.8%)
Other	1 (3.8%)	0 (0.0%)	0.001	1 (1.7%)
Shared Bath Towels				
Yes	5 (19.2%)	7 (21.9%)		12 (20.7%)
No	20 (76.9%)	25 (78.1%)		45 (77.6%)
Don’t know	1 (3.8%)	0 (0.0%)	0.113	1 (1.7%)
Shared Hand Towels				
Yes	20 (76.9%)	7 (22.6%)		47 (81.0%)
No	6 (23.1%)	24 (77.4%)	0.0001	11 (19.0%)

*Several participants opted not to answer several questions

Table 4-3: Participant exposure to potential *S. aureus* risk factors

	Livestock Contact (n=26)	**No livestock Exposure (n=33)**	**P-value**	**Full Cohort (n=59*)**
Team sports, past 3 months				
Yes	0 (0.0%)	4 (%)		4 (6.9%)
No	26 (100.0%)	28 (%)	0.12	54 (93.0%)
Gym use, past 3 months				
Zero	26 (100.0%)	25 (%)		51 (87.9%)
1-3 times	0 (0.0%)	0 (0.0%)		0 (0.0%)
4-6 times	0 (0.0%)	2 (%)		2 (3.4%)
7-9 times	0 (0.0%)	1 (%)		1 (1.7%)
<10 times	0 (0.0%)	4 (%)	0.011	4 (6.9%)
Visited a Correctional Facility				
Yes	1 (%)	1 (%)		2 (3.4%)
No	25 (%)	32 (%)	1.0	56 (96.6%)
Outpatient surgery in last 3 months?				
Yes	0 (0.0%)	1 (%)		1 (1.7%)
No	26 (100.0%)	31 (%)	1.0	57 (98.3%)
Visited a hospital or long-term care facility?				
Yes	14 (%)	10 (%)		24 (41.4%)
No	12 (%)	22 (%)	0.142	34 (58.6%)
Work/ volunteer in a healthcare facility?				
Yes	1 (%)	7 (%)		8 (14.0%)
No	25 (%)	24 (%)	0.59	49 (86.0%)

* One participant opted to not answer the questions related to potential risk factors.

Table 4-4: Livestock contact (n=26)

Animal	N (%)	Ave. Number Animals (range)	Ave. Days per week (range)	Ave. Hours per day (range)
Swine	18 (69.2%)	3,024 (8-10,000)	6 d (2-7)	2.5 h (0.5-10)
Cattle	12 (46.2%)	191 (4-850)	6.4 d (1-7)	1.6 h (0.5-3)
Poultry	4 (15.4%)	1,644 (20-6,500)	7 d	1.0 h (0.25-2)
Other				
Sheep	4 (15.4%)	28 (10-50)	6.5 d (6-7)	1.4 h (0.25-3)
Horses	2 (7.7%)	6.5 (1-12)	6.5 d (6-7)	5.5 h (3-8)
Goats	1 (3.8%)	10	7 d	2 h

CHAPTER 5: DISCUSSION

S. aureus is an important pathogen colonizing the nares and oropharynx. Here we have utilized culture-independent sequencing to characterize the nasal and oropharyngeal microbiomes of livestock workers with relation to *S. aureus* colonization in healthy adults. We enrolled participants from a pre-existing cohort of urban and rural Iowans as well as Iowans with livestock contact into a cross-sectional study. The population was comprised of primarily older (mean age of 54.6 years), Caucasian (98.3%) males (69.5%). Those with livestock contact were significantly older than those without livestock contact (59.1 years compared to 51.1 years) as well as more likely to be male (92.3% male compared to 51.5% male). While the livestock workers enrolled in this study were almost exclusively males, this represents the average farmer worker in the United States where a majority of farm workers are males [202]. In the majority of Iowa counties, including Keokuk County, less than 10% of farm workers are female. Furthermore, as of 2012 the average age of principal farmworkers was 58.3 years with 61% being between 35 and 64 years nationwide. The USDA predicts the average age of farm workers will continue to increase [202].

5.1 The livestock worker microbiome

Very little is known about the normal livestock worker nasal and oropharyngeal microbiomes. The majority of studies assessing the microbial communities related to livestock work have either been done in animals [203, 204] or have studied the aerosolization of microorganisms in and around livestock facilities [205-207]. Here we have described the nasal and oropharyngeal microbiomes of 26 livestock workers and 33 non-livestock workers in Iowa.

The importance of livestock contact on the human microbiome has been recognized in relation to respiratory diseases. Not surprisingly, livestock workers had significantly more diverse nasal microbiomes compared to non-livestock workers likely due to the inhalation of aerosolized microorganisms. Livestock workers are exposed to high levels of inhalable dust which contains microorganisms [205, 206]. A study by Nonnenmann et al. identified several genera of bacteria present in the air of poultry confinement facilities including the *Ruminococcaceae* family and *Lactobacillus* which were both found to be significantly more abundant in the nares of those participants with livestock contact than those lacking this exposure. *Lactobacillus* has been shown to be at least partially effective as a probiotic treatment of perennial allergic rhinitis in a clinical trial and was associated with a decrease in sneezing, itchy nose, and swollen eyes in children [208]. *Moraxella* – a human commensal also known to cause respiratory tract infections [209] – is a bacterial air contaminant in livestock houses [210]. Others have found organisms belonging to the *Aerococcaceae* family, *Dietzia,* and *Prevotella* in air surrounding livestock [207]. *Aerococcus* (a cattle pathogen belonging to the *Aerococcaceae* family [211]), *Dietzia,* and *Prevotella* were all found to be significantly more abundant in the anterior nares of those with livestock contact in our population leading to the conclusion that these organism may be being inhaled.

While the diversity of the livestock worker microbiome has been hypothesized to be protective, we identified several potential pathogens as more abundant in livestock workers' nares and oropharynx. One of the organisms found to be significantly more abundant in the livestock worker microbiome was *Dietzia*, a gram positive genus known to be an opportunistic pathogen and able to colonize skin and formerly classified as

Rhodococcus maris [212]. It is unsurprising that this genus is also able to colonize the anterior nares, as they are anatomically similar to the skin [52]. *Dietzia* is predominantly a zoonotic pathogen, but has been identified in invasive human infections as well [213-215]. Due to its similarity to *Rhodococcus* spp., it is often mistakenly identified as a contaminant [212, 216]. *Dietzia* was found to be roughly seven times more abundant in livestock workers compared to those with no livestock contact (2-fold change of -3.55) in our population. While *Dietzia* was found in the negative controls, it was found in few samples and likely was not a large enough contaminant to account for the large difference between the groups. *Dietzia* infection has been thought to be potentially related to prior livestock exposure in case reports [217] and has been identified in the air of poultry (duck) barns [218]. Due to its high prevalence in livestock workers, it may be a potential cause of difficult-to-diagnose infections in people with livestock contact, especially in the immunocompromised [219]; however, little information on *Dietzia* as an opportunistic pathogen exists. Other potential pathogens found in higher abundance in livestock workers were *Prevotella* [220-222], *Streptococcus* [223-225], *Moraxella* [226, 227], *Rothia* [228], and *Oscillibacter* [229].

Prevotella spp., particularly *P. ruminicola*, are difficult to culture microorganism prevalent in the gastrointestinal tracts of all livestock animals in addition to ruminants. It has been demonstrated *P. ruminocola* has the ability to transfer tetracycline resistance to other members of the Bacteroidetes phylum, particularly to other *Prevotella* species, in the host and that horizontal transfer of the *tetQ* gene among *Prevotella* spp. is common in the human and ruminant intestines as well as the human oral cavity [230]. While it was not significantly enhanced in the livestock worker microbiome, *P. ruminocola* was

present (OTU 3243) as were many oral-associated *Prevotella* species. *Prevotella* spp. are frequent causes of odontogenic infections associated with gram-negative, anaerobic bacteria [231, 232]. These organisms are also known to cause infections of the respiratory system, head, and neck [232]. This is of interest as tetracycline is still commonly used in agriculture as well as a treatment for periodontal disease [233, 234] and *Prevotella* spp. were very common in the nares and oropharynx in our population and significantly more abundant in the oropharynx of swine workers.

When considering the microbiomes of livestock workers by the type of animal the participant had contact with, there were several significant differences by animal type in both the nares and oropharynx. Several potentially pathogenic microorganisms were significantly more abundant in those who had swine contact such as *Prevotella* and *Haemophilus* while *Lactobacillus* – a genus found in many probiotics that may improve oral health [235] – was found to be more abundant in those with no swine contact.

As it is likely these organisms are being inhaled while working around livestock, it is possible their presence is nasal contamination and not true colonization. While there is little research surrounding nasal contamination vs. true colonization, several studies have been done with regard to livestock worker colonization with *S. aureus* and have found many livestock workers drop *S. aureus* carriage within 24 hours [236]. On average it had been roughly 30 hours since swine workers had their last contact with swine, 24 hours since cattle workers had their last contact with cattle, and 1.5 hours since poultry workers had their last contact with poultry at the time of swabbing. It is possible some of the organisms observed in the nasal microbiome were due to contamination from recently being around their livestock, especially in those with poultry contact. As many of the

swine and cattle workers were close to 24 hours since their last contact with animals, it is difficult to determine if the presence of these organisms is true colonization or temporary contamination without further longitudinal research.

The microbiome of livestock workers and farmers has been primarily studied with regard to asthma. The relationship between a farming lifestyle was found to be negatively-associated with asthma in adults and children. It has been suggested that the farm effect – the hypothesis that animal-associated microbes and plant materials may protect those raised on farms by stimulating the immune system – is protective against asthma. This is particularly true for children where it has been shown early life exposure to microbes and microbial components – such as endotoxins – prime the immune system by the upregulation of T-helper 1 cells and the downregulation of T-helper 2 cells reducing the risk of atopy [237]. Studies have shown having a parent in a farming occupation – particularly ones with livestock exposure – is significantly associated with lower rates of allergen disorders and allergy attacks and there is a dose response relationship with less atopy in children with parents who are full-time farmers [238, 239]. It is thought the high-diversity of microorganism – likely inhaled – outcompete the harmful bacteria that may promote asthma [240, 241]. While the strongest associations between farming, microbial exposure, and reduced atopy has been shown in children, it has also been studied in adults. In adults farmer's asthma is low (around 4%) as is atopy (14%); however, unlike in children, asthma rates are higher among those who work with livestock, particularly swine and cattle [242]. Interestingly, this study found asthma was more common in farmers without atopy than those with atopy and individuals with more than one type of animal exposure were at increased risk of non-atopic asthma. In this

study it appeared working with livestock reduced atopic asthma, but put farmers at risk of non-atopic asthma [242]. It has been shown microbial exposures induce neutrophilic airway inflammation followed by non-IgE mediated airflow obstruction leading to non-atopic asthma [243].

