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BY
RYAN LEE MORK

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Table of Contents

List of Figures

List of Tables

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Abstract

Staphylococcus aureus, a Gram-positive prokaryote, is the most common human pathobiont, capable of asymptomatic colonization and infection in the majority of the human population. *S. aureus* causes infections in both healthy and hospitalized individuals, with a markedly high rate of recurrence. Colonization of host by the pathogen serves as a primary risk factor for initial and recurrent infections. Host individuals can be colonized with *S. aureus* across multiple body sites, commonly shed the organism in their local environment, and transmit *S. aureus* to nearby contacts. Multiple epidemiological and statistical studies have indicated that efficient prevention of *S. aureus* skin and soft tissue infections (SSTIs), as well as invasive infections, requires a deeper understanding of *S. aureus* colonization dynamics in the community and especially in the household.

In order to provide the most comprehensive picture to date of *S. aureus* colonization dynamics, we have followed 150 households, comprising 692 individuals over a 12-month period. We recorded the history of *S. aureus* infection in these individuals, pairing information about host demographics and behavior with temporal colonization status at three body sites, pathogen environmental sampling, and molecular typing. We specifically traced the appearance and distribution of the *S. aureus* strains causing previous and interval SSTIs in the household and found these strains commonly present on household members and their local environment months after the initial infection. By combining this information with family pedigrees, we have further found that both colonization and infection risk are nearly evenly split between genetic and behavioral factors.

Through our analysis, three major household *S. aureus* colonization dynamics types have been delineated and defined: (1) *introductions,* the appearance of a novel strain types across one

or more individuals and environmental sites, (2) *transmissions*, the acquisition of a new strain type on a household member previously present within the household, and (3) *persistence events*, the continued presence of a strain type on an individual. These distinct phenomena share partially overlapping behavioral and hygiene-related factors, many of which were previously reported as risk factors for *S. aureus* colonization. We propose that colonization risk can be represented as the combination of individual susceptibility factors to these dynamics. Successful *S. aureus* colonization and infection intervention strategies must accordingly embrace a comprehensive set of prevention methods that are tailored to both individual and household attributes.

Chapter 1: Introduction

Scientific advancement in the 20th and 21st century is a story of unprecedented mastery over infectious disease—of the conquest of dozens of the most common ailments that have historically led to the suffering and death of a significant portion of humanity. The first major advancement was the vaccine in the early 1800's, which lead to the near eradication of smallpox, polio, as well as a dramatic reduction in influenza in the developed world within a century (Pasteur 1881; Van Sant 2007). Next, the discovery of antibiotics in the 1940's meant there was now a cure for a huge swathe of bacterial infections, such as pneumonia, strep throat and diarrhea, which previously were the most common cause of physical disabilities and death, especially in children and the elderly (Lesch 2007; Waksman and Woodruff 1942). When new diseases arise, scientific minds are quick to develop solutions. Within five years of the identification of the human immunodeficiency virus (HIV), the first life-saving treatment, azidothymidine (AZT), was developed, approved for treatment, and deployed to save the lives of thousands of patients in the United States (Kazanjian 2014; Mitsuya et al. 1985).

Still, numerous infectious diseases have eluded the comprehension of modern scientific medicine. One common class of diseases are those that, while treatable via antibiotics, result in chronic or recurrent infections. *Clostridium difficile*, *Pseudomonas aeruginosa* and *Escherichia coli* can all cause these infections, and often in critically ill or hospitalized individuals (Tang and Stone 2017; Gellatly and Hancock 2013; Furukawa, Kuchma, and O'Toole 2006). Another class are organisms capable of long-term, asymptomatic colonization and dissemination in healthy individuals. *S. aureus* infections fall into both of these categories, and although has been studied for over a century, is still among the most common causes of infections in both hospitalized individuals and in the community (Dayan et al. 2016; Berube and Bubeck Wardenburg 2013).

Treatment and eradication of *S. aureus* infections in the community is challenging in terms of the variety of disease manifestations, the myriad virulence factors employed by the bacterium to cause these infections, and critically, the capacity of the organism to persistently colonize human hosts and contaminate their local environment (Creech, Al-Zubeidi, and Fritz 2015; Sampedro and Bubeck Wardenburg 2017). These individuals and fomites serve as possible reservoirs that lead to numerous, subsequent infections in exposed individuals. Prevention and treatment of these infections, therefore, requires an understanding of how biological and sociological factors both contribute to acquisition and persistence of *S. aureus* colonization and infection amongst individuals and how intervention strategies can be crafted that successfully interrupt these processes in a real-world setting. Here, we specifically focus upon the study of *S. aureus* epidemiology in the household to develop personalized prophylactic measures that, by reducing the incidence of infection within individual households, will ultimately curtail serious disease both in the community and in the hospital setting.

S. aureus Colonization of Humans

Asymptomatic colonization of *S. aureus* is commonly observed in healthy humans across numerous body sites. *S. aureus* is most typically found in the anterior nares, but can also be found colonizing moist body sites, including the inguinal folds, axilla, and sparsely across the human skin (Human Microbiome Project Consortium 2012; Elizabeth A. Grice and Segre 2011). Throat colonization in the absence of nasal colonization has also been observed in both healthy individuals in the community as well as inpatients (Mertz et al. 2009).

The nares are recognized as the primary reservoir for *S. aureus* colonization in humans, since this represents the most common site of colonization and the site with the highest number of documented adaptations enumerated by *S. aureus* for successful colonization (Sakr et al.

2018; Brown et al. 2014); however, exclusive sampling of the nares has been estimated to miss 50% of those colonized (Loren G. Miller et al. 2012). Non-nasal colonization does significantly increase the risk of nasal colonization (Harbarth et al. 2000), indicating that there may be significant intra-site cross-colonization in humans.

Across the population, three major categories of nasal colonization status have been delineated: 'non-carriers', 'intermittent carriers', and 'persistent carriers' (Kluytmans, van Belkum, and Verbrugh 1997). *S. aureus* can be found transiently colonizing the nares of 20-30% across a healthy population, which has been considered a combination of intermittent and persistent carriers (Brown et al. 2014; B. J. Chen et al. 2017). In longitudinal studies, the observed incidence of persistent *S. aureus* colonization was 10-30%, and non-carrier status was 10-47% (Muthukrishnan et al. 2013; J. L. Nouwen et al. 2004; VandenBergh et al. 1999). However, the definition of a 'persistent carrier' varies significantly across studies, from a strict definition of colonization at all samplings to a loose definition of colonization at two consecutive samplings, with persistence duration observed from between 70d-8y (VandenBergh et al. 1999).

Evidence suggests that since persistent and intermittent carriers are colonized by similar *S. aureus* strains, a complex interaction of strain morphology and the health and genetic status of the carrier determines whether an individual is persistently colonized (Muthukrishnan et al. 2013). Artificial inoculation of *S. aureus* into the anterior nares found subsequent survival significantly divergent across non-carriers, intermittent carriers, and persistent carriers (4, 14, and 154d, respectively) (van Belkum et al. 2009). This same study also observed significant differences in humoral immune response to *S. aureus* antigens between persistent carriers and other carriers.

Acquisition of *S. aureus* primarily occurs as the result of physical contact with contaminated skin or fomites (Loren G. Miller and Diep 2008; M. F. Davis et al. 2012). This process can occur extremely early in life, with infants becoming colonized by maternal strains within two weeks after birth (Peacock et al. 2003). Contact with *S. aureus* does not necessarily lead to colonization nor persistent carriage. A minority of individuals remain colonized with *S. aureus* after recovery from *S. aureus*-associated infections (Loren G. Miller et al. 2012). Medical students exposed to *S. aureus* in the clinical setting was not observed to significantly increase *S. aureus* colonization (C.-S. Chen, Chen, and Huang 2012). Persistent *S. aureus* nasal carriage may further protect against the acquisition of new strains (Kluytmans, van Belkum, and Verbrugh 1997; J. Nouwen et al. 2004), although alterations of strain composition has been observed within persistent carriers longitudinally (Muthukrishnan et al. 2013).

Canonical, independent individual risk factors for *S. aureus* colonization include age, hospitalization, and skin conditions. Newborns and children tend to exhibit higher incidence of intermittent and persistent nasal carriage (Armstrong-Esther 1976), and newborns exhibit an initial carriage incidence of 40-50% which falls to 21% at 6mo (Peacock et al. 2003). Methicillin-resistant *S. aureus* (MRSA) colonization incidence has been found significantly higher in individuals with healthcare exposure or healthcare-associated risk factors (Salgado, Farr, and Calfee 2003). Individuals suffering from chronic skin conditions such as atopic dermatitis and psoriasis exhibit markedly higher incidence of *S. aureus* colonization (C. Y. Ng et al. 2017; Hill and Imai 2016; Totté, van der Feltz, Hennekam, et al. 2016; Breuer et al. 2002). Notably, there have been several studies that have connected specific genetic polymorphisms to increased incidence of *S. aureus* colonization, including the glucocorticoid receptor, vitamin D receptor, interleukin-4, and complement inhibitor proteins (Ruimy et al. 2010).

S. aureus Infections in the Community and the CA-MRSA Epidemic

Although *S. aureus* is associated with a wide variety of disease manifestations in the hospital setting, skin and soft tissue infections (SSTIs), including recurrent infections, are the most commonly observed in the community (community-associated, CA) (King et al. 2006). These infections are most commonly caused by MRSA, as opposed to methicillin-sensitive *S. aureus* (MSSA) (Dantes et al. 2013). Worldwide, *S. aureus* is the leading cause of skin and soft tissue, bacteremia, device-related, osteoarticular, pleuropulmonary, and surgical site infections (Tong et al. 2015). SSTI's account for the vast majority of CA-*S. aureus* infections, followed by respiratory tract and bloodstream infections (80, 93%, 13%, 6%, 4%, 1%, CA-MRSA and CA-MSSA, respectively, n=204) (S. L. Davis et al. 2007). *S. aureus* SSTI's are characterized by a cutaneous abscess, although other, more severe disease manifestations can occur, such as necrotizing fasciitis and pyomyositis (Tong et al. 2015). Although invasive MRSA versus MSSA infections have been associated with significantly higher mortality, it is unclear whether this is the result of increased lethality of MRSA versus individuals typically exposed to and colonized by MRSA exhibiting lower quality of health and an increased number of chronic health conditions (Sakr et al. 2018).

S. aureus colonization has been repeatedly demonstrated as a primary risk factor for *S. aureus* infections, both superficial and invasive in the hospital and community setting. In the hospital setting, *S. aureus*, and specifically MRSA, colonization is associated with a significant increased risk of bacteremia (Wertheim et al. 2004), surgical site infections (Kalmeijer et al. 2000; Muñoz et al. 2008), and ventilator-associated pneumonia (Honda et al. 2010). Forty-eight-hour *S. aureus* infection risk for colonized patients in a prospective cohort admitted to the ICU was estimated at 2.5-4.7-fold (Honda et al. 2010). Further, MRSA colonization compared to

MSSA colonization has been specifically associated with increased risk of invasive infection in patients (Safdar and Bradley 2008).

For healthy individuals, nasal *S. aureus* carriage was found to be a significant risk factor for *S. aureus* SSTIs (Chou et al. 2015) and recurrent furunculosis (Demos, McLeod, and Nouri 2012). A recent meta-analysis of 12 articles covering 6998 subjects further estimated *S. aureus* and MRSA infection risk of colonized individuals at 1.8 (95% Confidence Interval [*CI*] 1.2-2.9) and 7.1 (95% *CI* 4.6-10.8); however, MSSA specifically did not share such an association (M. W. Kim et al. 2018). The incidence of invasive disease compared to asymptomatic colonization has been estimated at 1:1000; indicating that while SSTI's are common, the most common modality and selective pressures experienced by *S. aureus* is colonization and dissemination between hosts (Laupland, Ross, and Gregson 2008, 2000–2006; Brown et al. 2014).

Recurrent infections are common in those suffering from *S. aureus* SSTI's, even when initial treatment is successful (Creech, Al-Zubeidi, and Fritz 2015). Risk factors for recurrence has been attributed to a complex interaction of individual, environmental, and strain-level factors, in addition to the therapy administered at initial infection (Creech, Al-Zubeidi, and Fritz 2015). *S. aureus* colonization at multiple body sites has been identified in multiple studies as a primary risk factor for recurrent infection, indicating that recurrent infection may be the result of a high degree of *S. aureus* exposure (Kaplan et al. 2014; Fritz, Hogan, Hayek, Eisenstein, Rodriguez, Epplin, et al. 2012). There is evidence that the severity of infection may also influence recurrence, with individuals suffering from invasive *S. aureus* infections exhibiting a more robust, protective immune response (Fritz et al. 2013). Critically, 90% of recurrent infections in a recent community pediatric population were attributed to the same strain type as

the primary infection (Al-Zubeidi et al. 2014), highlighting the need to develop intervention strategies beyond treatment of the primary infection to prevent subsequent infections.

The CA-MRSA Epidemic in the United States

The contemporary CA-MRSA 'epidemic' in the United States has led to a significant rise in both superficial and invasive infections in the community in the past 20 years (DeLeo et al. 2010). Starting in the 1990's, reports of infections associated with MRSA, traditionally isolated to individuals with healthcare-associated risk factors, appeared in the community, especially in children presenting with SSTI's (Tong et al. 2015). These strains were collectively dubbed 'CA-MRSA' by the scientific community, and although there are several strains (definitions varying by molecular typing technique employed), USA300 quickly became the dominant clone in the United States associated with CA-MRSA SSTI's (King et al. 2006). The success of this clone has been attributed to alterations to the structure and regulation of several virulence factors associated with increasing severity of both superficial and invasive disease (Tong et al. 2015). Since similar rises in CA-MRSA colonization and infections were not observed in other developed nations such as Switzerland and Europe in this time frame, the epidemic CA-MRSA phenomenon may be unique to the United States (David and Daum 2010).

There is significant evidence that rather than supplanting *S. aureus* colonization, CA-MRSA increased the overall burden of MRSA colonization and SSTI; with a ballooning of specifically MRSA infections and across several United States cities as well as MRSA colonization, even when overall *S. aureus* nasal carriage decreased (David and Daum 2010). USA300 specifically may be more transmissible in households compared to other *S. aureus* strains (Loren G. Miller et al. 2012). Compared to other MRSA strains, CA-MRSA has been found to specifically colonize non-nasal sites more frequently, including the axilla and inguinal

folds (Yang et al. 2010). While CA-MRSA, and USA300 specifically, remains a primary cause of SSTI's in the community, incidence in some parts of the United States is decreasing, which may be representative of an overall historical process of expansion and contraction of *S. aureus* clones (Planet 2017).

S. aureus Contamination of the Built Environment and Pets

While humans are recognized as the native carriers of *S. aureus*, numerous studies have reported *S. aureus* on environmental surfaces and household pets and thus may serve as putative reservoirs of *S. aureus* infections. These strains typically share the same molecular profile as those colonizing individuals in the immediate vicinity, and households with a history of MRSA infections are far more likely to have environmental MRSA contamination (Fritz et al. 2014). Environmental contamination of MRSA has also been associated with increased risk of SSTI in the hospital setting (S. J. Dancer 2009). A lack of close temporal resolution has limited the capacity to understand whether the environment serves as a reservoir for *S. aureus* or as an indirect measure of the degree of *S. aureus* shedding by carriers within the environment.

