Obesity and Comorbid Diseases as a Host Determinants of *Staphylococcus aureus* Colonization

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ABSTRACT

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The etiology of obesity is heterogeneous as are the cardio-metabolic complications, associated with it. The cardio-metabolic profile of obese individuals places them at risk of a range of chronic metabolic diseases including diabetes. Paradoxically, a subset of the population classified as obese based on established methods present with few metabolic abnormalities, whereas a subset classified as non-obese present with a wide range of abnormalities. The observed heterogeneity suggests not only that excess adiposity is likely one of many determinant of metabolic complications, but also that our methods of measuring obesity might not be fully capturing the underlying biological mechanisms at play. The heterogeneity by which obesity presents itself in the general population is becoming more pertinent to the field of infectious diseases as findings increasingly implicate obesity in impaired host defenses and increased susceptibility to a range of different infectious organisms, one of which is *Staphylococcus aureus*.

S. aureus is an opportunistic pathogen with significant infectious burdens in clinical, community as well as incarcerated settings. The organism also asymptomatically colonizes human mucosal surfaces, particularly the anterior nares. The anterior nares of approximately 25-30% of US adults are colonized at any given time, and prior colonization serves as a strong predictor of subsequent infection. Obese females have been consistently shown to be at elevated risk of *S. aureus* colonization, however,

findings amongst obese males have been inconsistent. The mechanism by which obesity increases risk of colonization remain unclear, however, many cite the underlying metabolic/immune dysfunction that frequently accompanies obesity. Given the global burden of obesity and increasing evidence that it impairs host defenses, understanding how obesity increases host colonization with *S. aureus* is imperative. The overall objective of this dissertation was therefore to evaluate the influence of obesity and metabolic abnormalities on *S. aureus* colonization among New York State Maximum-Security prison inmates. The objective of the dissertation was met using three aims.

First a systematic review was conducted to assess the different definitions used to define persistent *S. aureus* colonization in community dwelling adults, as well as the reported prevalence estimates associated with those definitions. The study demonstrated that a considerable amount of variation existed in the way persistent colonization was defined in the extant literature. Despite the variation however, the prevalence of persistent *S. aureus* carriage remained relatively consistent after categorizing the different definitions into four general groups. The review also demonstrated that two groups of persistent carriers might exist. Therefore, differentiating strain persistence carriers from species persistence carriers may reconcile some of the inconsistencies with regard to length of strain carriage reported in the literature.

Second, the influence of metabolic heath (a measure incorporating both body mass index (BMI) and metabolic abnormalities) was assessed. A significantly higher probability of *S. aureus* colonization of the anterior nares and/or oropharynx was observed among metabolically abnormal normal weight (BMI < 25 kg/m^2) as well metabolically abnormal obese (BMI ≥ 30 kg/m2) females when compared to

metabolically healthy females. No significant association was observed between the categories of metabolic health and the prevalence of *S. aureus* colonization among males. We did, however observe a significant decline in exclusive oropharyngeal colonization among obese male inmates with metabolic abnormalities.

Lastly, factors associated with persistent *S. aureus* carriage were evaluated in the third aim. Approximately 27% of the population was persistent carriers at the species level and 17% were persistent carriers at the strain level. Obesity was independently associated with species persistent carriage but not strain persistent carriage. Correspondence analysis evaluating strain compositional differences between exclusive persistent anterior nares carriers, exclusive persistent oropharynx carriers, exclusive persistent carriers at both the anterior nares and oropharynx and intermittent carriers suggested compositional differences existed between the different groups. More specifically, the relative abundance of certain *S. aureus* strains appeared more prominent among exclusive nasal carriers as compared to all other carriage/mucosal site types (i.e. exclusive oropharynx, both nasal and oropharynx).

Table of Contents

List of Tables and Figures

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CHAPTER 1: Introduction: The Utility of Sub-phenotypes of Obesity When Evaluating its Effects on Infectious Outcomes

<u>Background and Significance</u>

Obesity results from storage of excess energy in the form of triglycerides in the lipid droplets of adipocytes, the major cell type of adipose tissue.[1] To accommodate excess energy, adipose tissue either increases the number (hyperplasia) or the size (hypertrophy)[1] of adipocytes in adipose tissue depots. Two main subcategories of depots are recognized: subcutaneous adipose tissue (SAT) located between the dermis and fascia of muscle and visceral adipose tissue (VAT) located within the intra- and extra peritoneal spaces of the abdomino-pelvic cavity.[2] Adipose tissue deposition displays a significant amount heterogeneity across sex, age and race/ethnicity.[1,3] For example, males have a higher VAT/SAT ratio as compared to pre-menopausal females.[4] In addition, males have more pronounced depositions in their abdomen (android) as compared to the pronounced deposition in the hips and thighs (gynoid) observed in females.[5] Further, whereas females preferentially expand adipose tissue through hyperplasia when deposition is in the hips and thighs, and hypertrophy when deposition is in the trunk, males appear to expand adipose tissue primarily through hypertrophy.[1] VAT deposition compared to SAT, abdomen deposition compared to hips and thighs and expansion by hypertrophy compared to hyperplasia are all more closely associated with cardio-metabolic complications such as dyslipidemia, hyperglycemia, hyperinsulinemia and insulin resistance, collectively referred to as the metabolic syndrome.[1,6] Body mass index (BMI) does not capture these differences, and failing to account for them masks the heterogeneity associated in the observed relationship between obesity and cardio-metabolic disease.[7] Despite these observations, few studies

evaluating obesity and infections have taken these factors into account despite growing evidence implicating obesity in impaired host defenses and susceptibility to infections.[8-11]

Another factor that needs to be taken into account when measuring the effects of obesity on health outcomes, specifically host defenses is the dual role of adipose tissue as both a site of energy storage and an endocrine organ.[6] Obesity alters the endocrine functions of adipose tissue. For example, adipocytes secrete bioactive proteins (adipokines) that regulate metabolism, immunity and inflammation.[12] Expansion of adipocytes can dysregulate adipokine secretion leading to chronic subclinical inflammation characterized by increased levels of pro-inflammatory adipokines such as interleukin-6 and leptin, and decreased levels of anti-inflammatory adipokines particularly adiponectin.[1,6] In addition, changes in the cellular composition of adipose tissue due to changes in the absolute number and phenotype of the immune, vascular and structural cells found in adipose tissue could further impair endocrine function and additionally contribute to inflammation, which increases cardio-metabolic risk.[6] Given that approximately 1.9 billion adults worldwide are overweight and over 600 million are obese, it is imperative to elucidate the mechanism by which obesity places individuals at risk.[13]

Interestingly, not all obese individuals present with increased cardio-metabolic risk factors; a proportion are metabolically healthy. Recognition of this has led to the development of a sub-phenotype of obesity referred to as metabolically healthy obese (MHO) individuals characterized by their relative absence of metabolic complications as compared to their metabolically abnormal (MAO) counterparts.[14] Though, the use and utility of these sub-phenotypes is still controversial,[15,16] incorporating these sub-phenotypes has improved research related to metabolic diseases if for nothing else to demonstrate that heterogeneity exists in the subgroups (e.g. BMI category) used to define adiposity.[15] These sub-phenotypes, however, have

yet to be implemented in research evaluating the association between obesity and host defenses despite accumulating evidence implicating obesity and host susceptibility to infectious organisms[17-20] and inconsistencies in some of the findings.[21-23] This dissertation aims to utilize obesity sub-phenotypes to evaluate their utility in infectious diseases outcomes using colonization by *Staphylococcus aureus*, an organism that has been linked to obesity,[24-26] as the infectious disease outcome of interest.

The sub-phenotypes of obesity often described in the literature include MHO, MAO as well as their normal weight counterparts, metabolically healthy normal weight (MHNW) and metabolically abnormal normal weight (MANW). As discussed above, the development of these sub-phenotypes has shed some light on the complex and heterogeneous effect of obesity on health.[6] Whether these phenotypes represent a true subset of the population or are part of a continuum is still under debate.[14,27] Despite this controversy, MHO individuals have been shown to differ in significant ways from their metabolically abnormal (MAO) counterparts including having lower levels of inflammation.[1,14] In addition, their metabolic profile has been shown to be comparable to MHNW individuals and generally better than MANW individuals.[27] In populations where markers of immune/metabolic dysfunction are prevalent, sub-typing individuals may provide a more granular understanding of how obesity influences outcomes related not only to infection but also colonization with opportunistic pathogens. More specific to our purposes, sub-phenotypes of obesity based on the presence or absence of morbidities associated with metabolic and/or immune dysfunction may provide a mechanistic insight into the observed association between obesity and *S. aureus*. Obesity has been implicated in both infection and colonization with *S. aureus* and the organism could serve as a model by which we can explore the effects of sub-phenotypes of obesity on infectious outcomes.

S. aureus is one of the most frequently isolated human pathogens in health care settings, and is a leading cause of both community acquired and healthcare-associated infections.[28,29] Methicillin-resistant *S. aureus* (MRSA) isolates were initially largely confined to hospitals and other health care settings. However, since the mid-1990s community-associated MRSA (CA-MRSA) strains have emerged[28] and continue to be a leading cause of uncomplicated skin and soft tissue infection as well as invasive disease.[29] Asymptomatic colonization with *S. aureus* is a significant predictor of subsequent infection in both clinical[30] and community[31-33] settings, and also serves as a major reservoir for continued transmission.[31] In fact, in clinical settings, 80% of *S. aureus* infections have been attributed to the colonizing strain, suggesting that colonization directly contributes to the risk of infections.[30] The risk of infection is particularly pronounced among persistent carriers,[34] defined as individuals from whom *S. aureus* is consistently isolated upon multiple sampling of the anterior nares,[35-37] as compared to their intermittent carrier counterparts. Intermittent carriers are individuals from whom *S. aureus* is inconsistently or rarely isolated.[35] Factors that predispose individuals to colonization by *S. aureus* include youth and elderly, male sex, exposure to healthcare environments as well as inconsistent findings with regard to medical morbidities such as human immunodeficiency virus (HIV),[38,39] diabetes[25,40] and obesity[24-26]. More elusive are factors that differentiate persistent carriers from intermittent carriers, though limited evidence implicates factors related to both innate and adaptive immune function.[41-46]

Obesity as a host determinant of *S. aureus* colonization warrants further investigation, due not only to the potential public health impact given its high and increasing prevalence world wide, but also its unique and sometimes inconsistent association with *S. aureus* infection and colonization.[24-26] Obese individuals, have been shown to be susceptible to a variety of hospital-

acquired infections frequently caused by *S. aureus* including post-operative surgical site,[47,48] catheter-related,[49] bloodstream,[49] wound,[47] urinary tract infections and sepsis.[47] In addition, in non-clinical settings, obese individuals are vulnerable to skin and soft tissue infections[8] as well as bacterial complicated influenza virus infection[9,10] caused by *S. aureus*. With regards to colonization, obesity as a host determinant of colonization has been reported among patients'[50] as well as non-clinically institutionalized adults.[24-26] Findings among females consistently show an elevated prevalence/likelihood of colonization among overweight and obese females as compared to their normal weight counterparts.[24-26] Findings among males, however, have been inconsistent.[24-26]

Several factors contribute to the underlying theory that obesity is associated with *S aureus* colonization. First, *S. aureus* is a commensal that colonizes human epithelial surfaces without causing disease. Epithelial surfaces form the interface between the host and its exterior environment, and are therefore the main route of entry for microbial organisms.[51] Important changes in epithelial tissue have been observed amongst obese individuals including alterations in barrier function and integrity, impaired wound healing, and increased susceptibility to infection.[52] In addition, microbial composition of epithelial sites differ between obese and normal weight individuals, which has been posited to affect the pathogenicity of some of the residing opportunistic pathogens such as *S. auerus*.[53,54] These observations have led some to hypothesize that the underlying systemic immune dysfunction observed in obesity may also affect local immune function at epithelial sites, including the anterior nares, leading to vulnerability to organisms such as *S. aureus*.[52] The heterogeneity in the presentation of these characteristics among obese individuals, however, may preclude attempts to fully describe their influence on *S. aureus* colonization and explain the inconsistencies in findings among men.[24-26]

Incorporating sub-phenotypes of obesity in our measures may help differentiate obese individuals presenting with the biological factors that place them at risk. More importantly sub-phenotypes may help inform prevention efforts in populations such as the incarcerated where the burden of both obesity and *S. aureus* infection and colonization are high.

Incarcerated populations have high burden of *S. aureus* disease[55-58] and colonization,[59,60] particularly as it relates to MRSA. The reported prevalence of MRSA colonization in correctional settings ranges from 0.8%-15.8% as compared to 0.8%-1.5% in non-institutionalized settings.[25,58,60-65] Furthermore, high rates of chronic immune diseases such as HIV and hepatitis C virus,[66] as well as chronic non-communicable diseases have been reported. In fact, age adjusted estimates of a nationally representative sample of incarcerated individuals report elevated rates of chronic metabolic/immune conditions such as hypertension, asthma, myocardial infarction, and diabetes among the incarcerated as compared to the general population.[67,68]

A recently completed study amongst New York State maximum-security inmates funded by the National Institute of Allergy and Infectious Disease (5R01AI082536) confirmed the high rates of obesity and metabolically diseases[69] as well as *S. aureus* colonization.[65] The study also provides an opportunity to assess the role of sub-phenotypes of obesity on *S. aureus* colonization in a population with high burdens of both. The data comprised of over 2,800 prison inmates from one male and one female maximum-security prison in New York State. The study consists of three different cross sectional phases: inmates entering into prison, inmates during incarceration, and inmates being released into the community. Because a number of inmates were cultured two or more times during their prison stay, this study also provided a unique opportunity to evaluate how obesity may influence persistent carriage. Using these data, the analytical aims for this dissertation were therefore to assess the association between 1) obesity sub-phenotypes as

measured by body mass index category and the presence of metabolic/immune morbidities and *S. aureus* colonization (inclusive of both persistent and intermittent carriers) and 2) evaluate the association between obesity and persistent *S. aureus* carriage.

Conceptual Framework

Two existing conceptual frameworks were used to guide the work conducted in this dissertation. The first framework (Appendix A1.1A) frames our understanding of how underlying disease could influence *S. aureus* colonization by illustrating how host pathology can alter the epithelial microbial environment through effects of the core microbiome. This framework is rooted in ecology and was formulated to evaluate the effects of the human core microbiome on disease and vice versa.[70] This framework grounds our understanding of how obesity may influence epithelial surfaces such as the anterior nares and/or oropharynx leading to *S. aureus* colonization. The second builds on the conceptual model first described by Scrimshaw, Taylor and Gordon for the interaction of nutrition and infection.[71] This framework describes the synergistic and antagonistic influence of malnutrition on infections. When the framework was developed malnutrition was synonymous with under nutrition, but the definition was since broadened by Solomons to include obesity as a form of malnutrition due to accumulating findings implicating obesity in infectious outcomes.[72] The modified framework represented in (Appendix A1.1B) demonstrates the effects of obesity on the morphology and function of adipocytes, which in turn results in immune dysfunction characterized by chronic low-grade inflammation and reduced immune response of both the innate and adaptive pathways.

Figure 1.2 modifies the above-mentioned frameworks to accommodate obesity sub-phenotypes. In this conceptual model, excess caloric intake results in adipose tissue expansion, which in turn has a heterogeneous effect on both local and systemic immune/metabolic

function.[19,20] One well-described effect is chronic low-grade inflammation that impairs both innate and adaptive immune response pathways. The focus here is on host factors, specifically health factors that influence *S. aureus* colonization. Host metabolic/immune function is posited to influence *S. aureus* colonization, and is crudely captured by the presence of metabolic abnormalities defined in this dissertation as the presence of diabetes, hypertension and/or HIV. In the conceptual framework described here the sub-phenotypes should result in increasing prevalence of *S. aureus* colonization. We also posit that a more pronounced association will be observed between obesity and persistent *S. aureus* carriers as compared to intermittent carriers, given the underlying differences in innate and adaptive immunity observed amongst persistent carriers as compared to those intermittently colonized.

Summary of Dissertation Activities

Before embarking on the analytical aims that assessed the influence of obesity and its sub-phenotypes on *S. aureus* colonization and carriage, a systematic review of the literature was conducted to better understand how the definition of persistent carriage could impact observed estimates. Chapter 2 summarizes the results of this systematic review and also provides results of a meta-analysis summarizing the observed prevalence estimates for persistent carriage. The third chapter evaluates the association between obesity and markers of metabolic health on *S. aureus* colonization. To do so, individuals were categorized based on BMI category (normal weight, overweight and obese) and were then further stratified based on the presence or absence of one or more comorbidities (hypertension, diabetes and HIV). The association between these categories was then evaluated to determine if they were associated with *S. aureus* colonization of the anterior nares and or the oropharynx. Also evaluated in this chapter was the influence of the sub-phenotypes on site of colonization (exclusive anterior nares Vs. exclusive oropharynx Vs.

both anterior nares and oropharynx). In Chapter 4, the association between obesity and

persistent *S. aureus* carriage, defined as *S. aureus* culture positivity at each study visit, was

assessed. The diversity of *S. aureus* strains as assessed by Simpson's Index of Diversity as well

as by multivariate techniques was also evaluated to demonstrate the utility of complimenting

arithmetic measures of diversity with ordination techniques in an effort to more completely

account for composition. Lastly, in chapter 5 we summarize the dissertation findings within the

context of the extant literature.

Figure 1.1 Conceptual Frameworks Describing the Effect of Obesity Sub-phenotypes on Colonization/Infectious Outcomes
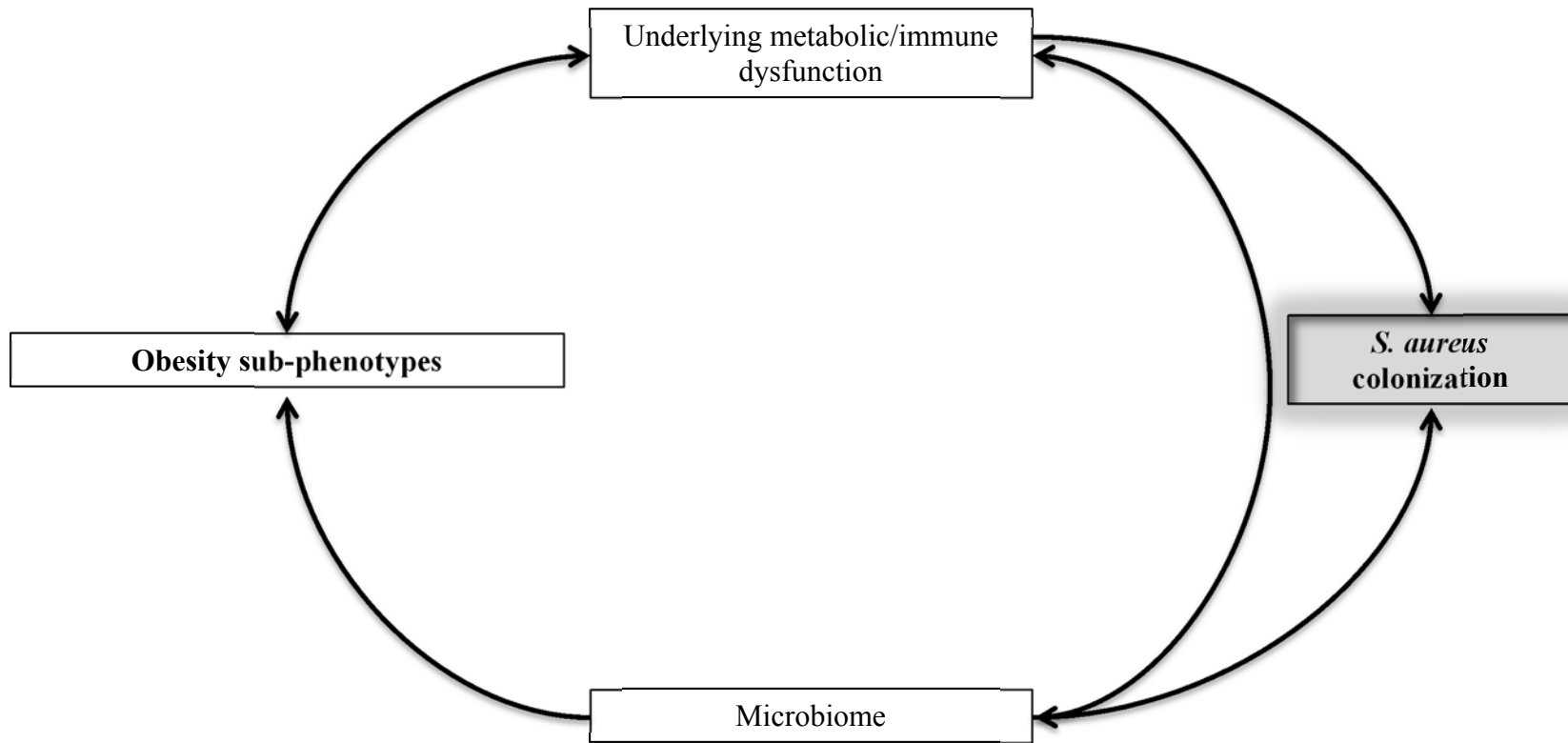
Figure 1.1 modifies the established frameworks illustrated in Appendix A1.1A and A1.1B to accommodate obesity sub-phenotypes, body mass index further stratified by metabolic health. In this conceptual model, adiposity and the comorbid conditions being assessed are characterized by underlying immune dysfunction and this underlying immune dysfunction may directly influence *Staphylococcus aureus* (*S. aureus*) colonization through impaired immune function which in turn compromises the clearance of *S.aureus* when

encountered leading to colonization. Obesity sub-phenotypes may also indirectly or directly cause shifts in the microbial composition of different body sites, which may facilitate *S. aureus* colonization

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Appendix 1

Figure A1.1. Established Framework Conceptualizing the Role of Obesity and Markers of Metabolic/Immune Dysfunction on

Infection and Colonization by Opportunistic Pathogens such as *Staphylococcus aureus*
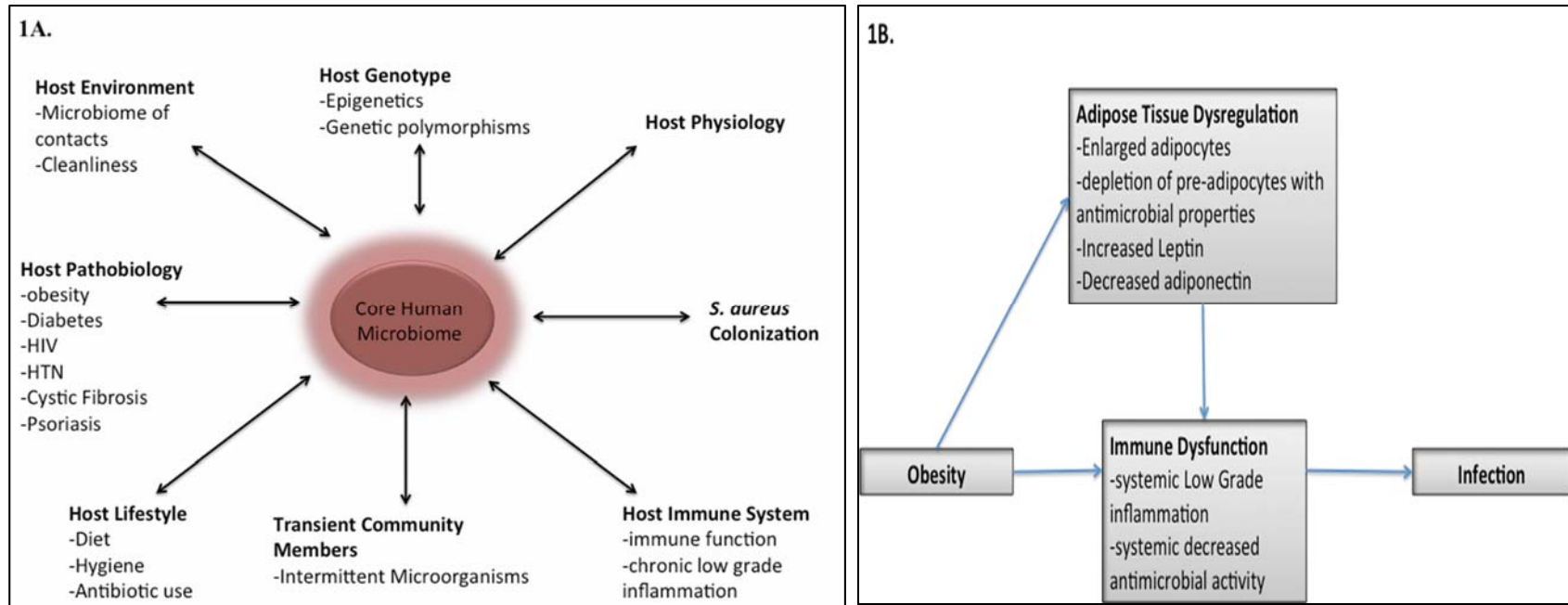
Definitions: HIV, human immunodeficiency virus; HTN, hypertension, *S. aureus, Staphylococcus aureus*

Figure A1.1A frames our understanding of how underlying disease could influence *S. aureus* colonization by illustrating how host

pathology can alter the microbial environment. This framework is rooted in ecology and was formulated to evaluate the effects of the

human core microbiome on disease. **Figure A1.1B** builds on the conceptual framework developed by Scrimshaw, Taylor and Gordon

for the interaction of nutrition and infection. In this treatise they describe the synergistic and antagonistic influence of malnutrition on

infections. At the time during which the framework was developed malnutrition was synonymous with under nutrition, but the definition has since broadened to include over-nutrition and obesity. The modified framework demonstrates effects of obesity on morphology and function of adipocytes results in immune dysfunction characterized by chronic low-grade inflammation and reduced immune response of both the innate and adaptive pathways.

CHAPTER 2: Measurement of Persistent Carriage of *Staphylococcus aureus* in Non-Institutionalized Adults: A Systematic Review

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is an important cause of both community acquired and healthcare-associated superficial and invasive infections.[1,2] Methicillin-resistant *S. aureus* (MRSA), *S. aureus* isolates resistant to all available penicillin and other β-lactam antimicrobial drugs, have historically been confined to hospitals and other health care settings. However, since the mid-1990s community-associated MRSA (CA-MRSA) strains have emerged among populations lacking the traditional healthcare associated risk factors.[2] As CA-MRSA continues to spread, particularly in high-risk populations such as the incarcerated[3,4] and reports of vancomycin-intermediate or resistant organisms emerge, it is critical to elucidate the pathways that place individuals at risk of *S. aureus* infections.[5,6] One such pathway, is asymptomatic colonization with *S. aureus*.

Approximately 25-30% of healthy adults in the United States (US) are asymptomatically colonized with *S. aureus*.[7,8] The most common niche for *S. aureus* asymptomatic colonization is the anterior nares, and colonization at this site is an established risk factor for *S. aureus* infections.[9,10] Nasal carriage has historically been classified into three distinct patterns, persistent, intermittent and non-carriers.[9] Broadly speaking, individuals classified as persistent carriers are those in whom *S. aureus* is consistently isolated,[11] however, variations of this definition exist.[12,13] An estimated 20% of individuals in the general population fall into this phenotypic category.[14] Intermittent carriers are individuals in whom *S. aureus* is inconsistently isolated and non-carriers are defined as individuals who are repeatedly culture negative for *S.*

aureus. Due to similarities in carriage time and clearance rate upon artificial inoculation,[15] intermittent and non-carriers are generally described as one phenotypic category.

Consistent culture positivity for *S. aureus* is not the only distinguishing factor of persistent carriage. Persistent carriers of *S. aureus* have also been shown to demonstrate less genotypic diversity among colonizing strains over time as compared to intermittent carriers.[9,14] For example, among persistent carriers, the colonizing strain remained identical or genetically similar after 8 years of follow up.[13] Furthermore, artificial inoculation studies among persistent and non-carriers have shown that persistent carriers revert back to their original colonizing strain after inoculation with a mixture of *S. aureus* strains whereas intermittent and non-carriers do not.[16] Secondly, the number of colony forming units (CFUs) among persistent carriers is higher than those found among intermittent carriers.[12] Third, persistent carriage demonstrates a protective effect against acquisition of new *S. aureus* strains in health care settings as compared to intermittent carriers.[17] Lastly, persistent colonization, as mentioned earlier, is associated with a higher risk of infection with *S. aureus*, but seems to also confer a degree of protection against a more severe disease course, particularly bacteremia.[10,18] The determinants of which phenotypic category an individual demonstrates are still largely unknown.