In our study we saw livestock workers had significantly greater diversity both within individuals and as a group compared to those with no livestock contact. A stronger understanding of the farmer microbiome is needed to understand the relationship between asthma and the protective effects livestock exposure may provide against atopy in children [241] as well as the negative effects it may have on adults. Microbes appear to play a significant role in asthma and the understanding of the beneficial and harmful organisms that may either prevent or exacerbate asthma is necessary for the development of future treatments [244], especially treatments focusing on non-atopic asthma [243]. By characterizing the nasal and oropharyngeal microbiomes of healthy livestock workers, we are able to begin to establish a baseline for the healthy livestock worker microbiome so comparisons can be made to microbes present in disease states.

5.1.2 Livestock exposure and participant behaviors

We observed three participant behaviors to be significantly different between those with and without livestock contact: type of soap used, sharing of hand towels, and the frequency of tooth brushing. However, none of these behaviors were significantly associated with alterations in either the nasal or oropharyngeal microbiomes. The most surprising of these was that frequency of tooth brushing, which was less frequent in the livestock workers, but was not associated with any differences in oral microbiota. One explanation for this is frequency of tooth brushing may not be an adequate marker of oral

hygiene. While we chose to assess oral health through a single question (frequency of tooth brushing) in this pilot study as the enrollment visit was already long and required participants to fill out up to three surveys, in future studies directed towards assessing oral health and the livestock worker microbiome, this will not be sufficient. A better marker for oral hygiene may have been to assess the number of dental carries, gingivitis, gum disease, and/or halitosis. In the future, it would be better to assess oral hygiene using a standardized survey, such as the NHANES Oral Health Survey [245]. As for the type of hand soap used and the sharing of bath towels, microbiota differences may be more likely to be seen in the skin microbiome [246, 247].

5.2 *S. aureus* colonization and the microbiome

Understanding the ecologic niche *S. aureus* lives in is an important step in understanding the biologic factors associated with *S. aureus* colonization and infection prevention. Here we have compared the microbiomes of the anterior nares and oropharynx and their relation to *S. aureus* in 59 healthy Iowans. Using 16s rRNA sequencing, we were able to assess what other bacteria may play an important role in *S. aureus* carriage in healthy adults. *S. aureus* colonization – as defined by the presence of *S. aureus* in the 16s rRNA sequencing data – occurred in the nares of 30 participants and the oropharynx of seven participants. While were unable to identify any OTUs significantly associated with either *S. aureus* presence or absence, there were several differences between carriers and non-carriers, particularly in the nasal microbiome.

S. aureus nasal carriers' microbiomes were significantly different from the non-carriers ($P = 0.002$), though no individual OTU was significantly different between the groups. However, as seen in Figure 19a, colonized individuals have more Firmicutes

compared to the non-colonized participants while the non-colonized participants have more Actinobacteria. This is similar to other studies which have found strong, significant inverse correlations between the presence of Firmicutes and Actinobacteria in the nares [53] and have found communities dominated by Actinobacteria – particularly *Propionibacterium* and *Corynebacterium* – have fewer staphylococci [141]. While the differences were not significant, we also found *Corynebacterium* to be more frequently present in non-carriers compared to carriers; however, *Propionibacterium* was very similar between the two groups. *Corynebacterium* is one of the most consistently negatively-associated genera with regard to *S. aureus* colonization [51, 53, 141, 248, 249]. *Corynebacterium* has been shown to inhibit the growth of *S. aureus* in culture, specifically with regard to *C. pseudodiphtheriticum* [51].

Several other studies have found *S. epidermidis* to be both positively [51, 250] and negatively associated with *S. aureus* colonization [141]. Here, we observed *S. epidermidis* to be present 100% of the time when *S. aureus* was present in both the nares and the oropharynx. *S. epidermidis* was the most prevalent *Staphylococcus* species present in 56 of the 59 nasal samples and 28 of the oropharyngeal samples. The study by Frank et al. that identified *S. epidermidis* as being negatively associated with *S. aureus* carriage ($P = 0.004$) was done on primarily patients in intensive care units (ICUs) (n= 42) and only 5 non-hospitalized participants. It has been shown that ICU stays can change the composition of the microbiome [251] with large changes to the ratios of Bacteroidetes and Firmicutes being observed in the gut microbiome [252]. It is possible the ICU environment is affecting the relationship between *S. aureus* and *S. epidermidis* in the Frank et al. study. It has also been hypothesized *S. epidermidis* will only inhibit *S. aureus*

colonization when *S. epidermidis* is able to express a serine protease, *esp* [253] and the positive association between *S. aureus* and *S. epidermidis* is only observed when the majority of the *S. epidermidis* strains present in the microbiome do not express *esp*. However, a recent study demonstrated while *esp* was ubiquitous in all *S. epidermidis* strains tested, there was no correlation with *S. aureus* inhibition [254]. Future studies are need comparing hospitalized patients to healthy persons to better understand the association between the two microorganisms.

In the oropharynx, we observed a great deal of genus richness which is consistent with other studies [53]. As with prior studies, we observed the oropharynx to be dominated by Firmicutes as well as a high prevalence of Bacteroidetes and Proteobacteria [113]. Our inability to see any differences in the oropharyngeal microbiomes between carriers and non-carriers is likely due to the small number of oropharyngeally colonized individuals (n=7).

5.2.1 *S. aureus* colonization in livestock workers

When comparing the colonized individuals, colonized livestock workers had twenty-four OTUs that were significantly more abundant than in the nasal microbiota of their colonized, non-livestock worker counterparts. Unsurprisingly, almost all of these OTUs belonged to the Firmicutes phylum and many were the same OTUs observed to be different between livestock workers and non-livestock workers in Aim 1. Only four of the 24 OTUs were not seen in Aim 1: *Psychrobacter, Staphylococcus cohnii, Atopostipes,* and *Clostridium sensu stricto*. It has been hypothesized that at least a portion of livestock workers identified as *S. aureus* carriers are not truly colonized, only contaminated and that while they are continuously in the presence of aerosolized *S. aureus, S. aureus* is

unable to adhere to the epithelial cells. van Cleef et al, showed up to 94% of those testing positive after working around livestock would test negative within 24 hours of the exposure [236]. A limitation of our study is its cross-sectional nature. It was not possible for us to determine the duration of colonization for those participants with livestock exposure. As many of them were not enrolled through the prior prospective cohort study, there is limited historical data available for this group. In the prior prospective study, those with livestock exposure were more likely to be intermittent colonizers, though the trend was not significant ($P = 0.07$) [42]. We did assess how long it had been since the livestock workers last contact with livestock. For swine and cattle, it had been around 24-30 hours since last contact, so it is likely any *S. aureus* presence is true colonization, not contamination. It is possible the microbial differences in the microbiomes of the colonized livestock workers and colonized non-livestock workers may have an influence on whether *S. aureus* is able to establish colonization.

We also assessed the differences between livestock workers who were colonized and not colonized in the nares and oropharynx to assess any microbial differences that may influence whether a livestock worker was a carrier or not. While there were no significant differences in the nares, colonized livestock workers were significantly more likely to carry *Porphyromonas gingivalis* in the oropharynx while non-colonized livestock workers were more likely to carry *Atopobium* in the oropharynx. Studies have shown that in the presence of *P. gingivalis*. *Streptococcus* spp. (which was significantly more abundant in colonized livestock workers) and other members of the oropharyngeal microbiome will aggregate with *S. aureus* meaning that even if *S. aureus* is a contaminant instead of a true colonizing species in the oropharynx, when *P. gingivalis* is present, *S.*

aureus may adhere to the biofilms created by the other microorganisms. *P. gingivalis* acts as a 'coaggregation bridge' mediating the attachment between two species that would not otherwise aggregate [255]. The presence of *P. gingivalis* in the oral cavity is associated with poor oral health and hygiene [256]. In our study, livestock workers were significantly less likely to brush their teeth daily (P <0.001) which has been linked to poor oral health and gingivitis [257]. However, we were not able to identify an association between oral hygiene and any differences in the microbiota in our study, likely do to small sample sizes and an inadequate measure of oral hygiene as described in section 5.1.2. While this observation requires a great deal of further study to better understand and quantify the relationship between *P. gingivalis* and *S. aureus* as our numbers are small, the improved oral hygiene may help reduce oropharyngeal *S. aureus* colonization.

5.2.2 *S. aureus* carrier states and the microbiome

As described in chapter 2.5, *S. aureus* carriage can be categorized into three states: persistent carriers (20%), intermittent carriers (30%), and non-carriers (50%). As to why some persons carry *S. aureus* persistently and others intermittently or never carry it remains unclear. The host factors and the host immune response play a role in *S. aureus* colonization, though little is known about the immune system and its impact on colonization. What is known is *S. aureus* is well equipped to evade the hosts immune response. *S. aureus* produces a polypeptide called staphylococcal complement inhibitor (SCIN) that binds complement C3 convertases C4b2a and C3bBb blocking the complement cascade resulting in decreased phagocytosis by human neutrophils [258]. The *Staphylococcal protein A* gene (*spa*) gene may also play a role in colonization. The

spa gene is able to block opsonophagocytosis by binding the Fc region of antibodies allowing for the bacteria to evade the hosts immune system [259].Increased expression of the *spa* gene by certain strains of *S. aureus,* particularly ST239, have been shown to increase the organisms ability to colonize and may increase the organisms ability to spread [260]. Persistent carriage of *S. aureus* may also be associated with having the IL-4 haplotype IL-4-524 C/C [261, 262] as well as the C-reactive protein single nucleotide polymorphism C2042T and C1184T [262]. However, the host immune response does not fully account for why some subjects carry *S. aureus* and other do not; microbial interference likely plays a role in colonization status.

A prior study by Yan et al. demonstrated differences between persistent carriers (defined as any subject with 2 consecutive *S. aureus* positive samples) from non-persistent carriers (including both intermittent carriers and non-carriers) in twelve healthy participants [51]. The study by Yan et al. is the only other study we were able to identify assessing the relationship between the different *S. aureus* carriage states and the microbiome. In this study it was found persistent carriers were more likely carry a large number of bacteria including *Propionibacterium acnes, Gemella,* and *Prevotella*. Persistent carriers also had higher abundances of all *Staphylococcus* species compared to non-carriers. The authors also observed increased diversity within the microbiomes of persistent carriers [51].