S. aureus Contamination of the Household Environment

The household environment is increasingly under investigation as a reservoir of *S. aureus* infections due to the inability of human colonization to account for the extent of *S. aureus* infections in the household (L. G. Miller et al. 2012; Marcela Rodriguez et al. 2014a) and the incomplete success of decolonization trials to eradicate the pathogen longitudinally (Fritz, Hogan, Hayek, Eisenstein, Rodriguez, Epplin, et al. 2012; Kaplan et al. 2014). Overall *S. aureus* contamination of sites in the built environment was estimated at 40% (95% *CI* 29–54%) across 20 studies, and MRSA at 9% (95% *CI* 5–13%) for 39 studies in a recent meta-analysis (Lin et al. 2016). Sites of highest reported *S. aureus* contamination include commonly handled objects, including toys, remote controls, bedding, hand towels, and doorknobs, and faucets, with cloth-

based items serving as the most prominent fomites in the household setting (M. F. Davis et al. 2012).

In households without a history of SSTI, *S. aureus* contamination of the environment is common, with 38-51% of households exhibiting at least one incidence of *S. aureus* colonization. Moreover, households with a history of SSTI are significantly more likely to have MRSA contamination present (32% versus 5% of control households) (Justin Knox et al. 2012; Anne-Catrin Uhlemann et al. 2011). In households with children with a history of CA-MRSA SSTI, environmental MRSA contamination was encountered in 23 of 50 households (46%), and colonized individuals exhibited a significantly higher proportion of MRSA-contaminated sites than noncolonized individuals (Fritz et al. 2014). Households with recurrent infection harbor significantly more environmental contamination of the associated clinical isolate compared to households without (Anne-Catrin Uhlemann et al. 2011). Environmental contamination has been significantly associated with increased body site colonization as well as a higher density of individuals within the household (Eells et al. 2014). Presence of the clinical isolate, and more generally MRSA fomite contamination, in the household environment was further associated with increased incidence of recurrent infection (Justin Knox et al. 2016b; Loren G. Miller et al. 2015)

S. aureus Contamination of Companion Animals

S. aureus rarely colonizes companion animals compared to humans and livestock (Morgan 2008). Pets exhibit infrequent *S. aureus* colonization in households; while 31% of humans were colonized with *S. aureus*, only 16% of dogs and 4% of cats were colonized in the same households (MRSA colonization 3.3% for people and 1.5% for people and dogs, respectively) (Hanselman et al. 2009). Identified risk factors for *S. aureus* colonization in pets

includes history of contact with veterinary clinics, antibiotic use, surgery, and contact with children (Lefebvre et al. 2009; J. Scott Weese and van Duijkeren 2010).

Pets in households with a history of CA-MRSA showed low incidence of MRSA colonization (12% of dogs, 7% of cats, 8% for mammalian pets), with the mouth and nose serving as the most sensitive site for overall *S. aureus* isolation (Fritz et al. 2014; Iverson et al. 2015). In these households, *S. aureus* (or MRSA) strain concordance between members and pets has been reported between 50-67% depending upon molecular typing method employed (Hanselman et al. 2009; Kottler et al. 2010). Due to the lack of longitudinal studies, it is difficult to resolve the directionality of transmission of these strains (M. F. Davis et al. 2012). A more recent longitudinal case study was unable to identify the dog or the household environment as a source of persistent colonization in the household members (M. F. Davis et al. 2015), casting doubt on the likelihood these animals act as reservoirs for *S. aureus* colonization and infection.

S. aureus Colonization and Infection in Households and Associated Biological and Demographic Factors

Numerous studies have highlighted the role of the household as a primary reservoir for *S. aureus* colonization and infection in the community (A.-C. Uhlemann et al. 2014; M. F. Davis et al. 2012; Macal et al. 2014; Alam et al. 2015). Households with a history of SSTI have consistently higher MRSA colonization compared to the general population, with this MRSA colonization high in both individuals with a history of SSTI as well as those without (Fritz, Hogan, Hayek, Eisenstein, Rodriguez, Krauss, et al. 2012a). *S. aureus* strains colonizing individuals within the same household tend to be genetically similar (Muthukrishnan et al. 2013; Marcela Rodriguez et al. 2014b). Studies have also observed transmission of CA-MRSA strains capable of invasive infection between healthy and infected household members (T. F. Jones et al. 2006a). The current hypothesis is that an initial CA-MRSA infection leads to subsequent

colonization of other household members and contamination of the household environment, which in turn potentiates recurrent infections (Loren G. Miller et al. 2015; Justin Knox et al. 2016a).

Colonization and transmission of *S. aureus* in households has canonically been tied with the degree of colonization observed and proximity measures of household members (Justin Knox, Uhlemann, and Lowy 2015a). In a meta-analysis of households from the United States, Australia, and the Netherlands, common risk factors for subsequent colonization amongst household contacts of MRSA-infected index patients included index patient colonization as well as the proportion of household members <18 (J. Knox et al. 2015). Towel sharing, international travel, antibiotic use, and participation in sports have also been reported as significant risk factors for serious SSTI (M. Miller et al. 2009). However, the interplay between colonization and SSTI is incompletely understood. While the degree of household contact MRSA colonization (measured as colonization pressure, the proportion of individuals colonized by MRSA) has been associated with persistent colonization, it was not found associated with recurrent SSTI (M. Rodriguez et al. 2013). In a randomized cohort study of 321 households, no significant association was found between *S. aureus* colonization and reported serious skin infection (*S. aureus* prevalence 25%; 24% of households reported serious skin infection) (M. Miller et al. 2009).

In addition to MRSA colonization of household contacts and environmental as primary risk factors for individual colonization and SSTI, hygiene, socioeconomic, and demographic measures have also been identified as risk factors, although their relationship between colonization, infection, and household contamination is not straightforward. Low socioeconomic status (Medicaid usage, African American race, low household income), history of complex

disease such as diabetes, hospitalization, and younger age have all been associated with increased *S. aureus* and MRSA colonization and infection (Ray, Suaya, and Baxter 2013; Loren G. Miller et al. 2015; Fritz, Hogan, Hayek, Eisenstein, Rodriguez, Epplin, et al. 2012). However, these same factors have not been significantly connected to household environmental contamination (Justin Knox et al. 2016a). Although contact sport participation is associated with increased risk of SSTI (Creech, Al-Zubeidi, and Fritz 2015), the opposite relationship has been found for recurrent infection in households (Loren G. Miller et al. 2015).

Conclusions of Statistical Models of *S. aureus* Colonization and Transmission

Statistical models of *S. aureus* colonization, infection, and transmission within hospitals and the community serve as invaluable tools for hypothesis testing and insight generation in contexts where comprehensive, longitudinal measurement of *S. aureus* colonization are cost-prohibitive (Chisholm et al. 2018). These studies have focused upon two major areas: estimating long-term trends of MRSA colonization and infection, and secondly, how these trends may be curtailed by various intervention strategies.

A large, agent-based simulation study of Chicago, simulating 3million agents across 32 separate runs over 10years, reported the household as the primary source of transmission events as well as asymptomatic colonization, rather than infection, as primary drivers of *S. aureus* transmission events (Macal et al. 2014). Another model of dynamic *S. aureus* colonization and transmission across the United States predicted that under the context of strong competition of colonization, MRSA may supplant MSSA over time (Hogea, van Effelterre, and Acosta 2014). Using Markov chain models to assess the steady-state colonization of nursing home residents, greater antibiotic stewardship was postulated to reduce MRSA acquisition incidence, and predicted that USA300 would remain a minority strain in these nursing homes (Batina et al.

2016), although another deterministic modelling study postulated that CA-MRSA will become the dominant MRSA strain in the hospital setting (Webb et al. 2010). Due to the capacity of MRSA to stably, asymptomatically colonize hosts to facilitate transmission, MRSA is postulated across numerous models to continue to present a significant healthcare burden.

Statistical models are especially useful in estimating the effectiveness of intervention strategies in hospital and community settings. Modeling studies in the hospital have examined the effectiveness of different screening approaches, and have reported the effectiveness of universal MRSA screening of adults upon hospital admission and rapid identification and treatment of those at risk for colonization, especially those with a history of antibiotic usage (Chamchod and Ruan 2012; Bruce Y. Lee et al. 2010). A Markov Chain Monte Carlo (MCMC) model of SSTI incidence in Maricopa County, Arizona found that decolonization strategies targeting infected individuals alone would be insufficient to reduce SSTIs and recurrent infections in the community (X. Wang, Panchanathan, and Chowell 2013). MCMC methods were also used to examine how the incidence of mupirocin-resistant *S. aureus* in hospitals increases with various treatment regimens, highlighting the trade-off between universal and selective decolonization approaches (Deeny et al. 2015). Another study found that vaccination against pathogens with a high degree of strain diversity typically only reduces the incidence of specific strains, resulting in the possibility of decreasing disease while increasing the incidence of asymptomatic carriage (Chisholm et al. 2018). Consistently across these studies is the noted effectiveness of hand hygiene, especially in healthcare workers, as well as the necessity of targeted decolonization of individuals colonized by MRSA to reduce subsequent transmission and infection.

Current Intervention Measures to Reduce *S. aureus* Colonization and Infection in Households

The strong association between *S. aureus* colonization with infection and recurrence has motivated the development of education paradigms and decolonization approaches as preventative strategies for recurrent infection. Subsequent studies have indicated that household-level approaches may be more effective in reducing recurrent infection. Due to the role *S. aureus* environmental contamination may play in transmission and subsequent recurrent infection, there is an increasing appreciation of the necessity of environmental decolonization regimens paired with individual decolonization methods (Creech, Al-Zubeidi, and Fritz 2015).

Hygiene measures have been associated with prevention of recurrent SSTI, and therefore education measures for improved hygiene practices in the household have become an integral part of intervention strategies. Educating individuals upon measures that may reduce *S. aureus* transmission in the household such as frequent handwashing and reduced sharing of hygiene items (i.e., towels) has been shown to significantly reduce subsequent SSTI (Kaplan et al. 2014). Daily changing of clothing and towels has also been advocated to reduce *S. aureus* colonization in homes (Creech, Al-Zubeidi, and Fritz 2015). However, the relative effectiveness of each of these hygiene measures in reducing *S. aureus* transmission in the household is incompletely understood, and such information would highly benefit the development of streamlined education policies in households with poor hygiene.

Several methods for *S. aureus* decolonization, defined as the employment of antimicrobial or antiseptic agents to eradicate *S. aureus* colonization (Liu et al. 2011), have been developed that target both nasal and extra-nasal colonization (McConeghy, Mikolich, and LaPlante 2009). Mupirocin applied to the nares is the most common method to eradicate *S. aureus* colonization in this site, although several rounds of treatment are required to ensure

complete clearance of the organism, and the duration of clearance varies (Laupland and Conly 2003). Although infectious disease physicians commonly prescribed decolonization to prevent recurrent infections, evidence for efficacy in households and children is sparse (Creech et al. 2008). Further, resistance to the antibiotic has been noted, which dramatically increases in accordance with its employment in the population (McNeil et al. 2011; J. B. Patel, Gorwitz, and Jernigan 2009).

Whole body decolonization methods include chlorhexidine body washes and dilute sodium hypochlorite (bleach) baths and have both been shown to significantly reduce *S. aureus* presence on the skin, although individuals with a higher number of colonized sites are more likely to remain colonized (Fisher et al. 2008; Wendt et al. 2007). Several studies have shown that decolonization with only antimicrobial body washes or mupirocin alone were insufficient to reduce SSTI incidence, and that body decolonization in addition to education was as effective as education alone in reducing subsequent SSTI (Kaplan et al. 2014; Ellis et al. 2007). While there has been concern that body decolonization may deplete the native microbial flora of the skin, a recent study has shown such an intervention only significantly impacts colonizing *S. aureus* (Burnham et al. 2016)

Household decolonization trials have attempted to determine to what extent these decolonization regimens reduce subsequent MRSA colonization and SSTI, and if such an effect increases when all individuals in the household are targeted for decolonization. A randomized control study of decolonization regimens in a cohort of 300 individuals suffering from CA-*S. aureus* infection with colonization found that while combined mupirocin and bleach bath decolonization significantly reduced *S. aureus* colonization up to four months past treatment compared to educated controls, recurrent SSTI remained the same between groups (Fritz,

Camins, et al. 2011). Decolonization with mupirocin and chlorhexidine of all household members versus only the individual with a history of SSTI was found to significantly reduce subsequent reported SSTI, although CA-MRSA colonization was not significantly reduced between groups (Fritz, Hogan, Hayek, Eisenstein, Rodriguez, Epplin, et al. 2012). Another randomized trial of households in Pennsylvania recapitulated this observation, finding that a regimen of mupirocin and chlorhexidine decolonization was as effective compared to education in promoting MRSA clearance (Cluzet et al. 2016).

Recent studies have shown that environmental cleaning approaches traditionally associated with reducing persistent environmental pathogens such as *Clostridium dificile* may also reduce MRSA colonization and infection incidence (Stephanie J. Dancer 2008; Loren G. Miller and Diep 2008; Boyce 2007). Typical disinfectant strategies employed in households are sufficient to eradicate *S. aureus* contamination in clothing and surfaces (Creech, Al-Zubeidi, and Fritz 2015), although the degree and frequency of cleaning in households was not associated with significantly less *S. aureus* environmental contamination (Fritz et al. 2014; Eells et al. 2014). Understanding the relative impact of varying degrees of environmental cleaning, especially when paired with individual decolonization, in *S. aureus* transmission and persistence in homes is necessary to develop comprehensive intervention strategies in households suffering from recurrent SSTIs.

Chapter 2: Comprehensive modeling reveals proximity, seasonality, and hygiene practices as key determinants of MRSA colonization in exposed households.

This chapter is adapted from the following publication:

Mork, Ryan L., Patrick G. Hogan, Carol E. Muenks, Mary G. Boyle, Ryley M. Thompson, John J. Morelli, Melanie L. Sullivan, et al. 2018. "Comprehensive Modeling Reveals Proximity, Seasonality, and Hygiene Practices as Key Determinants of MRSA Colonization in Exposed Households." *Pediatric Research*, July. https://doi.org/10.1038/s41390-018-0113-x.

Introduction

Staphylococcus aureus poses a threat to the health of individuals in the community and those requiring hospitalization. Methicillin-resistant *S. aureus* (MRSA), and particularly the USA300 clone, has become the predominant etiology of skin and soft tissue infections (SSTIs) in the United States (Dukic et al. 2013; Moran et al. 2006; Otto 2007; Justin Knox, Uhlemann, and Lowy 2015b; Alam et al. 2015). The high incidence of recurrent skin infections (Fritz, Hogan, Hayek, Eisenstein, Rodriguez, Epplin, et al. 2012), as well as increasing antibiotic resistance (Diep et al. 2008), demands novel strategies to curtail the epidemic.