To date, several literature reviews have been published summarizing both host and bacterial factors associated with *S. aureus* nasal colonization, and a number were focused primarily on persistent carriage. Specifically, five reviews of host and bacterial factors associated with persistent colonization have been published,[19-23] three reviews assessed factors associated with persistent colonization and the role it plays in infection,[9,10,24] one reviewed the role of the host immune response as it relates to *S. aureus* colonization[11] and one provided a comprehensive review of *S. aureus* carriage inclusive of both intermittent and persistent carriage.[25] Though the

published literature reviews performed a comprehensive narrative review of factors associated with persistent colonization, none were systematically conducted. Furthermore, many alluded to the variability in the measurement of persistent colonization across studies; however, none assessed the impact of the variability on the estimated prevalence of persistent colonization. This review differs from previous reviews in that it is a systematic and comprehensive synthesis of existing literature to evaluate persistent colonization with *S. aureus* among non-institutionalized adults with a specific focus on how persistence is defined and measured across studies. The secondary aims were to determine whether the variability in the measurement of *S. aureus* influenced the estimated prevalence of persistent colonization and to conduct a meta-analysis of prevalence to calculate a summary estimate of the population prevalence of persistent carriage.

METHODS

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[26]

Search Strategy and Selection Criteria

A literature search was performed to identify peer-reviewed articles that assessed persistent colonization with *S. aureus.* Relevant articles were identified using the following databases: PubMed, Medline and EMBASE. In collaboration with a trained medical librarian, an automated search strategy was conducted using the following key words:

1. "*Staphylococcus aureus*"[Mesh] OR *S. aureus* OR "methicillin-resistant *Staphylococcus aureus*"[Mesh] OR MRSA

2. "Carrier State"[Mesh] OR colonisation OR colonization OR carrier OR carriage OR flora

3. "Prospective Studies"[Mesh] OR persistent OR "prospective studies" OR "longitudinal studies" OR "longitudinal study" OR "follow-up study" OR "follow up study" OR "retrospective studies" OR "retrospective study" OR persistence OR observational

4. Search 1 AND search 2 as defined above

5. Search 4 AND search 3 as defined above

The search was limited to studies published in English in a peer-reviewed journal (conference presentations, abstracts and dissertations were excluded) between January 1, 2000 and November 12, 2015. Relevant studies identified using the electronic search criteria were recorded in a reference manager software program (Endnote, www.endnote.com). Any duplicate entries were removed.

A single reviewer (MB) screened all the titles identified using the above search strategy to identify titles for further review. Abstracts of articles selected based on the title review were subsequently screened independently by two reviewers (MB) and (SS) to determine whether the inclusion criteria were met. Inconsistencies between the two reviewers with regards to eligibility were discussed and resolved by consensus. Additional inclusion criteria considered during the title and abstract review included: 1) observational study conducted in community dwelling adults 18 years of age or older (studies conducted within health care or long term care settings were excluded); 2) assessed *S. aureus* colonization of the anterior nares longitudinally as a primary or secondary outcome. Studies conducted exclusively among health care workers/students, individuals that worked or handled livestock daily and those conducted among injection drug users were excluded as they represented a subset of the population that has been shown to have higher prevalence of *S. aureus* carriage.[25,27] The reference lists of the included

articles were reviewed to identify additional peer reviewed literature that may not have been captured.

Data Extraction

All data were extracted and entered onto a standard form by one reviewer (MB). The primary data extracted included design (sample size, origin, and setting), participants (age, gender, and race), exposure(s) assessed, outcome(s), definition of persistent colonization (i.e. how persistent colonization was measured) and the observed prevalence of persistent colonization. Although the primary required outcome for included studies was longitudinal assessment of *S. aureus* colonization in the anterior nares, all outcomes assessed by the included articles were extracted.

Risk of Bias in Individual Studies

Two reviewers (MB and SS) independently assessed the risk of bias of each included study using a modified version of the Cochrane risk of bias tool (Appendix A2.1).[28] The tool assessed the risk of bias on the following domains: study design, exposure assessment, outcome assessment and different sources of bias including but not limited to selection bias, attrition, misclassification or confounding. The risk of bias for each domain was assessed as low, unclear or high. Each reviewer then assigned each study an overall risk of the bias based on the performance on the different domains outlined above. Discrepancies were discussed and consensus achieved.

Data Synthesis and Analysis

Prevalence estimates for persistent *S. aureus* carriage were calculated by pooling the study specific estimates using a random effects meta-analysis that accounted for heterogeneity between studies. The pooled estimate excluded redundant studies that assessed different

exposures within the same population, studies evaluated as having a high risk of bias as well as twin studies.

The binomial proportion confidence intervals for prevalence estimates were calculated by first performing a double arcsine transformation advocated by Barendregt et al.[29] for not only restricting estimates between zero and one as the logit transformation accomplishes, but additionally producing more stable variances than the logit transformation.[29] Between study heterogeneity was assessed using the I^2 statistic,[30] which measures how much of the variance between studies can be attributed to true differences rather than sampling error/chance. Higher values of I^2 signify a greater degree of variation. Sub group sensitivity studies were conducted to determine whether a substantial change in the estimates would be observed by the general definition used or by geographic region. Finally publication bias of included studies was assessed using funnel plots and Egger's test for asymmetry.

Data were additionally synthesized by categorizing the definitions of persistent carriage used across the included studies based on commonalities between those definitions. The prevalence estimates of the different studies were then summarized by category to qualitatively assess variation in prevalence across the different definition categories. All statistical analysis was conducted using STATA 14.0.

RESULTS

The search strategy initially yielded 5,582 records (Figure 1.1). After excluding 2,442 duplicates, a total of 3,140 unique titles were screened for eligibility. Of the titles screened, 3,011 were subsequently excluded, commonly because they were conducted in hospitalized patients, long-term care facilities or health care professionals or were primarily a pediatric population. Titles were also excluded if they assessed *S. aureus* colonization at only one time point, did not

assess colonization in the anterior nares, or were an intervention study. The remaining 132 articles underwent abstract review by two reviewers and 104 were excluded primarily due to being conducted exclusively among healthcare professionals or individuals who regularly interacted with livestock. Studies were additionally excluded during the abstract review stage if the prevalence estimate of persistent carriage was a priori fixed based on study inclusion/exclusion criteria (e.g. case control study). Of the remaining 28 articles that underwent full article review, ten were excluded leaving a total of 18 articles that met the criteria for inclusion in the systematic review.

Study Characteristics

Eight cross sectional[18,31-37] (exposure and first outcome assessment were assessed concurrently) and ten longitudinal[12,38-46] studies were included in the study (Table 1.1). Two of the studies were conducted in the United States,[40,43] five in the Netherlands,[12,32,36,38,45] three in Germany,[18,41,46] two in Denmark,[31,33] two in Norway,[42,44] two in Hong Kong,[34,35] and one each in England[39] and French Guiana.[37] The sample size of the included studies ranged from 72 to 3,851 healthy individuals. In most (73.6%) of the included studies[12,31,33-35,37,39-44,46] standard methods for processing and confirming *S. aureus* through phenotypic and culture methods were used. Generally, samples were collected by rotating a moist or dry cotton tipped swab two to four times in the inner wall of both nostrils. Swabs were subsequently placed in transport medium and processed within three weeks of collection. Processing included incubation at 37°C for 24-48 hours, after which colonies morphologically identified as *S. aureus* were cultured on blood agar plates and subsequently confirmed as *S. aureus* using various methods, the predominant one being an agglutination test. Four (26%) studies[18,32,38,45] provided insufficient details as to the laboratory methodology used to identify and confirm *S. aureus* colonization.

<u>Measures and Prevalence of Persistent Carriage</u>

The definition of persistent carriage varied significantly across studies, however, common themes emerged that allowed for the categorization of the definitions into four different groups. The first major category defined persistence based on multiple assessments (> 2) of *S. aureus* carriage collected at least one week apart. Persistent carriers were *S. aureus* culture positive on all cultures.[39-41] Two[40,41] of these studies described culture positive results from the species level only, and one[39] additionally examined carriage at the strain level (*spa* type) and assessed persistence of the same strain over time. When culture positivity at the species level was considered the prevalence of persistent carriage ranged from 18%–24%. In the study that assessed strain level persistence in addition to species level persistence, 18% of the total population was colonized with *S. aureus* for the duration of the study and approximately 12% were colonized with the same *spa* type.[39]

The second category that emerged was a variation of the culture rule developed by Nouwen et al.[12] Nine[32,33,36,38,42-46] studies defined persistent carriage based on only two samples taken at least one week apart (time between samples ranged from 7-28 days). Eight[32,33,38,42-46] of the nine studies reported persistent carriage prevalence rates ranging from 18%–27%, and one[36], which had the smallest sample size (N=72) of all the included studies, reported a prevalence of 33%. Of those reporting persistent carriage rates ranging from 18%–27%, four used a semi-quantitative culture rule defined by culture positivity in both samples taken one week apart and a threshold of colony forming units in each sample, whereas the remaining studies conducted only qualitative assessments.

The third category of definitions used was a less rigorous implementation of the culture rule described above, and defined persistent carriage as two samples taken several months and in

some cases years apart as a method of assessing persistent carriage. Of the five[18,31,34,35,37] studies

using this rule, four[18,31,34,35] reported a persistent carriage rate ranging from 16%–21%, and one[37] reported a slightly higher rate of 26%.

The final definition of persistent carriage was related to Vandenberg et al. carrier index,[13] which divided the total number of positive *S. aureus* cultures by the total number of cultures taken. Individuals with an index of ≥80% were considered persistent carriers. Only one study[12] used this definition, and the reported prevalence of persistent *S. aureus* carriage was 29%.

Summary Measure of Persistent Carriage

After taking into account redundancy due to studies assessing different factors within the same study population as well as high risk of bias, 11 of the 18 studies included in the systematic review were included in the meta-analysis. The meta-analytic pooling of the prevalence estimates reported by each included study resulted in a summary estimate of 22% (95% confidence interval, 19% – 25%) (Figure 2.2), however, a substantial amount of between-study heterogeneity was observed ($I^2 = 90.6\%$ $P < 0.01$). Sensitivity analysis conducted by subgroup defined by region (Appendix Figure A2.1) and definition (Appendix Figure A2.2) demonstrated lower heterogeneity in certain groups, however prevalence estimates remained relatively consistent. Figure 2.3 depicts the prevalence of persistent carriage by the definition used and the size of the study. As is illustrated by the figure, a considerable overlap in the range of persistent carriage reported by definition can be observed across definitions.

Summary of Factors Associated with Persistent Carriage

Several demographic, behavioral and genetic factors were associated with persistent carriage. As has been observed in studies evaluating *S. aureus* colonization at one time point, male sex and younger age were found to be independently associated with a higher prevalence of

persistent carriage.[42,46] In addition to demonstrating the influence of age and sex on persistent carriage, Olsen et al. also reported that both smoking and serum 25-hdroxyvitamin D levels were associated with a lower likelihood of persistent carriage in men.[42] Zanger et al. additionally demonstrated that hormonal contraceptive use increased the prevalence of persistent carriage.[46] Women taking contraceptives had a higher likelihood of being persistently colonized and also appeared to carry the same strain for longer.[46]

Many of the included studies assessed the association between genetic factors previously shown to be associated with the immune response and persistent *S. auresus* carriage. For example, Nurjadi et al. demonstrated that genetic polymorphisms in the 5' UTR of the DEFB1 gene decreased mRNA expression of human β defensin 1 and 3 in experimentally wounded skin and correlated with persistent nasal carriage.[41] Similarly, polymorphisms in the glucocorticoid (GC) receptor gene were associated with persistent nasal carriage[45] as were single nucleotide polymorphisms in the IL4 and C-reactive protein gene.[38] Despite positive findings with regard to the GC receptor, cortisol levels were not associated with persistent carriage in a small study conducted in the Netherlands.[36] In addition, two studies assessing the heritability of persistent carriage failed to demonstrate an association between familial predisposition and persistent carriage.[31,43]

<u>Summary of Risk of Bias Assessment</u>

Given that the objective of this systematic review was to identify different definitions used to determine persistent *S. aureus* carriage among non-institutionalized adults and the resulting prevalence, the biggest threat to the validity of our summary estimate was the method in which persistent carriage was operationalized. Most studies had a low risk of bias with respect to the outcome given the studies definition (Appendix Table A2.1). However, a few

33

studies[18,31,32,34,35,40,41] failed to provide one or more of the following; 1) description of time between sample collection and processing 2) details on enrichment 3) method of confirming *S. aureus*. Additional factors that influenced the overall risk of bias scores were uncontrolled confounding and inadequate description of study and source population. Factors such as previous antibiotic use as well as smoking, which have been shown to influence intermittent colonization, were not consistently accounted for, nor were adequate descriptions of the study population or the source population from which they were sampled included. Despite these shortcomings, all the studies had well-defined exposed and comparison groups, and contributed to the scant literature assessing persistent carriage in non-institutionalized settings.

DISSCUSSION

This systematic review identified 18 studies that assessed persistent *S. aureus* carriage among non-institutionalized adults.[12,18,31-46] The definitions used by each study to assess persistent carriage were evaluated, as were the reported prevalence estimates associated with those definitions. The findings demonstrated that considerable variation existed in the way persistent colonization was defined. The definitions fell under four general categories: culture positive at each point of assessment with > 2 assessments occurring a few weeks to a few months from one another,[39-41] two positive cultures taken approximately one week apart,[32,33,36,38,42-46] two positive cultures taken several months to a few years apart[18,31,34,35,37] and lastly a carrier index of ≥ 80% among all assessments conducted.[12] The reported prevalence of persistent carriage ranged from 16%–33%. A summary estimate of eleven of the eighteen studies (excluding redundant studies and those with high risk of bias) resulted in a pooled persistent carriage prevalence of 22% with substantial between study heterogeneity. A summary of each individual study's findings as it relates to their exposure of interest and persistent carriage suggests that persistent

carriers harbor significantly different characteristics than intermittent carriers, regardless of how it is defined.

The results of the meta-analysis estimated the pooled prevalence of persistent carriage at 22%. It is important to note that the variability in defining persistence may have resulted in between-study heterogeneity above what would have been expected by chance. Our calculated heterogeneity was 91% and as a general rule an I^2 of $\geq 75\%$ is considered high.[30,48] Therefore, interpretation of the pooled estimate should be approached with caution. Even after sub-setting by region (Figure A2.1) or the definition (Figure A2.2), of persistence used, variation in the prevalence of persistence was still observed, and may therefore point to true differences across populations. Despite the heterogeneity observed, our estimate fall within the historically predicted range of 20-35%.[10,49] Even more striking is the consistency in the range of persistent carriage estimates reported across the broad categories of definitions identified. This suggests that identifying persistent carriers may be relatively robust to the definition used, and having at least two swabs may be sufficient to identify individuals at risk of carrying *S. aureus* for prolong periods of time. Future studies with multiple points of assessment should assess the performance of two samples taken at random time points as compared to three or more consecutive samples.

The importance of identifying individuals who are persistently colonized with *S. aureus* and the underlying prevalence of this phenotype in the general population rests in the observation that persistent nasal carriage not only increases the risk of infection[50] but also of transmission.[35] Several characteristics unique to persistent carriers likely contribute to these outcomes and were assessed by the included studies. More specifically, though inconsistencies were observed, the studies largely implicate factors that impair the host's innate and/or adaptive immune response. For example, glucocorticoids are well known immune suppressants and are used in the treatment

of inflammatory as well autoimmune diseases.[51] Functional polymorphisms in the glucocorticoid receptor genes have been shown to confer a degree of resistance to glucocorticoids leading to a more active innate immune response. Polymorphisms that conferred a degree of resistance to glucocorticoids were shown by Van den Akker et al.[45] to decrease the likelihood of persistent carriage. Similarly, functional polymorphisms in genes coding for interleukin-4,[38] which stimulates production of cytokines involved in *S. aureus* defense, and those coding for human β-defensin-1 promoter region,[41] which influences antimicrobial peptide secretion which are also involved in defense against *S. aureus* were also shown to influence persistent carriage. In addition serum 25-hyrdroxyvitamin D level,[42] a known modulator of innate and adaptive immune response, was also associated with persistent carriage, however the effect was only observed in non-smoking men. All of these factors along with the increased *S. aureus* colonization burden and increased time associated with strain clearance observed amongst persistent carriers as compared to intermittent/non-carriers all suggest the involvement of factors that influence the host's innate immune response in *S. aureus* carriage. It is important to note that these factors are likely multifactorial and complex as negative findings of known immune modulators were also observed. For example, polymorphisms in the vitamin D receptor gene, which have been shown to influence a host's susceptibility to infections and host's innate immunity were not associated with persistent carriage.[32] The latter study, however, did not stratify by sex, which could have precluded their ability to detect the sex dependent association observed by Olsen and colleagues.[42]

Other characteristics of the included studies as well as the extant literature also implicate factors that influence the innate and/or adaptive immune response in persistent carriage. Specifically, studies have shown that persistent carriage of *S. aureus*, frequently described at the

species level, can be distinguished from intermittent carriers by bacterial load, bacterial dispersion, and exchange rate of *S. aureus* clones. Persistent carriers have generally been shown to have a higher nasal bacterial load[12,15] than intermittent carriers. For example, Van Belkum et al. demonstrated that persistent carriers had significantly higher mean colony-forming units per culture than intermittent carriers.[15] Van Belkum's observation validated an observation made by one of the included studies[12]-- that the inclusion of both quantitative and qualitative assessment of *S. aureus* improved the prediction of persistent carriage. Increased bacterial load, as was observed by Van Belkum et al.[15] and Nouwen et al.[12], likely contributes to the increased bacterial dispersion observed in persistent carriers as compared to intermittent carriers.[52] Ho et al. further illustrated the increased likelihood of dispersion by persistent carriers by demonstrating that a substantial amount of the hand contamination with *S. aureus* observed among food handlers was similar/identical to strains cultured from persistent carriers.[35]

The last characteristic of persistent carriers demonstrated by studies included in this analysis as well as the extant literature is related to the exchange rate of *S. aureus* clones upon repeated culture. Van Belkum et al. reported a significantly lower exchange rate among persistent as compared to intermittent carriers.[15] In fact, after de-colonization, persistent carriers reverted back to their endogenous strain 50% of the time suggesting a symbiotic relationship between host and bacteria, and carried the strain for >154 days. Miller et al. observed similar findings, reporting *S. aureus* loss rates of 4-6 months (120-180 days) among persistent carriers as compared to two months among intermittent carriers.[39] The observations of Van Belkum et al.[15] as well as Miller et al.[12], however, were not supported by a three year longitudinal study conducted by Muthukrishnan et al.[40] The latter assessed whether a strain change occurred within the study period rather than an average of how long a strain was carried. Given the duration of

the study (3 years), it is likely that persistent carriers changed strain types several times, however the length of time during which they carried each colonizing strain may be the salient question rather than whether change occurred.

Another salient question that needs to be addressed is whether there is a need to distinguish "truly persistent" from "consistent carriers" as defined by Miller et al.[39] In one of the most extensive studies assessing persistent carriage Miller and colleagues argue that a "truly persistent" carriage phenotype does not exist. The authors suggest that the existence of this phenotype would be characterized by a loss rate of S. aureus reducing to zero some time after 24-30 months of follow up. Because loss decreased linearly throughout the study, the authors hypothesized that all of those being followed would eventually lose carriage. If the purpose of assessing persistence is to identify individuals at risk of infection and those at increased likelihood of transmitting the bacteria then the interest in a gradient of risk may be of little consequence. If the purpose, however, is to increase the precision of how persistence is measured, then understanding what proportion of individuals can be truly described as persistent carriers per Miller et al.[39] may be of some value. Demonstrating whether these individuals harbor significantly different characteristics from individuals they defined as consistent carrier would likely require large sample sizes and long follow up periods. Such studies are sill warranted as they may strengthen the evidence of an association between currently recognized characteristics and clarify some of the controversial findings that may have resulted from misclassification of phenotypes.

Another area in which precision in the measurement of persistent colonization might be relevant is in the assessment of the role of the nasal microbiome on S. aureus carriage. None of the included studies assessed the role of the nasal microbiome on S. aureus persistence, and to

date no study has assessed longitudinal carriage of *S. aureus* within the context of the entire

nasal ecology. Observations reported in a recent study conducted by Lu et al. suggested that the

nasal taxa determined *S. aureus* colonization (assessed at one time point) by influencing both its

presence as well as its absolute abundance.[53] Authors described seven major "community state

types", each characterized by a "uniquely high prevalence and proportional abundance of

specific nasal bacteria"[53]. Community state type 1 was characterized by the high prevalence and

relative abundance of *S. aureus*. Authors also demonstrate a negative correlation between

Corynebacterium and *Propionibacterium* abundance and *S. aureus* carriage, a finding that

mirrors others[54,55] in the literature assessing the microbiome and *S. aureus* carriage. A degree of

granularity could be introduced into these assessments should *S. aureus* carriage phenotypes be

taken into account.

This systematic review has several limitations. First, only 18 studies met the established

criteria for study inclusion. The low yield is likely due to the difficulty in assessing *S. aureus*

persistent carriage in non-institutionalized settings. Persistent carriage requires a longitudinal

assessment of carriage; therefore there is a higher risk of bias from differential loss to follow up

especially when conducted in non-controlled general population settings. The low yield could

also be due to some articles being overlooked during the literature search because the

assessments of persistent carriage were not indicated in the title or abstract. To mitigate this

potential limitation, a broad search was conducted in which 3,144 unique titles were screened as

well as 132 abstracts and references of included studies. Furthermore, a significant amount of

persistent carriage studies were conducted amongst individuals with a high risk of exposure such

as health care workers, livestock farmers, and patients. These populations were omitted in an

attempt to limit misclassification of persistent carriage due to frequent exposure as compared to

persistent carriage due to the biological description of the phenotype. Persistent carriage rates in these settings would be artificially increased and thereby artificially increase the study specific prevalence of persistence as well as the resulting summary prevalence. Second, the heterogeneity in the summary estimates was high suggesting a significant amount of heterogeneity across studies. Inconsistencies in how study variables were collected precluded the implementation of a meta-regression analysis based on study definition, as did the small number of unique study populations represented in each definition. The prevalence by persistent carriage across definitions, however, suggests that despite the high heterogeneity, persistent carriage rates were relatively consistent. Finally, as in all systematic reviews and meta-analysis there is a risk of publication bias, however the funnel plot and the accompanying egger statistic provided little evidence for publication bias (Appendix Figure A2.3).

Conclusion

In conclusion, varying methods have been implemented to define and identify persistent carriage among non-institutionalized adults. Despite the definitional differences implemented, the prevalence of persistent carriage reported was relatively comparable suggesting that measuring persistent carriage prevalence may be robust to the different methods currently being implemented. In addition, factors that influence the host's innate and/or adaptive immunity were heavily implicated in *S. aureus* persistent carriage. Known immune modulators, such as obesity, diabetes should be assessed.

Tables and Figures

Figure 2.1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Flow Chart Illustrating the Selection Process of Articles Included in the Systematic Review
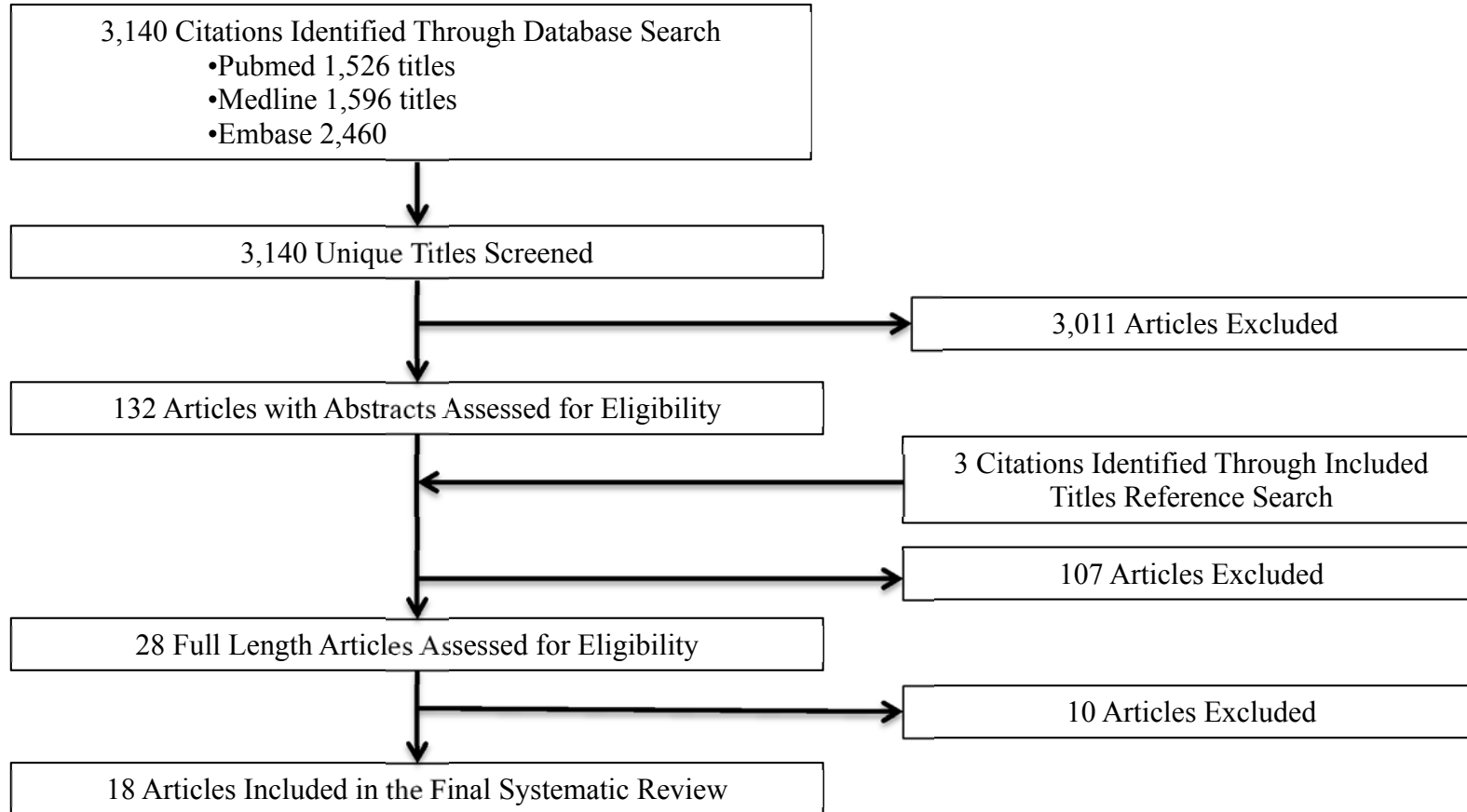
Flow diagram of search strategy and selection process for systematic review of studies that assessed persistent *Staphylococcus aureus* carriage among non-institutionalized adults

Table 2.1. Summary of Studies Assessing Persistent *Staphylococcus aureus* Carriage in Non-Institutionalized Adults, 2000-2015

Study	Country	Year(s) of Study	Sample Size	Definition of Persistent Carriage Used	Opportunities to Assess Carriage	Prevalence of Persistent Carriage
General Definition: All Positive Cultures (>2 Assessments)						
Muthukrishnan G. et al., 2013	US	NR	109	•All nasal cultures tested *S. aureus* positive for the duration of the study •median of 4 nasal samples (range 2-18)	18	•23.8%
Nurjadi D. et al., 2013	Germany	2009-2011	603	•Four consecutive positive nasal swabs (mean of 75 days first and last swab)	4	•20%
Miller R. et al., 2014	England	2008-2009	1,123	•Consistent Carriage Species Level= Individuals consistently colonized with *S. aureus* for duration of the study (~3 years) •Consistent Carriage *Spa* type Level= Individuals colonized with the same *S. aureus* spa-type for long time periods (~3 years)	14	• Species Level 17.6% • *Spa* type Level 12.6%
General Definition: Two Positive Cultures taken ~ 7-28 days Apart						
Claassen M. et al., 2005	Netherlands	1997-1999	3,851	•Two positive quantitative nasal swab cultures taken one-week apart with at least 8 colony-forming units of *S. aureus* per culture	2	• 18%
Van den Akker .et al., 2006	Netherlands	1997-1999	2,929	•Two positive nasal swabs taken one week apart	2	19.2%
Emonts M. et al., 2007	Netherlands	1997-1999	3,851	•Two positive quantitative nasal swab cultures taken one-week apart with at least 8 colony-forming units of *S. aureus* per culture	2	•18%
Fode P. et al., 2011	Denmark	NR	169	•Two successive positive nasal swabs taken one week apart	2	•20%
Roghman MC., et al. 2011	US	2008-2009	398	•Two positive cultures with an average of ≥1000 CFU on the two cultures	2	•18%