In the nasal microbiome, we observed no significantly differentially abundant OTUs between persistent, intermittent, and non-carriers. However, *Corynebacterium* and *Finegoldia* were more abundant in the non-carriers while *Propionibacterium* was

elevated in intermittent carriers. This is consistent with the study by Yan et al. who observed similar trends [51] with carriers having increased *Propionibacterium.*

In the oropharynx, we observed several OTUs that were significantly different between carrier states. Interestingly we found non-carriers had significantly more *Gemella* compared to intermittent carriers which is in contradiction with the Yan et al. study who found persistent carriers had increased *Gemella* abundance. There are several possible reasons for this difference. First both studies have small sample sizes with regard to persistent carriers, (n= 6 in Yan et al., n=7 in this study). Another difference may be in how the carrier state was defined. The Yan et al. study combined intermittent carriers and non-carriers into one group and compared them to persistent carriers where as we separated the non-carrier group into intermittent and non-carriers. We also defined carrier status based off of year-long weekly data compared the Yan study that defined carrier status as persistent after 2 consecutive swabs. It is possible the Yan study misclassified intermittent carriers as persistent carriers which could have accounted for the difference in our results.

We also observed *Corynebacterium* to be more abundant in persistent carriers which is a directly opposite finding compared to what was seen in the nasal microbiome comparing colonizers and non-colonizers (section 5.2.). This finding also contradicts what has been seen in prior studies who have found *Corynebacterium* to be negatively associated with *S. aureus* colonization. Again, there are several possible reasons for this finding. First, only one of the *Corynebacterium* OTUs in the oropharyngeal microbiome was more abundant in the persistent carriers. Additionally, this OTU was present in only one persistently colonized individual. The Yan et al. study also found *Corynebacterium*'s

association with *S. aureus* was strain dependent. They found *C. allocens* did not inhibit *S. aureus* growth while *C. pseudodiphtheriticum* did [51]. The OTU significantly more abundant in the persistent oropharyngeal carriers was *C. argentorantense.* It is possible this strain of *Corynebacterium* does not inhibit the growth of *S. aureus.*

The Yan et al. study also found S*treptococcus* to be more abundant in persistent carriers while our study found it to be more abundant in non-carriers. Again, this difference could be due to the differences in the study designs. One of the OTUs in the study was uncultured and the other was *S. mitis.* Other studies have found *S. mitis* to inhibit the growth of *S. aureus* likely due to the production of H_2O_2 [139]. In general, the *Streptococcus* spp. have been thought to inhibit *S. aureus* colonization [261, 263, 264] which supports our finding that *Streptococcus* was significantly more abundant in the non-colonized participants. Overall, more studies are needed to determine the relationship between the microbiome and *S. aureus* carrier states, especially in the oropharynx.

5.2.3 Culture of *S. aureus*

In order to better characterize the *S. aureus* carriers, we supplemented the 16s rRNA sequencing with traditional *S. aureus* culture Not surprisingly, t034 – a livestock associated strain – was the most prevalent *spa* type identified in our population. Of the 45 isolates positive for *S. aureus,* almost half (48.9%) were multidrug resistant. The highest resistance was to benzylpenicillin followed by erythromycin (53.3%) and tetracycline (37.8%). High levels of tetracycline resistance were not surprising as almost half of the population had livestock exposure; tetracycline is commonly used in animal husbandry, especially swine and cattle.

We performed a sensitivity and specificity analysis comparing *S. aureus* colonization between the 16s rRNA sequencing and the standard culture. The sensitivity and specificity were both low indicating there was not strong concordance between the two methods. One possible reason for this is because the culture swab was taken after the 16s rRNA swab. In the samples that were culture negative for *S. aureus*, but positive via sequencing, the relative abundance of *S. aureus* was very low. It is possible that the participants' only positive by sequencing had a very low bacterial load of *S. aureus* and there was not enough present to be collected on the second swab. Another possible explanation is the ability of DNA-based sequencing to detect dead (therefore unculturable) bacteria that may be picked up by the swab.

5.3 Study strengths and limitations

There are several limitations to our study. First, this study was cross-sectional. As such, we were unable to assess any change in the microbiome and how those changes may impact *S. aureus* colonization. It's also highly possible some of the intermittent *S. aureus* colonizers were mistakenly classified as not colonized [8] which could mask any differences in the microbiomes. It has been shown seven or more nasal cultures are necessary to distinguish intermittent carriers from noncarriers while one negative swab will exclude almost all persistent carriers [265]. Furthermore, it is possible the differential OTUs identified in our study could be transient and may not be significant when considering the microbiome over an extended period of time.

We were also unable to determine whether the OTUs found to be differentially abundant between the colonized and non-colonized individuals were able to inhibit the growth of *S. aureus.* This is of particular interest with the *Corynebacterium* OTUs. Prior

studies have found specific strains of *Corynebacterium* may inhibit *S. aureus* growth, while others do not [256]. A list of *Corynebacterium* species whose interactions with *S. aureus* have been studied is provided in Table 5-1. As seen in Table 5-1, *Corynebacterium* has typically been found to negatively inhibit *S. aureus* growth while we found *C. argentorantense* to be positively associated. To ensure this finding is not spurious and that *C. argentorantense* has an effect on *S. aureus*, we need to demonstrate in vitro whether it has the ability to inhibit *S. aureus* growth. The same is true for any bacterial species that has not had its growth relationship with *S. aureus* assessed.

Very few studies exist assessing the microbial differences of the nares and oropharynx of *S. aureus* carriers by carrier state. In our study we were able to use yearlong historical data for a subset of our population enrolled from a prospective cohort. The historical data was collected in between 2012 and 2013 so it is possible the participants' colonization status has changed which is not possible to assess in a cross-sectional study. However, a 9-year study of *S. aureus* colonization found the half-life of colonization was roughly 40 months [266]. Additionally, a meta-analysis of MRSA clearance rates showed 50% of MRSA colonized patients clearing colonization after 88 weeks [267]. As the sampling for this study was conducted in 2015, it is likely the participants' colonization status remained the same. Unfortunately, we were unable to assess any differences by carrier state in the livestock workers as the majority of them were not part of the prospective cohort study.

5.4 Future directions

While our study is the first we are aware of to assess the microbiome of livestock workers and there has been little research using next-generation sequencing technology to

assess the microbiome and *S. aureus* colonization, a great deal of additional research is needed. Future studies are needed both with regard to the healthy livestock worker microbiome as well as *S. aureus* colonization. Additionally, future studies are needed to assess the relationship between differentially abundant OTUs to determine their impact on the growth of *S. aureus* in vitro.

As discussed above, there is very little research on the livestock worker microbiome. More research is needed to better understand the relation of the livestock worker respiratory microbiomes and diseases such as asthma. Longitudinal studies need to be done to first characterize the livestock workers over time and at different stages of life. Animal-based studies are needed to more definitively assess the relationship between the core microbes of the livestock worker airways and their impact on asthma. Animal models are necessary for this research to be able to determine if the microbes encountered during early childhood exposure to farm-life may be able to prevent asthma.

Additional longitudinal studies of *S. aureus* colonization and the microbiome are also needed, especially with regard to difference in in carrier states. To date, we were only able to find one other study assessing microbial differences in the nares by carrier state. Our study found conflicting results with the Yan et al. study likely due to differing methodologies and small sample sizes. Both studies have weaknesses that limit the generalizability of the findings. Longitudinal studies sampling greater number of subjects to reconcile the differences in our findings and to provide a more robust understanding of the carrier states. These studies will help determine what may cause carriage to persist in certain people while it is transient in others. Furthermore, these studies may eventually lead to treatments to break colonization. As described in chapter 2.7, current

decolonization practices have high rates of treatment failure and may lead to antimicrobial resistance. Better treatments are needed to break colonization for those at high risk of infection.

As we begin to expand our understanding of the healthy microbiome, future research is needed to understand how stressors like hospitalization impact the normal flora. As mentioned above, hospitalization has been shown to alter the microbiome. Additional research is needed to determine how this impacts a person's susceptibility to acquiring MRSA during their hospital stay and what can be done to minimize the effects of shifts in the normal flora.

While it is important to understand what organism may constitute the healthy microbiome and how those may be altered during disease states, it is also necessary to understand the functional role these organisms play in relation to health and disease. Taxonomic variations between the microbiomes of healthy persons is common. Understanding community compositions is a necessary start, but does not provide a full picture of the relationship between the microbiome and disease states or colonization with pathogenic organisms such as *S. aureus.* Studies using shotgun metagenomics to assess the potential interactions between the host and the microbiome. Metagenomics has the ability to tell us the genomic potential of the microbiome, though metatranscriptomics studies are also necessary to understand the functional potential of the microbiome. "-omics" technologies are in their infancy and as the technology and bioinformatic tools are improved, studies beyond community composition are necessary to fully understand the role the microbiome plays in human health.

Table 5-1: The relationship between *S. aureus* and *Corynebacterium sp*.

Corynebacterium sp.	**Relationship**	**Evidence**
Co304	Competitive inhibition	In vivo replacement study healthy adults. *S. aureus* was eradicated from 71% (12/17) carriers by replacement with Co304 [249].
C. accolens	Competitive interaction	Culture of both organism together on agar plates shows *S. aureus* growth is enhanced by proximity to *C. accolens* [51].
C. pseudodiphtheriticum	Competitive inhibition	Culture of both organism together on agar plates shows *S. aureus* growth is inhibited by proximity to *C. pseudodiphtheriticum* [51, 268].
C. simulans	Possible inhibition	*C. simulans* may be associated with decreased MRSA colonization in hospitalized patients [139].
C. diphtheriae	Competitive inhibition	In culture, *S. aureus* strong zones of inhibition against *C. diphtheriae* while *C. diphtheriae* caused much weaker zones of inhibition against *S. aureus* [269].
C. striatum	Competitive inhibition	*S. aureus* grown in vitro with *C. striatum* resulted in less *S. aureus* growth and decreased virulence [270].
C. amycolatum	Competitive inhibition	*S. aureus* grown in vitro with *C. striatum* resulted in less *S. aureus* growth and decreased virulence [270].
C. glutamicum	Competitive inhibition	*S. aureus* grown in vitro with *C. striatum* resulted in less *S. aureus* growth and decreased virulence [270].