Asymptomatic human carriage is a critical reservoir for *S. aureus* infection (Justin Knox, Uhlemann, and Lowy 2015a; Wertheim et al. 2005a; Fritz et al. 2009). Overall, half of all individuals are colonized at least intermittently with *S. aureus* (J. L. Nouwen et al. 2004; Costello et al. 2009; Kluytmans, van Belkum, and Verbrugh 1997). Multiple members of the same household frequently experience MRSA infections, and a high proportion of household contacts of patients with MRSA infection are colonized (Justin Knox et al. 2012; Anne-Catrin Uhlemann et al. 2011; Fritz, Hogan, Hayek, Eisenstein, Rodriguez, Krauss, et al. 2012b; L. G. Miller et al. 2012). Measures to reduce the incidence of MRSA colonization and recurrent SSTI

in these households, including decolonization strategies (i.e., "the use of antimicrobial or antiseptic agents to suppress or eliminate *S. aureus* carriage" (Kaplan et al. 2014) such as mupirocin topical antibiotic or body washes with chlorhexidine or dilute bleach water), have yielded modest success (Kaplan et al. 2014; Fritz, Hogan, Hayek, Eisenstein, Rodriguez, Epplin, et al. 2012).

To date, factors associated with MRSA SSTI and colonization have included prior skin infections, antibiotic history, and the burden of colonization among household members (Fritz et al. 2008; M. Rodriguez et al. 2013; L. G. Miller et al. 2012). However, these studies were limited by examining a subset of household members, discounting hygiene practices and proximity to other household members, or incomplete molecular resolution of the strains in each household, especially the presence and distribution of the strain responsible for the initial SSTI.

In the present study, we measured *S. aureus* colonization in 150 households of children (index patients) presenting with MRSA infection. We observed how the household distribution of *S. aureus*, household demographics, health history, and individual hygiene practices influence overall *S. aureus* and MRSA colonization. Through molecular typing of available infecting isolates from the initial SSTI and all colonizing isolates collected from index patients and their household contacts, we determined the prevalence of carriage of infecting strains compared to other colonizing strains as well as strain concordance across household members. We sought to identify practices that correlate with colonization with *S. aureus* in general, and MRSA specifically (especially the infecting strain within households), to inform clinical practice guidelines and ultimately to mitigate the incidence of *S. aureus* infections in the community.

Results

Participant Demographics

A cohort of 150 pediatric patients (median age 3.0 years, range 0.1-18.6) with an MRSA infection from 150 distinct households and their household contacts (n=521, ranging from 1-12 contacts per household; median age 26.6 years, range 0.1-82.2) were enrolled. Index patients were primarily Caucasian (102, 68%) and African American (37, 25%). The groin/buttock was the most common site of enrollment infection (69, 46%). Notably, 33% (49) of index patients had eczema, and 58% (87) reported an SSTI in the year prior to the enrollment SSTI. Median household size was four (range 2-13) (**Table 1**).

Hygiene Practices and Physical Activities

Index patients and household contacts were administered a comprehensive survey regarding personal hygiene, laundering practices, and activities inside and outside of the home (**Table 1**). Many (58%) individuals engaged in bathing daily, while the remainder (42%) reported bathing at least 3-4 times per week; 3 reported bathing less than once per week. Nearly half of the participants (40%) reported washing their bedding weekly or more, while 22% washed bedding once a month or less. Most participants (63%) reported reusing bath towels before laundering them; 18% used their towel ≥5 times between washings. While towels were most often washed in hot (56%) or warm (29%) water, participants less often reported washing underwear, undershirts, pants, and outer shirts with hot water (26%, 24%, 16%, and 16%, respectively). On a 4-point cleanliness scale, interviewers found that 27% of households were very dirty or below average, 45% were average cleanliness, and 27% were rated above average.

Activities outside of the home included exercising at a gym (20%), sports participation (29%; 4% in a contact sport such as football, lacrosse, or wrestling), and public pool attendance

(36%) (**Table 1**). Over half (57%, 124/218) of children six years of age or younger were enrolled in daycare; 48% (72/149) of index patients attended daycare.

Colonization Overview

Of 671 participants, 275 (41%) were colonized with *S. aureus* at one or more body site(s) (24% specifically with MRSA). Index patients were equally likely as household contacts to be colonized with *S. aureus* and MRSA (p=0.45 and p=0.06, respectively). Overall, MRSA colonization was significantly higher in children <12 years of age (odds ratio [OR]=1.6, 95% confidence interval [*CI*] 1.1-2.3) compared to older participants, and lower in Caucasians (OR=0.7, 95% *CI* 0.4-1.0) than in other racial groups. At the household level, 125 (83%) and 89 (59%) households had at least one household member colonized with *S. aureus* and MRSA, respectively.

By anatomic site, 190 (28%), 72 (11%), and 127 (19%) participants were colonized by *S. aureus* in the nares, axillae, and inguinal folds, respectively (15%, 6%, and 11% with MRSA, respectively). Eighty-eight (13%) participants were colonized with *S. aureus* at multiple body sites (7% with MRSA at multiple sites). Of 481 participants not colonized with *S. aureus* in the nares, 85 (18%) were colonized with *S. aureus* at an extranasal site (7% with MRSA).

Antibiotic Susceptibility and Molecular Strain Typing

Of 389 colonizing isolates, 56% (218) were MRSA, the majority of which were SCC*mec* type IV (98%, 214), typical of U.S. epidemic CA-MRSA. Of 171 colonizing MSSA isolates, 40% (69) possessed the SCC*mec* element, with 25% (42) harboring a residual type III element and 12% (20) harboring a type II element. All infecting isolates available for molecular typing were MRSA SCC*mec* type IV. All infecting MRSA isolates were susceptible to trimethoprim-

sulfamethoxazole, rifampin, linezolid, ceftaroline, and mupirocin, while only 7% (6), 14% (13) and 43% (39) were clindamycin, erythromycin and ciprofloxacin-susceptible, respectively.

Household Strain Richness

Up to three strain types were found to colonize a single participant (of those colonized, median=1). Of 150 households, 59% (89) harbored at least one colonizing MRSA strain type; 47% (70) had at least one colonizing MSSA strain type. Only 23% (34) of households harbored both MRSA and MSSA strains. The median number of *S. aureus* strain types recovered per household was one (range 0-7), with an average of 0.37 unique strain types per person. Of 81 households with ≥2 members colonized, 75% (61) harbored ≥2 unique strain types (mean 2.3±1.2). Sampling season, household cleanliness rating, living in a house (vs. apartment/condominium/trailer), and home ownership (vs. renting) were not significantly associated with strain richness (p>0.05 by the Kruskal-Wallis test). However, households with ≥5 individuals exhibited a significantly increased number of strain types compared to smaller households (mean 1.8 vs. 1.2 strains, p<0.001, Wilcoxon signed-rank test). The number of individuals correlated with the number of unique strains (Spearman's ρ=0.35, p<0.001), and was even more significant when considering only colonized individuals (Spearman's ρ=0.77, p<0.001).

Colonization with the Index Patient Infecting Strain Type across Household Members

Ninety-one (61%) isolates from the infection prompting enrollment ("infecting strain") were acquired from the hospital laboratory and subjected to molecular typing. At enrollment (median 20 days from SSTI), 27 (30%) of these index patients were colonized with their infecting strain (**Figure 1**). Of 298 household contacts of index patients with an available infecting isolate, 62 (21%) were colonized with the infecting strain. Parents had the lowest

prevalence of colonization with the infecting strain (22% for mothers, 19% for fathers), while siblings were more commonly colonized (28%) with the infecting strain. Fifty-two (57%) households had at least one individual colonized with the infecting strain. In 41 (45%) households, the infecting strain was the most common strain type recovered from household members. Of the 64 (70%) households in which the index patient was not colonized with the infecting strain, at least one household contact was colonized with this strain in 22 (34%) households.

As the infecting strain was commonly present within households, we constructed a generalized linear negative binomial model examining the degree of anatomical site colonization (nares, axillae, inguinal folds) with the infecting strain across households with an infecting isolate available for typing, the 'Infecting Strain Prevalence' model. Seasonality (as measured by monthly low temperature at time of enrollment), specifically warmer months, was significantly associated with presence of the infecting strain (rate ratio per unit increase 1.6, 95% Credible Interval [*CrI*] 1.1-2.2). The proportion of household members reporting bathing at least daily was significantly associated with a reduced household burden of the infecting strain (rate ratio for daily bathing 0.3, 95% *CrI* 0.1-0.8). Time between index patient SSTI and enrollment (colonization sampling) and the number of household members reporting recent SSTI were not associated with increased infecting strain burden.

Factors Associated with Individual Colonization

To determine the relative impact of collective household versus individual behaviors upon individual colonization, three models measuring "household preventability" (proportion of variance explained through a household-level random effect) were constructed (K. Wang et al. 2017). Household preventability was lowest for *S. aureus* colonization (21%, 95% *CrI* 10-33%),

moderate for MRSA colonization (38%, 95% *CrI* 24-52%), and highest for infecting strain colonization (51%, 95% *CrI* 34-66%). These models indicate that individual attributes and behaviors primarily determined *S. aureus* colonization, while MRSA colonization and particularly colonization with the infecting strain type were equally driven by individual attributes (e.g., health status, age, hygiene) and household attributes (e.g., overall cleanliness, distribution and number of individuals).

Observing that individual practices governed a significant degree of individual *S. aureus* colonization, a univariate analysis of all individual covariates was conducted for index patients and household contacts (**Table 2**). Measures of proximity were most significantly associated with colonization across household members, with *S. aureus*- or MRSA-colonized individuals significantly more likely to share a personal hygiene item or bedroom with another *S. aureus*- or MRSA-colonized individual, respectively. Frequent bathing (≥1x/day) was significantly associated with decreased MRSA colonization. Anatomic site colonization pressure, measured as the proportion of sites (nares, axillae, inguinal folds) colonized across household members (excluding the person of interest), was significantly associated with increased individual colonization for both *S. aureus* and MRSA colonization. Household strain richness per person (i.e., the number of unique strains measured by repPCR normalized to the number of individuals in the household) was significantly associated with individual *S. aureus*, but not MRSA, colonization.

Colonization Pressure Model

We sought to understand the extent to which colonization pressure and strain richness, defined as the number of unique strain types by repPCR per person in the household, predict colonization risk. Increasing anatomical site *S. aureus* colonization pressure of household

contacts (Odds Ratio [*OR*] 1.4, 95% *CrI* 1.2-1.5) and renting (vs. owning) dwelling (*OR* 1.2, 95% *CrI* 1.0-1.5) were significantly associated with individual *S. aureus* colonization (**Table 3**). Higher household contact anatomical site MRSA colonization pressure (*OR* 1.8, 95% *CrI* 1.6-2.1) was also predictive of MRSA colonization. Antibacterial soap usage (*OR* 0.8, 95% *CrI* 0.6-0.97), increasing age (*OR* 0.9, 95% *CrI* 0.8-0.98), and increasing strain richness (*OR* 0.5, 95% *CrI* 0.3-0.9) were significantly associated with a lower incidence of individual MRSA colonization.

Colonization Proximity Model

We next assessed colonization in terms of "proximity models" to understand how sharing household spaces, such as a bedroom or bed, and objects, such as towels or personal hygiene items, with colonized individuals increases colonization risk. In these models, renting (vs. owning) dwelling (*OR* 1.5, 95% *CrI* 1.1-1.9) was significantly associated with *S. aureus* colonization. Sharing a bedroom with an MRSA colonized individual (*OR* 1.5, 95% *CrI* 1.1-2.2) and seasonality (as measured by monthly low temperature at enrollment; *OR* 1.2, 95% *CrI* 1.01-1.4 per unit increase) (Arguez et al. 2012) were significantly associated with MRSA colonization (**Table 3**). Frequent bathing was associated with decreased likelihood of MRSA colonization (*OR* 0.7, 95% *CrI* 0.6-0.98). Although considered, sharing hygiene items or a bed with colonized individuals were not selected in the final models.

Colonization Activity Model

While previous models focused primarily upon how the degree and distribution of *S. aureus* influences colonization, household demographics and individual activities may influence colonization. "Activity models" assessed modifiable practices that may reduce *S. aureus* colonization risk in the community while accounting for socioeconomic status (SES) and

demographics. In these models, renting dwelling (vs. owning) was significantly associated with both *S. aureus* colonization (OR 1.5, 95% *CrI* 1.1-2.0) and MRSA colonization (*OR* 1.4, 95% *CrI* 1.01-2.1) (**Table 3**). Of note, home ownership was significantly associated with other measures of SES including private health insurance and a college-educated mother. Antibacterial soap usage was associated with a significant reduction in *S. aureus* colonization (*OR* 0.8, 95% *CrI* 0.6-0.95), while antibacterial soap usage and bathing at least daily each were significantly associated with a reduction in MRSA colonization (OR 0.7, 95% *CrI* 0.5-0.97 and *OR* 0.7, 95% *CrI* 0.5-0.9, respectively). Seasonality was again associated with MRSA colonization (*OR* 1.2, 95% *CrI* 1.03-1.5 per unit increase), while it was not associated with overall *S. aureus* colonization in the activity model (**Table 3**).

Concordance of Colonization Strain Types among Household Members

Concordance of *S. aureus* colonization strain types between household members was considered in the context of shared environments and hygiene items. **Figure 2** depicts the frequency at which various pairs of immediate family members were colonized with at least one concordant strain type. Overall, colonization with concordant strains occurred in 12% of all pairs of individuals living within the same household; siblings (excluding the index patient) exhibited the highest strain concordance (17%), closely followed by sibling-parent pairs (15%). Of the 284 pairs of individuals both colonized with *S. aureus*, 64% (168) were colonized with concordant strain types. Colonized siblings exhibited the highest concordance with another colonized household member, followed by parents and then index patients at 62% (62), 61% (66), and 56% (32), respectively (all pairwise comparisons not significant). History of SSTI was not associated with strain concordance.

Factors that contributed to strain concordance were measured through considering pairs of individuals within households in a generalized linear mixed logistic regression model accounting for a household-level random effect. Strain concordance between immediate family members was significantly higher than pairs of unrelated individuals (*OR* 3.4, 95% *CrI* 1.1-10.5). Although sharing bath towels, bedrooms, beds, and personal hygiene items (e.g., razor, cosmetics) were considered in the models, only considering all of these objects in aggregate was significantly associated with colonization with concordant strains (*OR* 2.7, 95% *CrI* 1.2-6.6). Household preventability in this model was high at 71% (95% *CrI* 53-85%), meaning that household-level activities and properties (such as overall cleanliness and crowding), rather than behaviors or attributes shared by the two individuals (such as sharing a bed), may have heavily influenced whether two individuals were colonized by the same strain.

Discussion

S. aureus SSTIs are difficult to prevent and treat, both in terms of primary and recurrent infection, due to the pathogen's capacity to both stably colonize and reinfect the host over time. The development of future prevention strategies must address both the eradication of extant colonization as well as the prevention of recolonization and recurrent infection. The present study illuminates household *S. aureus* and MRSA colonization in terms of strain richness, strain concordance between household contacts, and critically, prevalence of the infecting strain in the household following index patient treatment.

Proximity to colonized individuals is a primary risk factor for *S. aureus* colonization, although it is unclear to what extent physical contact versus interaction with common fomites contributes to such risk. Previously, *S. aureus* colonization pressure was shown to be associated

with increased risk for colonization (M. Rodriguez et al. 2013), and while recapitulated in the present models, colonization pressure does not specify the routes contributing to colonization. Here, we have demonstrated that proximity to colonized individuals, through sharing a bedroom, was critical in predicting MRSA colonization. As only 42% of the individuals reporting sharing a bedroom also shared a bed, simply sharing a bedroom (even without sharing a bed) with a colonized individual may be sufficient to increase MRSA colonization risk. Our models have also highlighted that household members who share personal hygiene items have an increased likelihood of colonization with concordant *S. aureus* strains. Intervention strategies that promote discrete items (e.g., towels) for each household member may therefore decrease household contact colonization and augment the efficacy of household decolonization strategies.