Study	Country	Year(s) of Study	Sample Size	Definition of Persistent Carriage Used	Opportunities to Assess Carriage	Prevalence of Persistent Carriage
Sangvik M. et al., 2011	Norway	2007-2008	2,997	•Two positive *S. aureus* cultures taken within a few weeks of each other (median 28 days)	2	•24% among men •22% among women
Manenschijn L. et al., 2012	Netherlands	NR	72	•Two positive nasal swabs drawn two weeks apart	2	•33%
Olsen K. et al., 2012	Norway	2007-2008	2,780	•Two positive *S. aureus* cultures taken within a few weeks of each other	2	•34% among men •21% among women
Zanger P. et al., 2012	Germany	2009-2011	1,180	•Two positive nasal swabs taken one week apart	4	•22%
General Definition: Two Positive Cultures Taken a Few Months to Years Apart						
Hofltreter S. et al., 2006	Germany	NR	123	•Two *S. aureus* positive nose swabs (swabs were drawn at maximum 3 months apart)	2	•18%
Ruimy R. et al., 2010	French Guiana	2006, 2008	154	•Persistent carriers were culture positive *S. aureus* in both the 2006 and 2008 assessment	2	•26%
Andersen PS. et al., 2012	Denmark	2008-2011	617 twin pairs	•*S. aureus* colonization at both sampling points taken one year apart	2	• 21%
Ho J. et al., 2015	Hong Kong	2002, 2003, 2011	499	•*S. aureus* positive in both the 2002 and 2003 assessments	2	•16%
Ho J. et al., 2015	Hong Kong	NR	540	•Individuals colonized with *S. aureus* spa type at both time points assessed	2	•16%
General Definition: Carrier Index						
Nouwen JL. et al., 2004	Netherlands	1995-1998	• 51 for derivation cohort	•Persistent *S. aureus* carriage characterized as 9-10/10 cultures positive for *S. aureus*	12	•29% in the derivation cohort
			•106 for validation cohort			•30% in the validation cohort

Abbreviations: US, United States;

S aureus refers to *Staphylococcus aureus*

Spa type refers to molecular strain typing of *S. aureus* isolates based on the protein A

Figure 2.2. Meta-Analysis of the Prevalence of Persistent Carriage Among Studies Conducted in Non-Institutionalized Adults from 2000 to 2015
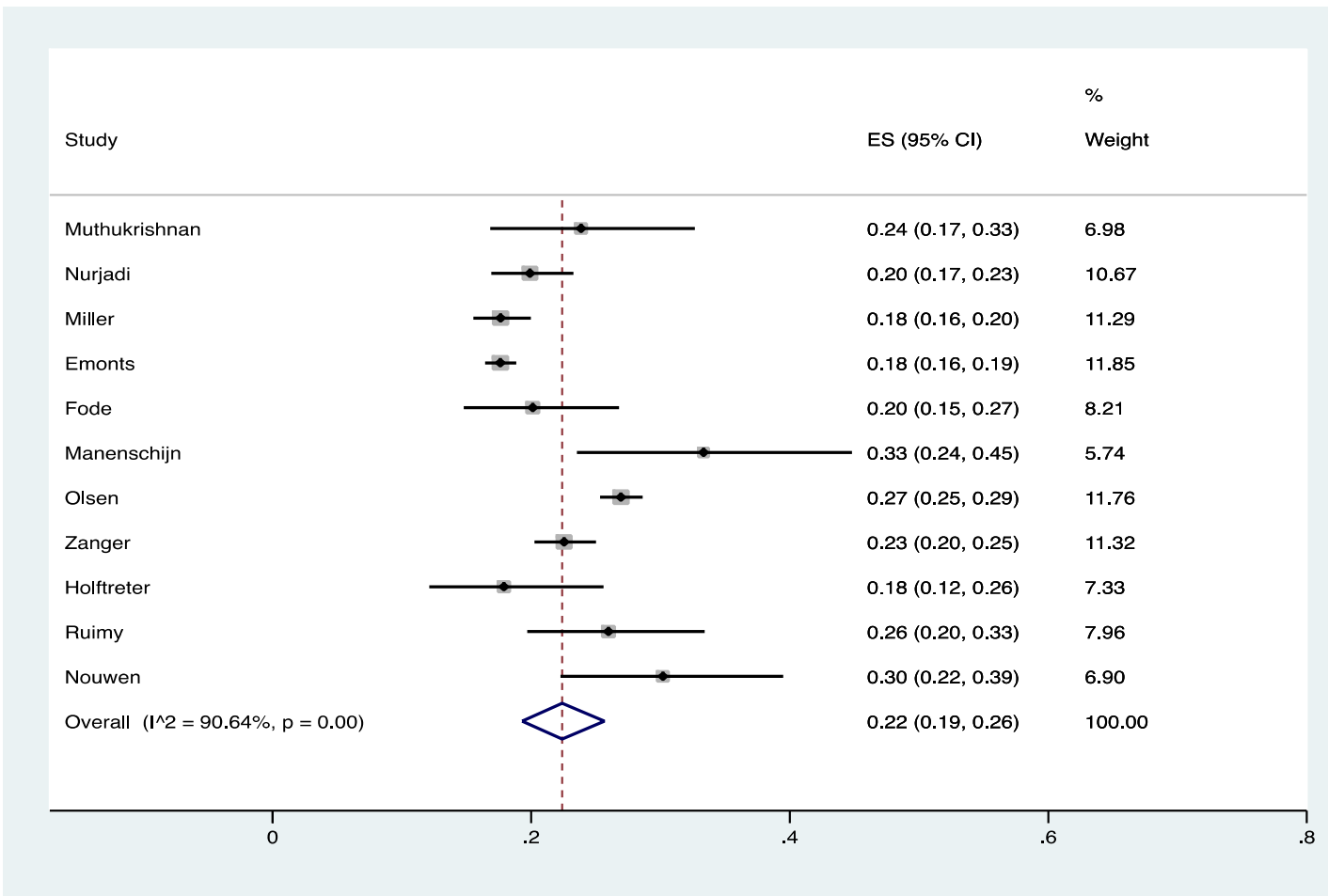
Abbreviations: ES, Prevalence Estimate, CI, Confidence Interval

I^2 refers to the heterogeneity across studies with heterogeneity of ≥ 0.75 reflective of significant between study heterogeneity

The dashed red line is representative of the summary estimate of persistent colonization

Figure 2.3. Prevalence of Persistent *Staphylococcus aureus* Carriage Reported by Studies Conducted from 2000 to 2015

According to the Definition of Persistence Used
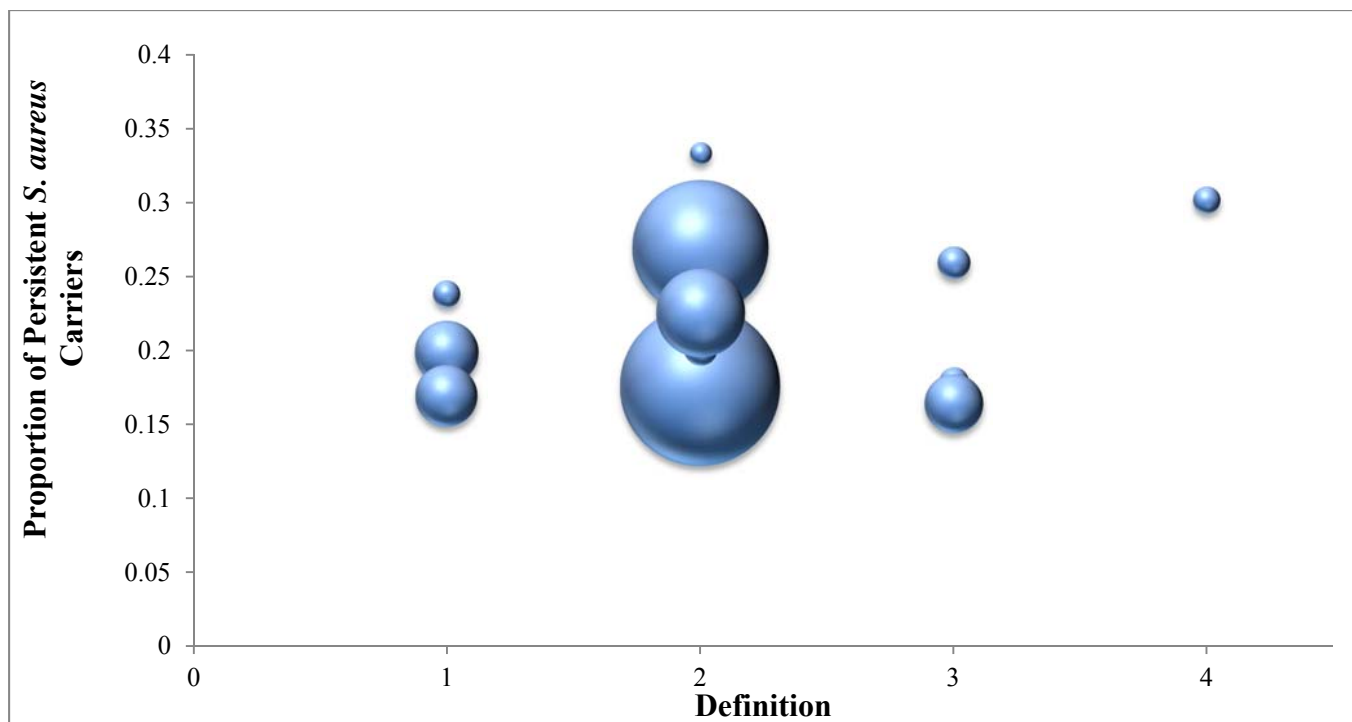
Figure 1. The bubble sizes shown are proportional to the number of participants enrolled in the study at baseline.

Definition 1: *S. aureus* culture positive for each sample taken a few weeks-months apart;

Definition 2: Two positive *S. aureus* cultures taken approximately one week apart;

Definition 3: Two positive *S. aureus* cultures taken several months to a few years apart;

Definition 4: A carrier index of ≥ 80% among all *S. aureus* swabs collected.

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Appendix 2

Table A2.1 Risk of Bias Assessment Tool

Domain	Description	Review Author's Judgment
Study Design	- Key elements of study design, namely type of design, setting, location, time frame and sample population (study population i.e. exposed/unexposed, cases/control do not pose a cause for concern) are appropriate for the study question - Eligibility criteria for study is appropriate - Biological specimen collected, if relevant are collected, stored and processed in manner that pose no threat to validity of the resulting data (amount of sample, nature of collecting procedures, time between sample collection if relevant should be explicitly stated)	Low Risk, Unclear Risk, High Risk
Exposure Assessment	- Exposure should be clearly defined - If molecular exposure is being assessed, the type of assay used, detection limit, assay timing after specimen collection or any standard used should be stated	Low Risk, Unclear Risk, High Risk
Outcome Assessment	- Outcome is clearly defined - Time frame between samples should be explicitly stated - In case of multi-site swabbing, it should be stated whether swabs were cultured separately or as a pooled sample on the same agar plate.	Low Risk, Unclear Risk, High Risk
Sources of Bias	- Loss to follow up, confounding, effect measure modification, missing data are addressed and acknowledged in the analysis and/or discussion	Low Risk, Unclear Risk, High Risk

Risk of Bias	Interpretation	Within a Study
Low risk of bias	Plausible bias that unlikely to seriously alter the results	Low risk of bias for all key domains
Unclear risk of bias	Plausible bias that raises doubt about the results	Unclear risk of bias for one or more key domains
High risk of bias	Plausible bias that seriously weakens confidence in results	High risk of bias for one or more key domains

Adapted from Cochrane Risk of bias assessment tool[28]

Table A2.2 Risk of Bias Assessment for Articles Included in the Systematic Review

Article	Overall Risk of Bias
Andersen *et* al. 2012	High risk
Claassen *et* al. 2005	Unclear risk
Emonts *et* al. 2008	Low risk
Fode *et* al. 2011	Low risk
Ho *et* al. 2015	High risk
Ho *et* al. 2015	High risk
Holtfreter *et* al. 2006	High risk
Manenschijn *et* al. 2012	Unclear risk
Miller *et* al. 2014	Low risk
Muthukrishnan *et* al. 2013	Unclear risk
Nouwen *et* al. 2004	Unclear risk
Nurjadi *et* al. 2013	Unclear risk
Olsen *et* al. 2012	Low risk
Roghmann *et* al. 2011	Low risk
Ruimy *et* al. 2010	Low risk
Sangvik *et* al. 2011	Low risk
Van den Akker *et* al. 2006	Unclear risk
Zanger *et* al. 2012	Low risk

Figure A2.1 Meta-analysis of the Prevalence of Persistent Carriage Stratified by Region of Assessment
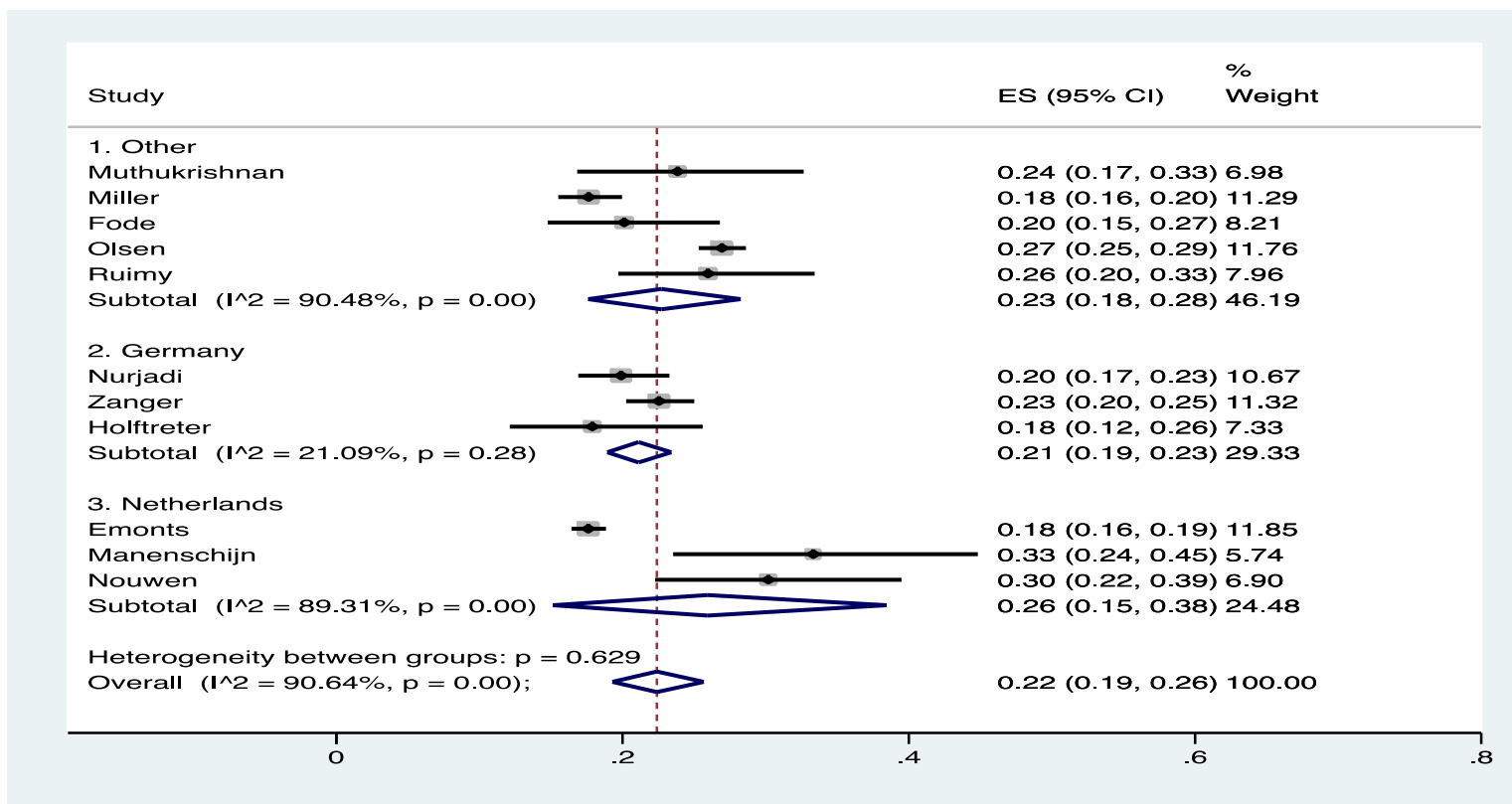
Group 1: studies conducted in the United States, England, Denmark, Norway & French Guiana; Group 2: Studies conducted in Germany;

Group 3: Studies conducted in the Netherlands

Abbreviations: ES, Prevalence Estimate, CI, Confidence Interval

I^2 refers to the heterogeneity across studies with a heterogeneity of ≥ 0.75 reflective of significant between study heterogeneity

The dashed red line is representative of the summary estimate of persistent colonization

Appendix A2.2 Meta-analysis of the Prevalence of Persistent Carriage Stratified by Definition of Persistence Used

Study	ES (95% CI)	% Weight
Definition_1		
Muthukrishnan	0.24 (0.17, 0.33)	6.98
Nurjadi	0.20 (0.17, 0.23)	10.67
Miller	0.18 (0.16, 0.20)	11.29
Subtotal (I^2 = 39.66%, p = 0.19)	0.19 (0.17, 0.22)	28.94
Definition_2		
Emonts	0.18 (0.16, 0.19)	11.85
Fode	0.20 (0.15, 0.27)	8.21
Manenschijn	0.33 (0.24, 0.45)	5.74
Olsen	0.27 (0.25, 0.29)	11.76
Zanger	0.23 (0.20, 0.25)	11.32
Subtotal (I^2 = 95.47%, p = 0.00)	0.23 (0.18, 0.29)	48.87
Definitions_3_and_4		
Holftreter	0.18 (0.12, 0.26)	7.33
Ruimy	0.26 (0.20, 0.33)	7.96
Nouwen	0.30 (0.22, 0.39)	6.90
Subtotal (I^2 = 60.44%, p = 0.08)	0.24 (0.18, 0.32)	22.18
Heterogeneity between groups: p = 0.173		
Overall (I^2 = 90.64%, p = 0.00);	0.22 (0.19, 0.26)	100.00

Abbreviations: ES, Prevalence Estimate, CI, Confidence Interval

Definition 1: *S. aureus* culture positive for each sample taken a few weeks-months apart;

Definition 2: Two positive *S. aureus* cultures taken approximately one week apart;

Definition 3: Two positive *S. aureus* cultures taken several months to a few years apart;

Definition 4: A carrier index of ≥ 80% among all *S. aureus* swabs collected.

I^2 refers to the heterogeneity across studies with a heterogeneity of ≥ 0.75 reflective of significant between study heterogeneity

The dashed red line is representative of the summary estimate of persistent colonization

Appendix A2.3 Funnel Plots of Studies Included in the Meta-Analyses of Persistent *Staphylococcus aureus* Carriage 2000-2015
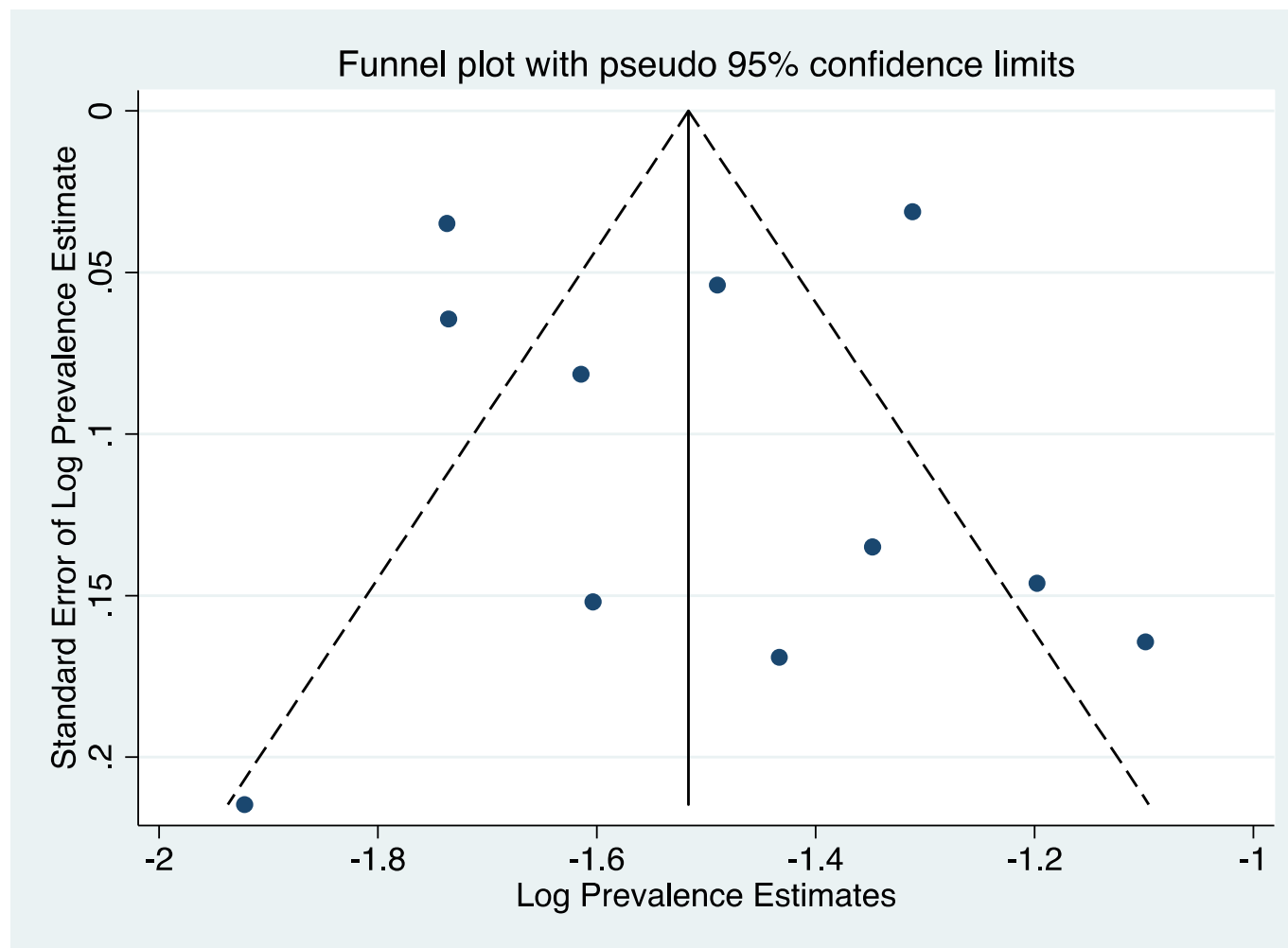
Funnel plots are graphical representations of the logarithm of each study's prevalence estimate versus the standard error of the logarithm of each study's prevalence estimate. The dotted lines are pseudo 95% confidence intervals.

CHAPTER 3: The Association between Obesity and Comorbid Conditions and Intermittent *Staphylococcus aureus* Colonization

INTRODUCTION

Obesity, characterized as a state of excess adipose tissue (fat), is a recognized chronic condition that has reached epidemic levels.[1,2] Approximately 1.9 billion adults worldwide are overweight and over 600 million are obese.[3] In the United States, over 30% of adults 20 years of age or older are obese, and rates are higher among Hispanics (42%) and non-Hispanic Blacks (48%).[4] It is now well established that obesity increases the risk of metabolic diseases such as type 2 diabetes, hypertension and ischemic heart disease[5]. However, evidence now also implicates obesity in impaired host defenses resulting in an increased susceptibility to clinical and community acquired infections.[6,7] One organism that has been linked to obesity is *Staphylococcus aureus*,[8-10] the most frequently isolated human pathogen in health care settings, and a leading cause of both community acquired and healthcare-associated infections.[11,12]

S. aureus is not only an opportunistic human pathogen, but also a transient and sometimes persistent component of the resident flora of the human anterior nares, the organism's ecological niche. Approximately 25-30% of US adults are colonized at this site at any given time,[9,13] and prior colonization serves as a strong predictor of subsequent infection[14] and transmission.[15] Identifying factors that predispose individuals to colonization has the potential to contribute substantially to the reduction of *S. aureus* disease burden in both healthcare and community settings, however, host determinants of colonization, and more particularly persistent colonization, are still largely unknown.[16] Factors that have been shown to influence *S. aureus* colonization include age, smoking, 25-hydroxyvitamin D levels,[17,18] female hormonal

contraceptive use,[19] and genetic polymorphisms in genes associated with immune function.[20-22] Unfortunately, many of the findings have yet to be reproduced, and in the case of 25-hydroxyvitamin D levels, supplementation did not significantly reduce *S. aureus* carriage.[23] One host factor that, however, appears to be consistently associated with *S. aureus* colonization is obesity.

Obesity as a host determinant of *S. aureus* colonization has been reported among patients,[24] non-institutionalized adults[8,9] and maximum-security inmates.[10] Though findings among females in non-clinical settings consistently show a higher prevalence among the obese,[8-10] results among males have been inconsistent, and only one study[9] demonstrated a significantly elevated prevalence among obese males. Mechanisms that might mediate the observed association between obesity and *S. aureus* colonization, particularly among females, have yet to be elucidated, however, the metabolic abnormalities and accompanying immune dysfunction frequently observed in obesity has been posited.[8,25] Interestingly, elevated glucose concentrations, an established marker of metabolic dysfunction,[26,27] is associated with *S. aureus* colonization,[9,28] suggesting that altered glucose metabolism could mediate the association between obesity and *S. aureus*. Unfortunately, the independent effects of other markers of metabolic dysfunction that frequently cluster with obesity including hypertension, low high-density lipoprotein-cholesterol (HDL-C) levels and elevated level of triglyceride have yet to be evaluated. Obese individuals that present with this clustering of metabolic complications are generally defined as having metabolic syndrome[29] or having metabolically abnormal obesity (MAO). There are, however, 20-30% of obese individuals that are metabolically healthy (MHO).[30] Conversely, a clustering of metabolic complications in the absence of obesity, frequently referred to as metabolically abnormal normal weight (MANW) [31,32] has also been

observed. The impact of these phenotypic categories of obesity and metabolic health on infectious disease outcomes have yet to be evaluated despite increasing evidence linking obesity with infectious outcomes[33,34] and calls to phenotype obesity.[35]

In an attempt to elucidate the association between obesity and *S. aureus* colonization, we assess here the influence of obesity and comorbidities associated with metabolic and/or immune dysfunction on *S. aureus* colonization. We hypothesize that obese individuals with metabolic abnormalities will have a higher prevalence of *S. aureus* carriage than obese individuals that are metabolically healthy.

METHODS

Setting and Sample

Data from an NIH-funded study (5R01AI082536) of *S. aureus* prevalence and risk factors in two NYS maximum-security prisons, Bedford Hills Correctional Facility (Female prison) and Sing Sing Correctional Facility (male prison), were used. Study data were collected in three distinct phases: (1) when inmates entered the facility (intake), (2) at any time point during incarceration (cross-sectional), (3) when inmates were being released back into the general population (discharge). Both participating facilities are located in Westchester county New York (NYS).

Participant Recruitment

Participant recruitment began in January 2009 and ended in February 2014. Inmate participation at both facilities was voluntary and compensation was not provided. Eligible participants were inmates ages 16 years or older capable of providing informed consent. The

Institutional Review Boards of Columbia University Medical Center (CUMC) and the New York State Department of Corrections and Community Supervision (DOCCS) approved the study.

To recruit participants for intake, inmates entering either the female or the male prisons were invited to participate in the study when they first entered the facility and while being processed. One of five trained research associates from CUMC invited the inmates to speak with them in a private room. At this time, inmates were provided with a brief description of the study and informed consent was solicited along with Health Insurance and Portability and Accountability Act (HIPAA) release. Recruitment for the cross-sectional phase varied by prison facility. In the female prison, inmates were called to the facility medical center and invited to participate. To recruit participants in the male prison trained research associates visited classrooms in the school, vocational training, and counseling buildings, and asked inmates if they were interested in participating in the study. At both facilities, interested inmates were subsequently invited to speak with a trained research associate from CUMC in a private room and were enrolled in the cross-sectional phase if informed consent was provided. Recruitment for the discharge phase was similar for both facilities; inmates scheduled for release back into the community were called to the facility medical center and invited to speak privately with a trained research associate.

Data Collection and Extraction

Trained research associates used a structured questionnaire to interview participants and collect demographic, behavioral and medical history information. During the interview, participants were cultured at the anterior nares and oropharynx, and culture swabs were later processed to assess asymptomatic colonization with *S. aureus*. In addition, research associates extracted data from the medical charts of inmates focusing on the NYS DOCCS Health Services

System problem list and the provider's progress notes. Medical conditions were included in an inmate's Health Services System problem list during the intake processing and/or anytime during incarceration. Upon entry into DOCCS, each inmate underwent a medical screening process that includes a detailed medical history, blood tests, dental and chest X-ray. In addition, every inmate is offered a human immunodeficiency virus (HIV) test at prison entry, and those at risk for hepatitis C virus (HCV) based on medical and drug history screenings were tested for the condition. Medical conditions of note at screening were included in the Health Services System problem list, or were included in the progress notes of an inmate's medical chart. In addition, any medical condition identified during incarceration was included in the problem list and /or the progress notes along with any medical procedures and/or laboratory tests conducted while incarcerated.