APPENDIX A: DESCRIPTION OF THE PROSPECTIVE COHORT

A majority of participants for this study were enrolled from a pre-existing cohort of Iowans. These individuals were enrolled into a prospective cohort study examining *S. aureus* colonization dynamics and familial transmission events. Ninety-five family units (totaling 263 persons) were enrolled into the study between October 2011 and January 2012 from Keokuk and Johnson Counties, Iowa and followed for up to 52 weeks.

Participants were recruited by two county specific methodologies. In Johnson County, participants were recruited via newspaper advertisements and the University of Iowa emailing list. Interested participants contacted the researchers either by phone or email to determine eligibility and schedule in-home study visits. Participants in Keokuk County were recruited from the Keokuk Rural Healthy Study. These participants were contacted first by letter and then by phone call. Inclusion and exclusion criteria were assessed over the phone for both groups before scheduling sampling visits.

To be included in the study, participants must have been at least 6 months of age at enrollment and must have been able to provide consent, assent, or have parents willing to provide consent. Additionally, participants must have been willing to complete the weekly follow-up questionnaires, provide weekly biologic samples (nares and oropharynx swabs from adults and nares swabs from minors), and provide a mailing address, phone number, and/or email address for follow-up purposes. Participants must also speak and read English. And lastly, participants must have shared a home with at least one other family member who was also willing to enroll in the study.

Participants were enrolled into a 52-week prospective cohort study. During the initial in-home study visit participants were consented and then each member of the

family filled out an enrollment questionnaire. One member of the family also filled out a household questionnaire on the number of persons living in the home, number of children, and several risk factors. Participants who had contact with either healthcare or livestock or both were asked to fill out subsequent questionnaires on their healthcare and/or livestock contact and risk factors. Samples were also taken from the anterior nares and oropharynx of each adult participant and the anterior nares of each minor participant to assess *S. aureus* colonization status at baseline. Participants were left with a package of swabs and questionnaires to be filled out weekly and mailed back to the CEID for analysis. All participants were instructed on proper self-swabbing technique.

Each week for 52 weeks participant self-swabbed their nose and throats and mailed back two questionnaires. The weekly questionnaire collected information on any potential *S. aureus* infections that occurred since the questionnaire was last filled out as well as additional meat consumption (another aim to the study). If any family member reported an infection an additional questionnaire on infection symptoms and treatment was filled out and a wound swab was mailed in.

The primary outcome of interest in this study was weekly colonization status to determine the average time a person was colonized during the study and to determine cut offs for non-carriers, intermittent carriers and persistent carriers of *S. aureus*. We were also interested in secondary transmission events to family members defined as a family member previously un-colonized or colonized with an unrelated strain acquired an *S. aureus* strain of a family member. We were also interested in any infections that occurred over the 52 weeks. Detailed information on colonization status over the duration of the

study as well as baseline risk factor information is available for all participants completing the study.

APPENDIX B: DATA COLLECTION QUESTIONNAIRES

SAMI Study – Enrolment Survey

Thank you for participating in this study. We are studying how the bacteria that live naturally in your nose and throat may impact another bacterium called *Staphylococcus aureus*, commonly known as "Staph." The survey should only take a few minutes to complete. Please answer the questions to the best of your knowledge and check the appropriate boxes. All of your responses will be kept private and confidential. You may skip any question you do not wish to answer.

Please mark boxes like this ☒ or ☑

PERSONAL INFORMATION

1. Today's date _____/_____/_____
   (month/ day / year written as YYYY)

2. Date of Birth: _____/_____/_____
   (month/ day / year written as YYYY)

3. What is your height? _____ (feet) _____ (inches)

4. What is your weight? _____ (lbs)

5. How would you describe your race (please choose all that may apply)?
   - ☐ American Indian/Alaska Native
   - ☐ Asian
   - ☐ Black or African American
   - ☐ Native Hawaiian or Other Pacific Islander
   - ☐ White
   - ☐ Other

6. How would you describe your ethnicity?
   - ☐ Hispanic or Latino
   - ☐ Not Hispanic or Latino

7. What is your biologic gender?
   - ☐ Male
   - ☐ Female

Please continue to the next page

Figure B-1: Enrollment survey

8. Which of the following categories best describes your family's net income (choose one)?
   - ☐ Less than $20,000
   - ☐ $20,000 - $39,999
   - ☐ $40,000 - $59,999
   - ☐ $60,000 - $79,999
   - ☐ $80,000 - $99,999
   - ☐ Over $100,000

9. Which of the following categories best describes the highest education level you have achieved (choose one)?
   - ☐ Less than high school graduate
   - ☐ High school graduate
   - ☐ Some college
   - ☐ College graduate
   - ☐ Graduate level
   - ☐ Professional level

10. What size would you say your home living space is?
   - ☐ less than 1500 square feet
   - ☐ more than 1500 square feet
   - ☐ Don't know

11. How many people over 18 years old live in your home? _____

12. How many people under 18 years old live in your home? _____

13. Do you have a water softener in your home?
   - ☐ Yes
   - ☐ No
   - ☐ Don't know

14. What type of water do you have at your home?
   - ☐ Well water
   - ☐ Public water
   - ☐ Don't know

15. How do you heat your home?
   - ☐ Central heating
   - ☐ Wood furnace
   - ☐ Other: _____

Please continue to the next page

Figure B-1: continued

16. How do you cool your home?
- ☐ Central air
- ☐ Wall unit air conditioner
- ☐ Ceiling or portable fan
- ☐ Other: _____

17. How often do you handle raw/uncooked pork products?
- ☐ I do not handle pork products
- ☐ Less than once a week
- ☐ Approximately once a week
- ☐ 2-3 times a week
- ☐ More than 4 times a week

18. How often do you eat pork products?
- ☐ I do not eat pork products
- ☐ Less than once a week
- ☐ Approximately once a week
- ☐ 2-3 times a week
- ☐ More than 4 times a week

19. How often do you handle raw/uncooked beef products?
- ☐ I do not handle beef products
- ☐ Less than once a week
- ☐ Approximately once a week
- ☐ 2-3 times a week
- ☐ More than 4 times a week

20. How often do you eat beef products?
- ☐ I do not eat beef products
- ☐ Less than once a week
- ☐ Approximately once a week
- ☐ 2-3 times a week
- ☐ More than 4 times a week

21. How often do you handle raw/uncooked chicken or turkey products?
- ☐ I do not handle chicken or turkey products
- ☐ Less than once a week
- ☐ Approximately once a week
- ☐ 2-3 times a week
- ☐ More than 4 times a week

Please continue to the next page

Figure B-1: continued

22. How often do you eat chicken or turkey products?
- ☐ I do not eat chicken or turkey products
- ☐ Less than once a week
- ☐ Approximately once a week
- ☐ 2-3 times a week
- ☐ More than 4 times a week

MEDICAL INFORMATION

23. Do you currently have any of the following conditions:

Asthma?
☐ Yes ☐ No ☐ Don't know

Emphysema?
☐ Yes ☐ No ☐ Don't know

Chronic Obstructive Pulmonary Disorder (COPD)?
☐ Yes ☐ No ☐ Don't know

Heart disease or diseases of your blood vessels, veins or arteries?
☐ Yes ☐ No ☐ Don't know

Diabetes?
☐ Yes ☐ No ☐ Don't know
if yes:
- ☐ Type 1
- ☐ Type 2

Kidney disease?
☐ Yes ☐ No ☐ Don't know

HIV/AIDS?
☐ Yes (STOP) ☐ No ☐ Don't know

Autoimmune disease?
☐ Yes (if so, describe: _____)
☐ No ☐ Don't know

Have you been diagnosed with cancer in the last five years?
- ☐ Yes (if so, describe_____)
- ☐ Yes, in remission (Remission year: _____)
- ☐ No, in remission (Remission year: _____)
- ☐ No
- ☐ Don't know

Please continue to the next page

Figure B-1: continued

Any other condition that may weaken the immune system?
- ☐ Yes (if so, describe: _____)
- ☐ No
- ☐ Don't know

24. Have you taken medications such as anti-cancer drugs, steroids (such as prednisone), or other drugs that weaken the immune system in the last 3 months?
- ☐ Yes (STOP)
- ☐ No
- ☐ Don't know

25. Do you currently smoke cigarettes?
- ☐ Yes (*continue to question 25a*)
- ☐ No (*continue to question 26*)

25a. How many cigarettes do you smoke in a day?
_____cigarettes _____ packs per day

25b. What year did you start smoking cigarettes? _____ (YYYY)

26. Have you ever smoked cigarettes in the past?
- ☐ Yes (*continue to question 26a*)
- ☐ No (*continue to question 27*)

26a. How long ago did you quit smoking cigarettes?
I quit smoking cigarettes ____ days ____weeks ____months ____years ago
For example, if I quit smoking 5 months and 2 weeks ago I would fill write a 0 next to days, a 2 next to weeks, a 5 next to months, and a 0 next to years.

27. Do you currently use smoke cigars?
- ☐ Yes (*continue to question 27a*)
- ☐ No (*continue to question 28*)

27a. How many cigars do you smoke in a week?
_____cigars per week

27b. In what year did you start smoking cigars? _____ (YYYY)

28. Have you ever smoked cigars in the past?
- ☐ Yes (*continue to question 28a*)
- ☐ No (*continue to question 29*)

28a. How long ago did you quit smoking cigars?
I quit smoking cigars ____ days ____weeks ____months ____years ago
For example, if I quit smoking 5 months and 2 weeks ago I would fill write a 0 next to days, a 2 next to weeks, a 5 next to months, and a 0 next to years.

Please continue to the next page

Figure B-1: continued

29. Do you currently use chew or pipe tobacco?
- ☐ Yes (*continue to question 29a*)
- ☐ No (*continue to question 30*)

29a. How many ounces or chew or pipe tobacco do you use in a day?
_____ounces per day

29b. What year did you start using chew or pipe tobacco? _____ (YYYY)

30. Have you ever used chew or pipe tobacco in the past?
- ☐ Yes (*continue to question 30a*)
- ☐ No (*continue to question 31*)

30a. How long ago did you quit using chew or pipe tobacco?
I quit smoking cigarettes ____ days ____weeks ____months ____years ago
For example, if I quit using chew 5 months and 2 weeks ago I would fill write a 0 next to days, a 2 next to weeks, a 5 next to months, and a 0 next to years.