Atmospheric temperature (modeled as monthly average low temperature) was associated with a significantly increased prevalence of the infecting strain in the household as well as individual MRSA colonization risk. In a recent study across 20 geographically diverse regions in the U.S., higher relative humidity, but not temperature, was significantly correlated with MRSA colonization of ICU patients (Blanco et al. 2017). However, two other studies evaluating the impact of meteorological factors upon MRSA SSTIs in outpatients in India and the southwest U.S. found significant correlation between higher temperature and incidence of MRSA SSTIs (X. Wang et al. 2013; Sahoo et al. 2014). Rather than climate directly increasing incidence of MRSA colonization and infection, it may be that increased physical activity and other behaviors associated with warmer months influence this phenomenon.

MRSA infecting strains were pervasive among household members following index patient treatment, representing the predominant strain in 45% of households. Overall, 57% of households had at least one person colonized with the infecting strain, and 34% of households

had at least one person other than the index patient colonized with the infecting strain. A study in New York also found 37% of household contacts colonized with the infecting strain (determined by *spa* typing) (Justin Knox et al. 2012). When considering family relationships, siblings of the index patient were most likely to harbor the infecting strain (30%), followed by parents (21%), reflecting individuals most likely to be in close proximity to the index patient. A study from Chicago and Los Angeles examining *S. aureus* skin infection in children and adults observed only 14% of household members colonized with the infecting strain over a similar timeframe between infection and colonization sampling (L. G. Miller et al. 2012). The present study provides evidence that, rather than a transient invader of the index patient, the infecting strain is capable of persistence and colonization of multiple individuals within the household after SSTI treatment.

Across a wide range of personal hygiene practices, health history, and household behaviors, only primary measures of hygiene (bathing frequency) and seasonality were highly significant in predicting colonization burden of the infecting strain across household members. A meta-analysis by Knox, et al. found that the number of household contacts colonized by the infecting strain was positively associated with the number of children present in the household and negatively associated with the number of days from index patient SSTI to collection of household contact colonization cultures (J. Knox et al. 2015). In our models, both of these covariates were examined and neither were found significant; of note, neither of the covariates found significant in our study (bathing frequency and seasonality) were examined in the meta-analysis. Further, this meta-analysis was conducted exclusively in a univariate fashion, examined studies that only sampled nares colonization, classified the infecting strain through *spa* typing, and surveyed households after a longer time from initial infection (33-114 days vs. 3-95 days in

the present study). This discrepancy highlights the need to study the longitudinal colonization patterns of the infecting strain when considering measures to reduce colonization and SSTIs.

Modifiable behaviors to reduce MRSA colonization (specifically infecting strain persistence and transmission) within the household are especially important to clinicians and patients to mitigate the burden of recurrent SSTI. Of the many hygiene behaviors examined in the present study, bathing frequency and using antibacterial soap (of note, several active ingredients of these agents have recently been banned by the FDA (Fischer 2016)) were significantly associated with lower colonization prevalence across multiple models, while sharing a bedroom correlated with higher prevalence of colonization. Nerby et al. reported a similar reduction in MRSA colonization with antibacterial soap usage (Nerby et al. 2011). In a laboratory study by Honisch et al., laundering *S. aureus*-contaminated test swatches in colder temperatures resulted in low staphylococcal clearance (<4 logs) and more frequent cross-contamination of sterile swatches (Honisch, Stamminger, and Bockmühl 2014). In our study, the reported water temperature used to launder clothing and linens was not significantly associated with individual *S. aureus* or MRSA colonization. These contrary results could reflect a high correlation of hygiene behaviors; for example, bathing at least daily could be an indicator of other healthy hygiene practices or antibacterial soap usage may indicate a desire for decolonization, which could ultimately reduce MRSA burden.

Strengths and Limitations

While our study evaluated strain-level resolution of individual *S. aureus* colonization within households, this is only a cross-sectional snapshot into what may be a highly variable, random system. Ongoing studies of longitudinal strain-level persistence and transmission will be informative. As each household was enrolled only after the index patient presented with an

MRSA infection, determining the directionality of transmission of the infecting strain was not feasible. Given that enrollment relied on at least one child presenting with an MRSA infection, our results must be considered within the context of households that include children. While specific hygiene practices have been correlated with colonization, randomized controlled trials are necessary to verify the efficacy of such practices prior to their widespread implementation. Crucial strengths of our study are the large sample size of 150 households (671 participants) in a geographically (urban and rural) and sociodemographically diverse region across metropolitan St. Louis, comprehensively surveyed for behavioral, hygiene, and health history combined with molecular identification of all available *S. aureus* isolates. These data allow for measures of strain richness, concordance, and infecting strain prevalence in households, as well as the delineation of possible intervention strategies to reduce MRSA colonization within the community.

Conclusions

Combining strain-level molecular typing with a comprehensive survey of personal hygiene, proximity, and physical activity measures provides a thorough analysis of both strain colonization and concordance across a large cohort while distilling key preventive measures to reduce MRSA colonization burden. We have found that the MRSA strain recovered from the index patient infection commonly persists among household members past initial treatment. These findings can inform the guidance provided by clinicians regarding bathing frequency and avoiding sharing personal hygiene items. As seasonality has also been associated with MRSA colonization across multiple studies, clinicians may want to emphasize preventive efforts, including decolonization, during the summertime in patients prone to recurrent SSTI. Future studies are necessary to understand how infectious strains persist in light of such hygiene

practices and decolonization strategies in order to reduce the incidence of infection in the community setting.

Chapter 3: Incidence and Risk Factors for Longitudinal *S. aureus* Transmission, Introduction in Households with History of SSTI

This chapter is adapted from a version of the manuscript: **Incidence and Risk Factors for Longitudinal *S. aureus* Transmission, Introduction in Households with History of SSTI** in preparation for submission at *Lancet ID*. The authors of this manuscript are as follows: Mork R, Hogan P, Muenks C, Boyle M, Thompson R, Sullivan M, Orscheln R, Gehlert S, Bubeck Wardenburg J, Burnham CD, Rzhetsky A, Fritz S.

Introduction

Staphylococcus aureus causes a wide spectrum of infections, ranging from asymptomatic colonization to invasive, life-threatening disease. The most frequent entity attributed to *S. aureus* is skin and soft tissue infections (SSTIs), particularly since the emergence of epidemic strains of community-associated methicillin-resistant *S. aureus* (CA-MRSA) in the late 1990s (e.g., ST1 in Australia, ST80 in Europe, and USA300/ST8 in the United States) (Mediavilla et al. 2012; Otter and French 2010). CA-MRSA poses a unique prevention challenge in its capacity to disseminate through asymptomatic colonization and to cause recurrent infections in healthy individuals in the community. Indeed, up to 70% of patients with CA-MRSA SSTI will experience a recurrent infection within a year (Fritz, Hogan, Hayek, Eisenstein, Rodriguez, Epplin, et al. 2012; Williams et al. 2011; Loren G. Miller et al. 2015). Thus, devising comprehensive control strategies to prevent recurrent infection and transmissions between individuals is of high priority.

Household contacts of patients with CA-MRSA infection have a high prevalence of MRSA colonization, frequently with a strain concordant with the index patient's infecting strain (M. Rodriguez et al. 2013; W. Ng et al. 2017; Nerby et al. 2011; Fritz, Hogan, Hayek, Eisenstein, Rodriguez, Krauss, et al. 2012b). As asymptomatic colonization poses risk for subsequent SSTI, a household approach to decolonization with topical antimicrobials was previously investigated

(Fritz, Hogan, Hayek, Eisenstein, Rodriguez, Epplin, et al. 2012). While a comprehensive approach to decolonization, comprising topical antimicrobials and improved hygiene measures, targeted at the individual with the CA-MRSA infection (index patient) and all household contacts significantly reduced subsequent SSTI incidence in the index patient and household contacts compared to decolonization of the index patient alone, this approach did not completely eliminate the problem of recurrent SSTI (Fritz, Hogan, Hayek, Eisenstein, Rodriguez, Epplin, et al. 2012). Thus, although *S. aureus* has traditionally been purported to be spread via person-to-person contact, other vectors, such as environmental sources, also warrant evaluation in CA-MRSA transmission dynamics. In healthcare and household settings, environmental surfaces and fomites can harbor MRSA for prolonged periods of time (Fritz et al. 2014; Kurashige, Oie, and Furukawa 2016; Desai et al. 2011; Justin Knox et al. 2012; Faires et al. 2012). While there are numerous studies detailing transmission within hospitals (Price et al. 2017; Tosas Auguet et al. 2018; Price et al. 2014), there is limited understanding of the role of environmental contamination in household MRSA transmission dynamics. Moreover, our understanding of CA-MRSA transmission dynamics, and in particular directionality, between humans and pets is incomplete.

To best devise targeted, effective infection prevention interventions and therapies, it is essential to understand how MRSA is introduced into households, and once established, the household transmission dynamics. While MRSA colonization among household members has been evaluated previously, studies to date have been limited by either assessing a single time point, collecting only limited epidemiologic data, not evaluating the household environment, exclusion of companion animals, or performing low-resolution strain typing (T. F. Jones et al. 2006b; Huijsdens et al. 2006; Nerby et al. 2011; Mollema et al. 2010). The objective of this

study was to comprehensively define longitudinal, strain-level *S. aureus* dynamics, including the introduction of new strains into the household and transmission of established strains between household members, environmental surfaces, and pets in households of pediatric patients experiencing CA-MRSA infections. These dynamics were assessed in the context of extensive demographic, hygiene, health, and activity characteristics to ultimately inform household-level interventions to interrupt MRSA transmission and prevent recurrent infections.

Results

Study Population, Environment, and Colonization Status

From 2012-2015, 150 children (median age 2 years, range 1mo-18.6yrs) presenting with a CA-MRSA infection (149 SSTIs, 1 invasive) and their household contacts (n=521, median age 26 years) were enrolled at the baseline visit; 21 additional participants who joined the household during the 12-month longitudinal study (e.g., a newborn infant) were also enrolled. Median household size was 4 (range 2-13). Of the 150 households enrolled, 135 (90%) completed the 12-month study visit. Of the 671 individuals sampled at baseline, 540 (80%) were sampled at all subsequent time points, and of the 692 individuals enrolled overall, 650 (94%) were sampled at least twice consecutively. Of the 150 environments sampled at baseline, 108 (72%) were sampled at least twice consecutively, and 98 (65%) were sampled at every follow-up visit. Of the 154 total pets sampled throughout the study, 106 (69%) were sampled at least twice consecutively, and 74 (56%) of pets sampled at baseline were sampled at all follow-up visits.

Over the 12-month study period, of these 650 individuals, 513 (74%) of individuals were colonized at least once with *S. aureus* and 319 (46%) with MRSA (**Table 4**). Of the 671 individuals participating in at least one follow-up visit, 173 (26%) reported an interval SSTI (75, 50% of index patients). Of the 154 pets sampled at least twice, 68 (44%) were colonized with *S.*

aureus at least once, 44 (29%) with MRSA. Throughout the 12-month study period, 136 (91%) of homes had at least one *S. aureus*-contaminated environmental site; 104 (69%) exhibited at least one instance of environmental MRSA contamination.

Incidence of Acquisitions (Introductions or Transmissions) in Household Members

Across the 650 household members sampled at least twice consecutively in 144 households, 703 total acquisitions, defined as an incidence in which an individual became colonized with a unique strain type that was not recovered from that individual at the sampling 3 months prior (**Figure 4**). Of these, 308 (44%) can be considered "introductions," as the strain was not found in the household at any prior samplings; 297 (42%) can be considered "transmissions," as the strain was recovered from another household member, pet, or environmental site at the prior sampling. The remaining 98 (14%) acquisitions involved strains present in the household at a previous sampling, but not the prior sampling, and it is unclear whether these were transmissions or re-introductions (i.e., "indeterminate"). Of these 650 participants, 246 (38%) experienced at least one introduction, 205 (32%) were transmission recipients and 265 (41%) were putative transmission sources (i.e. experienced at least one transmission). Exemplar families and associated acquisitions are illustrated in Error! Reference source not found.**.** While transmissions were equally split between MSSA and MRSA strain types (150 and 147, respectively, p=0.87), there were significantly more introductions associated with MSSA versus MRSA (209 vs 100, p<0.0001, respectively).

Exogenous Introductions

Introduction Incidence

Of the 341 strain introduction events across household members and the environment, the novel strain was found on at least one household member in 237 (70%) of these events, and in at least one environmental site in 180 (53%) (**Table 5**). The novel strain was exclusively present in

the environment at the time of the introduction event in 94 (28%) of these incidences (**Figure 6**). When an introduction event occurred overall (personal and/or environmental), a median 1 (range 0-4) household members became colonized and median 1 (range 0-10) environmental sites became colonized with the novel strain (**Figure 6**).

Factors Associated with Introductions

After observing the frequency of introductions, we next sought to determine specific factors from the demographic, health, hygiene, and activities information gathered through the baseline visit and follow-up interviews that were significantly associated with these incidents. In the univariate analyses, individuals who reported washing hands "sometimes" (or more) after preparing food (p=0.003, *OR*=0.2 (0.1-0.6) or "always" after using the bathroom (p=0.01, *OR*=0.7; 95% *CI* 0.5-0.9), were less likely to experience an introduction. Introductions were more likely to occur in children (p=0.005, *OR*=1.6; 95% *CI* 1.2-2.2) and daycare attendees (p=0.005, OR=1.6; 95% *CI* 1.2-2.2). Individuals spending fewer nights in the household were more likely to experience an introduction (p=0.04, Kruskal-Wallis). Temporally, individuals in households with a lower personal *S. aureus* colonization pressure (i.e., proportion of body sites colonized across household members) were more likely to experience introduction of a novel strain (median 11% Interquartile Range [*IQR* 20%] vs 17% [*IQR* 30%], p=0.01). Households reporting at least one household contact with an SSTI in the year prior to enrollment exhibited significantly fewer introductions (p=0.04, 0.36 vs 0.58 introductions per person-sampling, Kruskal-Wallis). Introductions were not significantly associated with healthcare or prison exposure, nor reporting an interval SSTI or hospitalization during the longitudinal study.

Temporal, Multivariable Model of Introductions

We assessed the influence relevant factors associated with introductions in a longitudinal, multivariable generalized mixed effects logistic regression model across household members.

Eligible individuals were those sampled at two consecutive visits that had completed the enrollment survey, for a total of 2363 observations of 640 individuals in 143 households across 4 follow-up samplings. Introductions were more common in colder months, and frequent handwashing (an aggregate variable defined as "always" washing hands after using the bathroom or before preparing food, and at least "frequent" handwashing before eating or after handling a diaper, when applicable) trended toward a reduction in introductions (**Table 7**). Although visiting public locations, such as hair salons, locker rooms, and pools were considered in the model selection process, none of these remained in the final model.