Assessment of Multiple Comorbidities (Exposure Assessment)

Body mass index (BMI) category was the index condition under study, and was assessed by first calculating the BMI of each inmate based on self-reported height and weight using the following equation $\left[\frac{mass\ (lb)}{(height(in)^2)} \times 703\right]$. Inmates were then categorized as normal weight, overweight or obese based on having a BMI of <25 kg/m^2, 25-29.9 kg/m^2 or ≥30 kg/m^2, respectively. After BMI category was defined, metabolically abnormal health status was then established for each participant. Metabolically abnormal health was defined as being diagnosed with one or more of the following conditions: diabetes, hypertension and/or HIV. These conditions were chosen based on established literature suggesting: 1) natural disease course affects and/or is affected by either innate and/or adaptive immune function and/or response 2) are also independently associated with obesity and/or fat distribution. Hypertension and diabetes are established markers of abnormal metabolic status.[32] We include HIV in this index due to its high

prevalence among incarcerated population, established affect on immune function and its association with the redistribution of fat both independently and as a result anti-retroviral therapy.[36,37] An inmate whose medical chart problem list or progress notes contained the diagnosis of HIV, diabetes and/or hypertension was defined as having the condition. The following categories were then created as defined in Table 3.1 based on BMI category and unhealthy metabolic health; metabolically healthy normal weight, metabolically abnormal normal weight, metabolically healthy overweight, metabolically abnormal overweight, metabolically healthy obese, metabolically abnormal obese. Similar methods have been previously used to measure metabolic health.[38]

Characterization of *S. aureus* (Outcome Assessment)

S. aureus characterization was carried out as previously described.[39] Swab samples obtained from the anterior nares and oropharynx of participants were incubated overnight in 6% sodium chloride-supplemented tryptic soy broth (Becton Dickinson) at 35°C. This particular broth was used to enrich *S. aureus* selection. Aliquots of the broth were then plated onto mannitol salt agar (Becton Dickinson) and incubated for 48 hours at 37°C. Individual colonies on the salt agar plates that were morphologically identified as *S. aureus* were streaked onto sheep blood agar plates and subsequently confirmed as *S. aureus* using the coagulase and protein A detection kit (Murex Staphaurex, Lenexa, KA). Confirmed isolates were then characterized by spa typing and compared using Ridom Staph Type software (Ridom GmBH). In order to determine the clonal relatedness of the different *S. aureus* strains identified in the population, spa types was clustered into spa clonal complexes using Based Upon Repeat Pattern (BURP) analysis.[40]

Covariates

Self-reported age, sex, race (non-Hispanic white, non-Hispanic Black, Hispanic and other), education (less then high school, high school grad, some college/college grad), antibiotic use in the past six months (yes/no), Current smoking (yes/no), number of showers per week, other underlying chronic conditions (liver disease, kidney disease, asthma) and history of drug use (marijuana use in the past 6 months, heroin use ever, crack/cocaine use ever) were assessed as potential confounding variables. Also assessed were systemic and/or topical antibiotic use in the past six months. These characteristics were assessed as potential confounding variables based on literature indicating an association with both the exposure and the outcome. For example, increasing age, non-Hispanic black and Hispanic race, education as well as smoking are associated with increased prevalence of diabetes and hypertension as well as their co-occurrence, and are additionally associated with *S. aureus* colonization.[5,41] [14]

Statistical Analysis

Means and standard errors were calculated for continuous variables and frequencies and percentages were calculated for categorical variables. Bivariate analyses were conducted using chi-squared tests or t-tests as appropriate. Multivariable analysis using log-binomial regression was then conducted to assess the association between the exposure, metabolic health and the likelihood of being colonized with *S. aureus*. Age, race, education and smoking status were included in the model as covariates based on a priori knowledge of their association with metabolic health status and *S. aureus* colonization. In addition covariates that were associated with both the exposure and outcome with a 10% level of significance and additionally changed the beta estimate for metabolic health status by 10% or more were retained in the final model. Both statistical and biological interaction by gender was assessed. To assess statistical interaction, cross product terms between comorbidity status and gender were included in the log-

binomial model, and a likelihood ratio test based on the -2 log-likelihood estimates of the full

(with the interaction term) and reduced (without the interaction term) models were assessed. To

assess biological interaction, the relative excess risk of regression as described by Rothman[42] and

95% confidence intervals as described Hosmer and Lemeshow[43] were calculated. Final models

were stratified by gender based on extant literature of the variation across gender with regards to

metabolic outcomes associated with obesity.[5,44]

To further elucidate the association between obesity and *S. aureus* colonization, we used

multivariable multinomial logistic regression to evaluate whether metabolic health status

influenced site of colonization among those colonized. Potential confounding factors associated

with metabolic health status and the multinomial outcome (anterior nares, oropharynx, both sites)

with a 10% level of significance, and additionally changed the beta estimates for metabolic

health status by 10% or more were included in the final model.

Several sensitivity analyses were conducted to evaluate how robust our results were to

misclassification and assumptions about/treatment of missing data. First we conducted a

quantitative bias analysis to examine how robust the findings were to misclassification of self-

reported height weight and metabolic health. We hypothesized that the results obtained would

not change appreciably after accounting for misclassification of BMI category and comorbidity.

Based on the literature we assumed perfect specificity[45] and any misclassification resulted from

under reporting of weight, over reporting of height or under diagnosis of medical comorbidities.

We also assumed that misclassification occurred only between adjacent cells. Monte Carlo

simulation studies with a uniform distribution and bootstrapping methods were then conducted.

To do so 10% of the population was randomly selected and their BMI category changed to

reflect what was presumed to be the true BMI category. Separately, 10% of the population was

also randomly selected and their comorbidity category changed to reflect what was presumably their hypothetically true category. Figure 3.1 illustrates the different steps used to obtain the estimates. The multivariable log-binomial regression model was then fitted to reflect the changes and the process repeated 1000 times. A summary measure reflecting the median estimate and standard error across all simulations was then obtained. The second sensitivity study evaluated our comorbidity construct for misclassification due to the inclusion of HIV.[37] Though HIV is associated with metabolic and immune dysfunction[46] not all individuals present with these symptoms. Furthermore, individuals in whom the only comorbidity is HIV may have risk factors that place them at elevated risk of colonization.[47] To assess the robustness of our construct, we excluded HIV from our measure, but included it as a covariate in our multivariable model. We hypothesized that our inferences would remain the same, but our standard errors would increase. Additionally, multiple imputation analysis was conducted to determine whether estimates changed substantially from the complete case analysis that was conducted as compared to available case and full data with all observations included. Lastly we conducted a sensitivity study assessing the influence of metabolic abnormalities within levels of BMI category, and another study assessing the influence of BMI category within levels of metabolic health. All statistical analyses were conducted using SAS, version 9.3 (SAS Institute, Inc., Cary, North Carolina) and STATA version 14.0.

RESULTS

Participation rates at both facilities were high for each phase of the study ranging from 81-87% for the female prison and 80-94% for the male prison. Characteristics of the total sample stratified by gender and *S. aureus* colonization status are presented in Table 3.2. The mean age of the 1,357 female and 1,472 male participants were 37 ± 11 and 37 ± 10, respectively.

Approximately 70% of all female and 65% of all male participants had a high school diploma or GED. Forty-three percent of all female participants were non-Hispanic black, 33% were non-Hispanic white, 16% were Hispanic and approximately 7% were of other or mixed race. Among male participants, 56% were non-Hispanic black, 10% were non-Hispanic white, 29% were Hispanic and 5% were of other or mixed race.

The mean BMI for female participants was 29.5 kg/m^2 ± 6.7. Approximately 41% of all female inmates were considered obese (BMI ≥ 30kg/m^2), and 20% were severely obese (BMI ≥ 35kg/m^2) (Table 3.3). The prevalence of obesity was highest among non-Hispanic black women (49%). Twenty seven percent of all female inmates reported one or more metabolic abnormalities. The prevalence of metabolic abnormalities among obese, overweight and normal weight women was 37%, 23% and 17%, respectively.

The mean BMI among male participants was 27.9 kg/m^2 ± 4.5 (Table 3.3). The prevalence of obesity (BMI ≥ 30kg/m^2) was approximately 26% and only 6% were severely obese (BMI ≥ 35kg/m^2). Twenty-seven percent of non-Hispanic male participants were obese. Twenty percent of all male participants reported at least one marker of metabolic abnormalities. When assessed across levels of BMI category, 32% of obese, 17% of overweight and 13% of normal weight males reported at least one marker of metabolic abnormality.

The prevalence of *S. aureus* colonization was 45% among all female participants, and was lowest (39.4%) among normal weight female participants with no recorded markers of metabolically abnormal health (Table 3.3). *S. aureus* prevalence rates increased to approximately 51% among normal weight female participants with at least one marker of metabolic abnormality. Prevalence was 46% and 41% among overweight female participants free of and those with markers of metabolically abnormal health, respectively. *S. aureus* colonization rates

were approximately 46% and 50% among obese female participants with and without markers of metabolically abnormal health status, respectively. Prevalence rates did not significantly differ by metabolic health status in unadjusted analysis (P=0.15) Table3.3. Colonization rates were 47% among normal weight men with no markers of metabolic abnormality, 42% among normal weight men with one or more metabolic abnormalities, 51% among overweight men with no metabolic abnormalities, 41% among overweight men with metabolic abnormalities, 52% among obese men with no metabolic abnormalities and 49% among obese men with metabolic abnormalities.

In the multivariable analysis (Table 3.4), the prevalence of *S. aureus* colonization among normal weight females with metabolic abnormalities was significantly higher than those of normal weight female participants with no metabolic abnormalities (PR=1.34, 95% confidence interval (CI) 1.01, 1.77). The multivariable model controlled for the effects of age, race, education, smoking, study phase, marijuana use in the past six months, heroin use ever, injection drug use, hepatitis C virus infection, systemic antibiotic use and topical antibiotic use. Obese female participants with metabolic abnormalities also had a higher likelihood of *S. aureus* colonization (PR=1.28, 95%CI 1.05-1.57) after controlling for the relevant covariates mentioned above. No statistically significant association was observed between metabolic health status and *S. aureus* colonization among male participants after controlling for covariates.

Results of the sensitivity studies (Appendix Table A3.1) demonstrated that our findings were somewhat robust for obese females with metabolic abnormalities but not for normal weight females with metabolic abnormalities with regard to misclassification of BMI category and metabolic health status. Furthermore, the effect estimates increased and remained significant when HIV was removed from the comorbidity estimate and controlled for in the model

69

(Appendix Table A3.2), and remained relatively unchanged between the complete, available and multiply imputed models assessing the impact of missing data (Appendix Figure A3.1). When we restricted our assessments to within metabolic health status (Appendix A3.5) or BMI category (Appendix A3.6) we observed no differences in the prevalence of *S. aureus* colonization across the different BMI categories, but observed an effect of metabolic health across levels of BMI. More specifically, a significant difference in the prevalence of *S. aureus* was observed when MANW was compared to MHNW (PR=2.15 95%CI 1.11, 4.18), but not when MAO was compared to MHO (PR=1.16 95%CI 0.79,1.72). Inferences in the results of the sensitivity studies in males (Appendix Table A3.1, Table A3.2, Figure A3.2, Table A3.5, Table A3.6) did not change from what was observed in our final model.

Table 3.5. provides the results of *S. aureus* colonization by site. Among the 612 female participants colonized with *S. aureus* 14% were colonized in the nares only, 17% were colonized in the oropharynx only and 13% were colonized at both sites (Table 3.3). Metabolic abnormalities were not significantly associated with site of colonization among female participants; however, exclusive nares colonization was 18%, 13% and 21% among normal weight, overweight, and obese female participants with metabolic abnormalities, respectively. Among the 722 males colonized with *S. aureus*, 12%, 23% and 14% were colonized exclusively in the anterior nares, exclusively in the oropharynx and both sites, respectively. Site of colonization was significantly associated with metabolic abnormalities among male participants in bivariate analysis (P< 0.01). In multivariable multinomial logistic regression analysis, obese male with metabolic abnormalities were significantly more likely (OR=2.40, 95%CI 1.13, 5.09) to be colonized exclusively in the anterior nares as compared to exclusive oropharynx colonization than normal weight males with no comorbidities after controlling for age, race, drug

70

use, and hepatitis C infection. No statistically significant difference by metabolic health status was observed for female participants with regards to site of colonization.

DISCUSSION

We demonstrated an effect of metabolic abnormalities on *S. aureus* colonization of the anterior nares and/or oropharynx among female maximum-security inmates. The prevalence of *S. aureus* colonization among female inmates who were normal weight (BMI < 25 kg/m^2) and had at least one marker of metabolic abnormal health status was 34% higher than the prevalence reported among individuals of normal weight with no metabolic abnormalities. A similar but slightly lower increase in prevalence (28%) was observed amongst obese (BMI $\geq$ 30 kg/m2) females with one or more metabolic abnormality(s). No significant association was observed amongst overweight metabolically abnormal females. Neither normal weight nor overweight nor obese comorbid males with metabolic abnormalities demonstrated a significantly higher prevalence of *S. aureus* colonization as compared to normal weight metabolically healthy males. We did, however observe a significant increase in exclusive anterior nares colonization among obese male inmates with metabolic abnormalities as compared to metabolically healthy normal weight males. These findings partially support our hypothesis that metabolic abnormalities may be driving the observed association between obesity and *S. aureus* colonization, particularly among women.

Our group[10] and others[8,9] have demonstrated an association between obesity and *S. aureus* colonization independent of diabetes. Olsen et al. was able to demonstrate an effect of obesity even after controlling for pre-diabetes.[8] We expanded on these analyses by stratifying individuals by not only BMI category, but also metabolic health status to crudely test the hypothesis that abnormal metabolic health may be driving the observed association between

obesity and *S. aureus* colonization. In doing so we were able to demonstrate an effect of abnormal metabolic health on *S. aureus* colonization in the presence and absence of obesity, but no effect of BMI category when stratified by metabolic abnormalities. These findings not only contribute to the extant literature addressing the influence of obesity on infectious disease outcomes, but also to the literature attempting to understand the concepts of metabolically healthy and unhealthy obesity.[48] As our study demonstrates, not all obese individuals present with increased cardio-metabolic risk; a proportion are metabolically healthy. This observation led to the development of a sub-phenotype of obesity referred to as metabolically healthy obese (MHO); characterized by their relative absence of metabolic complications.[49] The estimated prevalence of MHO ranges from 13-30% of all obese individuals depending on the population assessed.[30,49] Whether this phenotype represents a true subset or one end of a continuum is still under debate.[49] However, MHO individuals have been shown to differ from their metabolically abnormal obese (MAO) counterparts on several different biological endpoints. For example, MHO individuals store energy preferentially through hypertrophy as compared to hyperplasia, present with a higher subcutaneous adipose tissue to vascular adipose tissue ratio, and have lower levels of inflammation than their metabolically abnormal counter parts.[5,49] To our knowledge, we are the first to assess the influence of these obesity sub-phenotypes on an infectious disease outcome, and postulate that they could serve as mechanisms by which obesity impairs host defenses. Utilizing these sub-phenotypes in ID research as it relates to obesity might serve as a crude measure of the biological progression of obesity, and could introduce a degree of granularity in our estimates of the impact of obesity on infectious outcomes.

Our sensitivity study restricting our assessments of metabolic health within categories of BMI demonstrated a significant increase in colonization in MANW as compared to MHNW, but

not for MAO as compared to MHO. As discussed above, studies assessing differences between sub-phenotypes of obesity have observed significant differences in risk in MAO as compared to MHO in certain conditions[50,51] but not others.[50] One of two explanations can account for our observation. First, similar to observations made with regards to MHO individuals as it relates to chronic non-communicable diseases such as diabetes and cardiovascular disease, the difference in the prevalence of *S. aureus* among MHO females as compared to MHNW, though non-significant is not negligible, which is in accordance with previous observations.[8] Second, the imprecision in our measure of metabolic health may be precluding our ability to detect a difference between healthy and abnormal obese individuals, and more precise measures using molecular markers of metabolic health may clarify the nature of the association.

The mechanism by which metabolic abnormalities, both in the presence and absence of obesity, influence *S. aureus* colonization need to be elucidated. Many have cited increased levels of leptin and subsequent leptin resistance as potential pathways through which obesity influences infectious outcomes.[6,7,52,53] This pathway is also plausible within the context of *S. aureus* colonization, as leptin has been shown to enhance the production of the anti-microbial peptides (AMP) such as human β-defensin-2 in human keratinocyte cell, which are known to line the anterior nares as well as other mucosal surfaces.[54] Human β-defensin-2 is one of many AMPs that are constitutively expressed in keratinocytes that line the human nasal epithelium, and is one of a family of human β-defensin AMPs that have been shown to influence *S. aureus* colonization.[21,55,56] Other underlying chronic diseases such as HIV, diabetes and hypertension are also associated with leptin levels,[46] and could influence *S. aureus* colonization independently by this pathway or interact with one another to amplify the effect. Given that metabolic abnormalities are strongly associated with leptin resistance,[5] our observation of increased

prevalence of *S. aureus* colonization among metabolically abnormal individuals aligns with this hypothesized pathway, and supports previous observations in animals[57] implicating leptin in bacterial clearance from mucosal surfaces. It is important to note, that other factors such as 25(OH)D levels, which are associated with obesity[58] immune function,[59,60] as well as *S. aureus* persistence[18] may also play a role.

We observed differences in the association between metabolic health and *S. aureus* colonization between men and women. These differences may be due to the sexual dimorphism in body fat distribution observed in obesity, which results from storage of excess energy in the form of triglycerides in the lipid droplets of adipocytes, the major cell type of adipose tissue. Two main subcategories of adipose tissue depots are recognized: subcutaneous adipose tissue and visceral adipose tissue. Subcutaneous adipose tissue is located between the dermis and fascia of muscle and is more prevalent amongst overweight/obese premenopausal females. Visceral adipose tissue is located within the intra- and extra peritoneal spaces of the abdomino-pelvic cavity and is more prevalent in post-menopausal women and males.[44] Subcutaneous adipose tissue expresses higher levels of leptin, which could explain some of the sex differences observed.[5,44] Furthermore, factors such as circulating sex hormones, which are altered differently in obese men as compared to women could also play a role.[44] In an attempt to evaluate true biological differences of gender in the association between metabolic health and *S. aureus* colonization we used RERI methods to evaluate the possibility of biological synergism. No statistically significant evidence of biological synergism was observed. However, we were likely not sufficiently powered to detect true biological interaction.[61] Future studies that are sufficiently powered should incorporate RERI methodology to evaluate biological interaction, when multiplicative models are used to evaluate the main effect of an exposure on an outcome.[42] This

will not only help fully describe the associations under study, but provide useful indications of where interventions may have the most impact.[62] Though the differences by sex could be biological in nature, we are unable to rule out the differences in the precision by which BMI captures adiposity in men and women as a potential explanation.[63] For a given BMI, women have greater amounts of body fat than do men.[64] Furthermore, BMI potentially misclassifies individuals with high muscle mass as overweight or obese, which may make BMI an imprecise measure of total adiposity in our male population.[65] More granular measures of adiposity might clarify the underlying association between metabolic health and *S. aureus* colonization among both men and women.

Another potential pathway by which obesity and other underlying medical conditions may affect infection/colonization with *S. aureus* is by causing a shift in the composition and/or abundance of microbial species in the human microbiome. As a commensal organism, *S. aureus* colonizes human epithelial surfaces without causing disease. Epithelial surfaces form the interface between the host and its exterior environment, and are therefore the main route of entry for microbial organisms.[66] Important changes in epithelial tissue have been observed amongst obese individuals including alterations in barrier function and integrity, impaired wound healing, and increased susceptibility to infection.[67] Evidence of epithelial dysfunction lies in the observation that the microbial composition of epithelial sites differ between obese and normal weight individuals, which has been posited to affect the pathogenicity of some of the residing organisms.[68,69] Even more interesting, are recent findings suggesting microbial compositional changes at epithelial site observed in the presence of disease may be reflective of disease induced dysbiosis at other sites.[70] Specifically, Zhang et al. observed that dysbiosis in gut microbial composition in the presence of rheumatoid arthritis was reflected in both the oral and saliva

microbiome.[70] Furthermore, host genetic variation has been shown to influence microbial composition across different body sites[71] lending credence to the hypothesis that dysbiosis at one site could be an indicator dysbiosis at other sites. More specific to our topic is the idea that the dysbiosis we observe in gut microbiota in obesity, may be an indication of shifts in the microbiome of other epithelial surfaces such as the anterior nares and/or oropharynx making those surfaces more susceptible to *S. aureus* colonization. This is supported by the fact that studies assessing *S. aureus* colonization within the context of the entire microbial composition of the anterior nares have linked distinct phylotypes to the presence or absence of the organism.[72]

Results of the sensitivity analysis assessing the effect of misclassification of BMI category and comorbidity demonstrated that our findings for obese individuals with markers of metabolic abnormalities were somewhat robust to misclassification, however our findings for normal weight women with metabolic/immune dysfunction were not. The trend in the data was similar, but our effects were attenuated and failed to reach significance. Conversely, our sensitivity analysis removing HIV from our comorbidity construct support the robustness of our findings and demonstrate that markers of metabolic health alone could serve as proxies of metabolic dysfunction in the absence of more precise molecular measures such as elevated C-reactive protein levels and elevated white blood cell.[73]

We observed an association between site of colonization (exclusively anterior nares, exclusively oropharynx, both sites) and metabolic health status in men. This observation was surprising at first, given our findings of the main effect of metabolic health was restricted to women, however, they appeared more plausible when placed within the context of the existing literature. First, colonization site has been shown to differ by demographic factors, most notably age, race and gender.[74,75] Individuals of Hispanic race/ethnicity were significantly more likely to

be exclusively colonized in the pharynx as are individuals of younger age and male sex.[74,75] Our sample population differed also by clinical characteristics,[76,77] suggesting that a subset of the population may be preferentially exclusively colonized at extra nasal sites, particularly the oropharynx. Few studies have assessed factors associated with *S. aureus* colonization site,[74,75,77] and those that have, had limited sample sizes and did not evaluate the influence of health characteristics on site of colonization. Given our findings, factors that influence site of colonization may warrant further assessment. Host factors associated with significant deviations from the established norm may be useful in identifying individuals who should be additionally screened in their oropharynx, as current practice requires only surveillance of the anterior nares.

Finally, we are unable to rule out the fact that obese individuals, particularly those with comorbidities, may also engage in behavior that may predispose them to colonization such as less frequent or thorough hygiene due to limited mobility. Because overweight and obesity pose a significant public health concern in both adults and children, and the prevalence of metabolic abnormalities is higher among overweight and obese individuals, it is important that the pathway by which obesity and related chronic conditions influence *S. aureus* colonization be explicitly described so adequate measures can to be taken. This is especially pertinent in populations where the prevalence of overweight/obesity, metabolic chronic conditions and *S. aureus* colonization, is high.

The present study has several limitations. Because of the cross-sectional nature of the study design, we are unable to ensure temporality of the exposure and outcome. It is unlikely, however, that colonization of the anterior nares by *S. aureus* predisposes individuals to metabolic abnormalities, though as discussed above, one cannot rule out an unmeasured factor such as a predisposing microbiome phylotype that may be driving the association. We also were limited by

the use of self-reported height and weight in our calculation of BMI, which then governed our classification of metabolic health. Both the inmates and the trained interviewers were blinded to the colonization status of the inmate at the time height and weight was ascertained, therefore any misclassification of BMI category is likely non-differential. In addition, comorbidities were assessed using the medical chart of patients, and therefore undiagnosed diseases were not included, again resulting in non-differential misclassification. Results of our sensitivity study demonstrated that our findings for MAO vs MHNW females were somewhat robust to misclassification, but not our findings for MANW vs MHNW females. Corroboration in different populations with regards to all findings is needed to rule out chance. Corroboration using more granular measures of metabolic health namely, blood pressure, fasting glucose, triglyceride level and high-density lipoprotein cholesterol is of particular interest. Moreover, as discussed above, the precision of BMI as a measure of adiposity differs across levels of gender in addition to other factors. This may have influenced our ability to detect a difference if individuals that were biologically normal with respect to fat mass were classified as obese.

Despite the limitations, the study still had several strengths. This study was the first to crudely assess the influence obesity sub-phenotypes on an infectious outcome. Furthermore, the study population was relatively large with considerable variability in both BMI and metabolic abnormalities, which enabled us to make robust estimates of the association controlling for numerous confounders. We included an analysis on site of colonization, and in doing so demonstrated that males that were obese with metabolic abnormalities were significantly more likely to be exclusively colonized in the anterior nares and less likely to exclusively colonized in the oropharynx supporting previous research with the same observation.[74,76]

In conclusion, our results indicate that metabolic/immune abnormalities that frequently accompany obesity are associated with *S. aureus* colonization and may play a role in understanding the reported association between obesity and *S. aureus* colonization.

Table 3.1. Definition of Obesity Phenotypes

Characteristic	BMI	Metabolic/Immune Comorbidities
Metabolically Healthy Normal Weight	< 25 kg/m^2	Normal – None
Metabolically Abnormal Normal Weight[a]	< 25 kg/m^2	Abnormal – One or more
Metabolically Healthy Overweight	25-29.9 kg/m^2	Normal – None
Metabolically Abnormal Overweight	25-29.9 kg/m^2	Abnormal – One or more
Metabolically Healthy Obese	≥ 30 kg/m^2	Normal – None
Metabolically Abnormal Obese	≥ 30 kg/m^2	Abnormal – One or more

Definitions: normal weight; body mass index (BMI) ≤ 24.9 kg/m^2; overweight BMI 25-29.9 kg/m^2; obese BMI ≥ 30.0 kg/m^2

Normal metabolic Health refers to absence of all of the following; hypertension, diabetes and human immune-deficiency virus

[a]Abnormal metabolic Health refers to the diagnosis of one or more of the following; hypertension, diabetes and human immune-deficiency virus

Figure 3.1. Diagram Illustrating the Methodology Used for the Monte Carlo Quantitative Bias Analysis of Misclassification
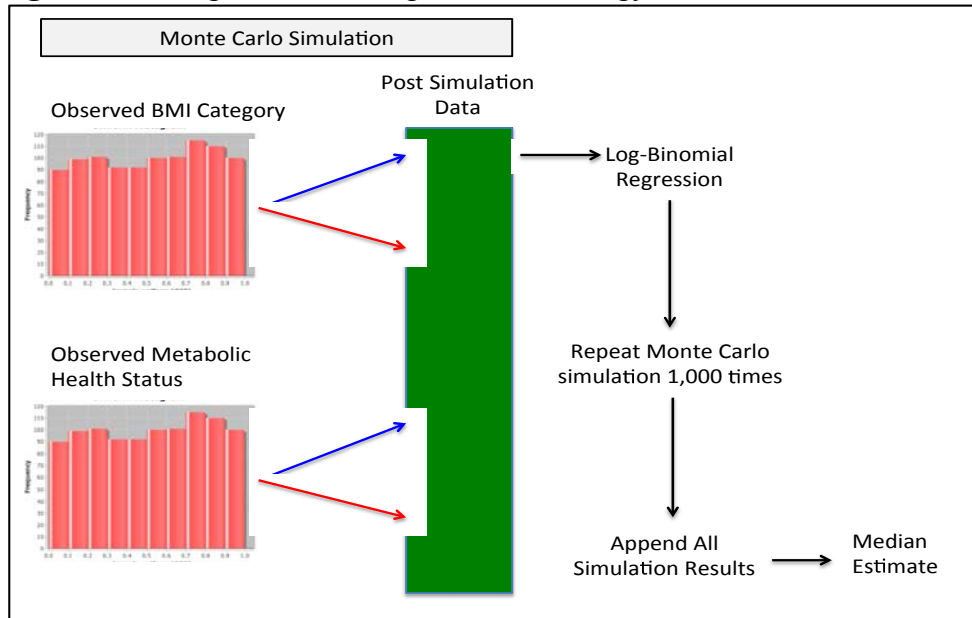
Figure 3.1 illustrates how the Monte Carlo simulation sensitivity study with bootstrapping methods assessing misclassification of body

max index (BMI) category and metabolic health status was conducted. Using a uniform distribution 10% of the population was

randomly selected and reclassified (blue arrow) independently for BMI category and metabolic health. The remaining individuals kept

their observed classification (red arrow). The sample population was then reconstructed to reflect the hypothesized true distribution of

both BMI category and metabolic health status (green column). The multivariable log-binomial regression model was subsequently

fitted and the process repeated 1000 times. A summary measure reflecting the median estimate and standard error across all

simulations was obtained.