31. Do you currently have an upper respiratory tract infection (infection in the throat, mouth, or nose), ear infection, or sinus infection?
- ☐ Yes (STOP)
- ☐ No

32. Have you taken antibiotics in the past 3 months?
- ☐ Yes (STOP)
- ☐ No

33. Have you had the nasal influenza (flu) vaccine, sometimes called FluMist or nasal flu spray, in the past month?
- ☐ Yes (STOP)
- ☐ No

34. Have you ever been told by a doctor that you have any of the following skin conditions (check any/all that apply)?
- ☐ Eczema
- ☐ Psoriasis
- ☐ Folliculitis
- ☐ Red bumps or pimples

35. Have you had a skin or soft tissue infection (such as an infection of the muscle, abscess, furuncle, impetigo) in the past 3 months?
☐ Yes ☐ No ☐ Don't know

Please continue to the next page

Figure B-1: continued

35a. Where on your body was the infection?

- ☐ Legs
- ☐ Arms
- ☐ Hands
- ☐ Neck/face
- ☐ Stomach/ waist
- ☐ Groin
- ☐ Back
- ☐ Other: _____

36. Have any of your friends or family had a skin or soft tissue infection (such as an infection of the muscle, abscess, furuncle, impetigo) in the past 3 months?

☐ Yes ☐ No ☐ Don't know

37. Have you been told by a doctor you have a Staph infection in the previous 3 months?

☐ Yes ☐ No ☐ Don't know

38. Have you been told by a doctor you have a MRSA (methicillin-resistant *Staphylococcus aureus*) infection in the past 3 months?

☐ Yes ☐ No ☐ Don't know

39. Have you used any type of probiotics in the past 3 months?

- ☐ Yes
  If yes, what type? _____
- ☐ No
- ☐ Don't know

40. Do you wear dentures?

- ☐ Yes
- ☐ No

41. How often do you brush your teeth (check all that apply)?

- ☐ Every morning
- ☐ Every evening
- ☐ Most mornings
- ☐ Most evenings
- ☐ Some mornings
- ☐ Some evenings
- ☐ I never brush my teeth in the morning
- ☐ I never brush my teeth in the evening
- ☐ After every meal

PUBLIC CONTACT

42. Have you participated in team and/or contact sports in the last 3 months?

☐ Yes ☐ No

Please continue to the next page

Figure B-1: continued

43. In the past month, approximately how many times have you gone to a gym to use the facility?
- ☐ Zero
- ☐ 1-3 times
- ☐ 4-6 times
- ☐ 7-9 times
- ☐ More than 10 times

44. Have you spent time in a jail or other correctional facility in the previous 3 months (as a visitor or inmate)?
☐ Yes ☐ No

45. Have you been hospitalized for more than 24 hours in the previous 3 months?
☐ Yes (STOP) ☐ No

46. Have you or any member of your household had outpatient surgery that did not require hospitalization lasting more than 24 hours in the previous 3 months?
☐ Yes ☐ No

47. Have you visited a patient in a hospital or long-term care facility (such as a nursing home) in the past 3 months?
☐ Yes ☐ No

JOB RELATED CONTACT

48. Do you work or volunteer in a hospital, physician's office, or long-term care facility?
☐ Yes ☐ No

49. Do you work or spend time in the following? (check all that apply)
- ☐ Occupation that involves close physical contact with animals
- ☐ Occupation that involves close physical contact with animal waste products
- ☐ Livestock or poultry processing plant

Please continue to the next page

Figure B-1: continued

OTHER CONTACT

50. In the past 3 months have you been in contact with any of the following types of **live animals?** **(check *yes* or *no* for each)** *(if you check yes to any of the following, continue to 50a)*

Chickens	☐ yes	☐ no
Cattle	☐ yes	☐ no
Swine (pigs)	☐ yes	☐ no
Horses	☐ yes	☐ no
Goats	☐ yes	☐ no
Sheep	☐ yes	☐ no
Turkeys	☐ yes	☐ no
Cats	☐ yes	☐ no
Dogs	☐ yes	☐ no

Other type of animal, please specify _____

50a. Have any of these animals been diagnosed with a skin or soft tissue infection in the past 3 months?

- ☐ Yes, please describe: _____
- ☐ No
- ☐ Don't know

51. Do you handle or have contact with animal manure (for use on crops or in gardens, for example?)

- ☐ Yes, less than one acre
- ☐ Yes, greater than one acre
- ☐ No

52. What types of hand soaps are used in your home (check all that apply)?

- ☐ Bar soap, not antibacterial (Dove, Ivory, etc)
- ☐ Bar soap, antibacterial (Dial, Lever 2000, etc)
- ☐ Liquid soap, not antibacterial
- ☐ Liquid soap, antibacterial
- ☐ Other (describe:_____)

53. Are bath towels shared in your household without being washed in between users?

- ☐ Yes
- ☐ No
- ☐ Don't know

54. Are hand towels shared in your household without being washed in between users?

- ☐ Yes
- ☐ No
- ☐ Don't know

Thank you for completing this survey!

Figure B-1: continued

SAMI Study – Healthcare Occupations Survey

Thank you for participating in this study. We are studying how the bacteria that live naturally in your nose and throat may impact another bacteria called *Staphylococcus aureus*, commonly known as "Staph." The survey should only take a few minutes to complete. Please answer the questions to the best of your knowledge and check the appropriate boxes. All of your responses will be kept private and confidential. You may skip any question you do not wish to answer.

Please mark boxes like this ☒ or ☑

CONTACT WITH HEALTHCARE FACILITIES

1. Do you work or volunteer in a healthcare facility? (Hospital, physician's office, outpatient clinic, assisted living facility, nursing home, hospice, etc.?)
   - ☐ Yes
   - ☐ No (END OF SURVEY)

2. Which type of facility do you work or volunteer at? Please check all that apply.
   - ☐ Large hospital (approx. number of beds: _____)
   - ☐ Medium hospital (approx. number of beds: _____)
   - ☐ Small hospital (approx. number of beds:_____)
   - ☐ Prison healthcare facility
   - ☐ Physician's office (Number of physicians in clinic: _____)
   - ☐ Assisted living facility
   - ☐ Elder care facility
   - ☐ Outpatient clinic
   - ☐ Hospice
   - ☐ Urgent care clinic
   - ☐ Inpatient detoxification program
   - ☐ Dental office
   - ☐ Outpatient surgery center
   - ☐ Patient transport
   - ☐ Other (please specify) _____

Please continue to the next page

Figure B-2: Healthcare occupations survey completed by participants with healthcare exposure.

3. What type of work or volunteering do you currently perform? Check all that apply.
   - ☐ Direct patient care
   - ☐ Nurse (RN, LPN, NA, MA)
   - ☐ Respiratory therapist
   - ☐ Physical therapy, occupational therapy, musical therapy (or related)
   - ☐ Social worker
   - ☐ Hospital chaplain
   - ☐ Dietician
   - ☐ Physician assistant
   - ☐ Food worker (preparing food, delivering food trays to patients, cafeteria work, etc)
   - ☐ Engineering or building maintenance
   - ☐ Nurse midwife
   - ☐ Pharmacist or pharmacy technician
   - ☐ EMT
   - ☐ Dentist
   - ☐ Dental hygienist
   - ☐ Diagnostics/lab microbiology
   - ☐ Gift shop worker or volunteer
   - ☐ Hospital volunteer
   - ☐ Library worker or volunteer
   - ☐ Administration or other position with no patient contact
   - ☐ Other:_____

4. How long have you been working or volunteering in the healthcare industry? (Please total all years in current and previous healthcare-related jobs.)
   _____ (years)

5. How long have you worked or volunteered in your current position?
   _____ (years)

6. What is the average number of patients you have contact with in a typical day?
   _____ patients

7. Please write in one of the following spaces how long it has been since your last patient contact.
   _____ (hours) _____ (days) _____ (weeks) _____ (months)
   For example, if my last patient contact was 2 weeks ago I would fill write a 0 next to hours, a 0 next to days, a 2 next to weeks, a 0 next to months..

8. On average, how many days per week do you work or volunteer in direct contact (meaning in the same area) with patients? _____ (days/week)

Please continue to the next page

Figure B-2: continued

9. On days that you work or volunteer with patients, how many hours (on average) do you work in direct contact with them? _____ (hours/day)

10. Do you care for patients with diagnosed MRSA infections or handle their specimens?
- ☐ Yes (*continue to 10a*)
- ☐ No (*skip to question 11*)
- ☐ Unknown

10a. Is your contact:
- ☐ Direct patient care
- ☐ Diagnostics/ lab microbiology
- ☐ Other: _____

10b. If you care directly for MRSA-infected patients, how frequently do you do so?
- ☐ Daily
- ☐ A few times per week
- ☐ Once per week
- ☐ Less than once per month
- ☐ A few times per year

PERSONAL PROTECTIVE EQUIPMENT

11. Do you use any of the following types of eye protection? *Check all that apply*
- ☐ Glasses
- ☐ Goggles
- ☐ Other (describe: _____)
- ☐ I do not use eye protection

12. In the last 3 months how often have you used eye protection on the job?
- ☐ Always
- ☐ Rarely
- ☐ Most of the time
- ☐ Never (*skip to question 14*)
- ☐ Some of the time
- ☐ I am unsure

13. How frequently do you clean your eye protection?
- ☐ Wash them daily
- ☐ I do not wash them
- ☐ Wash them 1-3 times/week
- ☐ I do not use eye protection
- ☐ Wash them less than once/week

14. Do you use any of the following types of masks? *Check all that apply*
- ☐ Dust mask
- ☐ Filtered mask
- ☐ Surgical mask
- ☐ I do not use a mask
- ☐ Other (describe: _____)

Please continue to the next page

Figure B-2: continued

15. In the last 3 months how often have you used a protective mask?

- ☐ Always
- ☐ Most of the time
- ☐ Some of the time
- ☐ Rarely
- ☐ Never (*skip to question 17*)
- ☐ I am unsure

16. How frequently do you replace your mask?

- ☐ Dispose of daily
- ☐ Dispose of weekly
- ☐ I do not use a mask
- ☐ Dispose of after every patient
- ☐ Use until unusable