Transmissions

Transmission Incidence

Across 205 observed household members ("recipients") that became colonized through one of the 297 transmissions (some individuals were transmission recipients at multiple samplings), there were 545 putative transmission paths from other household members ("putative sources") (**Figure 4**). Of these 297 transmissions, 138 (46%) were associated with a sole transmission source. The household environment served as a putative transmission source in 178 transmission paths to recipients, and in 62 (35%) of these cases, this strain was exclusively found contaminating environmental sites (**Table 5**). These transmissions were nearly equally split between MRSA (283, 52% of all putative transmissions) and MSSA (262, 48%). Transmissions were most common between siblings (112, 21%) and offspring to parents (101, 19%). Comparatively, cohabitating parents rarely transmitted strains between each other (25, 5%).

When considering transmissions stratified by age and gender, 10% of male children (89 of 898 individuals sampled twice with at least one *S. aureus* strain type present within the household), 9% of male adults (53 of 602), 8% of female children (79 of 942), and 7% of female adults (66 of 881) were transmission recipients (**Figure 7**). Across these transmission paths, the

normalized transmission risk (See Transmission Risk definition, **98**) was calculated, representing the average proportion of putative source sites colonized with a given strain when transmitted versus not transmitted to a given recipient. Adult females were significant transmission sources to male adults and male children (**Figure 7**). Bathroom surfaces were the significant sources of transmission to adult males, adult females, and male children. Kitchen surfaces were also significant transmission sources to adult females, male children, and female children. Surveyed electronics were significant transmission sources for female adults and female children.

Factors Associated with Transmission

Sources and recipients of transmissions may exhibit different household dynamics. Thus, we ran separate analyses for each cohort to determine factors that influence the success rate of colonized individuals ("sources") in transmitting their colonized strains and eligible individuals becoming colonized ("recipients") with the transmitted strains. Source and recipient pairs were significantly more likely to share a bedroom (p<0.0001, *OR*=2.2, 95% *CI* 1.6-2.9) and/or bed (p<0.0001, *OR*=2.1, 95% *CI* 1.5-2.9), towel (bath, face, or hand; p=0.001, *OR*=1.7; 95% *CI* 1.2-2.4), and hygiene items (e.g., razor, hairbrush; p=0.02, *OR*=1.4, 95% *CI* 1.1-2.0) compared to a random sampling of pairs of household members. At the household level, significantly more transmissions occurred in houses with a lower cleanliness score (p=0.004, median transmissions per person-sampling: 0.5 [*IQR* 0.8] "dirty" vs 0.25 [*IQR* 0.6] "clean" houses), those rented by the participants (p=0.03, median transmissions per person-sampling: 0.6 [*IQR* 1.0] rented vs 0.29 [*IQR* 0.5] owned houses), and those with a higher number of individuals per square foot (p=0.003, Spearman's ρ=0.25). Across samplings, households with higher personal *S. aureus* colonization pressure (p=0.008, ρ=0.16), higher environmental *S. aureus* colonization pressure (p=0.006, ρ=0.14) and a higher number of strain types across the environment and household members (p<0.0001, ρ=0.27) were all associated with significantly more transmissions.

Transmission Recipients – Univariate Analyses

Hygiene practices, such as showering versus bathing (p=0.007, *OR*=0.6; 95% *CI* 0.4-0.9), brushing teeth at least daily (p=0.01, *OR*=0.6; 95% *CI* 0.3-0.9), and employing antibacterial hand soap (p=0.004, *OR*=0.6; 95% *CI* 0.4-0.8) were significantly associated with reduced incidence of receiving a transmitted strain. Transmission recipients were more likely to be children (p=0.02, *OR*=1.5; 95% *CI* 1.1-2.1) and individuals that shared a bath towel (p=0.02, *OR*=1.5; 95% *CI* 1.1-2.2) or cosmetics (p=0.03, *OR*=2.0; 95% *CI* 1.1-3.6). Transmission recipients were more likely to report experiencing an SSTI during the same interval as the transmission, (p=0.04, *OR*=1.5; 95% *CI* 1.0-2.2), and live in households with a higher personal *S. aureus* colonization pressure (p<0.001, *S. aureus* colonization pressure: 24% [*IQR* 20%] recipients vs 20% [*IQR* 20%] non-recipients).

Transmission Recipients – Temporal, Multivariable Model

In the transmission recipient model, eligible individuals included all those that had completed the enrollment interview, had been sampled at the prior and current sampling, had the environment sampled at the prior sampling, and lived in households with at least one *S. aureus* strain present at the prior sampling. This led to a total of 2952 observations across 603 household members in 134 homes across the four follow-up visits. In this model, transmission was associated with increasing environmental strain type colonization pressure and sharing a bedroom with an individual colonized with the transmitted strain. Reporting an SSTI since the prior sampling was also significantly associated with transmission. Conversely, the likelihood of successful transmission of a given strain type was significantly reduced by increasing environmental contamination pressure of all other strain types in the household and dwelling ownership.

Transmission Sources – Univariate Analyses

Across colonized individuals, those sharing a bedroom (p=0.04, *OR*=1.6; 95% *CI* 1.0-2.4) or bath towel with another individual (p=0.003, 1.9; 95% *CI* 1.2-2.8) or employing bar soap for hand washing (p=0.03, *OR*=1.8; 95% *CI* 1.1-2.9) were significantly more likely to successfully transmit their colonizing strain at least once. However, colonized individuals employing antibacterial hand soap were significantly less likely to transmit a strain (p=0.03, *OR*=0.6; 95% *CI* 0.4-0.9). The number of anatomic sites colonized with a given strain type on a source was not associated with transmission of this strain type (p=0.77, both successful and unsuccessful transmission sources: mean 1.25 anatomic sites).

Transmission Sources – Temporal, Multivariable Model

For the transmission source model, eligible individuals included all those that had completed the enrollment interview, were colonized with at least one *S. aureus* strain at the prior sampling, and lived in households with other individuals not colonized with this strain, for a total of 1,125 observations of 477 individuals in 139 households across the four follow-up samplings. Although many hygiene behaviors were examined, such as hand washing and bathing frequency, and proximity measures to other individuals, such as sharing a bed or bedroom, sharing a towel (bath, face, hand) was the only behavior significantly associated with increased likelihood of transmission. An increasing number of individuals per bathroom was significantly associated with an increase in the number of successful transmissions in a household (**Table 7**).

Incidence and Factors Associated with Pet Introductions and Transmissions

A total of 106 pets were sampled at least twice consecutively to assess transmission dynamics across 57 households. Nineteen pets, 14 dogs and 4 cats, were associated with 22 introductions (**Figure 8**). In 10 of these 24 introductions, this strain type did not colonize any household members nor contaminate the environment (i.e., pet-exclusive introduction event).

Fifteen pets served as putative transmission sources across 26 transmission paths to people; in 3 of these 26 transmission paths, the pet was the only source within the household. Only 7 of 26 paths were transmissions to the primary caretaker, while the other 19 were to other household members. Thirty-five pets (33%) were transmission recipients. Of the 67 transmission paths from household members to pets, 13 were associated with the primary caretaker as a possible transmission source. Pets associated with introductions and transmissions were not more likely to share a bed with another household member, exhibit signs of poor health, or report a history of SSTIs.

Discussion

To best target interventions to interrupt *S. aureus* transmission in households, it is essential that we understand the acquisition of novel strains, their source, and how these sources differentially influence the incidence of acquisition. Previous studies addressing *S. aureus* in households have been limited in their ability, either due to a lack of temporal resolution or molecular typing with insufficient discriminatory power, to identify whether a strain originates from within the household or the greater community (Nerby et al. 2011; Mollema et al. 2010; Papastergiou and Tsiouli 2018; Osterlund et al. 2002; L'Hériteau et al. 1999). The design of the present study affords the ability to truly discern household transmission dynamics: 96% of eligible household members enrolled, colonization samples were obtained at 5 time points longitudinally over one year from people, environmental surfaces, and pets, and molecular typing was performed on all recovered *S. aureus* isolates. Here, we have demonstrated that household MRSA acquisition is equally driven by both introductions of novel strains into the household and transmissions within the household, and that household environmental contamination serves as a key reservoir for transmission risk. This longitudinal study has demonstrated that prior correlates of colonization (e.g., history of SSTI and household

cleanliness), as well as correlates of transmission (e.g., sharing personal hygiene items), are connected indeed specifically to transmission likelihood, rather than simply colonization or general acquisitions, and therefore future interventions must inclusively target household members and the environment.

S. aureus acquisitions occurred by transmissions and introductions, with MRSA equally associated with introductions and transmissions and MSSA associated with more introductions than transmissions. Ng et al. reported that in households with MRSA-infected index patients, of the 68 households in which household contacts were not colonized with MRSA at initial screening, at least one household contact acquired subsequent colonization in 27 (40%) households over the following 3 months (W. Ng et al. 2017). Our study suggests that these observed acquisitions were equally likely to be introductions or transmissions; further, that MRSA acquisition in these households is driven not by one strain type, but possibly multiple strain types acquired from the exogenous environment. Here, we have found transmission risk specifically associated with history of SSTI and sharing fomites with colonized individuals, while poor hand washing practices and daycare attendance were exclusively associated with introductions. These associations were previously reported for *S. aureus* colonization risk (Fritz, Hogan, Hayek, Eisenstein, Rodriguez, Krauss, et al. 2012b; Creech, Al-Zubeidi, and Fritz 2015; Mork et al. 2018; Marimuthu, Pittet, and Harbarth 2014; Braga et al. 2014; J. A. Patel et al. 2015). Introductions from sources outside the household and transmissions within households are distinct mechanisms by which an individual can become colonized by *S. aureus*, each with disjoint risk factors previously considered correlates of overall *S. aureus* colonization.

To identify mechanisms by which *S. aureus* is introduced into households, we queried a wide variety of factors and activities exogenous to the household (e.g., occupation, using

personal service establishments, exercise facilities). We found that introductions significantly occurred in children. Indeed, the only exogenous activity associated with acquisition for children was daycare attendance, a factor which has been previously found to significantly reduce time to *S. aureus* acquisition in infants during the first 6 months of life (J. A. Patel et al. 2015). Contact sports have been associated with MRSA colonization (Jiménez-Truque et al. 2017), and MRSA has been recovered from exercise equipment at fitness centers (Mukherjee et al. 2014). In the present study, sports participation and gym attendance were not significantly associated with introductions. Further, locations and professions associated with high colonization risk, such as working in schools (Hanselman et al. 2008) or healthcare facilities (Legrand et al. 2015; Peters et al. 2018), were not significantly associated with introductions in adults. Importantly, hand hygiene was shown to be particularly protective against introductions. Combined, these data may indicate that quality hand hygiene techniques may protect an individual from acquisitions in daily life even when colonization incidence is high. As these individuals had been working or attending these places before study enrollment, it is possible that the strain types acquired at these locations had already been established in the household prior to study enrollment and thus did not appear to be introductions during our study. Interestingly, exclusive environmental introductions (i.e., the novel strain was only recovered from an environmental surface) comprised one-quarter of all household introduction incidences. Potential mechanisms for these introductions include household members who had spontaneously resolved their personal colonization prior to sampling, household visitors, or contaminated fomites brought into the home such as shoes and clothing, which may serve as reservoirs for such strain types.

To identify targets for intervention, the present study also sought to discern strain-level transmission dynamics within households. Individuals acquiring a strain via transmission

("transmission recipients") often shared personal hygiene items or towels with strain-colonized individuals. While sharing a bedroom and/or towels has been associated with increased individual colonization risk (Mork et al. 2018), the longitudinal and strain-level detail afforded by this study reveals that these act as reservoirs, or indicators of reservoirs, of transmission. Additionally, a high burden of the given *S. aureus* strain in the household environment was highly predictive of transmission. This reinforces the concept that environmental contamination serves as a strong indicator specifically of transmission success, rather than solely through personal contact or a high degree of *S. aureus*/MRSA colonization among household members, as previously reported in hospitals and households (Popoola et al. 2013; M. Rodriguez et al. 2013). Further, the significant number of times that transmission paths included environmental sources, and exclusively in 35% of instances, indicates that household fomites serve as sources of *S. aureus* transmission. Lastly, transmission recipients experienced significantly higher incidence of SSTI. Given the sampling interval of 3 months, we are not able to discern this "chicken or egg" phenomenon, but it is plausible that acquisition of a new strain through a transmission event led to development of an SSTI.

Transmission of MRSA between pets and their owners or veterinary personnel has been described, including reports of healthy pets colonized with MRSA in households with persistent human colonization or recurrent infections, though the directionality of transmission is unclear (Hanselman et al. 2006; J. S. Weese et al. 2006; van Duijkeren et al. 2004; Boost, O'Donoghue, and James 2008; Loeffler and Lloyd 2010; Ferreira et al. 2011). A recent study by Shahbazian et al., associated the presence of pets with increased risk of household environmental multidrug-resistant MRSA contamination (Shahbazian et al. 2017). In the present study, indoor pet dogs and cats contributed to the overall transmission dynamics of *S. aureus* within households, though

these events were not associated with pet age, overall health, or recent boarding. In this study, one-third of pets were transmission recipients over 12 months; approximately one-third of these transmission paths to pets were associated with the primary caretaker or someone sharing a bed with the pet. In contrast, pets were rarely the exclusive source of transmission events; only three transmission events occurred in which the pet was the only putative source. Similarly, Davis et. al., (2015) determined that a pet in a household of an individual with a recent MRSA infection was not implicated as the source of transmission to the human, as discerned by whole genome sequencing (M. F. Davis et al. 2015). These findings support traditional view that humans are more commonly the source of *S. aureus* to their pets, who are not believed to be natural hosts for *S. aureus*. Indeed, pets are more likely passive carriers who spontaneously resolve colonization, though while colonized, may serve as reservoirs for transmission or reacquisition (M. F. Davis et al. 2012; Cohn and Middleton 2010; Morris et al. 2012). Future research will illuminate whether decolonization of people and/or the household environment will affect pet colonization.

A principle focus of this study was to identify potential targets to prevent introduction of MRSA into the home, interrupt *S. aureus* transmission, and prevent recurrent SSTIs. We determined that transmission recipients were more likely to report interval SSTIs; as sharing personal hygiene items was associated with transmission among household members, measures that reduce transmission, including providing separate towels and hygiene items for each family member, may also reduce subsequent SSTI. In addition, consistent handwashing was protective against *S. aureus* introduction into the household, and thus, improved handwashing in daily life, especially in children attending daycare, may reduce *S. aureus* acquisition. Indeed, several trials conducted in households and childcare centers demonstrate that providing hand hygiene education materials, increased use of alcohol-based hand sanitizers, and implementation of

protocols for handwashing decrease the risk of subsequent gastrointestinal and respiratory illnesses (Sandora et al. 2005; Azor-Martinez et al. 2018). While the importance of hand hygiene may seem trite, compliance in controlled settings, such as hospitals, where the stakes are high, is sub-optimal, ranging from 13-68%, despite the staff having ready access to educational materials and hand hygiene products (Randle, Arthur, and Vaughan 2010; Karaaslan et al. 2014; Wetzker et al. 2016; A. Lee et al. 2011).

Individuals in the community face additional barriers, including limited resources. Potentially straight forward public health programs to implement effective hand hygiene measures and techniques in the community could have far reaching benefits to prevent a broad spectrum of infections (Prater et al. 2016; McGoldrick 2017). Lastly, *S. aureus* household environmental contamination was a significant predictor of transmission. As enhanced environmental disinfection in healthcare settings has been demonstrated to reduce pathogen transmission and acquisition, further research is needed in household settings to directly test the effectiveness of surface decontamination in reducing *S. aureus* transmission and SSTI incidence (Datta et al. 2011; Anderson et al. 2017).