Table 3.2 Descriptive Statistics of Factors Demographic, Behavioral Clinical Factors Associated with *Staphylococcus aureus*

Colonization among Male and Female Maximum-Security Inmates

	Female			Male			All		
Characteristic	Colonized N=612 (45.1%)	Not Colonized N=745 (54.9)	P value	Colonized N=722 (49.1%)	Not Colonized N=750 (51.0)	P value	Colonized N=1,334 (47.2%)	Not Colonized N=1,495 (52.9)	P value
DEMOGRAPHICS									
Age mean ± SD	36.4 ± 10.6	36.9 ± 10.9	0.34	34.7 ± 9.6	38.2 ± 10.8	<0.01	35.5 ± 10.1	37.5 ± 10.8	<0.01
Female Gender	N/A	N/A	N/A	N/A	N/A	N/A	612 (45.8)	745 (49.8)	0.04
Race			0.72			0.68			0.40
Non-Hispanic White	200 (32.7)	249 (32.7)		78 (10.8)	76 (10.1)		278 (20.8)	325 (21.7)	
Non-Hispanic Black	261 (42.7)	328 (44.0)		390 (54.0)	429 (57.2)		651 (48.8)	757 (50.6)	
Hispanic	108 (17.7)	114 (15.3)		216 (29.9)	209 (27.9)		324 (24.3)	323 (21.6)	
Other	43 (7.0)	54 (7.3)		38 (5.3)	36 (4.8)		81 (6.1)	90 (6.0)	
Education			0.04			<0.01			0.34
< High school	203 (33.2)	200 (26.9)		235 (32.6)	276 (36.8)		438 (32.8)	476 (31.8)	
High School/Equivalent	195 (31.9)	257 (34.5)		288 (39.9)	324 (43.2)		483 (36.2)	581 (38.9)	
> High school	214 (35.0)	288 (38.7)		199 (27.6)	150 (20.0)		413 (31.0)	438 (29.3)	
BEHAVIOR									
Smoking	415 (67.8)	518 (69.5)	0.50	437 (60.5)	470 (62.3)	0.44	852 (63.9)	988 (66.1)	0.22
Marijuana Use in Past 6 Months	75/610 (12.3)	64/739 (8.7)	0.03	93/717 (13.0)	81/747 (10.8)	0.21	168/1,328 (12.6)	145/1485 (12.6)	0.02
Crack/Cocaine Use	309/609 (50.7)	362/741 (48.9)	0.49	182/720 (25.3)	208/749 (27.7)	0.28	491/1,329 (37.0)	570/1,490 (38.3)	0.47
Heroin Use Ever	152/610 (24.9)	133/743 (17.8)	0.00	78/719 (10.9)	98/749 (13.1)	0.19	230/1,329 (17.3)	231/1492 (15.5)	0.19
History of IDU	91/606 (15.0)	84/731 (11.5)	0.06	31/717 (4.3)	46/745 (6.2)	0.11	122/1323 (9.2)	130/1,476 (8.8)	0.70
Shares Personal Items	93 (15.2)	93 (12.5)	0.15	53 (7.3)	58/749 (7.7)	0.77	146 (11.0)	151/1494 (10.1)	0.47
< 7 Showers per Week	49/609 (8.1)	65/737 (8.8)	0.61	406 (56.2)	393/749 (52.5)	0.15	455/1,331 (34.2)	458/1486 (30.8)	0.06
Prison time (days) N (mean ± SD)	612 (691 ± 1,432)	744 (676 ± 1,315)	0.83	717 (1,364 ± 1,824)	746 (1,781 ± 2,257)	<0.0001	1,326 (1,054 ± 1,689)	1,493 (1,229 ± 1,928)	0.01

MEDICAL

BMI mean ± SD	29.8 ± 6.6	29.3 ± 6.8	0.13	27.9 ±4.4	27.8 ± 4.6	0.78	1,334 (28.5 ± 5.6)	1,495 (28.5 ± 5.8)	0.29
Diabetes	64 (10.5)	63 (8.5)	0.21	33 (4.6)	28 (3.7)	0.42	97 (7.3)	91 (6.1)	0.21
Hypertension	129 (21.1)	133 (17.9)	0.13	107 (14.8)	142 (18.9)	0.04	236 (17.7)	275 (18.4)	0.63
HIV	36 (5.9)	45 (6.0)	0.90	17 (2.4)	17 (2.3)	0.91	53 (4.0)	62 (4.2)	0.69
Abnormal Metabolic Health	177 (28.9)	193 (25.9)	0.21	133 (18.4)	166 (22.1)	0.08	310 (23.2)	359 (24.0)	0.63
HCV	84 (13.7)	92 (12.4)	0.45	39 (5.4)	67 (8.9)	0.01	123 (9.2)	159 (10.6)	0.18
Liver Disease	95 (15.5)	97 (13.0)	0.19	46 (6.4)	77 (10.3)	0.01	141 (10.6)	174 (11.6)	0.37
Asthma	221 (36.1)	278 (37.3)	0.65	195 (27.0)	185 (24.7)	0.30	416 (31.0)	463 (31.1)	0.90
Kidney Disease	15 (2.5)	21 (2.8)	0.67	8 (1.1)	15 (2.0)	0.17	23 (1.7)	36 (2.4)	0.20
Systemic Antibiotic Use	225/589 (38.2)	278/734 (37.9)	0.90	136/701 (19.4)	184/738 (24.9)	0.01	361/1290 (28.0)	462/1472 (31.4)	0.05
Topical antibiotic Use	122/586 (20.8)	119/733 (16.2)	0.03	112/696 (16.1)	92/737 (12.5)	0.05	234/1282 (18.3)	211/1470 (14.4)	0.01

INCARCERATION CHARACTERISTICS

Length of Stay			<0.01			<0.01			<0.01
≤ 20 days	214 (35.0)	189 (25.4)		84 (11.6)	42 (5.6)		298 (22.3)	231 (15.5)	
21-3,000 Days	351 (57.4)	515 (69.1)		530 (73.4)	550 (73.3)		881 (66.0)	1,065 (71.2)	
> 3,000 Days	47 (7.7)	41 (5.5)		108 (15.0)	158 (21.1)		155 (11.6)	199 (13.3)	
Study Phase			<0.01			<0.01			<0.01
Intake	194 (31.7)	176 (23.6)		225 (31.2)	160 (21.3)		419 (31.5)	336 (22.5)	
Prevalence	263 (43.0)	349 (46.9)		340 (47.1)	430 (57.3)		603 (45.2)	779 (52.1)	
Discharge	155 (25.3)	220 (29.5)		157 (21.8)	160 (21.3)		312 (23.4)	380 (25.4)	

Definitions: normal weight; body mass index (BMI) ≤ 24.9 kg/m^2; overweight BMI 25-29.9 kg/m^2; obese BMI ≥ 30.0 kg/m^2; IDU: injection drug use; HIV; human immunodeficiency virus; HCV: hepatitis C virus

[a] "Other" category includes Asian, Native Americans, Pacific Islander and persons of or two or more racial/ethnic groups

[b] Abnormal metabolic health: diagnosis of one or more of the following; hypertension, diabetes and/or human immune-deficiency virus

Table 3.3. Descriptive Statistics of Demographic and *Staphylococcus aureus* Colonization Status of Male and Female Maximum-

Security Inmates Stratified by Comorbidity Status

	Normal weight Metabolically Healthy[b] N=302 (22.2%)	Normal weight Metabolically Abnormal[b] N=61 (4.5%)	Overweight Metabolically Healthy[b] N=334 (24.6%)	Overweight Metabolically Abnormal[b] N=100 (7.4%)	Obese Metabolically Healthy[b] N=351 (25.9%)	Obese with Metabolically Abnormal[b] N=209 (15.4%)
Female						
Age mean age ± SD	33.7 ± 10.4	45.0 ± 10.2	33.6 ± 10.2	43.8 ± 9.7	35.1 ± 9.9	42.8 ± 9.1
Race						
Non-Hispanic White	138 (45.7)	22 (36.1)	124 (37.1)	24 (24.0)	101 (28.8)	40 (19.1)
Non-Hispanic Black	87 (28.8)	29 (47.5)	132 (39.5)	54 (54.0)	162 (46.1)	125 (59.8)
Hispanic	45 (14.9)	6 (9.8)	56 (16.7)	17 (17.0)	66 (18.8)	32 (15.3)
Other[a]	32 (10.6)	4 (6.6)	22 (6.6)	5 (5.0)	22 (6.3)	12 (5.7)
Education						
< High school	83 (27.5)	12 (21.3)	88 (26.3)	30 (30.0)	114 (32.5)	75 (35.9)
High School/Equivalent	106 (35.1)	24 (39.3)	107 (32.0)	33 (33.0)	124 (35.3)	58 (27.7)
> High school	113 (37.4)	24 (39.3)	139 (41.6)	37 (37.0)	113 (32.2	76 (36.4)
Smoking	217 (71.8)	38 (62.3)	217 (65.0)	73 (73.0)	240 (68.4)	148 (70.8)
***S. aureus* Colonization**						
Colonized	119 (39.4)	31 (50.8)	154 (46.1)	41 (41.0)	162 (46.1)	105 (50.2)
Exclusively anterior nares	34 (28.6)	11 (35.5)	46 (29.9)	13 (31.7)	46 (28.4)	43 (40.9)
Exclusively oropharynx	50 (42.0)	8 (25.8)	66 (42.9)	13 (31.7)	69 (42.6)	30 (28.6)
Both sites	35 (29.4)	12 (38.7)	42 (27.3)	15 (36.6)	47 (29.0)	32 (30.5)
Male						
Age N (mean ± SD)	32.3 ± 9.4	45.6 ± 11.5	35.0 ± 9.6	44.6 ± 10.4	36.5 ± 9.2	42.2 ± 9.1
Race						
Non-Hispanic White	28 (8.9)	4 (8.5)	66 (11.0)	16 (12.6)	28 (10.8)	12 (9.6)
Non-Hispanic Black	180 (57.0)	26 (55.3)	310 (51.7)	81 (63.8)	137 (53.1)	85 (68.0)
Hispanic	89 (28.2)	16 (34.0)	183 (30.5)	27 (21.3)	84 (32.6)	26 (20.8)

Other[a]	19 (6.0)	1 (2.1)	40 (6.7)	3 (2.4)	9 (3.5)	2 (1.6)
Education						
< High school	125 (39.6)	21 (44.7)	196 (32.7)	35 (27.6)	95 (36.8)	39 (31.2)
High School/Equivalent	130 (41.1)	14 (29.8)	260 (43.4)	52 (40.9)	105 (40.7)	51 (40.8)
> High school	61 (19.3)	12 (25.5)	143 (23.9)	40 (31.5)	58 (22.5)	35 (28.0)
Smoking	204 (64.6)	32 (68.1)	362 (60.4)	74 (58.3)	169 (65.5)	66 (52.8)
***S. aureus* Colonization**						
Colonized	149 (47.1)	20 (42.5)	307(51.3)	52 (40.9)	133 (51.5)	61 (48.8)
Exclusively anterior nares	24 (16.1)	9 (45.0)	67 (21.8)	17 (32.7)	34 (25.6)	27 (44.3)
Exclusively oropharynx	82 (55.0)	8 (40.0)	20 (38.5)	20 (38.5)	53 (39.9)	26 (42.6)
Both sites	43 (28.9)	3 (15.0)	15 (28.9)	15 (28.9)	46 (34.6)	8 (13.1)

Definitions: normal weight; body mass index (BMI) $\leq$ 24.9 kg/m^2; overweight BMI 25-29.9 kg/m^2; obese BMI $\geq$ 30.0 kg/m^2

[a] "Other" category includes Asian, Native Americans, Pacific Islander and persons of or two or more racial/ethnic groups

[b] Comorbidities assessed included hypertension, diabetes and human immune-deficiency virus

Table 3.4. Multivariable Log Binomial Regression depicting the Independent Effect of Comorbidity Status on *Staphylococcus aureus* Colonization

Metabolic Health Status	BMI Status	Prevalence Ratio (95% Confidence Interval) Colonization with Staphylococcus aureus			
		Female		Male	
		Unadjusted	Adjusted	Unadjusted	Adjusted
Healthy	Normal Weight	Reference	Reference	Reference	Reference
	Overweight	1.17 (0.97, 1,40)	1.14 (0.95, 1.36)	1.09 (0.94, 1.24)	1.09 (0.95, 1.25)
	Obese	1.17 (0.98, 1.40)	1.12 (0.94, 1.36)	1.09 (0.93,1.29)	1.15 (0.98, 1.35)
Abnormal	Normal Weight	1.29 (0.97, 1.71)	1.34 (1.01, 1.77)	0.90 (0.63,1.28)	1.18 (0.83, 1.67)
	Overweight	1.04 (0.79, 1.37)	1.11 (0.84, 1.47)	0.86 (0.68, 1.10)	1.03 (0.81, 1.30)
	Obese	1.27 (1.05, 1.55)	1.28 (1.05, 1.57)	1.03 (0.84, 1.28)	1.21 (0.97, 1.51)

Abbreviations: PR, Prevalence ratio; 95%CI, 95% confidence intervals

Definitions: normal weight; body mass index (BMI) ≤ 24.9 kg/m^2; overweight BMI 25-29.9 kg/m^2; obese BMI ≥ 30.0 kg/m^2

[a]Controlling for age, race, education, smoking, study phase, marijuana use in the past six months, heroin use, injection drug use, systemic and topical antibiotic use

[b]Controlling for age race, education, smoking, phase, drug use ever, hepatitis C virus infection, systemic and topical antibiotic use

[c]Metabolically abnormal health was defined as the diagnosis of one or more of the following: hypertension, diabetes and human immune-deficiency virus

Table 3.5. The Role of Comorbidity Status on Site of *Staphylococcus aureus* Colonization among Colonized New York State Maximum-Security Inmates

Metabolic Health Status	BMI Status	Adjusted Odds Ratio (95% Confidence Interval)			
		Colonization with *Staphylococcus aureus*			
		Female[a]		Male[b]	
		Nose Vs Throat	Both Sites vs. Throat	Nose vs Throat	Both Sites vs. Throat
Healthy	Normal Weight	Reference	Reference	Reference	Reference
	Overweight	1.04 (0.57, 1.88)	0.90 (0.50, 1.62)	1.23 (0.71, 2.15)	0.97 (0.60, 1.15)
	Obese	0.82 (0.45, 1.48)	0.93 (0.52, 1.67)	1.66 (0.86, 3.20)	1.55 (0.88, 2.73)
Unhealthy	Normal Weight	1.20 (0.41,3.45)	1.75 (0.62, 5,01)	2.24 (0.70, 7.19)	0.63 (0.15, 2.65)
	Overweight	0.93 (0.37, 2.36)	1.49 (0.60, 3.68)	1.69 (0.73, 3.96)	1.28 (0.57, 2.89)
	Obese	1.29 (0.65, 2.57)	1.28 (0.63, 2.60)	2.40 (1.13, 5.09)	0.61 (0.25, 1.53)

Abbreviations: OR, odds ratio; 95% CI, 95% confidence intervals

Definitions: normal weight; body mass index (BMI) $\leq$ 24.9 kg/m^2; overweight BMI 25-29.9 kg/m^2; obese BMI $\geq$ 30.0 kg/m^2

[a]Controlling for age, phase, drug (marijuana, crack/cocaine, and or heroine) use ever & hepatitis C virus infection

[b]Controlling for age race, drug (marijuana, crack/cocaine, and or heroine) use ever & hepatitis C virus infection

[c]Abnormal Metabolic health defined by the diagnosis of two or more of the following: hypertension, diabetes and/or HIV

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Appendix A3

Table A3.1 Sensitivity Analysis Assessing 10% Misclassification of Body Mass Index Category and Independently 10% Misclassification of Metabolic Health Status in the Association between Metabolic Health Status and *Staphylococcus aureus* colonization

Metabolic Health Status[c]	BMI Status	Prevalence Ratio (95% Confidence Interval)	
		Female[a]	Male[b]
		Adjusted	Adjusted
Healthy	Normal Weight	Reference	Reference
	Overweight	1.15 (0.89, 1.42)	1.11 (089, 1.33)
	Obese	1.15, (0.90, 1.42)	1.15 (0.91, 1.40)
Abnormal	Normal Weight	1.19 (0.84, 1.53)	1.08 (0.68, 1.49)
	Overweight	1.15 (0.81, 1.50)	1.07 (076, 1.36)
	Obese	1.28 (1.00, 1.56)	1.18 (0.89, 1.47)

Abbreviations: BMI; body mass index

Definitions: normal weight BMI $\leq$ 24.9 kg/m^2; overweight BMI 25-29.9 kg/m^2; obese BMI $\geq$ 30.0 kg/m^2

[a]Controlling for age, race, education, smoking, study phase, marijuana use in the past six months, heroin use, injection drug use, systemic and topical antibiotic use

[c]Metabolically abnormal health was defined by the diagnosis of one or more of the following: hypertension, diabetes and human immune-deficiency virus

Figure A3.1 Sensitivity Analysis Assessing the Impact of Missing Data on Study Parameters Using Multiple Imputation Methods among Female Maximum-Security Inmates

BMI Category & Metabolic Health Status	PR (95%CI)
Complete	
Metabolically Healthy Normal Weight	1.00 (1.00, 1.00)
Metabolically Abnormal Normal Weight	1.34 (1.01, 1.78)
Metabolically Healthy Overweight	1.14 (0.95, 1.36)
Metabolically Abnormal Overweight	1.11 (0.84, 1.47)
Metabolically Healthy Obese	1.12 (0.94, 1.35)
Metabolically Abnormal Obese	1.28 (1.05, 1.57)
Available	
Metabolically Healthy Normal Weight	1.00 (1.00, 1.00)
Metabolically Abnormal Normal Weight	1.36 (1.03, 1.80)
Metabolically Healthy Overweight	1.16 (0.97, 1.38)
Metabolically Abnormal Overweight	1.11 (0.84, 1.47)
Metabolically Healthy Obese	1.16 (0.97, 1.39)
Metabolically Abnormal Obese	1.30 (1.06, 1.60)
Full	
Metabolically Healthy Normal Weight	1.00 (1.00, 1.00)
Metabolically Abnormal Normal Weight	1.32 (1.01, 1.74)
Metabolically Healthy Overweight	1.12 (0.94, 1.34)
Metabolically Abnormal Overweight	1.08 (0.81, 1.43)
Metabolically Healthy Obese	1.14 (0.95, 1.36)
Metabolically Abnormal Obese	1.26 (1.03, 1.54)

Abbreviations: PR, Prevalence Ratio; 95%CI, 95% confidence intervals

Definitions: normal weight; body mass index (BMI) ≤ 24.9 kg/m^2; overweight BMI 25-29.9 kg/m^2; obese BMI ≥ 30.0 kg/m^2

All models controlling for age, race, education, smoking, study phase, marijuana use in the past six months, heroin use, injection drug use, systemic and topical antibiotic use

Complete: Only observations with complete data on outcome, exposure and covariates were included

Available: Only observations with complete exposure and outcome data were assessed imputing missing covariate where required

Full: All observations included in the model, imputing all missing data with regards to exposure, outcome and covariates

Figure A3.2 Sensitivity Analysis Assessing the Impact of Missing Data on Study Parameters Using Multiple Imputation Methods among Male Maximum-Security Inmates

BMI Category & Metabolic Health Status	PR (95%CI)
Complete	
Metabolically Healthy Normal Weight	1.00 (1.00, 1.00)
Metabolically Abnormal Normal Weight	1.18 (0.83, 1.67)
Metabolically Healthy Overweight	1.09 (0.95, 1.25)
Metabolically Abnormal Overweight	1.03 (0.81, 1.30)
Metabolically Healthy Obese	1.15 (0.98, 1.35)
Metabolically Abnormal Obese	1.21 (0.97, 1.51)
Available	
Metabolically Healthy Normal Weight	1.00 (1.00, 1.00)
Metabolically Abnormal Normal Weight	1.18 (0.83, 1.67)
Metabolically Healthy Overweight	1.10 (0.96, 1.26)
Metabolically Abnormal Overweight	1.03 (0.81, 1.30)
Metabolically Healthy Obese	1.15 (0.98, 1.36)
Metabolically Abnormal Obese	1.23 (0.99, 1.53)
Full	
Metabolically Healthy Normal Weight	1.00 (1.00, 1.00)
Metabolically Abnormal Normal Weight	1.13 (0.81, 1.59)
Metabolically Healthy Overweight	1.11 (0.97, 1.26)
Metabolically Abnormal Overweight	1.04 (0.83, 1.30)
Metabolically Healthy Obese	1.14 (0.98, 1.34)
Metabolically Abnormal Obese	1.20 (0.97, 1.48)

.5   1   1.8

Abbreviations: PR, Prevalence Ratio; 95%CI, 95% confidence intervals

Definitions: normal weight; body mass index (BMI) ≤ 24.9 kg/m^2; overweight BMI 25-29.9 kg/m^2; obese BMI ≥ 30.0 kg/m^2

All models controlling for age, race, education, smoking, study phase, marijuana use in the past six months, heroin use, injection drug use, systemic and topical antibiotic use

Complete: Only observations with complete data on outcome, exposure and covariates were included

Available: Only observations with complete exposure and outcome data were assessed imputing missing covariate where required

Full: All observations included in the model, imputing all missing data with regards to exposure, outcome and covariates

Table A3.2 Multivariable Log Binomial Regression depicting the Independent Effect of Abnormal Metabolic/Immune Status (Hypertension & Diabetes) on *Staphylococcus aureus* Colonization

		Prevalence Ratio (95% Confidence Interval)			
Metabolic Health Status[c]	**BMI Status**	Colonization with *Staphylococcus aureus*			
		Female[a]		Male[b]	
		Adjusted	Unadjusted	Adjusted	Unadjusted
Healthy	Normal Weight	Reference	Reference	Reference	Reference
	Overweight	1.11 (0.93, 1.33)	1.11 (0.934, 1.33)	1.07 (0.93, 1.22)	1.07 (0.94, 1.24)
	Obese	1.10 (0.91, 1.31)	1.08 (0.91, 1.29)	1.09, 0.92, 1.29)	1.14 (0.97, 1.34)
Abnormal	Normal Weight	1.23 (0.89, 1.71)	1.39 (1.01, 1.91)	0.85 (0.57,, 1.28)	1.11 (0.74, 1.66)
	Overweight	1.04 (0.77, 1.39)	1.12 (0.84, 1.52)	0.87 (0.68, 1.11)	1.04 (0.82, 1.32)
	Obese	1.26 (1.05, 1.54)	1.33 (1.09, 1.64)	1.03, 0.82, 1.27)	1.19 (0.96, 1.48)

Abbreviations: BMI; body mass index

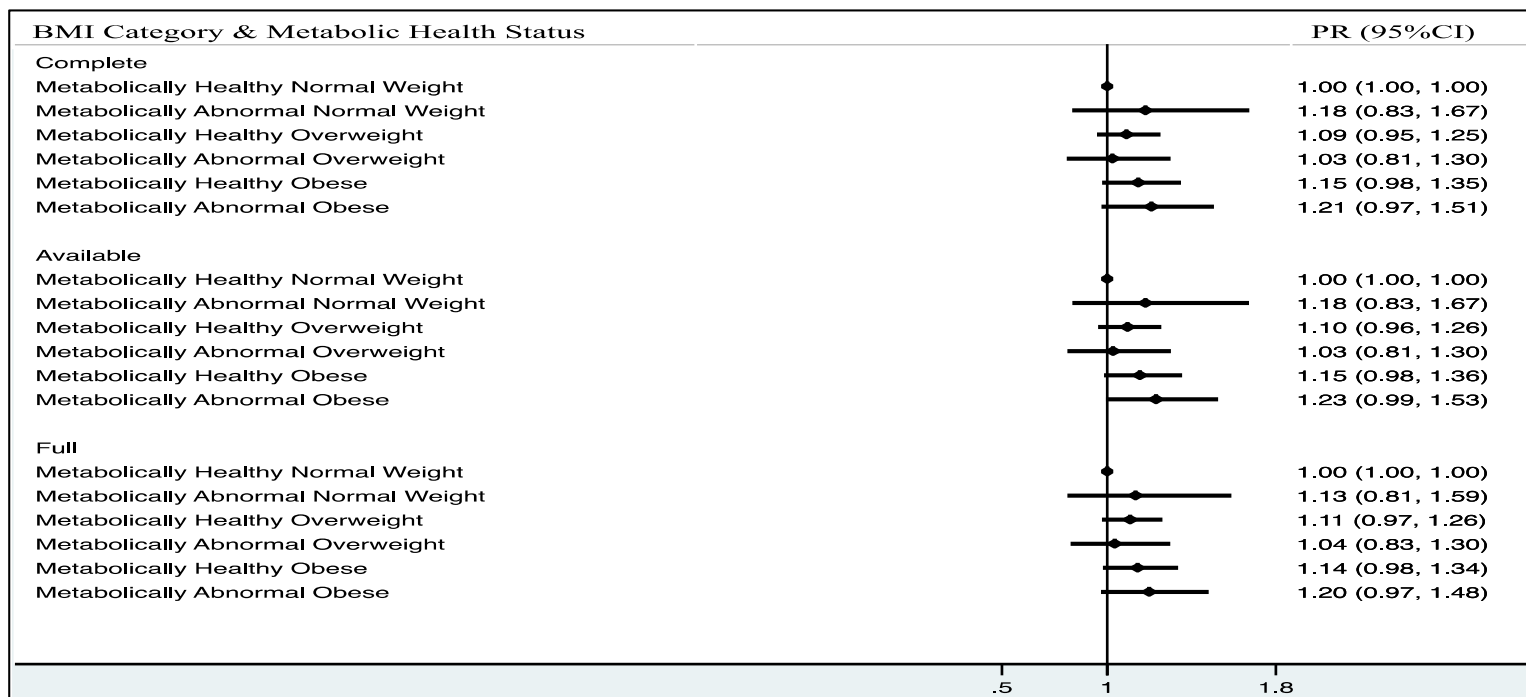Definitions: normal weight BMI $\leq$ 24.9 kg/m^2; overweight BMI 25-29.9 kg/m^2; obese BMI $\geq$ 30.0 kg/m^2

[a]Controlling for age, race, education, smoking, study phase, marijuana use in the past six months, heroin use, injection drug use, systemic, topical antibiotic use and Human Immunodeficiency Virus

[b]Controlling for age race, education, smoking, phase, drug use ever, hepatitis C virus infection, systemic, topical antibiotic use and Human Immunodeficiency Virus

[c]Abnormal Metabolic health defined by the diagnosis of hypertension and/or diabetes

Table A3.3 Description of the All Possible Clusters of Comorbidity Observed in the Data

	Hypertension Only	Diabetes Only	HIV Only	Hypertension & Diabetes	Hypertension & HIV	Diabetes & HIV	Hypertension, Diabetes, & HIV	Total
Female								
Normal Weight & Comorbid	28 (48.3)	8 (13.8)	15 (25.9)	3 (5.2)	4 (6.9)	0	0	58
Overweight & Comorbid	4 (44.7)	15 (16.0)	17 (18.1)	12 (12.8)	5 (5.3)	2 (2.1)	1 (1.1)	94
Obese & Comorbid	97 (48.0)	25 (12.4)	17 (8.4)	47 (23.3)	7 (3.5)	5 (3.5)	4 (2.0)	202
Total	167	48	49	62	16	7	5	354
Male								
Normal Weight & Comorbid	27 (58.7)	5 (10.9)	9 (19.6)	1 (2.2)	3 (6.5)	0	1 (2.2)	46
Overweight & Comorbid	91 (71.6)	11 (8.7)	10 (7.9)	11 (8.7)	4 (3.1)	0	0	127
Obese & Comorbid	86 (70.5)	10 (8.2)	4 (3.3)	19 (15.6)	1 (0.8)	1 (0.8)	1 (0.8)	122
Total	204	26	23	31	8	1	2	

Definitions: normal weight; body mass index (BMI) ≤ 24.9 kg/m^2; overweight BMI 25-29.9 kg/m^2; obese BMI ≥ 30.0 kg/m^2; HIV, human immune-deficiency virus

[a] "Other" category includes Asian, Native Americans, Pacific Islander and persons of or two or more racial/ethnic groups

[b] Comorbidities assessed included hypertension, diabetes and HIV

Table A3.4 Multivariable Log Binomial Regression depicting the Independent Effect of Abnormal Metabolic/Immune Status (2+ Comorbidities) on *Staphylococcus aureus* Colonization