17. Do you use any of the following protective clothing? *Check all that apply*

- ☐ Apron
- ☐ Lab coat
- ☐ Other (describe: _____)
- ☐ Coveralls

18. In the last 3 months how often have you used protective clothing?

- ☐ Always
- ☐ Most of the time
- ☐ Some of the time
- ☐ Rarely
- ☐ Never (*skip to question 20*)
- ☐ I am unsure

19. How frequently do you replace or launder your protective clothing?

- ☐ After every patient
- ☐ Weekly
- ☐ Daily
- ☐ Use until unusable

20. Do you use any of the following gloves? *Check all that apply*

- ☐ Disposable latex, vinyl, or nitrile
- ☐ Leather
- ☐ Other (describe: _____)
- ☐ Cloth

21. In the last 3 months how often have you used protective gloves?

- ☐ Always
- ☐ Most of the time
- ☐ Some of the time
- ☐ Rarely
- ☐ Never (*skip to question 23*)
- ☐ I am unsure

22. How frequently do you replace or launder your gloves?

- ☐ After every patient
- ☐ Weekly
- ☐ Daily
- ☐ Use until unusable

Please continue to the next page

Figure B-2: continued

HAND HYGEINE

23. During the work day, when do you wash your hands or use a hand hygiene product containing alcohol? *Check all that apply*

- ☐ After every patient
- ☐ Hourly
- ☐ Prior to meals
- ☐ Other (describe: _____)
- ☐ Moving between wards/units
- ☐ Several times during the day
- ☐ After using the bathroom

24. Do you use any of the following hand hygiene products? *Check all that apply*

- ☐ Triclosan-based hand sanitizers
- ☐ Alcohol-based hand sanitizers (such as Purell)
- ☐ Hand sanitizer, unsure of active chemical
- ☐ Liquid soap, antibacterial (such as Dial liquid or Softsoap)
- ☐ Liquid soap, not antibacterial
- ☐ Liquid soap, unsure if it contains antibacterial ingredients
- ☐ Bar soap, antibacterial (such as Dial or Lever 2000)
- ☐ Bar soap, not antibacterial
- ☐ Bar soap, unsure if it contains antibacterial ingredients
- ☐ Other (describe: _____)

Thank you for completing this survey!

Figure B-2: continued

THE UNIVERSITY OF IOWA

SAMI Study – Farming Occupations Survey

Iowa

Thank you for participating in this study. We are studying how the bacteria that live naturally in your nose and throat may impact another bacteria called *Staphylococcus aureus*, commonly known as "Staph." The survey should only take a few minutes to complete. Please answer the questions to the best of your knowledge and check the appropriate boxes. All of your responses will be kept private and confidential. You may skip any question you do not wish to answer.

Please mark boxes like this ☒ or ☑

CONTACT WITH SWINE (HOGS/PIGS)

1. Do you work, or have you previously worked, in a swine production facility (swine farm or swine processing plant)? (*Please mark all that may apply.)*
   - ☐ Yes—currently work in a swine farm
   - ☐ Yes—currently work in a swine processing plant
   - ☐ Yes—previously worked in a swine farm
   - ☐ Yes—previously worked in a swine processing plant
   - ☐ No (***SKIP TO QUESTION 8***)

2. What type of work do you currently perform in the plant/farm? (*Please mark all that may apply.)*
   - ☐ I do not currently work with swine (SKIP TO QUESTION 8)
   - ☐ Breeding
   - ☐ Farrowing
   - ☐ Working with nursery pigs
   - ☐ Finishing
   - ☐ Wean to finishing
   - ☐ Cleaning swine barn or trucks
   - ☐ Slaughtering and/or butchering swine
   - ☐ Packaging raw pork products
   - ☐ Cooking pork
   - ☐ Packaging cooked pork products
   - ☐ Transporting swine
   - ☐ Disposing swine waste
   - ☐ Examining and treating swine
   - ☐ Gathering blood or other specimens from swine
   - ☐ Cleaning and disinfecting equipment and areas exposed to swine, swine products, or swine waste
   - ☐ Administrative, but occasionally I enter areas where swine, raw pork products, or swine waste is
   - ☐ Administrative, I never enter areas where swine, raw pork products, or swine waste is
   - ☐ Other: _____

Please continue to the next page.

Figure B-3: Farming occupations survey completed by those with livestock exposure

3. How long have you been working with swine? (Please total all years in current and previous jobs)
_____ (years)

4. What is the average number of swine you have contact with in a typical day?
_____swine

5. Please write in one of the following spaces how long it has been since your last contact with swine.
_____ (hours) _____ (days) _____ (weeks) _____ (months)

6. On average, how many days per week do you work in direct contact (meaning in the same area) with swine?
_____ (days/week)

7. On days that you work with swine, how many hours (on average) do you work in direct contact with swine?
_____ (hours/day)

CONTACT WITH CATTLE

8. Do you work, or have you previously worked, on a cattle farm, production facility or dairy? (*Please mark all that may apply.)*
   - ☐ Yes—currently work in a beef or dairy farm
   - ☐ Yes—currently work in a beef processing plant
   - ☐ Yes—previously worked in a beef or dairy farm
   - ☐ Yes—previously worked in a beef processing plant
   - ☐ No (***SKIP TO QUESTION 15***)

9. What type of work do you currently perform in the cattle plant/farm? (*Please mark all that may apply.)*
   - ☐ I do not currently work with cattle (SKIP TO QUESTION 8)
   - ☐ Breeding
   - ☐ Calving
   - ☐ Weaning
   - ☐ Feeding
   - ☐ Working cattle (vaccinating, castrating, worming, pouring, implanting, dehorning, or pregnancy checking)
   - ☐ Milking
   - ☐ Cleaning cattle barns or trucks
   - ☐ Slaughtering and/or butchering cattle
   - ☐ Packaging raw beef products
   - ☐ Cooking beef
   - ☐ Packaging cooked beef products
   - ☐ Transporting cattle
   - ☐ Disposing cattle waste
   - ☐ Examining and treating cattle
   - ☐ Gathering blood or other specimens from cattle
   - ☐ Cleaning and disinfecting equipment and areas exposed to cattle, cattle products, or cattle waste
   - ☐ Administrative, but occasionally I enter areas where cattle, raw beef products, or cattle waste is
   - ☐ Administrative, I never enter areas where cattle, raw beef products, or cattle waste is
   - ☐ Other: _____

Please continue to the next page.

Figure B-3: continued

10. How long have you been working with cattle? (Please total all years in current and previous jobs)
_____ (years)

11. What is the average number of cattle you have contact with in a typical day?
_____ cattle

12. Please write in one of the following spaces how long it has been since your last contact with cattle.
_____ (hours) _____ (days) _____ (weeks) _____ (months)

13. On average, how many days per week do you work in direct contact (meaning in the same area) with cattle?
_____ (days/week)

14. On days that you work with cattle, how many hours (on average) do you work in direct contact with cattle?
_____ (hours/day)

CONTACT WITH CHICKENS

15. Do you work or have you previously worked on a chicken farm (eggs and/or broilers) or production facility?
- ☐ Yes—currently work in a chicken farm
- ☐ Yes—currently work in a chicken processing plant
- ☐ Yes—previously worked in a chicken farm
- ☐ Yes—previously worked in a chicken processing plant
- ☐ No (***SKIP TO QUESTION 22***)

16. What type of work do you currently perform in the cattle plant/farm? (*Please mark all that may apply.)*
- ☐ I do not currently work with live chickens (SKIP TO QUESTION 22)
- ☐ Caring for live chickens
- ☐ Cleaning of chicken house, cages or trucks
- ☐ Slaughtering and/or butchering chicken
- ☐ Packaging raw chicken products
- ☐ Cooking chickens
- ☐ Packaging cooked chicken products
- ☐ Transportation of chickens
- ☐ Chicken waste disposal
- ☐ Examining and treating chickens
- ☐ Gathering blood or other specimens from chickens
- ☐ Gathering eggs
- ☐ Cleaning and disinfecting equipment and areas exposed to chickens, chicken products or chicken waste
- ☐ Administrative, but occasionally I enter areas where chickens, raw chicken products or chicken waste is
- ☐ Administrative I never enter areas where chickens, raw chicken products or chicken waste is
- ☐ Other: _____

Please continue to the next page.

Figure B-3: continued

17. How long have you been working with chickens? (Please total all years in current and previous jobs)
_____ (years)

18. What is the average number of chickens you have contact with in a typical day?
_____ chickens

19. Please write in one of the following spaces how long it has been since your last contact with chickens.
_____ (hours) _____ (days) _____ (weeks) _____ (months)

20. On average, how many days per week do you work in direct contact (meaning in the same area) with chickens?
_____ (days/week)

21. On days that you work with chickens, how many hours (on average) do you work in direct contact with chickens?
_____ (hours/day)

JOB RELATED CONTACT WITH OTHER ANIMALS

22. Do you work with other live animals not mentioned above? (Including horses, sheep, goats, turkeys, etc.)
☐ Yes – please specify the type and approximate number of animal(s)
_____
☐ No (***SKIP TO QUESTION 29***)

23. What type of work do you currently perform with the animal(s) listed in question 22?
_____
_____
_____

24. How long have you been working with the animal(s) listed in question 22?
_____ (years)

25. What is the average number of animal(s) described in question 22 you have contact with in a typical day?
_____ animals

26. Please write in one of the following spaces how long it has been since your last contact with the animal(s) described in question 22.
_____ (hours) _____ (days) _____ (weeks) _____ (months)

27. On average, how many days per week do you work in direct contact (meaning in the same area) with the animals described in question 22?
_____ (days/week)

Please continue to the next page.

Figure B-3: continued

28. On days that you work with the animal(s) described in question 22, how many hours (on average) do you work in direct contact with them?
_____ (hours/day)

If you have job-related contact with live animals (answering "yes" to questions 1, 8, 15, or 22), please complete the questions below.