Strengths and Limitations

Longitudinal sampling of household members, their environment, and pets, combined with comprehensive molecular typing and personal and household epidemiologic data, have allowed for the novel delineation of transmission versus introduction acquisitions within the context of the household and factors associated with these acquisitions. This study does have several limitations. As the isolate from interval SSTIs was unavailable for many reported interval infections, it was impossible to associate these SSTIs with a specific transmission or introduction. While repPCR allows for a high degree of strain discrimination (Marcela

Rodriguez et al. 2015), it is not as comprehensive as whole genome sequencing (WGS), and precludes the analysis of specific genomic signatures associated with transmission. While a 3-month sampling interval may be sufficient to identify acquisition events, it may miss the numerous, transient colonization events commonly found when performing high temporal resolution sampling that can exactly identify transmission sources.

Conclusions

In this comprehensive investigation of households, we discerned that individuals acquire MRSA through both introductions from exogenous sources and transmissions within the household, which exhibit distinct hygiene and behavioral etiologies, and these transmissions are significantly associated with interval SSTIs. Introductions may be mitigated through frequent hand washing while transmissions were associated with sharing behaviors and high burdens of strains in the household environment. Thus, prospective, longitudinal studies of at-risk populations are necessary to investigate targeted decolonization regimens for sources and protective hygiene practices for their cognate recipients combined with environmental surface decontamination.

Chapter 4: Strain-Excluding Persistent *S. aureus* Colonization Modulates Recurrent SSTI Risk in Exposed Households

This chapter is adapted from a version of the upcoming manuscript: **Strain-Excluding Persistent *S. aureus* Colonization Modulates Recurrent SSTI Risk in Exposed Households**, in preparation for submission at *Clinical Infectious Diseases*. The authors of this manuscript are as follows: Hogan P, Mork R, Thompson RT, Muenks CE, Boyle MG, Sullivan ML, Orschelm RC, Gehlert SJ, Bubeck Wardenburg J, Burnham CD, Rzhetsky A, Fritz S.

Introduction

Staphylococcus aureus presentation ranges from asymptomatic colonization to invasive, life-threatening disease, and is the most frequent cause of skin and soft tissue infection (SSTI) in the United States (Loren G. Miller and Diep 2008; Kaplan 2006). The emergence of epidemic community-associated methicillin-resistant *S. aureus* (CA-MRSA) in the last two decades has created unique infection prevention challenges and caused widespread economic burden by spreading via asymptomatic colonization and establishing a niche in community reservoirs (B. Y. Lee et al. 2013; Otto 2007; Dukic et al. 2013). CA-MRSA is a disease of households (Fritz, Hogan, Hayek, Eisenstein, Rodriguez, Epplin, et al. 2012; Fritz, Hogan, Hayek, Eisenstein, Rodriguez, Krauss, et al. 2012b; M. Rodriguez et al. 2013). Up to 70% of patients with CA-MRSA SSTI will experience a recurrent infection within a year, and household contacts of those with SSTI are at increased risk of colonization and subsequent infection (Fritz, Hogan, Hayek, Eisenstein, Rodriguez, Epplin, et al. 2012; Fritz, Hogan, Hayek, Eisenstein, Rodriguez, Krauss, et al. 2012a; Loren G. Miller et al. 2015; Nerby et al. 2011; W. Ng et al. 2017). In addition to personal *S. aureus* colonization, household reservoirs include environmental contamination and pet carriage (W. Ng et al. 2017; Fritz et al. 2014; Justin Knox et al. 2016a). These CA-MRSA reservoirs can serve as chief contributors to persistent colonization and recurrent infection in the community setting.

States of *S. aureus* colonization include non-carriage, intermittent colonization, and persistent colonization (J. L. Nouwen et al. 2004; Sollid et al. 2014). Colonization can resolve spontaneously or through use of topical antimicrobials (McConeghy, Mikolich, and LaPlante 2009). Persistently colonized individuals serve as a constant reservoir for transmission to others and their environment and are thus of particular interest. One six-month study found that colonization persisted for a median duration of 140 days following MRSA SSTI with concurrent MRSA colonization, despite the majority of patients receiving systemic antibiotics and some receiving topical antimicrobials (Cluzet et al. 2015). At the household level, baseline MRSA environmental contamination has been shown to be a risk factor for recurrent environmental contamination (Eells et al. 2014) and human body colonization (M. Davis et al. 2017) in the three months following infection.

S. aureus colonization is a known risk factor for infection (Fritz et al. 2009). Recurrent infections, and infections in close contacts of infected individuals, are particularly concerning. Two recent studies demonstrated significant incidence of recurrent infection in index cases, with 43-51% reporting recurrence in 6 months, along with subsequent SSTI in 13% of household contacts (Loren G. Miller et al. 2015; Justin Knox et al. 2016a). Baseline MRSA environmental contamination was a risk factor for index case recurrent SSTI. These studies did not, however, assess the link between human colonization, or specifically persistent colonization, and recurrent SSTI.

To abate the household *S. aureus* reservoir and ultimately prevent recurrent SSTI, we must understand how and where *S. aureus* persists in households, and what relationship this persistence has to subsequent SSTI. Previous studies have provided insights into persistent colonization and recurrent infection separately, but there is a paucity of literature regarding the

interplay between these two phenomena. Our study connects persistent *S. aureus* colonization with recurrent SSTI by incorporating a household-level, high-resolution molecular typing methodology with comprehensive sampling of household members, environmental surfaces, and pets at 5 time points over 1 year, and examines persistence events at both a personal and environmental level.

We sought to identify factors associated with persistent *S. aureus* colonization and recurrent SSTI over one year in households of children with CA-MRSA SSTI, to examine the relationship between persistent colonization and recurrent SSTI, and to identify targets for intervention to interrupt the *S. aureus* household reservoir contributing to persistence and recurrent SSTI.

Results

Study Population and Longitudinal Colonization Status

From 2012-2015, 150 households were enrolled in a 12-month longitudinal study. Index patients (n=150, median age 2 years, range 1mo-18.6yrs) presenting with a CA-MRSA infection, their household contacts (n=542, median age 26 years), and indoor companion animals (n=154, dogs (n=116) and cats (n=38)) were enrolled. Median household size was 4 (range 2-13). Of the 150 households enrolled, 135 (90%) completed the 12-month study visit. Longitudinally, 540 (78%) participants were sampled at all five time points and 650 (94%) were sampled at least twice consecutively; 98 (65%) environments were sampled at all five time points and 108 (70%) were sampled at least twice consecutively; and 74 (48%) pets were sampled at all five time points and 106 (69%) were sampled at least twice consecutively. Longitudinally, 513 (74%) participants were colonized at least once with *S. aureus*, 319 (46%) with MRSA; 136 (91%) household environments were colonized at least once with *S. aureus*, 104 (69%) with MRSA;

and 68 (44%) participants were colonized at least once with *S. aureus,* 44 (29%) with MRSA (**Table 4**).

Individual Persistent Colonization Overview

Of the 650 household members across 147 households sampled consecutively, there were 471 total persistent colonization events (i.e., colonization with the same strain type at consecutive samplings); 245 (52%) were MRSA strain types. Site-specific persistent colonization was observed in 324 (69%), 112 (24%), and 28 (6%) of these events in the nares, groin, and axilla, respectively. Of the 540 participants completing all 5 samplings (every 3 months for a year), 254 (47%) were colonized at least twice consecutively with *S. aureus*; of these, 127 (50%) were persistently colonized with the same strain type (**Table 8**). Of the 117 individuals colonized consecutively with MRSA, 109 (93%) were persistently colonized with the same strain type. Index patients were not significantly more likely to be persistently colonized with MRSA than household contacts (p=0.07, 33 (26%) vs 76 (18%), respectively). The degree of persistent colonization (i.e. the number of consecutive times colonized) between individuals persistently colonized with MRSA versus MSSA was equivalent (p=0.8, median 3.0, 2.0, IQR 1.0, 2.0, respectively). Compared to household members, environmental surfaces rarely exhibited persistence events (**Table 10**).

When considering the 91 households in which the strain type of the isolate recovered from the index patient's enrollment infection ("infecting strain type") was identified, index patients were significantly more likely to have persistent colonization events of the infecting strain type compared to contacts (p=0.02, 7.6% of events versus 3.8% of events, respectively) (**Figure 9**); index patients and household contacts did not exhibit significant differences in the number of MSSA and non-wound MRSA persistence events.

Factors Associated with Individual Persistence

A univariate analysis across hygiene and demographic factors was next conducted to identify characteristics that may reduce individual persistence risk. At the individual level, eczema diagnosis (p=0.03, *OR*=1.77; 95% *CI* 1.1-2.9) and sharing a bath towel (p=0.005, *OR*=1.8; 95% *CI* 1.2-2.7) were significant risk factors for persistence. While mupirocin decolonization of the nares significantly reduced persistence incidence (p=0.0002, *OR*=0.39; 95% *CI* 0.2-0.7), body washes with bleach baths or chlorhexidine did not (p=0.8). Individuals in households that owned their dwelling (p=0.0005, 0.62 vs 1.12), had a mother with at least a high school education (p=0.005, 0.75 vs 1.71), or were rated 'clean' by the interviewers from the household cleanliness scale (p=0.03, 0.72 vs 0.98) exhibited significantly fewer persistence events. The number of individuals per square foot was also significantly correlated with the number of persistence events observed in the household (p=0.004, ρ=0.24), as well as the monthly average low temperature at sampling (p=0.02, ρ=0.11); further, both *S. aureus* personal colonization pressure and environmental colonization pressure at prior sampling significantly correlated with the number of persistence events (p<0.0001, 0.0001; ρ=0.43, 0.26, respectively).

Multivariable Model of Individual Strain Persistence

To delineate factors that contribute to strain type persistence on individuals longitudinally, a multivariable generalized linear mixed effects logistic model was constructed that evaluated whether an individual colonized with a given strain type maintained colonization with the same strain type at the subsequent sampling versus clearing colonization of this strain type (albeit the individual could become colonized with a new strain type). This model evaluated a total of 905 time points across 443 individuals in 133 households longitudinally. Individuals performing frequent handwashing (an aggregate variable defined as "always" washing hands after using the bathroom or before preparing food, and at least "frequent" handwashing before

eating or after handling a diaper, when applicable) were significantly more likely to suffer from persistent colonization, while those reporting interval decolonization of the nares were significantly less likely to experience a persistence event (**Table 12**). At the household level, increasing environmental strain colonization pressure was associated with increased individual persistence, while home ownership and increasing personal colonization pressure of other strain types in the household were both protective against persistent carriage. Notably, occurrence of an SSTI since the previous sampling was considered during model selection but was not retained in the final model.

Incidence and Risk Factors of Persistence on Pets

Of the 154 pets enrolled within the study, 106 (53 dogs, 53 cats) were sampled consecutively at least once throughout the study period across 57 households. Longitudinally, 9 pets (6 dogs and 4 cats) exhibited a total of 13 persistence events. The primary caretaker or the individual that shared a bed with the pet was also colonized with this persistent strain type at the previous sampling in 7 (54%) and 3 (23%) of these 13 events, respectively. Pet age, health status, history of SSTI, sharing a bed with a caretaker, and overall environment and person *S. aureus* colonization pressure were not significantly associated with persistence events in pets ($p>0.05$).

Household Strain Persistence

Across the 129 households in which household members and environmental surfaces were sampled consecutively at least once throughout the 12-month longitudinal study, 81 (63%) had at least one *S. aureus* strain type persist across ≥3 consecutive samplings (54, 42% had at least one MRSA strain type persist) when considering *S. aureus* strain types observed at enrollment and 3 months (**Table 9**).

In 9 of 54 (17%) households with persistent MRSA colonization across ≥3 consecutive samplings, this strain type persistently colonized exclusively one person, while in 33 (61%) this

strain type colonized ≥ 3 individuals longitudinally. The majority of these households (51, 94%) had this persistent MRSA strain type contaminating the environment. Across households and longitudinal visits, the infecting strain showed a higher degree of household persistence than recovered MSSA or non-infecting MRSA strains (p<0.0001, infecting strain recovered 1.78 average higher number of consecutive samplings vs. all other MSSA or MRSA strains recovered) (**Figure 9**). Moving during the study period was associated with a significant reduction in strain type persistence across household members, pets, and the environment (p=0.049, *OR*=0.5; 95% *CI* 0.2-1.0).

Multivariable Logistic Models of Strain Persistence

To determine significant risk factors for longitudinal strain type household persistence, a generalized linear logistic mixed model was constructed to predict whether a given strain type persists from a given sampling to the next on at least one individual, environmental site, or pet. This model incorporates 792 strain type-time points within 124 households. Strain types with a higher personal colonization pressure or that were MRSA (as opposed to MSSA), and households that exhibited a higher strain richness exhibited a higher likelihood of strain type persistence (**Table 12**). Households with a higher personal colonization pressure of the non-reference strain or crowding were less likely to have strain type persistence.

Factors Associated with Longitudinal Strain Contamination and Persistence on Environmental Sites

The impact of specific hygiene behaviors upon longitudinal environmental *S. aureus* contamination and persistence was considered by examining how the cleaning frequency of specific environmental surfaces accorded with the degree of longitudinal strain type CP and persistence. Increasing number of people per bathroom (p=0.02, ρ=0.2) was associated with increasing strain type environmental persistence, while the number of people per square foot was

positively associated with the degree of longitudinal strain type environmental CP (p=0.02, ρ=0.2) but not strain type persistence (p=0.4). Increasing proportion of individuals exhibiting quality hand hygiene significantly reduced longitudinal strain type environmental CP (p=0.05, ρ=-0.2), as well as index patients employing antibacterial hand wipes (p=0.02, Relative Risk [*RR*]=0.8), but not antibacterial soap nor alcohol-based hand sanitizer (p=1.0, 0.9, respectively). Towels in households washing towels in hot water (p=0.01, *RR*=0.6) or after at least seven usages (p=0.002, *RR*=0.3) had significantly less longitudinal strain type contamination.

The frequency of cleaning numerous environmental surfaces, such as the kitchen sink and counter, refrigerator door handles, bathroom doorknob, or bathroom counter, did not significantly reduce the longitudinal strain type contamination observed on these surfaces (p=0.2, 0.6, 0.8, 0.5, 0.7, respectively). The household cleanliness score rating assigned by the study team was also not significantly associated with longitudinal strain type environmental CP nor persistence (p=0.2, 0.3, respectively).

Markov Model of Household Strain Persistence

A first order Markov Model was constructed to capture the persistence dynamics of *S. aureus* strains in households with adults, children, and the environment (**Markov Model of Household Strain Persistence**). In this model, strains move longitudinally between the five possible colonization states (colonizing at least one child [C], at least one adult [A], both [C+A] (all with or without environmental contamination [±E]), solely environment [E], or not present in household [X]) (**Figure 10**). Strain types in both the adult and child states tend to stay in these states (self-transition frequencies 58%, 57%; 95% *CI* 51-65%, 50-63%, respectively), indicating that strain types most commonly persist within households by two strategies: persistent colonization of one individual, or via transmissions within adults or within children. The equal

transition probabilities between children and adults (32%; 95% *CI* 26-37% for both C->A and A->C) further indicate that persistence via transmission between these household members is highly bidirectional and not biased in terms of transmissions between adults to children nor children to adults. Strain in the environment only state showed the lowest persistence compared to children or adults (self-transition frequency 45%; 95% *CI* 35-53%) as well as the highest likelihood of transitioning to the loss state (34%; 95% *CI* 27-41%).