Metabolic Health Status[c]	BMI Status	Prevalence Ratio (95% Confidence Interval)			
		Colonization with *Staphylococcus aureus*			
		Female[a]		Male[b]	
		unadjusted	Adjusted	unadjusted	Adjusted
Healthy	Normal Weight	Reference	Reference	Reference	Reference
	Overweight	1.09 (0.92, 1.28)	1.09 (0.93, 1.28)	1.06 (0.93, 1.22)	1.07 (0.94, 1.22)
	Obese	1.10 (094, 1.29)	1.09 (0.93, 1.28)	1.07 (0.92, 1.25)	1.14 (0.99, 1.32)
Abnormal	Normal Weight	1.35 (0.70, 2.60)	1.58 (0.79, 3.12)	1.28 (0.62, 2.65)	1.81 (0.86, 3.80)
	Overweight	0.83 (0.45, 1.52)	0.91 (0.49, 1.68)	0.85 (0.46, 1.61)	1.01 (0.54, 1.90)
	Obese	1.31 (1.02, 1.69)	1.40 (1.067, 1.84)	1.17 (0.79, 1.74)	1.31 (0.89, 1.92)

Abbreviations: BMI; body mass index

Definitions: normal weight BMI ≤ 24.9 kg/m^2; overweight BMI 25-29.9 kg/m^2; obese BMI ≥ 30.0 kg/m^2

[a]Controlling for age, race, education, smoking, study phase, marijuana use in the past six months, heroin use, injection drug use, systemic and topical antibiotic use

[b]Controlling for age race, education, smoking, phase, drug use ever, hepatitis C virus infection, systemic and topical antibiotic use

[c]Abnormal Metabolic health defined by the diagnosis of two or more of the following: hypertension, diabetes and/or HIV

Table A3.5 Alternative Analysis Assessing the Association between Abnormal Metabolic Health and Colonization with

Staphylococcus aureus: The Influence of BMI within Metabolic Health Categories

		Odds Ratio (95% Confidence Interval)	
Metabolic Health Status[c]	**BMI Category**	**Colonization with *Staphylococcus aureus***	
		Female[a]	Male[b]
Model 1 - Healthy	Normal Weight	Reference	Reference
	Overweight	1.31 (0.94, 1.82)	1.27 (0.95, 1.70)
	Obese	1.28 (0.92, 1.78)	1.38, 0.97, 1.97)
Model 2- Unhealthy	Normal Weight	Reference	Reference
	Overweight	0.70 (0.35, 1.40)	0.85 (0.41, 1.77)
	Obese	0.90 (0.49, 1.67)	1.13 (0.53, 2.36)

Abbreviations: BMI; body mass index

Definitions: normal weight BMI $\leq$ 24.9 kg/m^2; overweight BMI 25-29.9 kg/m^2; obese BMI $\geq$ 30.0 kg/m^2

[a]Controlling for age, race, education, smoking, study phase, marijuana use in the past six months, heroin use, injection drug use, systemic and topical antibiotic use

[b]Controlling for age race, education, smoking, phase, drug use ever, hepatitis C virus infection, systemic and topical antibiotic use

[c]Abnormal Metabolic health defined by the diagnosis of two or more of the following: hypertension, diabetes and/or HIV

Among women: N total = 1,310, N among healthy = 956 (425 staph positive), N among unhealthy = 354 (173 staph positive)

Among men: N total = 1,142, N among healthy = 1,142 (573 staph positive) N among unhealthy = 296 (131 staph positive)

Table A3.6 Alternative Analysis Assessing the Association between Abnormal Metabolic Health and Colonization with

Staphylococcus aureus: The influence of Metabolic Health within BMI Categories

		Prevalence Ratio (95% Confidence Interval)	
BMI Category	Metabolic Health Status[c]	Colonization with *Staphylococcus aureus*	
		Female[a$]	Male[b&]
Model 1 - Normal Weight	Healthy	Reference	Reference
	Unhealthy	2.15 (1.11, 4.18)	1.47 (0.71, 3.05)
Model 2 - Overweight	Healthy	Reference	Reference
	Unhealthy	0.93 (0.54, 1.59)	0.87 (0.56, 1.34)
Model 3 - Obese	Healthy	Reference	Reference
	Unhealthy	1.16 (0.79, 1.72)	1.00 (0.62, 1.63)

Abbreviations: BMI; body mass index

Definitions: normal weight BMI $\leq$ 24.9 kg/m^2; overweight BMI 25-29.9 kg/m^2; obese BMI $\geq$ 30.0 kg/m^2

[a]Controlling for age, race, education, smoking, study phase, marijuana use in the past six months, heroin use, injection drug use, systemic and topical antibiotic use

[b]Controlling for age race, education, smoking, phase, drug use ever, hepatitis C virus infection, systemic and topical antibiotic use

[c]Abnormal Metabolic health defined by the diagnosis of two or more of the following: hypertension, diabetes and/or HIV

Normal weight women N total=343, Normal weight men N total = 351; Overweight women total = 420, Overweight men = 712;

Obese women total = 547, Obese men total N = 372

[$]P for interaction between metabolic health and normal weight among women was marginally significant (0.083) and not significant for obese (0.26)

&P for interaction between metabolic health and normal weight among men was marginally significant (0.026) and not significant for obese (0.56)

CHAPTER 4: Obesity and Persistent Colonization with *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus remains an important human pathogen, causing relatively benign infections such as skin and soft tissue infections (SSTIs) as well as serious conditions such as necrotizing pneumonia and endocarditis.[1] Despite its capacity to cause invasive diseases, the organism can also benignly reside as a transient and sometimes persistent component of the resident flora of different human mucosal sights, including the anterior nares,[2] oropharynx,[3,4] skin, gastro-intestinal tract[5] and inguinal areas.[6] Colonization of these mucosal sites, particularly the anterior nares, is associated with subsequent infection by the colonizing strain,[7] and individuals that are persistently colonized are at elevated risk of infection.[7] Studies also show that persistently colonized individuals or persistent carriers have a higher nasal bacterial load than intermittent or non carriers,[5,8,9] thereby contributing more substantially to the organisms dispersion and transmission.[10] The mechanisms by which *S. aureus* becomes a persistent component of the nasal microbial flora are still largely unknown. Identifying modifiable host factors that contribute to persistent carriage will not only identify individuals at risk of infection, but also help identify those that more efficiently disseminating the organism thereby placing others at risk of infection.

Although the definition varies, persistent carriers are most frequently described as individuals in whom *S. aureus* is isolated in over 80% of cultures taken weekly over a period of approximately three months,[11] or individuals with two positive *S. aureus* culture taken one week apart.[8] An estimated 20% of individuals in the general population are considered persistent carriers,[12] and the remainder are defined as Intermittent carriers. The Intermittent carriage

phenotype is comprised of individuals in whom < 80% of cultures obtained are positive for *S. aureus*, or those historically classified as intermittent carriers, as well as those who are consistently culture negative, or those historically defined as non-carriers.[9,11] Individuals historically classified as intermittent carriers or non-carriers now comprise one phenotypic group because they demonstrate similar characteristics that are distinct from those demonstrated by persistent carriers. For example, upon artificial inoculation, the rate of *S. aureus* clearance among intermittent and non-carriers were similar and also statistically significantly different than persistent carriers.[13] Furthermore, despite being inoculated with a mixture of different strains, persistent carriers reverted back to their original colonizing strains whereas intermittent and non-carriers displayed no affinity to a particular strain.[13] Lastly, persistent carriers displayed a lower exchange rate of *S. aureus* clones over time as compared to intermittent or non-carrier either in artificial inoculation environments or in strictly observational settings.[13-15] These data demonstrate that a small but substantial subset of the population interacts with *S. aureus* differently and reasons for those differences need to be elucidated.

Demographic and behavioral factors such as younger age[16,17], male sex[16] and contraceptive use [18] are all positively associated with persistent carriage whereas smoking[16] has been shown to be protective. More importantly, a number of local and systemic immune factors have been shown to influence *S. aureus* carriage type. For example, antimicrobial peptide levels, which are both constitutively and inducibly expressed in the anterior nares as well as other mucosal sites are lower in persistent carriers as compared to intermittent.[19] In addition, serum 25hydroxyvitaminD (25(OH)D) levels are significantly lower among persistent carriers, though this observation was restricted to males.[16] Also associated with persistent carriage are polymorphisms in genes that control the expression of the antimicrobial peptide β-defensin 1

messenger RNA expression[20] as well as polymorphisms in genes expressing C-reactive protein and Interleukin-4[21] (IL-4).

Many of the above systemic and local immune factors associated with persistent carriage are also altered in obesity. For example, levels of circulating IL-4, which is associated with an anti-inflammatory Th2 immune profile,[22-24] is lower in obese individuals. In addition 25(OH)D is currently viewed as central to a host's defense against infection,[25] however, circulating levels have been shown to significantly lower among obese as compared to normal weight individuals.[26] Lastly, leptin, an immune-modulatory adipokine that regulates antimicrobial peptide secretions both in vitro and in vivo has been shown to influence intestinal microbial composition in mouse models[27] and its receptor are expressed in the human nasal mucosa.[28] Leptin dysfunction in both adipose tissue and peripheral blood is a hallmark of obesity[29] and it has been implicated in the impaired host defenses observed amongst the obese.[30]

The data clearly demonstrates that both innate and adaptive immunity are associated with persistent *S. aureus* carriage, particularly in the anterior nares, and many of these factors are also independently associated with obesity. The extent to which these responses are influenced by obesity has yet to be investigated. Furthermore, the influence of ancillary colonization sites such as the oropharynx on the carrier patterns observed has not been extensively assessed in non-clinical settings.[31-33] Recent findings have highlighted the importance of persistent *S. aureus* carriage of the GI tract[5,34] as well as the oropharynx to subsequent disease. It is, therefore, increasingly important to assess bacterial and host factors that contribute to the organism's persistence not only in the anterior nares but other frequently colonized mucosal sites.

In an attempt to characterize *S. aureus* persistence in both the anterior nares and the oropharynx we used data from a recently completed study characterizing *S. aureus* colonization

among New York state inmates to determine the prevalence of persistent carriage among this high risk group.[35] Our primary aim was to examine the association between obesity and persistent *S. aureus* carriage as well as to characterize differences in persistent nasal as compared to oropharynx carriage. We hypothesized that obesity, which others[36] and our group[37] have shown to be prevalent in incarcerated population, more particularly NY state inmates,[37] will be associated with persistent *S. aureus* carriage. We further described the carriage phenotypes by assessing strain diversity and compositional differences across the different carriage types using Simpson's Index of diversity as well as correspondence analysis.

METHODS

Setting and Sample

Data from an NIH-funded study (5R01AI082536) of *S. aureus* prevalence and risk factors in two NYS maximum-security prisons, Bedford Hills Correctional Facility (women's prison) and Sing Sing Correctional Facility (men's prison), were used. Both participating facilities are located in Westchester county New York (NYS). The female prison is located in the town of Bedford Hills and is the only female maximum-security prison in NYS. It has the capacity to house approximately 900 inmates, and serves as the only female prison reception center in NYS and therefore all females entering into the NYS prison system are admitted to this facility before being transferred to their designated confinement facility. In contrast, the male prison is one of 15 male maximum-security prisons in NYS, and has the capacity to house approximately 1800 inmates. It is located in the town of Ossining and most inmates that reside in the facility have been transferred from other prisons. Study data were collected in three distinct phases: (1) when inmates entered the facility (intake), (2) at any time point during incarceration

112

(cross-sectional), (3) when inmates were being released back into the general population (discharge).

Participant Recruitment

Participant recruitment began in January 2009 and ended in February 2014. Inmate participation at both facilities was voluntary and compensation was not provided. Eligible participants were inmates ages 16 years or older capable of providing informed consent. The Institutional Review Boards of Columbia University Medical Center (CUMC) and the New York State Department of Corrections and Community Supervision (DOCCS) approved the study.

To recruit participants for intake, inmates entering either the female or the male prisons were invited to participate in the study when they first entered the facility and while being processed. One of five trained research associates from CUMC invited the inmates to speak with them in a private room. At this time, inmates were provided with a brief description of the study and informed consent was solicited along with Health Insurance and Portability and Accountability Act (HIPAA) release. Recruitment for the cross-sectional phase varied by prison facility. In the female prison, inmates were called to the facility medical center and invited to participate. To recruit participants in the male prison trained research associates visited classrooms in the school, vocational training, and counseling buildings, and asked inmates if they were interested in participating in the study. At both facilities, interested inmates were subsequently invited to speak with a trained research associate from CUMC in a private room and were enrolled in the cross-sectional phase if informed consent was provided. Recruitment for the discharge phase was similar for both facilities; inmates were called to the facility medical center and invited to speak privately with a trained research associate.

Data Collection and Extraction

Trained research associates used a structured questionnaire to interview participants and collect demographic, behavioral and medical history information. During the interview, participants were cultured at the anterior nares and oropharynx, and culture swabs were later processed to assess asymptomatic colonization with *S. aureus*. In addition, research associates extracted data from the medical charts of inmates focusing on the NYS DOCCS Health Services System problem list and the provider's progress notes.

Medical conditions were included in an inmate's Health Services System problem list during intake processing and/or anytime during incarceration. Upon entry into DOCCS, each inmate undergoes a medical screening process that includes a detailed medical history, blood tests, dental and chest X-ray. In addition, every inmate is offered a human immunodeficiency virus (HIV) test at prison entry, and those at risk for hepatitis C virus (HCV) based on medical and drug history screenings are tested for the condition. Medical conditions of note at screening are included in the Health Services System problem list, or are included in the progress notes in an inmate's medical chart. In addition, any medical condition identified during incarceration was included in the problem list and /or the progress notes along with any medical procedures and/or laboratory tests conducted while incarcerated.

Study Design

The sample population used to assess the association between obesity and persistent *S. aureus* carriage represents a subset of the entire study sample described above. Specifically, though the parent study was not designed to be a longitudinal study, 274 inmates were interviewed and cultured on at least two different occasions during the course of the study. This subset was used to generate a longitudinal data set reflecting inmates' carriage patterns over time. This data set was then used to examine factors associated with persistent colonization.

<u>Outcome Assessment: Persistent *S. aureus* Colonization</u>

S. aureus characterization for each time of assessment was carried out as previously described.[31] Swab samples obtained from the anterior nares and oropharynx of participants were incubated overnight in 6% sodium chloride-supplemented tryptic soy broth (Becton Dickinson) at 35°C. This particular broth was used to enrich *S. aureus* selection. Aliquots of the broth were then plated onto mannitol salt agar (Becton Dickinson) and incubated for 48 hours at 37°C. Individual colonies on the salt agar plates that were morphologically identified as *S. aureus* were streaked onto sheep blood agar plates and subsequently confirmed as *S. aureus* using the coagulase and protein A detection kit (Murex Staphaurex, Lenexa, KA). Confirmed isolates were then characterized by *spa* typing and compared using Ridom Staph Type software (Ridom GmBH). In order to determine the clonal relatedness of the different of *S. aureus* strains identified in the population, *spa* types were clustered into spa clonal complexes (spa-CC) using Based Upon Repeat Pattern (BURP) analysis.[38] All *S. aureus* isolates from which *spa* types were obtained and further had five or more repeats will be included in the BURP analysis. BURP analysis results in the assignment of each spa-type included in the analysis to a specific spa-CC. The spa-CC a specific *S. aureus* strain is assigned is dependent on all the *spa* types included in the analysis.

The outcome of interest was persistent colonization (yes/no) with *S. aureus*, which was defined two ways. The first method of assessing persistent *S. aureus* colonization was at the species level, i.e., inmates colonized with *S. aureus* at the anterior nares or oropharynx at all time points (usually two) in which they were assessed. Table 4.1 describes the different patterns of colonization that were observed over time and the nomenclature used to describe the pattern. The second method of assessing persistent *S. aureus* colonization was at the strain level, i.e., inmates

colonized with same *S. aureus* strain at the anterior nares or oropharynx at all of the time points in which they were assessed. We assessed both methods based on findings in chapter one of this dissertation suggesting that two different populations of persistent carriers may exist. Species persistence captures both populations in that that it is inclusive of individuals who are consistently colonized due to frequent exposure or decreased ability to prevent or clear carriage as well as those who display the biological characteristics (i.e. higher bacterial load, longer strain carriage, symbiotic relationship with colonizing strain) frequently associated persistent carriage. Strain persistence restricts our attention to the latter group. The majority of inmates included in this data set were assessed a maximum of two times, and therefore only two swabs cultures were used to determine persistent colonization in these individuals. This method has been frequently used to assess persistent *S. aureus* colonization, resulting in similar rates of persistent carriage across studies.[8,17,39,40]

<u>Exposure Assessment: Obese</u>

Obesity at each time of assessment was assessed by first calculating the BMI of each inmate based on self-reported height and weight using the following equation $\left[\frac{\text{mass (lb)}}{(\text{height(in)}^2)} \times \right.$ $\left. 703 \right]$. Inmates were then categorized as being obese defined as having a BMI $\geq$ 30 kg/m^2 or non-obese inmates (BMI < 30 kg/m^2). Individuals found to be obese at one point of assessment and obese or at risk of being obese (BMI > 25 kg/m^2) at subsequent time points were defined as being exposed, those that did not meet this criteria were defined as being non-exposed

<u>Covariates</u>

In addition to assessing whether obesity serves as a predictor of persistent colonization, relevant demographic, clinical and behavioral factors were also assessed. Specifically the diagnosis of HIV, diabetes and/or hypertension will be controlled for in the final model.

Additionally, self-reported age, sex, race (non-Hispanic white, non-Hispanic Black, Hispanic and other), antibiotic use in the past six months, Current smoking (yes/no), number of showers per week, shared personal items, history of IDU (yes/no) will also be assessed as potential confounding factors.

<u>Statistical analysis</u>

Means and standard errors were calculated for continuous variables and frequencies and percentages were calculated for categorical variables. Bivariate analyses were conducted using chi-squared tests and t-tests as appropriate. Multivariable analysis using log-binomial regression was then conducted to assess the association between the exposure, obesity, and the likelihood of being persistently colonized with *S. aureus*. Age and gender were included in the model as covariates based on a priori knowledge of their association with comorbidity status and persistent *S. aureus* carriage. In addition covariates that were associated with both the exposure and outcome with a 20% level of significance and additionally changed the beta estimate for comorbidity status by 10% or more were retained in the final model. Statistical interaction by gender was assessed by including a product term for obesity and gender in the final model and assessing whether the beta coefficient associated with the product term was statistically significantly associated with the outcome. Multiple imputation methods were used to impute missing covariate data. A total of 20 imputed data sets were generated using the Markov Chain Monte Carlo method of imputation to impute missing covariate data. Demographic, behavioral as well as self-reported medical data were used to impute missing chart based medical records and self-reported drug use behavior. The relative efficiencies of all imputed data exceeded 99%. All the imputed data sets were subsequently analyzed to determine whether obesity as defined above was significantly associated with persistent *S. aureus* colonization.

117

A sensitivity analysis was also conducted to evaluate how robust the findings were to misclassification of the exposure (obesity). Based on the literature we assumed perfect specificity[41] and that any misclassification resulted from under reporting of weight and over reporting of height. Monte Carlo simulation studies with a uniform distribution and bootstrapping methods were then conducted under the assumption that 10% and 15% of the population was misclassified, respectively. These individuals were then randomly selected and their obesity category changed to what was presumably their "true" obesity category. We additionally ran a sensitivity analysis using generalized estimating equations (GEE) as opposed to log binomial regression, to evaluate the robustness of our findings given how obesity was operationalized.

To further characterize and distinguish persistent carriers from intermittent carriers we calculated the alpha diversity of *S. aureus* strains among persistent as compared to intermittent carriers using Simpson's Index of Diversity. We additionally took compositional differences across the various carriage types into account by performing simple and multiple correspondence analyses. Correspondence analysis is a multivariate technique that uses a distance metric to extract underlying factors that drive the data distribution. These underlying factors are then used to orient the data on a two dimensional plot based on their similarities and dissimilarities.[42] The principal result of the correspondence analysis is a geometric map or biplot that illustrates how the relative frequencies of *S. aureus* strain types changed with the different carriage types (persistent Vs. intermittent) and by carriage type-stratified mucosal site (nares persistent Vs. oropharynx persistent Vs. intermittent). To interpret the results, the map origin corresponds to the centroid (average) of each variable. The closer a point on the graph is to the origin the closer it is to the average observed in the data. Also the closer points are to one another the more similar they are with regards to the variables included in the analysis. The spread of the data points from top to bottom and

118

left to right provide an indication of how similar or dissimilar the data are with the center of the axis representing the most common characteristics found in the data. All statistical analyses were conducted using SAS, version 9.3 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

Participation rates at both facilities were high for each phase of the study ranging from 81-87% for the female prison and 80-94% for the male prison. Characteristics of the total sample stratified by *S. aureus* phenotypic carriage types are presented in Table 4.2. The mean age at baseline for study participants was 35 ± 10 years. Approximately 49% of all study participants were female, and a little more than two-thirds held a high school diploma or a General Education Development test (GED). Forty-four percent of all participants were non-Hispanic black, 26% were non-Hispanic white, 24% were Hispanic and approximately 5% were of other or mixed race.

Seventy-four (27%) individuals were persistently colonized in the anterior nares, oropharynx or both sites at the species level (Table 4.2). When only the anterior nares were considered, 15% of the study participants were persistent carriers, 22% were intermittent carriers and 63% were non-carriers. When carriage phenotype assessment was restricted to the oropharynx, 17% were persistent carriers, 31% were intermittent and 52% were non-carriers. At the strain level 48 (17%) individuals were considered persistent carriers. Approximately 10% of participants met the definition of persistent carriage by strain at each mucosal site when assessed separately.

In bivariate analysis, age was the only demographic factor associated or marginally associated with *S. aureus* species and strain persistence. Over 70% of study participants were current smokers and smoking was not significantly associated with *S. aureus* species or strain

119

persistence in bivariate analysis (P> 0.05) (Table 4.2). Though only 12% reported marijuana use in the previous six months, 46% had used crack/cocaine in their lifetime 28% had used heroine, and 13% had a history of injection drug use. Heroin use appeared to differ across carriage types in bivariate analysis with higher usage among intermittent carriers in both species (p value=0.03) and strain (p-value=0.08) assessments. None of the medical data appeared to differ significantly across *S. aureus* carriage phenotypes when considering species or strain persistence (Table 4.2). Though obesity rates were slightly higher among persistent carriers at the species level (49% vs. 39%) the association was not significant at the 5% level in bivariate analysis, and prevalence rates were essentially the same across carriage phenotypes when strain persistence was considered.

Results of the multivariable model are shown in Table 4.3. In adjusted models, obesity was associated with species persistence (Prevalence Ratio (PR)=1.65 95% confidence interval (CI)1.09, 2.51) after controlling for age, gender, systemic antibiotic use, diabetes, HIV and hypertension. It was, however, not associated with strain persistence (PR=1.37 95% CI 0.78, 2.46) after controlling for age, gender, systemic antibiotic use, diabetes, HIV, hypertension and crack/cocaine use. Younger age was also significantly associated with species persistence (p value < 0.05) and marginally associated with strain persistence (p value=0.052). For every one-year increase in age the prevalence of *S. aureus* species persistence decreased by approximately 2.4% (PR=0.97 95% CI 0.95, 0.99).

Figures 4.1 and 4.2 illustrate the diversity of the 334 different isolates representing 104 different *S. aureus spa* types. The most frequent spa type representing 15.4% of all isolates was *spa* type t008. The alpha diversity (Table 4.4) of *spa* types as assessed by Simpson's Index of Diversity did not significantly differ between persistent (95.3%, 95% CI 93.5%, 97.0%) and

intermittent (95.6%, 95% CI 93.3%, 97.8%) carriers. Similar results with wider confidence intervals were obtained when persistence was stratified by mucosal site (Table 4.4). When strain composition is taken into account using correspondence analysis a different picture is, however, painted. Figures 4.3 and 4.4 depict the results of the multiple correspondence analyses assessing the distribution of *S. aureus* strain types by mucosal site and carriage type. Figure 4.3 demonstrates the biplot when all the different strains isolated are plotted and as is demonstrated by the biplot a distinct difference can be observed amongst individuals that are anterior nares persistent as compared to all other carriage types. This distinction becomes even more apparent when we restrict the analysis to strains that appear in our data set more than once (Figure 4.4). We were able to show that certain strains such as t571 seem to be more represented amongst individuals that are persistently colonized in the anterior nares, and therefore may favor this mucosal environment. We also observed considerable overlap amongst strains colonizing intermittent and oropharynx carriers.

DISCUSSION

Host and bacterial factors associated with persistent carriage have not been well elucidated, particularly in a population such as the incarcerated where *S. aureus* carriage and disease are high, but the opportunity to longitudinally assess carriage patterns are scarce. In the current study, *S. aureus* carriage patterns among NYS maximum-security prison inmates were characterized, and the independent effect of obesity on both species and strain persistence of *S. aureus* in the anterior nares and/or oropharynx was assessed. The results demonstrated that approximately 27% of the population was persistent carriers at the species level and 17% were persistent carriers at the strain level. Obesity was independently associated with species persistence but not strain persistence. Diversity of *S. aureus* strains as assessed by Simpson's

Index of Diversity did not differ between persistent as compared to intermittent carriers. Correspondence analysis, however, demonstrated differences in strain composition among persistent nasal carriers as compared to all other carriage/mucosal site types.

We were able to demonstrate a prevalence of *S. aureus* species persistence similar to what has been observed in published literature.[14,43] Even when restricting the assessment to persistence in the anterior nares our prevalence of approximately 15% remains similar to published results in the general population.[5] The inclusion of the oropharynx carriage in our measure of persistence, however, demonstrates what proportion of individuals would have been misclassified as intermittent/non-carriers had this mucosal site not been taken into account. Recent studies have shown the importance of ancillary mucosal site on *S. aureus* disease in the absence of nasal carriage.[3-5,44] In a study assessing both anterior nares and oropharynx colonization, 22% of *S. aureus* carriage was exclusively nasal as compared to 38%, which were exclusively oropharynx.[45] Furthermore, 13% of oropharyngeal carriers were considered persistent carriers after six years of follow up, and exclusively oropharyngeal and exclusively nasal carriers demonstrated genetically distinct clonal clusters. Persistent colonization of the oropharynx may partially explain anterior nares decolonization failures in medical settings, as well as infections among those that are culture negative at the anterior nares.[46] Even more interesting are recent findings regarding colonization of the GI tract and its contribution to on going disease outbreaks within a clinical setting.[5] Expanding our assessment of *S. aureus* persistence to include other mucosal sites would improve our understanding of persistence and perhaps introduce some granularity in bacterial factors that might influence *S. aureus* colonization, particularly persistent colonization.

Our study demonstrated a significant association between obesity and persistent *S. aureus* carriage at the species level, but not at the strain level. With regard to *S. aureus*, obesity has been shown to influence both infection and colonization.[37,47-49] Obesity is a risk factor for pre-operative colonization among surgical patients[49] as well as colonization among healthy community dwelling adults.[37,47,48] Among community dwelling adults, a modest association was observed between obesity and *S. aureus* colonization in both men and women using data from the National Health and Nutrition Survey (NHANES).[47] Their findings were partially corroborated by a Norwegian population-based survey[16] as well as our group[37] where an association among women but not men was observed. One postulated mechanism of action is that increased adiposity interferes with the immune functionality of the tissue thereby facilitating infection and/or colonization.[30,50] A recent assessment in mouse models seems to support this hypothesis.[51] An alternative hypothesis is that obesity may cause chronic-low grade inflammation, which is associated with impaired innate and adaptive immune response.[30,52] This hypothesis is particularly relevant given consistent findings with regards to impaired immune function and *S. aureus* persistent carriage. For example, the activity of antimicrobial peptides molecules that bind to proteins and regulate their biological activity in the innate immune response is diminished in the nasal secretions of persistent carriers as compared to non-carriers.[19,20,53,54] Other immune related factors that have been shown to be associated with persistence include polymorphisms in the glucocorticoid receptor gene,[55] single nucleotide polymorphisms in the IL4 and C-Reactive protein genes[21] as well as lower circulating levels of 25(OH)D.[16] Furthermore, nasal carriage of *S. aureus* has been described as a "low-grade" infectious process that triggers the innate immune response, which if impaired may not be able to adequately respond, thereby resulting in persistent colonization.[56] Several underlying medical

conditions have been shown to affect both the innate and adaptive immune response, including obesity.[50,57]

We were unable to detect a significant difference in the prevalence of *S. aureus* strain persistence among obese individuals as compared to non-obese. The implementation of this definition of persistence was intended to more accurately capture individuals who more closely displayed the biological characteristics now frequently associated with persistence including but not limited to longer length of time carrying the same strain.[9,14] Recent findings do suggest, however, that frequent strain replacement does occur even among persistent carriers, however, they still generally carry strains for longer periods of time than intermittent carriers.[15] Miller et al. observed a carriage time of four months or greater among species persistent carriers, however 12% of their sample carried the same strain for the duration of the study (3 years). Whether biological differences (e.g. bacterial load) exist between these long-term strain persistent carriers as compared to shorter-term strain persistent carriers remains to be determined.