PERSONAL PROTECTIVE GEAR

29. Do you use any of the following eye protection?
- ☐ Glasses
- ☐ Goggles
- ☐ Other (describe :_____)
- ☐ I do not use eye protection

30. In the last 3 months, how often have you used eye protection on the job?
- ☐ Always
- ☐ Rarely
- ☐ Most of the time
- ☐ Never
- ☐ Some of the time
- ☐ I am unsure

31. How frequently do you clean your eye protection?
- ☐ Wash them daily
- ☐ Do not wash them
- ☐ Wash them 1-3 times/week
- ☐ I do not use eye protection
- ☐ Wash them less than once/week

32. Do you use any of the following types of protective masks?
- ☐ Dust mask
- ☐ Filtered mask
- ☐ Surgical mask
- ☐ I do not use a protective mask
- ☐ Other (describe :_____)

33. In the last 3 months, how often have you used a protective mask?
- ☐ Always
- ☐ Rarely
- ☐ Most of the time
- ☐ Never
- ☐ Some of the time
- ☐ I am unsure

34. How frequently do you replace you protective mask?
- ☐ Dispose of daily
- ☐ Dispose of after every sick animal
- ☐ Dispose of weekly
- ☐ Use until unusable
- ☐ I do not use a mask

35. Do you use any of the following protective clothing?
- ☐ Apron
- ☐ Coveralls
- ☐ Protective clothing
- ☐ I do not use protective clothing
- ☐ Other (describe :_____)

Please continue to the next page.

Figure B-3: continued

36. In the last 3 months, how often have you used protective clothing?

- ☐ Always
- ☐ Most of the time
- ☐ Some of the time
- ☐ Rarely
- ☐ Never
- ☐ I am unsure

37. How frequently do you replace or launder your protective clothing?

- ☐ Daily
- ☐ Weekly
- ☐ I do not wear protective clothing
- ☐ After every sick animal
- ☐ Use until unusable

38. Do you use any of the flowing foot/shoe coverings?

- ☐ Disposable shoes
- ☐ Sandals
- ☐ Sneakers
- ☐ Washable boots
- ☐ I do not use foot/shoe coverings
- ☐ Other (describe :_____)

39. In the last 3 months, how often have you used foot/shoe coverings?

- ☐ Always
- ☐ Most of the time
- ☐ Some of the time
- ☐ Rarely
- ☐ Never
- ☐ I am unsure

40. How frequently do you replace or launder your footwear?

- ☐ Daily
- ☐ Weekly
- ☐ I do not use protective footwear
- ☐ After every sick animal
- ☐ Use until unusable

41. Do you use any of the following types of protective gloves?

- ☐ Disposable latex or vinyl
- ☐ Leather
- ☐ Other (describe :_____)
- ☐ Cloth
- ☐ I do not use protective gloves

42. In the last 3 months, how often have you used protective gloves?

- ☐ Always
- ☐ Most of the time
- ☐ Some of the time
- ☐ Rarely
- ☐ Never
- ☐ I am unsure

43. How frequently do you replace or launder your gloves?

- ☐ Daily
- ☐ Weekly
- ☐ I do not use protective gloves
- ☐ After every sick animal
- ☐ Use until unusable

Please continue to the next page.

Figure B-3: continued

CONTACT WITH OTHER ANIMALS

44. Do you have any of the following animals on your property and/or within your home?

Chickens	☐ Yes	☐ No
Swine	☐ Yes	☐ No
Cats	☐ Yes	☐ No
Dogs	☐ Yes	☐ No

Other type of animal, please specify _____

HAND HYGIENE

45. When do you wash your hands during the work day? *(Check all that apply)*

- ☐ After every sick animal
- ☐ Hourly
- ☐ Prior to meals
- ☐ Moving between buildings
- ☐ Several times during the day
- ☐ After using the bathroom
- ☐ Other (describe: _____)

46. Do you use any of the following hand hygiene products? *(Check all that apply)*

- ☐ Triclosan-based hand sanitizers
- ☐ Alcohol-based hand sanitizers
- ☐ Hand sanitizer, unsure of active chemical
- ☐ Liquid soap, antibacterial
- ☐ Liquid soap, not antibacterial
- ☐ Bar soap, antibacterial
- ☐ Bar soap, not antibacterial
- ☐ Other (describe: _____)

Thank you for taking this survey!

Figure B-3: continued

APPENDIX C: 16S rRNA SEQUENCING

Table C-1: Primer sequences used for 16s rRNA sequencing

Primer	Step	Sequence
Meta V1 27F*	16s rRNA amplification	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**AGAGTTTGATCMTGGCTCAG**
Meta V3 534R*	16s rRNA amplification	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**ATTACCGCGGCTGCTGG**
Forward index§	Library coding	**AATGATACGGCGACCACCGA**GATCTACAC[i5]TCGTCGGCAGCGTC
Reverse index§	Library coding	**CAAGCAGAAGACGGCATACGA**GAT[i7]GTCTCGTGGGCTCGG
Nextera adapter sequence	Read 1 post-run trimming	CTGTCTCTTATACACATCTCCGAGCCCACGAGACNNNNNNNNATCTCGTATGCCGTCTTCTGCTTG
Nextera adapter sequence	Read 2 post-run trimming	CTGTCTCTTATACACATCTGACGCTGCCGACGANNNNNNNNGTGTAGATCTCGGTGGTCGCCGTATCATT

* 16S-specific portion of primer sequence is in bold.
§ [i5] and [i7] refer to the index sequence codes used by Illumina. The flow cell adapters are in bold.

C.1 PCR Cycling Conditions

The following cycling conditions were used for the first PCR (amplification) using the Meta_V1_27F and Meta_V3_534R primer pair. Five minutes at 95°C followed by 15-25 cycles of: 98° for 20 seconds, 55°C for 15 seconds, and 72°C for 60 seconds; and holding at 4°C. After the first round of amplicon PCR, PCR products were diluted 1:100 and 5µl of 1:100 amplicon PCR product was used for the second round of PCR (PCR 2). Cycling conditions for PCR 2 were: 95°C for five minutes, 10 cycles of: 98° for 20 seconds, 55°C for 15 seconds, and 72°C for 60 seconds; and holding at 4°C.

APPENDIX D: RESULTS

Table D-1: Differentially abundant OTUs in the nares between livestock workers and non-livestock workers (n=26)

OTU	2-fold Change	P-value	Adjusted p-value*	Phylum	Genus
OTU_221	-8.62	6.05E-18	1.41E-15	Firmicutes	*Clostridium XI*
OTU_53	-9.12	8.67E-17	1.01E-14	Firmicutes	*Streptococcus*
OTU_130	-7.78	7.50E-15	4.37E-13	Firmicutes	*Lactobacillus*
OTU_34	-8.13	6.32E-15	4.37E-13	Firmicutes	*Clostridium sensu stricto*
OTU_40	-7.83	1.40E-13	6.54E-12	Firmicutes	*Turicibacter*
OTU_108	-7.04	4.27E-12	1.66E-10	Firmicutes	*Clostridium sensu stricto*
OTU_154	-7.76	1.73E-11	5.76E-10	Firmicutes	*Clostridium sensu stricto*
OTU_747	-6.99	7.65E-09	2.23E-07	Bacteroidetes	*Prevotella*
OTU_291	-5.97	1.50E-08	3.87E-07	Firmicutes	*Aerococcus*
OTU_209	-6.34	3.83E-08	8.92E-07	Firmicutes	*Clostridium XI*
OTU_802	-5.66	6.37E-07	1.35E-05	Bacteroidetes	*Prevotella*
OTU_282	-6.07	9.18E-07	1.78E-05	Firmicutes	*Lactobacillus*
OTU_1058	-5.62	2.80E-05	5.02E-04	Firmicutes	*Oscillibacter*
OTU_435	-5.69	4.36E-05	7.26E-04	Firmicutes	*Coprococcus*
OTU_507	-4.96	1.15E-04	1.79E-03	Firmicutes	*Clostridium sensu stricto*
OTU_17	-4.97	1.47E-04	1.90E-03	Proteobacteria	*Moraxella*
OTU_483	-5.34	1.36E-04	1.90E-03	Bacteroidetes	*Prevotella*
OTU_610	-4.82	1.40E-04	1.90E-03	Firmicutes	*Blautia*
OTU_2648	-5.08	1.66E-04	1.94E-03	Firmicutes	*Clostridium sensu stricto*
OTU_337	-5.27	1.65E-04	1.94E-03	Actinobacteria	*Rothia*
OTU_1177	-5.02	1.95E-04	2.06E-03	Firmicutes	*Megasphaera*
OTU_28	2.55	1.93E-04	2.06E-03	Cyanobacteria/ Chloroplast	*Streptophyta*
OTU_365	-5.07	2.50E-04	2.53E-03	Bacteroidetes	*Prevotella*
OTU_918	-3.55	3.18E-04	3.08E-03	Actinobacteria	*Dietzia*
OTU_534	5.33	6.40E-04	5.97E-03	Cyanobacteria/ Chloroplast	*Streptophyta*
OTU_880	-4.55	7.77E-04	6.96E-03	Firmicutes	*Ruminococcus2*

* Benjamini-Hochberg corrected p-value

Table D-2: Microbiota differentially abundant between livestock workers with and without swine contact in the nares (n=45)

OTU	2-fold Change	P-value	Adjusted p-value*	Phylum	Genus
OTU_34	11.94	2.96E-20	7.63E-18	Firmicutes	*Clostridium sensu stricto*
OTU_209	10.46	5.51E-14	7.10E-12	Firmicutes	*Clostridium XI*
OTU_130	9.42	4.72E-13	4.06E-11	Firmicutes	*Lactobacillus*
OTU_154	9.26	3.80E-10	2.45E-08	Firmicutes	*Clostridium sensu stricto*
OTU_747	8.27	4.01E-08	2.07E-06	Bacteroidetes	*Prevotella*
OTU_53	6.28	1.50E-06	6.43E-05	Firmicutes	*Streptococcus*
OTU_282	7.72	1.93E-06	7.12E-05	Firmicutes	*Lactobacillus*
OTU_17	6.78	3.78E-05	1.22E-03	Proteobacteria	*Moraxella*
OTU_1058	6.66	8.13E-05	2.10E-03	Firmicutes	*Oscillibacter*
OTU_610	6.57	7.91E-05	2.10E-03	Firmicutes	*Blautia*
OTU_507	6.26	1.02E-04	2.38E-03	Firmicutes	*Clostridium sensu stricto*
OTU_435	6.73	1.27E-04	2.73E-03	Firmicutes	*Coprococcus*
OTU_802	5.56	2.03E-04	4.03E-03	Bacteroidetes	*Prevotella*
OTU_90	-7.07	2.48E-04	4.58E-03	Actinobacteria	*Actinomyces*
OTU_1177	6.17	2.85E-04	4.65E-03	Firmicutes	*Megasphaera*
OTU_2648	6.17	2.88E-04	4.65E-03	Firmicutes	*Clostridium sensu stricto*
OTU_365	6.20	3.13E-04	4.75E-03	Bacteroidetes	*Prevotella*
OTU_483	6.35	3.44E-04	4.93E-03	Bacteroidetes	*Prevotella*
OTU_880	5.77	6.65E-04	8.57E-03	Firmicutes	*Ruminococcus2*
OTU_993	5.52	6.59E-04	8.57E-03	Firmicutes	*Anaerotruncus*
OTU_14	-5.62	9.24E-04	1.14E-02	Bacteroidetes	*Prevotella*
OTU_1959	5.55	9.88E-04	1.14E-02	Firmicutes	*Blautia*
OTU_2997	5.60	1.06E.02	1.14E-02	Bacteroidetes	*Unclassified Porphyromonada-ceae unclassified*
OTU_368	-8.03	1.02E-03	1.14E-02	Actinobacteria	*Actinomycetales*
OTU_1397	5.49	1.18E-03	1.22E-02	Bacteroidetes	*Prevotella*
OTU_815	-7.85	1.34E-03	1.32E-02	Proteobacteria	*Diplorickettsia*
OTU_108	4.17	1.39E-03	1.33E-02	Firmicutes	*Clostridium sensu stricto*