Infecting Strain Household Persistence

Of particular interest were factors that contributed to persistence of the strain recovered from the index patient's enrollment infection within the subset of households where this strain type was known. A model was constructed to assess whether subsequent SSTIs and decolonization influenced the persistence of the infecting strain beyond enrollment (168 infecting strain type-samplings across 71 households) when controlling for socioeconomic status and density. Although the proportion of individuals reporting a mupirocin, chlorhexidine, or bleach bath decolonization measure was not significantly associated with reducing the persistence of the infecting strain, the proportion of household members reporting an SSTI was significantly associated with a higher likelihood of infecting strain persistence (**Table 12**).

Incidence of Recurrent SSTI and Factors Associated with Longitudinal SSTI

Across the 144 index patients who completed at least one follow-up visit over the 12-month study period, 75 (52%) reported at least one recurrent SSTI, with 37 (26%) reporting ≥2 subsequent SSTIs (**Table 4**). The 523 household contacts reported 133 SSTIs, 98 (19%) reporting at least one. Across the 256 reports of SSTIs (excluding the index enrollment SSTI), the etiological agent was available for molecular characterization in 19 (7%) of these incidences (**Table 11**); 16 (84%) were MRSA and 15 (79%) were index patient recurrent infections. Of 14 households in which the enrollment infecting strain and subsequent SSTI were available, 11

(79%) of the isolates were identical strain types. This infecting strain type was commonly found in the household at the previous sampling (14, 74%), often in the environment (9 of 17 sampled environments, 52%).

When considering the relationship between colonization status and follow-up SSTI, individuals exhibiting a higher degree of strain type MRSA persistence (i.e. number of consecutive samplings exhibiting colonization of the given strain) reported significantly more follow-up SSTIs ($p<0.0001$, $\rho=0.29$), while a higher strain type MSSA persistence degree was associated with fewer SSTIs ($p=0.02$, $\rho=-0.12$) (**Table 8**). When considering the most persistently colonized individual within households, the degree of the most persistently colonized MRSA individual was not only correlated with significantly more individual reports of SSTI ($p<0.0001$, $\rho=0.34$), but also more SSTIs on other household members ($p=0.001$, $\rho=0.28$). The opposite relation was observed for the degree of MSSA persistence on the most persistently colonized individual; these individuals reported significantly fewer SSTIs ($p=0.01$, $\rho=-0.23$) and their contacts also reported significantly fewer SSTIs ($p=0.04$, $\rho=-0.18$).

Factors Associated with SSTI

Hygiene, demographic, and colonization status were next considered in a univariate analysis to identify factors associated with SSTI across participants. Individuals reporting a history of SSTI in the year prior to enrollment ($p<0.001$, *OR*=5.9; 95% *CI* 4.0-8.7) and children ($p=0.008$, *OR*=1.6; 95% *CI* 1.1-2.3) were significantly more likely to report at least one interval SSTI. Hygiene measures, such as showering (vs. bathing, $p=0.006$, *OR*=0.6; 95% *CI* 0.4-0.8), washing hands after using the bathroom ($p=0.03$, *OR*=0.7; 95% *CI* 0.5-0.96) or preparing food ($p=0.03$, *OR*=0.3; 95% *CI* 0.1-0.8), and at least daily teeth brushing ($p=0.03$, *OR*=0.6; 95% *CI* 0.4-0.9), were significantly protective against follow-up SSTI. Temporally, those colonized with

MRSA at the prior sampling (p<0.0001, *OR*=2.9; 95% *CI* 2.1-3.8) or sharing a towel with an MRSA-colonized individual (vs. not sharing a towel or sharing a towel with a non-colonized individual p=0.003, *OR*=1.5; 95% *CI* 1.2-1.9) were at a significantly higher risk for reporting an interval SSTI. The number of body sites colonized by MRSA at the prior sampling was associated with significantly higher interval SSTI risk (p=0.004, *RR*=1.3). Conversely, individuals colonized with MSSA (p=0.01, *OR*=0.6; 95% *CI* 0.5-0.9), or sharing a room (p=0.01, *OR*=0.6; 95% *CI* 0.4-0.9) or towel (bath, hand, and/or face p=0.04, *OR*=0.8; 95% *CI* 0.6-0.99) with an MSSA-colonized individual at the prior sampling, were all at a significantly lower risk of interval SSTI.

Considering household-level activities, hygiene, and colonization status and SSTI risk, frequent cleaning of refrigerator handles was associated with significantly fewer interval SSTIs (p=0.03, 0.05 vs 0.09 SSTIs per person-sampling). Households with at least one household contact reporting a history of SSTI in the year prior to study enrollment were associated with significantly more interval SSTIs (p=0.004, 0.11 vs 0.06 SSTIs per person-sampling). When considering colonization status of household members and the environment, higher personal (p<0.0001, ρ=0.22) and environmental (p=0.005, ρ=0.13) MRSA colonization pressure were associated with significantly more interval SSTI reports across household members, while increasing personal MSSA colonization pressure was significantly associated with fewer interval SSTIs (p=0.0005, ρ=-0.15). Although investigated, gym attendance, sport (and contact sport specifically) participation, and public shower and pool usage was investigated, none of these were significantly associated with interval SSTI.

Multivariable Model of Interval SSTI Incidence

To assess factors associated with interval SSTI, a multivariable logistic time series generalized linear mixed model was constructed for each follow-up visit. This model evaluated 640 individuals in 142 households longitudinally for a total of 1952 samplings. Individuals who reported a history of SSTI in the previous year at enrollment (including index enrollment infection) as well as index patients were significantly more likely to report an interval SSTI (**Table 12**). At the household level, increasing environmental MRSA CP at the prior sampling was significantly associated with interval SSTI. Although frequent handwashing (see Multivariable Logistic Models of Strain Persistence), sharing a bedroom, a hygiene item (e.g. a razor, brush, comb, toothbrush, and/or deodorant) or t6owel with an MRSA-colonized individual, interval strain type persistence, and MRSA colonization status at the prior sampling were considered in model selection, none of these covariates were retained in the final model.

Discussion

Prevention of *S. aureus* SSTI within the community is challenging due to the high incidence of recurrence in individuals, potentiated by an inability of the immune system to develop a protective response (Fritz et al. 2013), as well as asymptomatic colonization in providing a reservoir of infectious organisms to susceptible individuals (Chisholm et al. 2018). Increasingly, we are appreciating these factors lead to the perception of *S. aureus* SSTI as a disease of households, necessitating household-wide changes to behavior to prevent recurrent disease.

As a result of the CA-MRSA epidemic, increasingly high-resolution studies have been conducted to elucidate how MRSA colonization results in recurrent SSTI and how decolonization practices may prevent such outcomes (Fritz, Hogan, Hayek, Eisenstein, Rodriguez, Epplin, et al. 2012; Cluzet et al. 2016; Fritz, Camins, et al. 2011). Here, we have

connected longitudinal sampling of both household members and their environment to subsequent SSTI to identify how individual and household colonization dynamics lead to SSTI, and through careful molecular typing, the source of likely etiological agents of these infections.

Our study examines persistent colonization at an unprecedented level—longitudinally, at three body sites (nares, groin, and axilla), and at high molecular resolution of all *S. aureus* strain types encountered (Eells et al. 2014). The majority of individuals exhibiting persistence were colonized in the nares, and decolonization of the nares was specifically protective against persistent colonization in both the univariate analysis and the multivariable model. Strain type environmental CP was also a dominant predictor of individual persistence and opposed by the strain type CP of all other strains in the household in the multivariable model, indicating that strain types persist within households partially through continued re-exposure of individuals through the environment. However, since the Markov persistence model showed exclusive environmental colonization to be the least stable persistence state, it may be that persistently colonized individuals commonly shed their strain types to the environment, reinforced by the observation that close proximity to other individuals (in terms of sharing towels or individuals per square foot) also increases persistence risk. Longitudinal persistence is thus both an individual and household-level phenomenon, and individual persistence may be the result of continual re-colonization by exposure to other colonized individuals and contaminated environmental sites.

By pairing longitudinal colonization status with interval SSTI reports, this study both reinforces previous observations of the prevalence of recurrent SSTI and reveals the household as the source of the strains causing these infections. As with previous studies, we have found that individuals suffering from an SSTI are likely to develop subsequent SSTIs, and are in close

proximity to others with a history of SSTI (Loren G. Miller et al. 2015). When the etiological agent of the SSTI was known, it was present within the household 74% of the time, and when known, this strain type was usually (78%) concordant to the enrollment infecting strain type (a strain type that had already caused at least one infection in the household). This strain type, moreover, was equally likely to be found colonizing the individual reporting the SSTI and household contacts (8 versus 9 of 19 interval SSTIs). These SSTIs were typically recurrent infections in individuals with a history of MRSA colonization and/or high household MRSA exposure. SSTIs are a problem of households: their sources are primarily already present within the household, and therefore household-level interventions are integral to reducing incidence of recurrent SSTI.

Considering MRSA colonization from an individual, household, and environmental context longitudinally has reinforced its role in individual SSTI risk and revealed that environmental contamination serves as a primary risk factor for SSTI. *S. aureus*, specifically MRSA, colonization has been cited in numerous studies as a significant risk factor for SSTI; individuals with a history of colonization are at significantly higher risk for the SSTI in both a hospital setting and the community (Kalmeijer et al. 2000; Otto 2007; Fritz et al. 2009; Ellis et al. 2004). Here, prior MRSA colonization was associated with subsequent interval SSTI in the univariate analysis, and the degree of MRSA colonization further correlated with the number of subsequent SSTIs, replicating previous observations. Persistence of the infecting strain was also significantly associated with interval SSTI. This underlies a 'vicious cycle' of MRSA in households: persistent MRSA colonization potentiates SSTI, and SSTI increases the exposure of individuals to MRSA, increasing the likelihood of transmission to other household members and persistent colonization.

MRSA environmental contamination was significantly correlated with interval SSTIs in both univariate and multivariable analysis. Further, the environment was the location most commonly contaminated with strain types (the majority of which were MRSA) subsequently identified as the etiological agent of interval SSTI, which was found in a previous study to be a significant risk factor for recurrent SSTI (Justin Knox et al. 2016a). Since the environment clearly serves as a reservoir of MRSA readily able to cause infections within the home, environmental decolonization measures and practices may be concluded as essential in preventing SSTI.

Since environmental MRSA contamination was strongly associated with both persistence and interval SSTI, identifying hygiene practices that significantly reduce *S. aureus* contamination of the environment are particularly germane to the long-term prevention of recurrent infection. Our study provides a particularly powerful analysis by analyzing the effectiveness of specific cleaning behaviors with their respective site. However, we found no specific cleanliness behavior associated with significantly reducing persistent colonization, although more frequent replacement of kitchen hand towels and hotter wash temperature for bathroom towels both significantly reduced overall observed *S. aureus* colonization. These cleanliness behaviors may be particularly relevant since sharing contaminated bathroom towels was a significant risk factor for both interval SSTI and persistent colonization. High handwashing score was also significantly correlated with reduced *S. aureus* environmental contamination. The frequency of cleaning specific sites was not found to significantly correlate with reduced *S. aureus* colonization, especially in commonly-handled surfaces such as fridge handles or electronics. Possible reasons for this include incongruency in exactly how individuals clean between households, or the fact that the handling frequency of these objects leads to rapid

contamination that outpaces cleaning frequency. Effective household decontamination, therefore, must be paired with increased hand hygiene and frequent replacement of kitchen and bathroom towels, with carefully specified regimens for surfaces.

Our study observed a striking difference in SSTI risk associated with individuals and households experiencing significant MRSA versus MSSA colonization. While the majority of SSTIs are the result of MRSA in the community, MSSA is still commonly observed as an etiological agent; accordingly, *S. aureus* colonization (MRSA or MSSA) is considered a significant risk factor for SSTI (Albrecht et al. 2015; Tong et al. 2015). While this trend was also found in our study, measures of MSSA colonization and proximity to colonized individuals (sharing bedroom or towels, as well as MSSA environmental and personal CP) were protective of interval SSTI. In households with a specific history of MRSA, MSSA colonization may measure the degree other *S. aureus* strains can outcompete the MRSA strain types perpetuating SSTIs, preventing further SSTIs.

From our in-depth analysis of individual and household factors, we can postulate that SSTI prevention and intervention measures must be targeted at both the individual and household level, with specific care made to decontaminate environmental surfaces that were found to commonly harbor strains associated with subsequent SSTI. At the individual level, the univariate analysis indicates that attention to handwashing, especially after using the bathroom, as well as general hygiene (measured indirectly through daily oral hygiene) were impactful in reducing SSTI. Mupirocin decolonization may be most effective in preventing persistent colonization of a noxious strain in individuals suffering from recurrent SSTIs. Pairing decolonization with environmental decolonization efforts, such as frequent cleaning or replacement of frequently-touched surfaces (kitchen handles, bathroom handles, towels) is also

necessary to reduce MRSA contamination and thus SSTI, since interviewer cleanliness rating was associated with significantly fewer persistence events. Our study highlights that individual decolonization in the absence of measures taken to reduce environmental MRSA contamination may be ineffective in preventing recurrent SSTI.

Future Directions

Our study clearly indicates that recurrent SSTI is a problem that encompasses the entire household, both individuals and environmental surfaces, typically caused by the same strain type. While this observational study was successful in providing this perspective, future case-control studies are necessary to elucidate how specific combinations of individual, household contact, and environmental decolonization efforts are more or less effective in preventing recurrent SSTI. Our study also indicates that MSSA strains are protective in preventing SSTIs, and that the relative degree of different strain types can destabilize a given strain type's capacity to persist longitudinally on an individual. These data highlight the possibility that nonpathogenic, engineered strains that can outcompete *S. aureus* may be capable of significantly reducing transmission and subsequent SSTIs within households. Future studies are necessary to explore the utility and efficacy of a 'competitive strain' in combination with traditional decolonization methods.

Conclusions

Understanding how *S. aureus* colonization and SSTI develops in households and the role the household milieu dictates incidence of persistence and recurrent infection is necessary in developing streamlined, effective prevention measures. Here, we have paired longitudinal sampling of household contacts and their environment with high-resolution molecular typing that tracks strain type dynamics and assess their contribution to subsequent SSTI. This has allowed for the understanding that the household is the primary source of recurrent SSTI, and that

specific strain types commonly cause multiple subsequent infections in the same individual. Individual-level decolonization must be paired with environmental decontamination efforts to eradicate these strain types within the household that perpetuate recurrent SSTI.

Chapter 5: *Staphylococcus aureus* Colonization and Infection are Heritable in Humans

This chapter is adapted from a version of the submitted manuscript: ***Staphylococcus aureus* Colonization and Infection are Heritable in Humans** in preparation for submission. The authors of this manuscript are as follows: Ryan Mork, Kanix Wang, Patrick Hogan, Juliane Bubeck Wardenburg, Stephanie Fritz, Andrey Rzhetsky.