In addition to the influence of obesity on persistent carriage, this study also demonstrated an independent effect of age on persistent carriage. This finding corroborates published literature with regard to younger age and species persistent carriage.[17] The mechanism by which chronologic age influences persistent carriage has yet to be elucidated. Unfortunately, we were unable to see an effect of gender or smoking as has been observed in studies evaluating *S. aureus* persistence.[16,18] We additionally did not observe an influence of diabetes, hypertension nor HIV on persistent carriage at the species level, however, given the small number of individuals diagnosed with each of these conditions, we were likely underpowered to detect a true difference.

Simpson's Index of Diversity revealed no significant differences in alpha diversity in persistent carriers as compared to intermittent carriers, even when stratified by mucosal site. Diversity is a measure of species/strain richness (number of species in a given population) and evenness of distribution.[58] This simple arithmetic measure of diversity measures the probability that two observations taken at random from a population will be of the same type.[58,59] Its utility has been shown in determining differences in diversity between methicillin susceptible *S. aureus* strains and methicillin resistant *S. aureus* strains[59] as well as differences in *S. aureus* diversity across populations,[60] however, it appears to be limited in its ability to differentiate between populations with high diversity.[59] This limitation is highlighted in our data and underscores the importance of taking into account not only diversity but also composition when assessing the differences between two populations. Simpson's Index also does not account for site-specific differences when evaluating diversity nor does it reflect the complex relationships that might be present in a given habitat. Using correspondence analysis we were able to demonstrate that certain strains were over represented among individuals persistently colonized in the nose. We were also able to demonstrate a substantial overlap in strains colonizing individuals that were persistently colonized in the oropharynx and intermittent carriers. These observations can help inform future hypotheses as to bacterial factors that might delineate affinities for certain mucosal sites. Ordination techniques such as correspondence analysis may serve as a good alternative or compliment to arithmetic measures of diversity that do not fully account for composition.

It is important to note that correspondence analysis is an exploratory technique and confirmation of these observations; which can be conducted using canonical correspondence analysis, the hypothesis driven alternative to multiple correspondence analysis, should be addressed in future studies.[42] Though correspondence analysis is rarely used in molecular

epidemiologic research, it has the potential to bolster molecular analyses for both exploratory and hypothesis testing endeavors. We recommend the use of this analytical tool particularly with the growing interests in microbial composition at different body sites and their interaction with their environment.

There were several limitations to this study. First, because of a small sample size there was limited power to evaluate factors associated with persistent colonization in this setting and therefore only factors with large effects on the outcome and a high prevalence in the setting were identified in the analysis. Despite the limited sample size, we were able to show that our exposure of interest, obesity, was associated with species persistent carriage, and our sensitivity study in which GEE analysis (Appendix Table A4.2) was used to take into account differences in covariate values across study visits demonstrated similar results as our primary analysis. Another limitation is the self-reported nature of our exposure of interest as well as some of the covariates included in the final mode. The outcome of interest however, is persistent colonization, and it is unlikely that inmates were aware of their carriage status and therefore any bias is likely non-dependent and non-differential and therefore attenuated the observed effect given the dichotomous nature of our outcome and the majority of our covariates. The quantitative bias analysis assessing misclassification of obesity supports these assertions and demonstrated that our results were robust to 10% misclassification and marginally robust to 15% misclassification (Appendix Table A4.1). Due to limitations in sample size, we were also unable to further stratify our exposure category by the presence or absence of markers of metabolic dysfunction, which we demonstrated in chapter 3 of this dissertation had an effect on *S. aureus* colonization. This likely attenuated our ability to detect a difference in both species and strain persistence as some obese individuals likely presented with no metabolic abnormalities. In addition, the arithmetic

measure of genetic composition provides a means of assessing statistical differences in *S. aureus* community structure across carriage types, however it did not take into account differences in environment across the different types. We were, therefore, able to determine whether the richness and abundance of *S. aureus* strains differ between the groups but were unable to distinguish reasons why. The multivariate analysis using correspondence analysis begins to answer this question. However, because it served as an exploratory tool rather than a hypothesis driven one, primarily because the chi-squared metric is not tested against an underlying distribution, we were unable to draw statistical inferences about the results. Lastly, our measure of persistent carriage may have led to some individuals being misclassified as persistent carriers. This misclassification would have made the two groups similar and thereby attenuate our ability to determine a significant difference in the covariates in our model. We were, however, able to demonstrate a significant influence of obesity on persistence, and additionally show that age, a factor that has been shown to be associated with persistent carriage was also significant, not only at the species, but also the strain level.[16] These finding suggest that our definition of persistence is likely capturing the underlying biology that differentiates persistent carriers from intermittent and non-carriers.

Despite the limitations listed above, this study had several strengths. First, it is the first study to our knowledge to evaluate persistent colonization with *S. aureus* within the correctional setting. This is particularly relevant given the burden of *S. aureus* colonization and infection within the correctional system. Furthermore, few studies have evaluated persistent *S. aureus* colonization in both the anterior nares and the oropharynx outside of health care settings, and this study will add to the broader literature trying to identify factors associated with persistent colonization. This study was also the first to evaluate both *S. aureus* genetic composition along

with host factors as predictors of persistent carriage in a prison setting. Though we were unable to draw inferences from this analysis, it was able to capture subtle differences in community structure were identified that could inform future studies that can be empirically tested. Finally, we evaluated the influence of obesity on persistent carriage, and show that it may have an influence in this setting.

In conclusion, the determinants of which phenotypic category of carriage an individual demonstrates are still largely unknown. The limited studies that have been conducted in non-clinical settings demonstrate that the phenomenon is multi-faceted involving environmental, genetic, as well as host immune factors. This study suggests that obesity may serve as a host factor that may influence persistent carriage, however, due to its small sample size and crude measure of persistent carriage based on few points of assessment per observation, corroboration is required. Future studies should evaluate the influence of obesity taking not only into account anthropometric measures of obesity but also methods that capture the underlying biology that places obese individuals at elevated risk of a range of conditions, including infections. The study also demonstrates the importance of assessing persistence at other mucosal sites. Approximately 10% of persistent carriage population would have been missed had we assessed only the anterior nares. In assessing the oropharynx the study further demonstrates using multivariate techniques that persistent nasal carriage is not only more closely associated with obesity than other types, but is associated with different *S. aureus* strains than other mucosal sites.

Table 4.1. Different patterns of *Staphylococcus aureus* Colonization Observed Over Time and the Nomenclature Used to Describe the Pattern

	Time Point 1 Anterior Nares	Time Point 1 Oropharynx	Time Point 2 Anterior Nares	Time Point 2 Oropharynx	
Pattern 1	✔	✔	✔	✔	Persistent both sites
Pattern 2	✔	X	✔	X	Anterior nares persistent
Pattern 3	X	✔	X	✔	Oropharynx persistent
Pattern 4	✔	X	X	✔	Intermittent
Pattern 5		✔	✔	X	Intermittent
Pattern 6	✔	✔	X	X	Intermittent
Pattern 7	X	X	✔	✔	Intermittent
Pattern 8	✔	X	X	X	Intermittent
Pattern 9	X	✔	X	X	Intermittent
Pattern 10	X	X	✔	X	Intermittent
Pattern 11	X	X	X	✔	Intermittent
Pattern 12	X	X	X	X	Non carrier

X = No isolate identified

✔ = *S. aureus* isolate identified

Table 4.2. Baseline Demographic, Behavioral and Medical Characteristics of New York State Maximum-Security Inmates Stratified by Carriage Phenotype of *Staphylococcus aureus* at the species and strain level, 2009-2014

	Species Persistent			Strain Persistent		
	Persistent Carriage[a] N=74 (27.0%)	Intermittent/Non Carriage N=200 (73.0%)	P Value	Persistent Carriage[a] N=48 (17.5)	Intermittent/Non carriage N=226 (82.5)	P Value
DEMOGRAPHICS						
Age mean age ± SD	34.2 ± 10.5	36.6 ± 10.1	0.0836	33.1 ± 9.6	36.6 ± 10.3	0.03
Female Gender	36 (48.7)	97 (48.5)	0.9826	21 (43.8)	112 (49.6)	0.46
Race						
Non-Hispanic White	19 (25.7)	52/198 (26.8)	0.715	11 (22.9)	61/224 (27.2)	0.92
Non-Hispanic Black	33 (44.6)	89/198 (45.0)		23 (47.9)	99/224 (44.2)	
Hispanic	20 (27.0)	45/198 (22.7)		12 (25.0)	53/224 (23.7)	
Other[b]	2 (2.7)	11/198 (5.6)		2 (4.2)	11/224 (4.9)	
Education			0.8635			0.70
< High school	21 (28.4)	61 (30.5)		16 (33.3)	66 (29.2)	
High School/Equivalent	30 (405)	74 (37.0)		19 (39.6)	85 (37.6)	
> High school	23 (31.1)	65 (32.5)		13 (27.1)	75 (33.2)	
BEHAVIOR						
Smoking	56 (75.7)	142 (71.0)	0.4427	35 (72.9)	163 (72.1)	0.91
Marijuana Use in Past 6 Months	11/73 (15.1)	20/198 (10.1)	0.2544	11/73 (15.1)	20/198 (10.1)	0.25
Crack/Cocaine Use Ever	29/72 (40.3)	98/199 (49.3)	0.1913	29 (40.3)	98 (49.3)	0.19
Heroin Use Ever	13 (17.6)	60 (30.0)	0.0388	8 (16.7)	65 (28.7)	0.08
History of Injection Drug Use	8/71 (11.3)	28 (14.4)	0.5144	5/45 (11.1)	31/221 (14.0)	0.60
Shares Personal Items	18 (15.8)	18 (11.3)	0.2729	5 (10.4)	31 (13.7)	0.54
< 7 Showers per Week	22 (29.7)	66 (33.0)	0.6067	13 (27.1)	75 (33.2)	0.41
Mean Time Between 1st and 2nd Interview (mean ± SD)	256 ± 239.9	298 ± 275.0	0.2577	182 ± 172.2	309 ± 277.4	0.00
MEDICAL						

Diabetes	7 (9.5)	14 (7.0)	0.4968	4 (8.3)	17 (7.5)	0.85
Hypertension	9/70 (12.9)	27/189 (14.3)	0.7679	6 (13.3)	30 (14.0)	0.90
HIV	2 (2.7)	13 (6.5)	0.2199	2 (4.2)	13 (5.8)	0.66
Liver Disease	8 (10.8)	37 (18.5)	0.1272	5 (10.4)	40 (17.7)	0.21
Kidney Disease	2 (2.7)	8 (4.0)	0.611	2 (4.2)	8 (3.5)	0.83
Systemic Antibiotic Use in Past 6 Months	31 (41.9)	67 (33.5)	0.1982	17 (35.4)	81 (35.8)	0.96
Topical Antibiotic Use in Past 6 Months	16 (21.6)	36/198 (18.2)	0.5209	11 (22.9)	41/224 (18.3)	0.46
Exposure of Interest						
Obese (BMI ≥ 30 kg/m^2)	36 (48.7)	78 (39.0)	0.1502	20 (41.7)	94 (41.6)	0.99

Definitions: obese BMI ≥ 30.0 kg/m^2; HIV, Human Immunodeficiency Virus

[a]Persistent carriage is defined by colonization of the anterior nares and/or oropharynx with *Staphylococcus aureus* at all time points assessed

[b]"Other" category includes Asian, Native Americans, Pacific Islander and persons of or two or more racial/ethnic groups

Table 4.3. Multivariable Model Assessing the Independent Effect of Being Obese On *Staphylococcus aureus* Species and Strain

Persistent Carriage among New York State Maximum-Security Inmates

	Species Persistent[A]		Strain Persistent[B]	
	Unadjusted PR (95%CI)	Adjusted PR (95%CI)	Unadjusted PR (95%CI)	Adjusted PR (95%CI)
BMI < 30kg/m2	Ref	Ref	Ref	Ref
BMI >= 30 kg/m2	1.33 (0.90, 1.96)	1.54 (1.01, 2.38)	1.00 (0.59, 1.68)	1.15 (0.60, 2.05)

[A]Controlling for age at baseline, gender, antibiotic use in the past six months, diabetes, hypertension and HIV

[B]Controlling for age at baseline, gender, antibiotic use in the past six months, diabetes, hypertension, HIV and crack/cocaine use

Persistent carriage is defined by colonization of the anterior nares and/or oropharynx with *Staphylococcus aureus* at all time points assessed

Species persistence is defined by colonization with any *Staphylococcus aureus* strain at all time points assessed

Strain persistence is defined by colonization with the same *Staphylococcus aureus* strain at all time points assessed

Definitions: PR, Prevalence ratio; 95%CI, 95% Confidence interval

Figure 4.1. Relative Abundance of the Fifty most Frequently Isolated *Staphylococcus aureus Spa* Types Isolated From New York

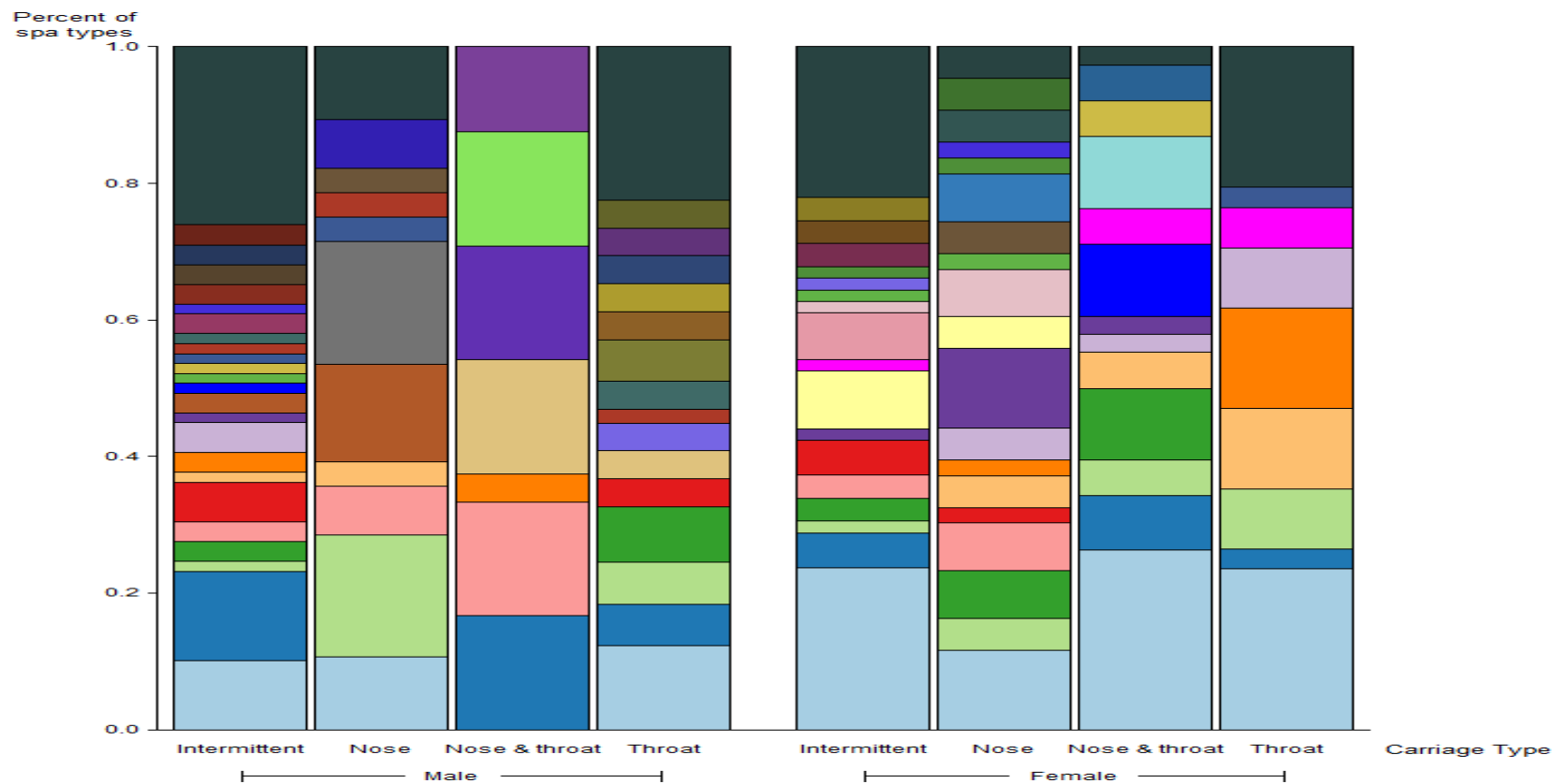State Maximum Security Inmates by Carriage Type

Figure 4.1. Illustrating the diversity of *spa* types isolated from participants assessed for longitudinal carriage of *Staphylococcus aureus*

Figure 4.2. Relative Abundance of the Fifteen most Frequently Isolated *Staphylococcus aureus Spa* Types From New York State Maximum Security Inmates by Carriage Type
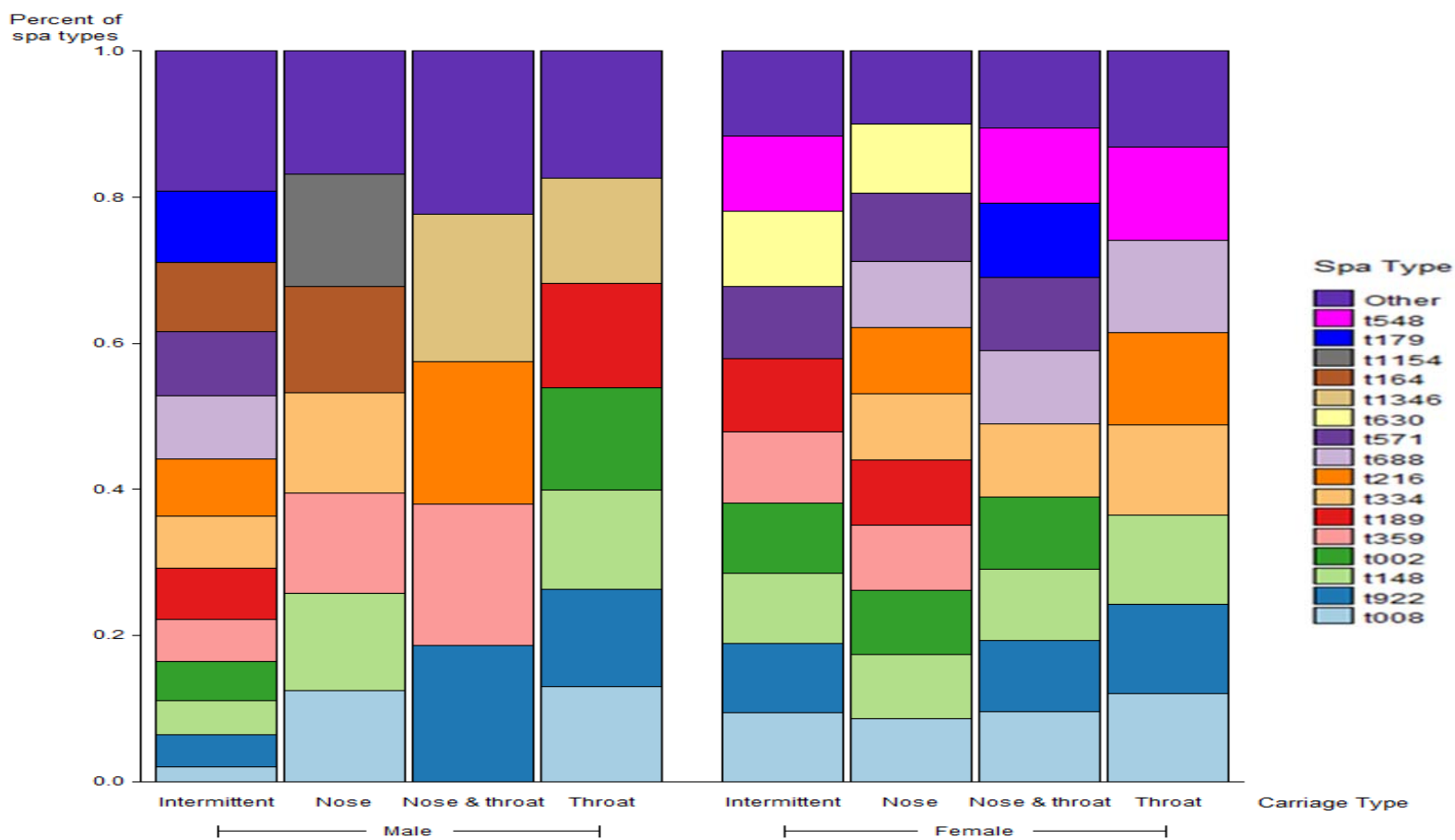

Figure 4.2. Illustrates the relative abundance of *Staphylococcus aureus spa* types isolated five or more times among participants assessed for longitudinal carriage of *Staphylococcus aureus*

Table 4.4. Alpha Diversity of *Staphylococcus aureus* Strains Stratified by Persistent Carriage Defined Globally and by Mucosal Site

	Simpson's Diversity Index	95% CI[b]
Global Persistence[a]		
Persistent	95.25%	(93.49, 97.02)
Intermittent	95.56%	(93.3, 97.76)
Persistence by Mucosal Site		
Colonized at Both Sites Persistently	92.35%	(88.15, 96.55)
Anterior nares Persistent	94.43%	(91.51, 97.34)
Oropharynx Persistent	93.54%	(89.86, 97.20)
Intermittent	95.56%	(93.37, 97.76)

[a]Global Persistence refers to individuals colonized with *Staphylococcus aureus* at the same mucosal site (anterior nares or oropharynx)

at every point of assessment time

[b]Definition: 95%CI, 95% Confidence Interva

Figure 4.3. The Distribution of *Staphylococcus aureus spa* Types by Persistent Carriage Type

Figure 2. Geometric biplots from a multi-way correspondence analysis assessing the distribution of *spa* clonal complex across levels of carriage type. The figure demonstrates that persistent nasal colonization is slightly different than other carriage types with regards to colonizing strains.

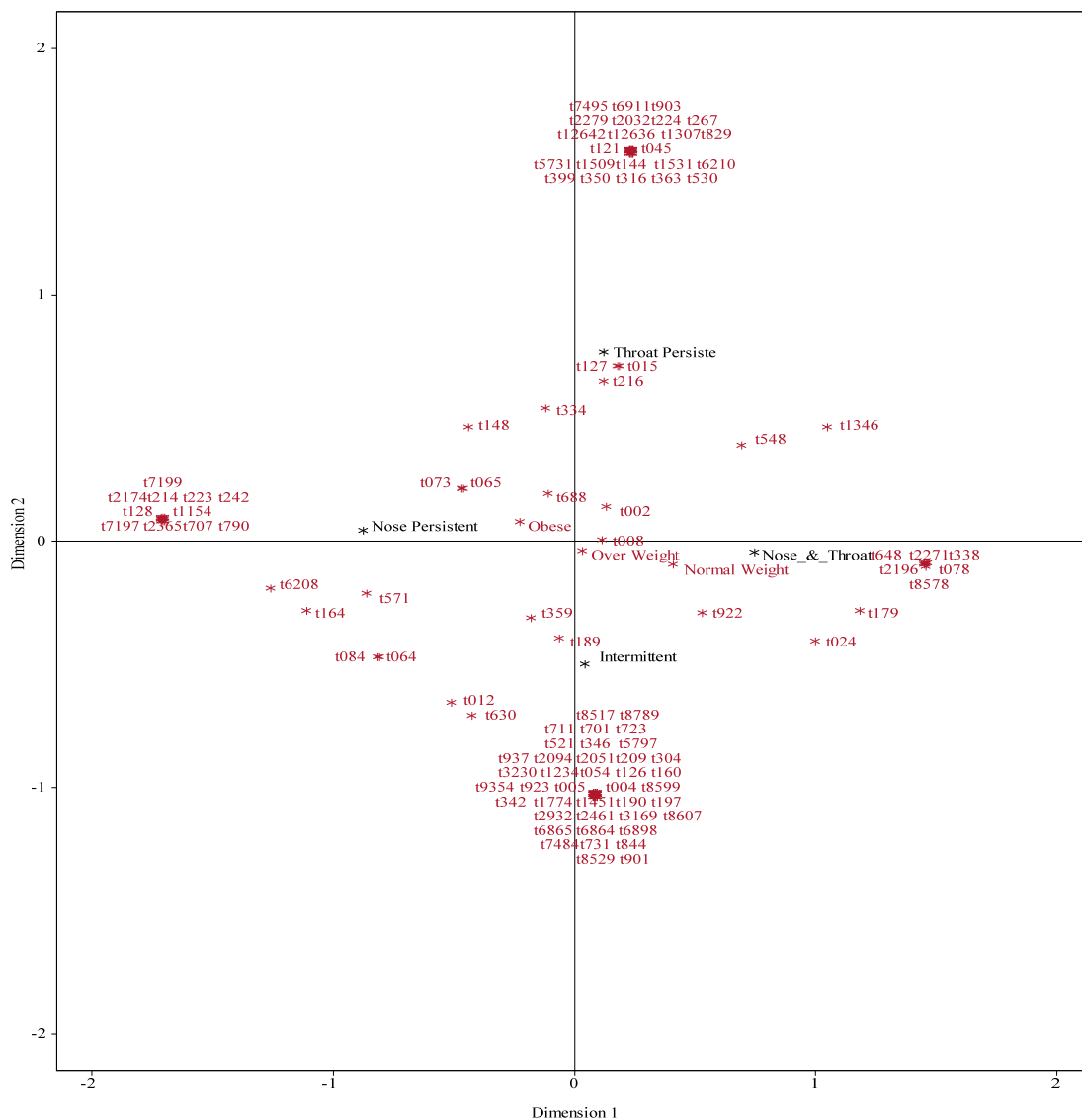**Figure 4.4.** The Distribution of the most frequent *Staphylococcus aureus spa* Types by Persistent Carriage Type
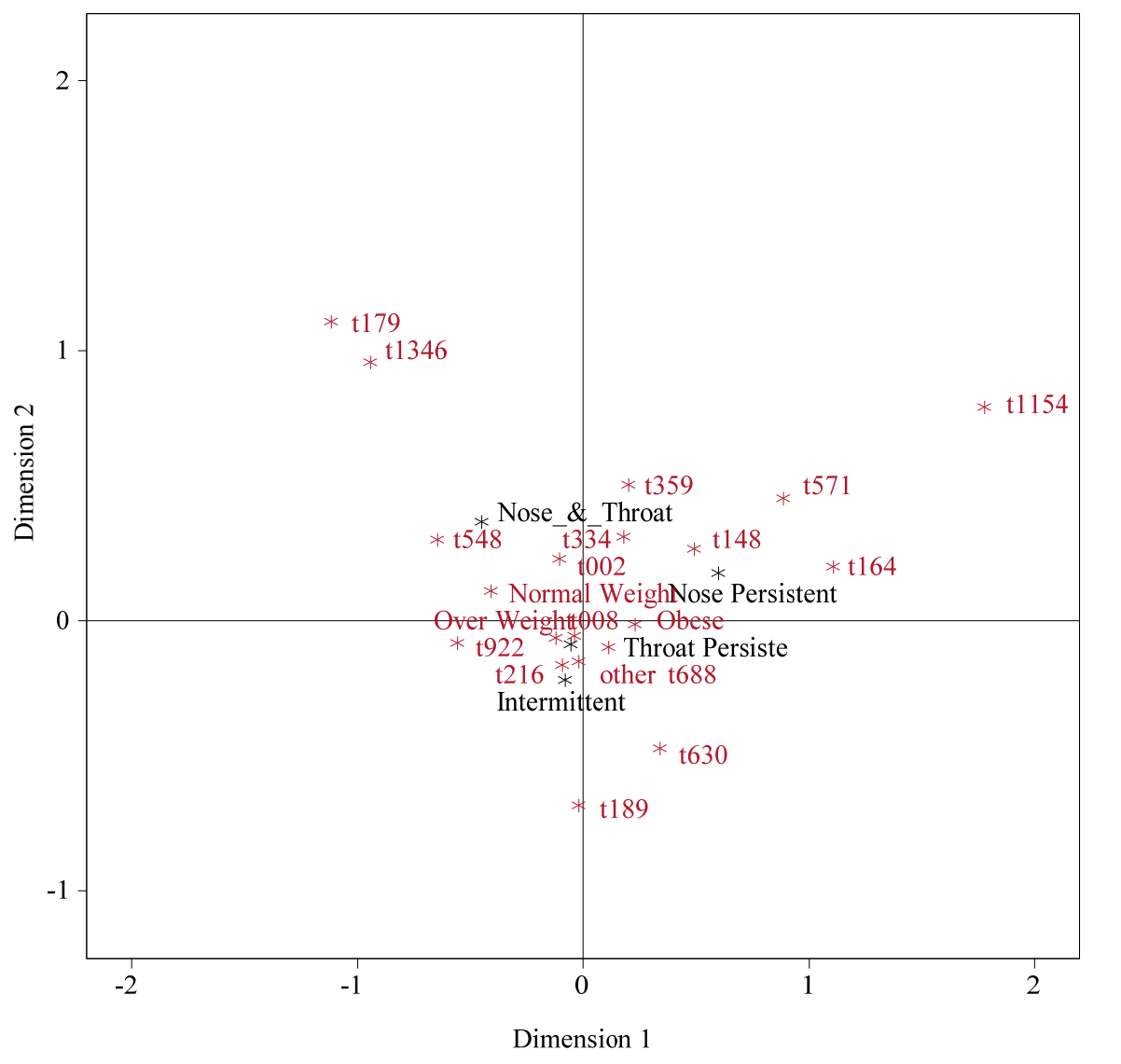
Figure 4. Geometric biplots from multi-way correspondence analysis assessing the distribution of spa types across levels of carriage

type. Dimension 1 corresponds to the most important underlying factor driving the distribution of the data points and dimension 2

corresponds to the second most important factor. The asterisks correspond to where specific *spa* types fall with respect to the

dimensions extracted and how they relate to the carriage type. To interpret the results, the broad direction of spread of the points from

top to bottom and left to right provide an indication of how similar or dissimilar the data points are with the center of the axis, which

represents the most common characteristics found in the data. The results suggest that individuals that are colonized in the nose are

distinct from other carriage types with regard to the *Staphylococcus aureus* colonizing strain

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Appendix 4

Table A4.1. Sensitivity Analysis Assessing the Influence of Misclassification of Body Mass Index Category on the Association between Obesity and Persistent *Staphylococcus aureus* Carriage

Characteristic	Observed	10% Misclassification[a]	15% Misclassification[a]
	Species Persistent[b] Adjusted PR (95%CI)	Species Persistent[b] Adjusted PR (95%CI)	Species Persistent[b] Adjusted PR (95%CI)
BMI < 30kg/m2	Ref	Ref	Ref
BMI >= 30 kg/m2	1.54 (1.01, 2.38)	1.44 (1.01,1.89)	1.42 (0.99, 1.84)

Total sample size for misclassification assessment was 263/274, due to missing covariate data

[a]Controlling for age at baseline, gender, antibiotic use, diabetes, hypertension and HIV

Persistent carriage is defined by colonization of the anterior nares and/or oropharynx with *Staphylococcus aureus* at all time points assessed

[b]Species persistence is defined by colonization with any *Staphylococcus aureus* strain at all time points assessed

Definitions: PR, Prevalence ratio; 95%CI, 95% Confidence interval

Table A4.2 Sensitivity Analysis Using Generalized Estimating Equations to Account for

Differences in Observed characteristics Across Visits.