* Benjamini-Hochberg corrected p-value

Table D-2: continued

OTU	2-fold Change	P-value	Adjusted p-value*	Phylum	Genus
OTU_646	5.55	1.47E-03	1.35E-02	Firmicutes	*Solobacterium*
OTU_424	-7.74	1.55E-03	1.38E-02	Actinobacteria	*Haloechinothrix*
OTU_126	-7.61	1.86E-03	1.60E-02	Bacteroidetes	*Prevotella*
OTU_476	5.52	2.13E-03	1.77E-02	Firmicutes	*unclassified Clostridia*
OTU_10	-6.84	2.62E-03	2.10E-02	Proteobacteria	*Haemophilus*
OTU_1521	5.29	2.69E-03	2.10E-02	Firmicutes	*unclassified Lachnospiraceae*
OTU_999	-7.30	2.84E-03	2.15E-02	Actinobacteria	*Saccharopolyspora*
OTU_733	5.38	3.05E-03	2.25E-02	Firmicutes	*Butyricicoccus*
OTU_1178	5.29	4.54E-03	3.22E-02	Firmicutes	*Aerococcus*
OTU_12	-5.03	4.72E-03	3.22E-02	Fusobacteria	*Leptotrichia*
OTU_763	5.23	4.75E-03	3.22E-02	Firmicutes	*Oscillibacter*
OTU_3242	4.91	5.50E-03	3.56E-02	Bacteroidetes	*Prevotella*
OTU_832	5.27	5.52E-03	3.56E-02	Firmicutes	*Coprococcus unclassified*
OTU_644	4.81	5.69E-03	3.58E-02	Firmicutes	*Lachnospiraceae*
OTU_1511	4.83	6.21E-03	3.81E-02	Firmicutes	*Ruminococcus*
OTU_1743	4.83	7.60E-03	4.56E-02	Firmicutes	*Faecalibacterium*
OTU_1627	5.05	8.32E-03	4.88E-02	Firmicutes	*Oscillibacter unclassified*
OTU_1245	4.72	8.62E-03	4.94E-02	Firmicutes	*Lachnospiraceae*

* Benjamini-Hochberg corrected p-value

Table D-3: Microbiota differentially abundant between livestock workers with and without swine contact in the oropharynx (n=12)

OTU	2-fold Change	P-value	Adjusted p-value*	Phylum	Genus
OTU_213	5.44	4.71E-06	4.25E-04	Firmicutes	*Streptococcus*
OTU_23	5.24	5.29E-06	4.25E-04	Proteobacteria	*Neisseria*
OTU_642	6.90	3.28E-05	1.76E-03	Bacteroidetes	*Prevotella*
OTU_470	6.30	1.43E-04	4.60E-03	Fusobacteria	*Leptotrichia*
OTU_5	3.39	1.19E-04	4.60E-03	Fusobacteria	*Fusobacterium*
OTU_130	-7.85	5.23E-04	1.40E-02	Firmicutes	*Lactobacillus*
OTU_35	5.45	6.53E-04	1.50E-02	Proteobacteria	*Haemophilus*
OTU_1250	-7.06	1.82E-03	3.25E-02	Bacilli	*Lactobacillus*
OTU_201	5.49	1.65E-03	3.25E-02	Bacteroidetes	*Capnocytophaga*
OTU_2524	-6.98	2.03E-03	3.26E-02	Firmicutes	*Lactobacillus*
OTU_251	5.29	2.33E-03	3.42E-02	Bacteroidetes	*Alloprevotella*
OTU_294	5.14	2.91E-03	3.91E-02	Firmicutes	*peptostrepotococcus*

* Benjamini-Hochberg corrected p-value

Table D-4: Microbiota differentially abundant between nasally colonized livestock workers and non-livestock workers (n=24)

OTU	2-fold Change	P-value	Adjusted p-value*	Phylum	Genus
OTU_221	-8.44	7.02E-12	8.42E-10	Firmicutes	*Clostridium XI*
OTU_53	-8.45	2.89E-11	1.73E-09	Firmicutes	*Streptococcus*
OTU_34	-7.66	2.32E-10	9.30E-09	Firmicutes	*Clostridium sensu stricto*
OTU_209	-8.23	5.66E-10	1.70E-08	Firmicutes	*Clostridium XI*
OTU_108	-7.49	5.96E-09	1.43E-07	Firmicutes	*Clostridium sensu stricto*
OTU_130	-7.31	3.56E-08	7.12E-07	Firmicutes	*Lactobacillus*
OTU_40	-6.91	1.27E-07	2.18E-06	Firmicutes	*Turicibacter*
OTU_291	-5.96	2.55E-06	3.82E-05	Firmicutes	*Aerococcus*
OTU_747	-6.52	8.49E-06	1.13E-04	Bacteroidetes	*Prevotella*
OTU_154	-5.99	1.89E-05	2.27E-04	Firmicutes	*Clostridium sensu stricto*
OTU_802	-5.51	4.17E-05	4.55E-04	Bacteroidetes	*Prevotella*
OTU_282	-6.23	5.64E-05	5.64E-04	Firmicutes	*Lactobacillus*
OTU_713	-5.59	1.72E-04	1.59E-03	Proteobacteria	*Psychrobacter*
OTU_17	-5.30	3.09E-04	2.65E-03	Proteobacteria	*Moraxella*
OTU_93	-3.12	1.08E-03	8.68E-03	Firmicutes	*Staphylococcus*
OTU_610	-5.04	1.36E-03	1.02E-02	Firmicutes	*Blautia*
OTU_483	-4.97	1.67E-03	1.18E-02	Bacteroidetes	*Prevotella*
OTU_337	-5.08	1.93E-03	1.29E-02	Actinobacteria	*Rothia*
OTU_1177	-4.70	2.88E-03	1.82E-02	Firmicutes	*Megasphaera*
OTU_1058	-4.40	5.39E-03	3.08E-02	Firmicutes	*Oscillibacter*
OTU_2648	-4.62	5.30E-03	3.08E-02	Firmicutes	*Clostridium sensu stricto*
OTU_365	-4.51	5.95E-03	3.24E-02	Bacteroidetes	*Prevotella*
OTU_966	-4.33	6.24E-03	3.26E-02	Firmicutes	*Atopostipes*
OTU_507	-4.23	7.61E-03	3.80E-02	Bacteroidetes	*Clostridium sensu stricto*

* Benjamini-Hochberg corrected p-value

Table D-5: Microbiota differentially abundant between nasally non-colonized livestock workers and non-livestock workers (n=11)

OTU	2-fold Change	P-value	Adjusted p-value*	Phylum	Genus
OTU_130	-7.2047	2.23E-06	1.90E-04	Firmicutes	*Lactobacillus*
OTU_40	-7.7268	1.29E-06	1.90E-04	Firmicutes	*Turicibacter*
OTU_53	-9.3034	1.82E-06	1.90E-04	Firmicutes	*Streptococcus*
OTU_221	-7.2642	1.04E-05	6.68E-04	Firmicutes	*Clostridium XI*
OTU_34	-7.166	3.14E-05	1.61E-03	Firmicutes	*Clostridium sensu stricto*
OTU_154	-6.7871	0.00035	1.49E-02	Firmicutes	*Clostridium sensu stricto*
OTU_108	-5.444	0.00064	2.33E-02	Firmicutes	*Clostridium sensu stricto*
OTU_113	4.48924	0.00132	4.00E-02	Actinobacteria	*Unclassified Moraxellaceae*
OTU_20	4.149	0.00172	4.00E-02	Actinobacteria	*Unclassified Actinomycetales*
OTU_435	-5.8809	0.00151	4.00E-02	Firmicutes	*Coprococcus*
OTU_747	-5.8665	0.00172	4.00E-02	Firmicutes	*Prevotella*

* Benjamini-Hochberg corrected p-value

Table D-6: Microbiota differentially abundant between oropharyngeal intermittent carriers and non-carriers (n=3)

OTU	2-fold Change	P-value	Adjusted p-value*	Phylum	Genus
OTU_22	5.84	0.00	0.00	Firmicutes	*Gemella*
OTU_7	4.52	0.00	0.04	Firmicutes	*Streptococcus*
OTU_213	6.67	0.00	0.04	Firmicutes	*Streptococcus*

* Benjamini-Hochberg corrected p-value

Table D-7: Microbiota differentially abundant between oropharyngeal intermittent carriers and persistent carriers (n=9)

OTU	2-fold Change	P-value	Adjusted p-value*	Phylum	Genus
OTU_38	4.26	3.74E-04	0.03	Proteobacteria	*Haemophilus*
OTU_69	6.20	4.05E-04	0.03	Bacteriodetes	*Soonwooa*
OTU_76	-2.65	1.62E-03	0.04	Firmicutes	*Moryella*
OTU_42	-3.34	1.69E-03	0.04	Bacteriodetes	*Alloprevotella*
OTU_86	5.60	1.39E-03	0.04	Bacteriodetes	*Porphyromonas*
OTU_145	5.34	1.81E-03	0.04	Bacteriodetes	*Capnocytophaga*
OTU_1124	5.28	2.03E-03	0.04	Firmicutes	Unclassified Firmicutes
OTU_233	5.36	2.18E-03	0.04	Bacteriodetes	*Bacteroides*
OTU_4048	5.54	1.53E-03	0.04	Actinobacteria	*Corynebacterium*

* Benjamini-Hochberg corrected p-value

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