	Species Persistent[a]		Strain Persistent[b]	
	Unadjusted PR (95%CI)	Adjusted PR (95%CI)	Unadjusted PR (95%CI)	Adjusted PR (95%CI)
BMI < 30kg/m2	Ref	Ref	Ref	Ref
BMI >= 30 kg/m2	1.33 (0.90, 1.96)	1.59 (1.10, 2.31)	1.10 (0.61, 1.76)	1.38 (0.83, 2.30)

Definitions: obese BMI $\geq$ 30.0 kg/m^2; HIV, Human Immunodeficiency Virus

[a]Controlling for age at baseline, gender, antibiotic use, diabetes, hypertension and HIV

[b]Controlling for age at baseline, gender, antibiotic use, diabetes, hypertension, HIV and

crack/cocaine use

Tables A4.3 Demographic, Behavioral, and Medical Factors of the Persistent Carriage Cohort as Compared to the Full Study

Population

	Persistent Carriage Subset N=274	**Entire Study Population N=2,829**	**P Value**
DEMOGRAPHICS			
Age mean age ± SD	35.9 ±10.2	36.6 ± 10.6	0.3529
Female Gender	133 (48.5)	1,357 (47.9)	0.8562
Race			
Non-Hispanic White	72 (26.3)	603 (21.3)	0.1911
Non-Hispanic Black	122 (44.5)	1,408 (49.8)	
Hispanic	66 (24.1)	647 (22.9)	
Other[a]	14 (5.1)	171 (6.0)	
Education			0.6733
< High school	82 (29.9)	914 (32.3)	
High School/Equivalent	104 (38.0)	1,064 (37.6))	
> High school	88 (32.1)	851 (30.1)	
BEHAVIOR			
Smoking	181/273 (66.3)	1,840 (65.0)	0.6766
Marijuana Use in Past 6 Months	31/274 (11.4)	313/2,813 (11.1)	0.8761
Crack/Cocaine Use Ever	107/268 (39.9)	1,061 (37.6)	0.4605
Heroin Use Ever	63/272 (23.2)	461/2,821 (16.3)	0.0042
History of Injection Drug Use	30/265 ((11.3)	252/2,799 (9.0)	0.2123
MEDICAL			
BMI mean ± SD	28.5 ± 5.2)	28.6 ±5.6)	0.7485
Diabetes	21/268 (7.8)	188 (6.6)	0.4578
Hypertension	46/68 (17.2)	511 (18.1)	0.7142
HIV	14/268 (86)	115 (4.1)	0.3641
Liver Disease	44/268 (16.4)	315 (11.1)	0.0098

Kidney Disease	10/268 (3.7)	59 (2.1)	0.081
Systemic antibiotic use	98 (35.8)	870/2,818 (30.9)	0.0954
Topical antibiotic use	52 (19.1)	468 (16.7)	0.3029
Outcome of Interest			
Colonized	132 (48.2)	1,334 (47.2)	0.7466

Definitions: obese BMI $\geq$ 30.0 kg/m^2; HIV, Human Immunodeficiency Virus

[a] "Other" category includes Asian, Native Americans, Pacific Islander and persons of or two or more racial/ethnic group

CHAPTER 5: Conclusions

Despite its ability to cause invasive disease such as necrotizing pneumonia,[1] at any given time *Staphylococcus aureus* is most commonly a commensal and comprises part of the resident flora of the human anterior nares in 25-30% of individuals worldwide.[2] In the majority of these individuals *S. aureus* colonizes their anterior nares persistently, whereas the remainders are only intermittently colonized.[2,3] Individuals colonized with *S. aureus* are at elevated risk of infection. In fact, in clinical settings, approximately 80% of *S. aureus* infections have been attributed to the endogenous colonizing strain,[4] and persistently colonized individuals appear to be at elevated risk.[5] Factors that influence *S. aureus* colonization are thought to be multifactorial and are host, bacterial as well as environmentally determined. Host determinants of *S. aureus* carriage include demographic (younger age, male sex),[6] behavioral (smoking,[6] injection drug use,[7,8] contraception use[9]), host environment (previous exposure to health care settings), genetic (genetic polymorphisms in genes that code for interleukin-4 (IL-4),[10] C-reactive protein (CRP), [10] and β-defensin 1[11]), immunological (antimicrobial peptide secretion,[12,13] and serum 25hyrdroxyvitaminD levels[6]) as well as host pathology (obesity,[14-16] HIV,[7] diabetes[16-18]). Unfortunately, the mechanisms by which many of these factors influence carriage are still poorly understood, and factors that determine carriage type (persistent vs. Intermittent) are still largely unknown. Despite these uncertainties, studies have consistently implicated both the innate and adaptive immune response in *S. aureus* carriage, and are thought to be a significant host determinant of colonization.[19]

Interestingly, many of the innate and adaptive immune factors that have been shown to influence *S. aureus* colonization are also impacted in obesity.[20-22] For example, circulating levels of 25(OH)D, which has a significant influence on the immune response,[22] are decreased among

obese individuals.[21] Similarly, IL-4, a cytokine whose levels are generally associated with an anti-inflammatory immune profile,[23] levels are also decreased in obese individuals.[24] Furthermore, obesity has been shown to influence *S. aureus* colonization,[14-16] however, the mechanism by which it elevates risk remains elusive and its influence on persistent *S. aureus* colonization, prior to this dissertation work, had yet to be evaluated. Among studies that have assessed the influence of obesity on *S. aureus* colonization, two pervading hypothesis have emerged. The first postulated mechanism of action suggests that the expansion of adipocytes due to obesity decreases the immune functionality of the tissue thereby facilitating infection and/or colonization.[25,26] An alternative hypothesis is that obesity may cause chronic-low grade inflammation, which is associated with a systemic impaired immune response.[26,27] These hypotheses are not mutually exclusive, and may be working in tandem to influence colonization. This dissertation begins to address the question of mechanism by addressing fundamental issues of measurement that may limit not only our ability to detect an association between obesity and *S. aureus* colonization, be it intermittent or persistent, but also our ability to differentiate among hypotheses about the mechanism by which it acts. It does so by not only recognizing the importance of granularity in our measurement of obesity, as has been advocated by many,[28] but also granularity in our measurement of colonization, particularly persistent colonization.[29]

Guided by a conceptual framework that interwove the effects of metabolic abnormalities and immune dysfunction frequently observed in the presence of obesity, this dissertation had two primary goals. The first was to determine whether sub-phenotypes of obesity based on body mass index (BMI) category and metabolic abnormalities would begin to elucidate the mechanisms by which obesity influence *S. aureus* colonization. The second goal was determine qualitatively whether a pronounced association would be observed between obesity and

persistent *S. aureus* carriage, a characteristic defined primarily by a compromised innate and/or adaptive immune response. These goals were accomplished by first conducting a systematic review to evaluating whether the chosen measure of persistent carriage affected the yield of persistent carriers in a sample population. We then used regression methods to isolate the effects of body mass index (BMI) category (normal weight, overweight, obese) stratified by metabolic health status on *S. aureus* colonization (inclusive of both intermittent and persistent carriers) in two maximum-security prisons in New York State. We then integrated our findings in Chapter one of this dissertation to our understanding of persistent carriage and defined persistence at both the species as well as the strain level, hypothesizing that the two measures were capturing distinct yet overlapping subpopulations of persistent carriers. Regression and multivariate ordination techniques were then used to evaluate the impact of obesity on *S. aureus* carriage type (persistent Vs intermittent) as well as explore any difference in *S. aureus* strain composition that may exist between persistent and intermittent carriers.

Summary of Study Findings

To better understand how to evaluate the influence of obesity on colonization (inclusive of both persistent and intermittent carriers) and persistent carriage exclusively, we first sought to determine whether a specific measure of persistent carriage captured the underlying biological characteristic better than others. To do this we conducted a systematic review to identify studies that evaluated persistent *S. aureus* carriage in non-institutionalized adults. The primary aim of the review was to assess the different methods used to measure persistent *S. aureus* carriage, and to determine whether variation in the measure affected the prevalence of persistent carriage observed. Chapter two of this dissertation summarized the results of the review. In short, the

review resulted in 19 articles that demonstrated considerable variation in how persistent *S. aureus* colonization was measured in non-institutionalized settings. The various definitions were collapsed into four general categories: culture positivity on all samples taken a minimum of one week and a maximum of a few months from one another,[11,29,30] two positive cultures taken approximately one week apart (culture rule[31]),[6,9,10,32-37] two positive cultures taken several months and sometimes a few years apart[38-42] and lastly culture positivity in at least 80% of all cultures taken[7,31] (carrier index rule[43]). Despite the variation in the definitions used, the prevalence of persistent carriage ranged from 16-34%. Even more interesting was the consistency in the range of persistent carriage estimates reported across the broad categories identified. A summary estimate of 11 of the 19 studies (excluding redundant studies and studies with a high risk of bias) resulted in an estimated prevalence of persistent carriage of 22% (95% CI 19%-25%). The results of the systematic review suggests that identifying persistent carriers may be relatively robust to the definition used, and having at least two swabs may be sufficient to differentiate persistent carriers from intermittent carriers. More importantly, the review suggested that two sup-populations of persistent carriers may exist and evaluating species persistence casts a wide net that captures all individuals carrying *S. aureus* consistently, whereas strain persistence restricts our assessment to those who may be particularly long term carriers of a given strain.

The third chapter evaluated whether BMI category (normal weight vs. overweight vs. obese) further stratified by presence and absence of comorbidities (diabetes, hypertension and HIV) was associated with *S. aureus* carriage, inclusive of both intermittent and persistent carriers. The primary hypothesis of this study was that abnormal metabolic health, defined by the presence of comorbidities, was partially driving the association between obesity and *S. aureus*

that we[14] and others[15,16] have described. Interestingly, our findings mirrored what has been described in the literature with regards to the sub-phenotypes of obesity and metabolic disease[44] in that normal weight individuals with metabolic abnormalities (MANW) had a higher likelihood of colonization than obese individuals without metabolic abnormalities. More specifically, the study demonstrated that MANW females had a significantly higher prevalence of *S. aureus* colonization than metabolically healthy normal weight (MHNW) females. A slightly lower but still significant increase in *S. aureus* prevalence was also observed among metabolically abnormal obese (MAO) females as compared to MHNW females. No association was observed among overweight females with or without markers of metabolic abnormalities or among metabolically healthy obese (MHO) females. Though the results partially supported our hypothesis, the negative finding among MHO females was surprising, given the reported association independent of diabetes[14-16] and pre-diabetes.[15] These studies, however, could have been further refined to control for hypertension, which may be interacting with other factors to elevate risk. It is also important to note that when the influence of metabolic abnormal health was evaluated by BMI category we observed a significant increase in prevalence in MANW females as compared to MHNW females, but did not observe a significant difference in MAO females as compared to MHO females. This suggests that though not statistically significant, the prevalence of *S. aureus* in MHO as compared to MHNW is likely not negligible. Other factors may, therefore, also be at play that place obese individuals at elevated risk of colonization. No association between normal weight, overweight, or obese males with or without markers of metabolic abnormalities and the prevalence of *S. aureus* colonization was observed.

A secondary aim addressed in the third chapter was the impact of the sub-phenotypes of obesity on site of colonization. We hypothesized that increasing comorbidity would result in

significant increase in exclusive anterior nares colonization. This analysis was based on preliminary results suggesting that anterior nares colonization was driving the observed association between obesity and the prevalence of *S. aureus* colonization among females.[14] The findings among female participants did not support our hypothesis; however, we did observe a marginally significant increase in exclusive anterior nares colonization among obese male inmates with metabolic abnormalities as compared to normal weight males with no metabolic abnormalities. The results of both the primary and secondary aims addressed in chapter three suggest that metabolic abnormalities might be driving the observed association between obesity and the prevalence of *S. aureus* colonization. More importantly, they suggest that sub-phenotypes of obesity might refine our ability to capture the underlying biological processes that are more prevalent among obese individuals and presumably drive its association with a multitude of conditions.

After determining the influence sub-phenotypes of obesity on *S. aureus* colonization, inclusive of both intermittent and persistent carriers, we then evaluated the influence of obesity on persistent carriage in the fourth chapter. This analysis was conducted to further characterize the association between obesity and the prevalence *S. aureus* colonization and the extent to which obese individuals are at risk. The results of our systematic review served as reassurance that two samples would perform well in capturing persistence. We attempted to assess persistence not only at the species levels, but also at the strain level under the assumption that the two measures were capturing slightly different but overlapping groups of persistent carriers. Under the guidance of the framework, we hypothesized that obese individuals would be at elevated risk of persistent carriage in either the anterior nares and/or oropharynx. The results demonstrated that approximately 27% of the population were persistent carriers at the species

level and 17% were persistent carriers at the strain level. Had our assessment been restricted to the anterior nares, 10% of individuals that were persistently colonizes in the oropharynx would have been misclassified as intermittent/non-carriers. Obesity was independently associated with species persistence but not strain persistence.

The fourth chapter additionally addressed the distribution of *S. aureus* strains as differentiated by spa typing across carriage types. Specifically, carriage patterns were further characterized by evaluating the molecular characteristics of the colonizing strains using two different methods. The first method, Simpsons Index of Diversity focused primarily on richness (i.e. number of different strains represented in the sample), whereas the second method, correspondence analysis, focused primarily on composition. Diversity of *S. aureus* strains as assessed by Simpson's Index did not differ among persistent as compared to intermittent carriers. When carriage was further stratified by site of colonization (nares persistent, oropharynx persistent, intermittent), the differences remained null. Correspondence analysis, however, demonstrated differences in strain composition among persistent nares carriers as compared to all other carriage/mucosal site types. The assessment also demonstrated that when restricted to strains that appear at least twice in the data set, persistent oropharynx carriers were similar to intermittent carriers at any mucosal site. Given the *a priori* exploratory nature of the correspondence analysis as a hypothesis-generating tool, future studies need to confirm findings, but the overall results suggest that correspondence analysis might be better suited to evaluate compositional differences in sub-populations with highly diverse strains.

Implications and Future Directions

The results of this study suggest that metabolic abnormalities, which are more prevalent in obese individuals, may partially account for the association observed between obesity and *S. aureus* carriage. The association appears to get stronger when persistent carriage is assessed, and may have differential effect on the different epithelial sites frequently assessed for *S. aureus* colonization. The overall study results have implications regarding how obesity is measured and operationalized in infectious disease research, the importance of addressing different mucosal sites, the importance of molecular biology in our assessments, and lastly to the extant literature evaluating sub-phenotypes of obesity

Individual and Public Health Implications

First and foremost, the study suggests that MANW and MAO individuals, particularly females, are at elevated risk of *S. aureus* colonization, thereby by placing them at risk of infection by the organism. The public health impact on MAO individuals, however, will be more pronounced. The above observation is due to the fact that only a moderate relative increase in carriage prevalence was observed for both MANW and MAO individuals, however, the relative prevalence of the two phenotypes are very different. Current estimates suggest that 1.9 billion adults worldwide are overweight and over 600 million are obese.[45] In the United States, over 30% of adults 20 years of age or older are obese, and rates are higher among Hispanics (42%) and non-Hispanic Blacks (48%).[46] The prevalence of MAO among all individuals defined as obese, primarily based on BMI, ranges from 51-70%.[47,48] Given the absolute number of individuals who are obese or at risk of being obese and the relative prevalence of MAO among obese individuals, a substantial number of individuals are therefore at risk of carriage and therefore subsequent infection. In contrast, only 23%[48] of normal weight adults are considered MANW and rates as low as 7% have been reported. Should the study findings be validated in

another population, the relative prevalence of the different sup-phenotypes should be taken into account if any public health interventions are subsequently assessed.

The relative increase in *S. aureus* carriage among MANW and MHO individuals demonstrated by the study results may also have global implications for the microbial composition of epithelial surfaces in these individuals. Epithelial surfaces form the interface between the host and its exterior environment, and are therefore the main route of entry for microbial organisms.[49] Important changes in epithelial tissue have been observed amongst obese individuals, and the microbial composition at these sites has been shown to differ between obese and non-obese individuals.[50-52] Composition has also been used to distinguish individuals with impaired glucose control from those with no impairment,[53] providing further evidence of compositional differences at epithelial sites in the presence and absence of disease. Though this evidence for microbial compositional changes in the presence of obesity have focused primarily on the gut microbiome, studies are accumulating that suggest compositional shifts observed in one epithelial surface might be reflective of compositional shifts in other areas. Specifically, Zhang *et al.* observed that dysbiosis in gut microbial composition in the presence of rheumatoid arthritis was reflected in the oral and saliva microbiome as well.[54] Furthermore, host genetic variation, which has limited effects on the microbiome as compared to diet, the environment and disease, does influence microbial composition across different body sites[55] lending credence to the hypothesis that dysbiosis at one site could be an indicator of dysbiosis at other sites. More specific to our topic and the implications therein is the idea that the dysbiosis we observe in gut microbiota in obesity may be an indication of shifts in the microbiome of other epithelial surfaces such as the anterior nares and/or oropharynx making those surfaces more susceptible to *S. aureus* colonization as well as other opportunistic pathogens. This hypothesis is supported by

161

the fact that studies assessing *S. aureus* colonization within the context of the entire microbial composition of the anterior nares have linked distinct phylotypes to the presence or absence of the organism.[56] Future studies should include assessments of the entire microbiome of the epithelial sites being assessed.

Research Implications

In addition to the individual and public health implications of these findings are those relevant to how obesity is evaluated in research settings, and the most salient to this particular study is its operationalization in studies related to infectious outcomes. The biology of obesity is complex and displays a heterogeneous effect on health. The methods used to measure obesity may account for the variability, as each measure makes an implicit assumption about the distribution or composition of adipose tissue that may not be consistently met. For example, BMI makes an implicit assumption of even distribution of adipose tissue. However, research demonstrates that body fat distribution varies at the minimum by sex and age.[57] Though the use of BMI to report secular trends in obesity is likely appropriate, its use to estimate the effects of obesity on different disease states might be flawed due to its imprecision in capturing the biological processes driving the disease.[58] Studies assessing the influence of obesity on metabolic complications take these factors into account, however to date, no study assessing the influence of obesity on susceptibility to infectious organisms has addressed the limitations of BMI. Understanding the heterogeneity associated with each measure of obesity as it relates to the host's susceptibility to infectious organisms might elucidate some of the mechanisms driving the relationships.

Our study provides proof of concept that sub-phenotyping obesity might be relevant in its assessment of infectious disease outcomes. Future studies assessing obesity and infectious

outcomes should implement a method of sub-phenotyping to not only provide support for the underlying biological framework, but to add a degree of granularity so that small but important associations are not overlooked. Even more important, but much more costly would be the incorporation of methods that measure body composition[59] in an attempt to fully describe the differences observed in this and other studies[14,15] between males and females with regards to excess adiposity and carriage.

Another methodological implication highlighted by these results is the importance of not only considering species/strain richness but also composition when evaluating molecular biology as it relates to specific outcomes. Diversity is a measure of species/strain richness (number of species in a given population) and evenness of distribution.[60] Many studies use simple arithmetic measures of diversity such as Simpson's Index, which measures the probability that two observations taken at random from a population will be of the same type, to evaluate strain diversity within a given population.[60-62] Simpson's Index is easily calculated and is the most frequently used arithmetic indices of diversity. Unfortunately, like other arithmetic diversity indices, it does not take into account site-specific differences when evaluating diversity nor does it reflects the complex relationships present in a given habitat. Multivariate ordination techniques such as correspondence analysis have the flexibility to take into account these complexities.[63] These techniques have been used to evaluate species/strain composition in the field of ecology for some time, and are only now being implemented in microbiology to evaluate strain composition.[64-66] The goal of correspondence analysis, as well as other ordination techniques, is not to test a hypothesis about the data structure, but to merely describe the data structure more concisely. Correspondence analysis was able to show genetic compositional differences across carriage types (i.e. anterior nares persistent vs. oropharynx persistent vs. Intermittent), where

163

Simpson's Index of Diversity was not able to detect differences in strain diversity by carriage type.

The final research implication is in how persistent *S. aureus* carriage is defined. With respect to this issue two salient points need to be addressed. The first point relates to the relative benefit of assessing strain persistence as compared to species persistence. In her comprehensive study evaluating the acquisition and loss of *S. aureus* carriage over a three-year period, Miller et al. questioned the existence of a truly persistent carriage type.[29] The primary reasoning behind their conclusion, despite the 12% of individuals that carried the same strain throughout the three-year period, was the consistent and linear rate of carriage loss observed throughout the study. A recent study challenged their conclusion suggesting that strain replacement is not uncommon even among persistent carriers; however, the length of time in which strains are carried is significantly longer in persistent carriers as compared to intermittent carriers.[67] This and many other factors make them distinctly different from individuals described as intermittent carriers. The level of granularity used to measure persistence, we argue is likely dependent on the question being assessed. If the goal is to identify individuals at risk of infection, then casting a wide net to include individuals who are consistently colonized, i.e. primarily colonized due to frequent exposure or other biological factors, as well as "true" persistent carriers, i.e. individuals that demonstrate higher bacterial loads and longer carriage times, is warranted. In this instance species persistence may suffice. If, however, the research aim is to determine factors associated with the biological characteristics of persistent carriage, then granularity in the measure such as refining the definition of persistence to at the strain level, may be warranted. However, the need to restrict the definition to capture individuals carrying the same strain indefinitely may overlook

important differences that have already been described between long time carriers at the species level and intermittent carriers.

The second issue that requires attention is the need for more studies evaluating *S. aureus* persistent carriage at other mucosal sights. By including an oropharyngeal assessment we were able to not only increase our detected prevalence of persistence, but also show that compositional differences may exist in strains that colonize persistent nasal carriers as compared to oropharyngeal carriers. Only one other study was identified that evaluated persistence in the oropharynx in non-clinical settings. However, direct comparison with our findings was hindered due to the inclusion of children, whose epidemiology of *S. aurues* differs substantially from that of adults, in their sample population.[68]

Broader Implications of study findings

The study findings also have broader implications with regard to controversies surrounding the practice of using sub-phenotypes of obesity to assess health outcomes. Critics of the use of sub-phenotypes argue that a lack of consensus in how the sub-phenotypes, particularly MHO, are defined hinders comparison of results across studies.[69] For example a study implementing three definitions of MHO reported an observed prevalence ranging from 8.5% to 44% across the definitions.[70] In addition, inconsistencies in study findings, particularly with regards to the association between MHO with all-cause mortality, have been observed across the numerous definitions.[71-73] Our study adds to this complexity, in that we were unable to utilize established measures of metabolic health, but relied on the diagnosis of metabolic diseases to capture the biological processes of interest. In doing so we likely captured individuals on the tail end of what is likely a spectrum of risk rather than identifying MHO as a benign condition as implied by the designations. Never the less, we were able to show that introducing these sub-

phenotypes offered new insights for our infectious outcome. We also provide support for the argument[74] that, though imperfect, these sub-phenotypes not only highlight the physiological differences represented in what we view as homogenous condition, but that these differences have a measurable influence on health outcomes.

The fluidity in the concept of MHO is another aspect of the controversy that warrants mention and plays a significant role within the context of infectious outcomes. We use fluidity here to describe the mounting evidence suggesting the transiency of the MHO phenotype.[75,76] Indeed, in long term follow up studies, a large proportion of individuals described as MHO at baseline transition to MAO and eventually demonstrate similar health outcomes as those described as MAO from baseline.[75,76] The fluidity is particularly pronounced among young adults and appears to become more stable in older adults.[75] Critics have used this fluidity when MHO is assessed in the long term to argue against its use.[69] It is important to emphasize, however, that short term differences have been observed.[75] More importantly, in the case of infectious outcomes, particularly acute infections/outcomes where the short term risks are of specific interest, factors that add a degree of granularity in our measures may improve our ability to detect subtle differences that would otherwise be overlooked. In addition, interventions to prevent MHO individuals from becoming MAO may be of substantial utility.

A parallel debate in which these results play into is the concept of cardio respiratory fitness (CRF) as compared to fatness and health outcomes. Advocates of the fitness hypothesis argue that CRF is more important in determining poor health outcomes such as heart failure[77] than fatness as defined by BMI. Even when fitness is assessed within categories of obesity sub-phenotypes, an effect of fitness is observed.[78] The concept of fitness provides an even more nuanced view of adiposity and sheds further light on the degree of heterogeneity encompassed in

166

what is traditional viewed as a benign concept (i.e. obese vs. not obese). Whether additionally assessing fitness would further improve research on health outcomes as they relate to infectious disease remains to be determined, and warrants investigation.

In conclusion, the epidemiology of obesity is evolving. Though traditionally considered a risk factor for metabolic conditions such as insulin resistance and chronic disease, evidence now also indicates that obesity increases vulnerability to colonization and infection by infectious organisms such as *S. aureus*.[15,16] Inconsistencies, however, with regard to *S. aureus* as well as other infectious organisms have been observed. Reconciling these inconsistencies would provide the field with a clearer path forward, and one way to reconcile the inconsistencies is to introduce a degree of granularity in our measures of obesity. Sub-phenotypes of obesity could introduce the necessary granularity, and should therefore be incorporated in research assessing obesity and infections. We provide a proof of concept with this dissertation, and also discuss several factors that need to be taken into account in future work. Given the growing prevalence of obesity and the increasing evidence suggestive of its interaction with both the benign and opportunistic pathogens that comprise the micro flora of different human mucosal sites, understanding how obesity may increase vulnerability to these organisms is critical.

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