Introduction

Development of multicellular species has been sculpted throughout evolutionary eons by a multitude of environmental factors, including exposure to pathogenic microorganisms (Zilber-Rosenberg and Rosenberg 2008). As an animal's innate ability to withstand microbial attacks is directly linked to its fitness, we expect that patterns of human interaction with common microbial species are highly heritable. Curiously, our collective understanding of the interplay between the environment and host genetics in shaping human responses to microbial symbionts and pathogens remains cursory (Davenport 2016).

Although the Human Microbiome Project (Methé et al. 2012) considered 4 skin sampling sites and the anterior nares of their 18 total sampling sites as critical for understanding human health and disease, the majority of studies examining the genetic underpinnings of the microbiome have focused specifically upon the gut microbiome. Several human genetics studies focusing on human gut microbiota abundance and obtained high heritability estimates for the abundance of several taxa in human stool samples (Goodrich et al. 2014, 2016) and associated specific single nucleotide polymorphisms (SNPs) in the host genome to the abundances of these microbes (Davenport et al. 2015).

Expanding beyond the human gut microbiome, one study explored human sequencing data from 93 individuals in the pan-USA Human Microbiome project and found nearly a hundred

associations between states of human coding-sequence SNPs and genus-level microbial taxa composition across ten body sites, primarily the anterior nares and cheeks (Blekhman et al. 2015). Focusing on the skin microbiota, a relatively small study of 45 Korean twins found evidence of high heritability for skin microbial abundance for a number of common bacterial taxa, including *Corynebacteriaceae* (Si et al. 2015). Although there is clear evidence for a considerable genetic component shaping the diversity of the human microbiome, longitudinal, species-level resolution across multiple body sites is currently lacking.

To assess how human genetic variation may influence colonization and infection by a specific microbe, we turned our attention to *Staphylococcus aureus*, one of the most common colonizers of human skin and a prominent member of the skin microbiome. *S. aureus* is a prime example of a 'pathobiont,' a bacterium capable of both long-term, asymptomatic colonization and cutaneous and invasive infection. *S. aureus* typically colonizes moist areas of the human epithelium protected from the sunlight, including the anterior nares, axilla, and inguinal folds (E. A. Grice et al. 2009; Elizabeth A. Grice and Segre 2011; Costello et al. 2009). Three general patterns exist for *S. aureus* colonization: persistent, intermittent, and non-carriage at 20%, 30%, and 50% of the US population, respectively, which lead to various definitions of longitudinal colonization (J. L. Nouwen et al. 2004; Eriksen et al. 1995; Fritz, Krauss, et al. 2011). *S. aureus*, specifically methicillin-resistant *S. aureus* (MRSA), is also the most common cause of hospital-associated infections (Sampedro and Bubeck Wardenburg 2017; Dantes et al. 2013; David et al. 2012; National Nosocomial Infections Surveillance System 2004) and skin and soft tissue infections (SSTIs) in the community setting (Justin Knox, Uhlemann, and Lowy 2015b; Otto 2007; Moran et al. 2006). Much evidence now points to a combination of host-associated factors, such as immunological status (L. S. Miller and Cho 2011; Rigby and DeLeo 2012; DeLorenze et

al. 2016), demographic background (Wertheim et al. 2005b), and microbiome composition, as well as strain-associated factors, including presence and expression of specific virulence factors (H. K. Kim, Missiakas, and Schneewind 2014), that together shape the incidence and severity of staphylococcal interactions with humans (Mulcahy and McLoughlin 2016; Sampedro and Bubeck Wardenburg 2017). *S. aureus* therefore represents a clinically relevant model for dissecting interactions among host genetics and environmental factors that influence the skin microbiome.

In this study, we monitored *S. aureus* colonization in a large set of demographically diverse, multi-generational St. Louis households over a 12-month period to evaluate the influence of host genetics upon bacterial colonization. Using Bayesian generalized mixed-effect regression models, we measured the relative influence of shared genetic and environments have upon antibiotic resistant, epidemic MRSA and methicillin-susceptible *S. aureus* (MSSA) colonization and SSTI longitudinally across several definitions of colonization. We then independently validated our heritability estimates with a very large *Truven* MarketScan health insurance dataset describing 156,133 US families with 602,500 unique individuals. To the best of our knowledge, we provide the first systematic analysis of the relative importance of environmental and genetic factors in determining the species-level longitudinal susceptibility to colonization and infection by a prominent representative of the human skin microbiome.

Results

Household S. aureus Colonization and Demographics

The focus of this study was to observe *S. aureus* colonization longitudinally within households with known exposure to MRSA. From 2012–2015, 150 households with a child (0-17yrs) that visited a regional St. Louis medical center presenting with a culture-verified MRSA

SSTI were enrolled in the study (692 participants, including all household members) (**Figure 3**). Within two months of presenting with the SSTI, *S. aureus* colonization status of all household members, pets, and household objects was assessed; simultaneously, a questionnaire concerning household practices was filled out by the household residents. *S. aureus* colonization was then re-assessed four additional times in 3-month intervals along with the administration of an additional questionnaire concerning health changes and recent infections. 80% (540/671) of individuals from enrollment were sampled at all sites and time points, while 90% (135/150) of households completed the 12-month study. At a population level, *S. aureus* and MRSA colonization did not significantly change across the sampling period (41-44% and 18-24%, respectively). Reported SSTIs in follow-up interviews also did not significantly change among household contacts and index patients (9-13%).

The study population was a highly heterogeneous sample of St. Louis households. Because study enrollment was contingent upon the presence of a child within the household, the study population primarily consists of 'nuclear families' with two married parents and their several children. Since many of the study hospitals were located near the center of the urban St. Louis area, the study population was racially and culturally diverse and primarily consisted of urban dwellers sampled from all socioeconomic strata.

'Family' was defined as all individuals residing overnight in the household >4 days per week at the time of enrollment. Relatedness of household inhabitants was assessed through interviews. Aside from one instance in which the fathers of two households were brothers, inhabitants of all houses were unrelated to one another. 13% (19/150) of households were multigenerational with respect to the index patient, featuring adult siblings of the parents, aunts, uncles, and grandparents. Stepfamilies were markedly common in the sample population at 18%

(27/150) with a total of 61 (12% of total household contacts) half-siblings. 8% (5) households contained individuals completely unrelated to anyone else. These factors contributed to the complexity of pedigrees describing our sample population.

Due to selection of households with recent MRSA SSTI, the study population exhibited an extremely high incidence of MRSA colonization and contact with MRSA-colonized individuals. While typical colonization rates for MRSA in the greater St. Louis area estimated with random sampling range <2% (Dulon et al. 2014), the study population exhibited an average of 21% colonization across sampling periods (**Table 4**). Overall *S. aureus* colonization was congruent with prior sampling at approximately 42% across participants (Eriksen et al. 1995; J. L. Nouwen et al. 2004). Of the households sampled at all time points, 98% (126/129) contained at least one positive culture of *S. aureus* and 81% (104/129) for MRSA from at least one household member at one or more time points (**Table 4**). It can therefore be safely assumed that all members of the population were at some point exposed to *S. aureus* and specifically MRSA in their daily life.

Colonization Heritability Models

S. aureus colonization was modeled to assess heritability of host-bacterial interactions across a diverse sample population while considering household enrollment and temporality of the dataset. Further, our focus is on the overall heritability of *S. aureus*, rather than the heritability of specific *S. aureus* strains within families. Due to the complex pedigrees of the study population and its robustness for estimating binary traits (de Villemereuil, Gimenez, and Doligez 2013), Bayesian generalized mixed-effect regression modeling (Kruuk 2004; Postma and Charmantier 2007) was employed for all subsequent heritability estimates.

Longitudinal colonization is a complex biological phenomenon that may arise as the result of a large number of individual and environmental factors, all of which may change over time. As a result, consistent, longitudinal definitions of colonization are currently lacking and variable across studies. Rather than choose a specific definition, we explored several formulations based upon the available colonization data and their respective models. Individuals exhibited between 0-5 colonization incidences across three body sites (nares, axillae, inguinal folds) in a time series, by both MRSA and MSSA. These data led to three major colonization definitions:

Burden model: the number of colonization incidences across time describes temporal colonization.

Fixed Probability model: temporal colonization is described by considering each time point an independent sampling of an underlying, fixed, colonization probability.

Variable Probability model: temporal colonization is a temporally variable probability that changes as the host and host environment changes.

Rather than stating which is the superior definition, the focus of the following analysis is to show how, across the definitions of temporal colonization, specific genetic and environmental components consistently contribute to describing colonization. See Methods for the comprehensive statistical formulations of these definitions.

Accounting for various shared environments is also critical in both understanding the relative contribution environments may play in colonization and infection as well as ensuring accurate heritability estimates (referred to as *G*, shared genetics). Accordingly, we also considered the following three environments in model development: *C*, the environment shared

by cohabitating spouses, *S*, the environment shared by cohabitating siblings or adults with a common upbringing, and *H*, the environment shared by all household members.

The proportion of total phenotypic variance these environments explained by *C*, *S*, and *H* are called 'preventabilities,' since, in comparison to genetics, one's shared environments can be altered through various interventions, and were also estimated for each colonization definition (see Methods) (K. Wang et al. 2017). For example, in couple preventability, the couple could engage in washing linens more frequently or use separate towels, and this may accordingly 'prevent' the influence of this environment upon an individual's risk for colonization or infection. Household preventability captures the degree by which household-level practices such as cleaning regimens explain phenotypic variance across all individuals within the household. Similar to how heritability describes the proportion of phenotypic variance is attributable to additive genetic effects, these preventabilities are percentages, and the larger percentage means more phenotypic variance can be contributed to a given environment, and possibly the greater impact interventions at this environmental level may alter the incidence of a given phenotype.

For each colonization phenotype (*S. aureus*, MSSA, and MRSA in one or more body site, and nares, axillae, and inguinal folds colonization), we designed and tested all possible 2^4 models that include or exclude each of these four genetic and environmental components (G, C, S, H). Deviance Information Criterion (DIC) (Spiegelhalter et al. 2002) was employed to determine which linear combination of genetic and environmental effects were most effective in fitting the data. For this method, the model with the lowest minimum DIC across repeated runs is considered the top model, preferring simpler models when the difference in DIC is not significant (see **Chapter 7: Microbiological and Statistical Methods** for model selection). Across the six phenotypes and three colonization definitions (burden, fixed probability, and

variable probability), *G* was present in 94% (17/18) of the top models, followed closely by *C*, present in 89% (16/18) of top models. Memory of colonization was then assessed through autoregressive (AR) models.

Burden of Colonization Model

The simplest model for human-bacterial interaction uses the number of colonization incidences exhibited by an individual over time as a measure of susceptibility to bacterial colonization, which we call the burden of colonization model. Considering colonization from the perspective of degree of temporal colonization using an ordinal model, model selection highly favored both *G* and *C* in the models with the lowest DIC, and disregarded *H* (**Figure 11**). When examining the top model for *S. aureus* colonization, shared genetics (*G*, 53%, 95% *CrI* 40-66%) and couple preventability (*C*, 24%, 95% *CrI* 11-36%) dominated the explained phenotypic variance, especially in colonization by MSSA and by site in the nares and inguinal folds (**Figure 12**).

Fixed Probability Colonization Model

Rather than count colonization events, we can instead model the expected probability of individual colonization at each assessment epoch as a Bernoulli trial. In this definition, we have five observations of each person (which were collapsed into a single measure between 0-5 in the burden model). Assuming that this probability of colonization is constant over the five colonization assessment trials, we obtain a binomial-response model where the expected probability of colonization is influenced by the heritability of colonization and various household-specific preventability factors. As shown in **Figure 11** and **Figure 12**, the general trends and results obtained from the ordinal models were replicated with this fixed probability model: estimates of heritability and spouse preventability were principle factors across models

with 23–35% and 9-21%, respectively. MRSA colonization was the only model to exhibit a significant influence from household preventability. Axilla colonization was nearly completely driven by heritability (*G*, 32%, 95% *CrI* 21-40%).

Variable Probability Colonization Model

We can further relax the assumption of constant probability of colonization during the year to accommodate the observed seasonality effects upon staphylococcal disease (Leekha, Diekema, and Perencevich 2012) and arrive at the variable probability colonization model. Further, this allows us to introduce and estimate the individual-environment preventability (*I*) since we now have several repeated measures for each individual. We addressed this by designing a threshold regression model controlling for sampling season (**Figure 11**, **Figure 12**). Individual preventability (*I*) accounted for a significant portion of the variance for nasal and axilla colonization (19% and 21%, respectively), and in the latter case completely dominated over heritability. Axilla colonization may therefore be better explained through personal hygiene practices rather than genetic predisposition. MRSA colonization was also the only model in our study to suggest a dominant role for household preventability at 23.6%, a trend consistent with the fixed probability model.

Autoregressive Colonization Estimation

All of our previous colonization definitions have made the simplifying assumption that each colonization trial is independent of previous time points and thus cannot account for a 'memory' of past colonization. To explore how memory may play a part in the determination of individual colonization, AR threshold models were constructed, accounting for earlier colorizations as predictors of later ones. For example, an AR1 model considers the previous colonization measure 3 months ago, while AR3 considers the previous three time points, all as separate fixed effects at 3,6, and 9 months. Since only five time points were sampled, and

increasing the order of the AR model decreases the sample size of the data, AR3 models were the highest order explored to ensure a sample size of at least 1000 person-time points. Considering the AR3 model with the lowest DIC, previous colonization information was highly informative, significantly increasing risk for subsequent 3-month intervals by 2.1–3.1-fold (**Table 13**). Between 3-month intervals, with the exception of axilla colonization, the risk decreased by ~33% on average across site, with the previous 9month colonization risk averaging 1.54-fold. Axilla colonization was the clear outlier, showing the opposite trend of increasing risk looking farther back in time. Considering the results from the previous fixed and variable probability models (**Figure 12**), this reinforces the concept that axilla colonization is heavily driven by long-term individual environmental variation. *S. aureus* colonization generally exhibits a high degree of repeatability that decays over a 9-month period.

SSTI Heritability Estimated in the St. Louis Study Population

Since *S. aureus* colonization exhibited a high degree of heritability, we wanted to examine whether such a trend also existed for SSTIs reported by the study population at follow-up sampling visits. The study population pursued medical attention for 55% (146/266) of these infections. Due to medical practitioners pursuing clinically appropriate empiric therapy in the absence of identifying microbial etiology of infection, patients forgetting this information, or researchers unable to recover these medical records, SSTIs reported cannot be verified as caused by *S. aureus*. However, due to the high degree of MRSA colonization, prior MRSA SSTI exposure in the study population, and MRSA as the dominant etiology of SSTIs in previous studies at 59–65% (Talan et al. 2011; Rajendran et al. 2007), it was plausible that the majority of the infections were caused by MRSA. The study population reported 257 SSTIs (250 skin abscesses, 5 cellulitis cases, and 2 impetigo cases) and no bloodstream or invasive infections across the study period. Since 9-13% of the population reported an SSTI (**Table 4**) across follow

up visits, a yearlong threshold generalized linear mixed-effect model was employed to capture the relative contributions of genetic and environmental components to SSTI incidence. As in previous models, 'index patient' was employed as a binary fixed effect to account for study construction and recent history of SSTI, and all 2^4 combinations of genetic and environmental components were considered in model selection.

SSTI was found to be highly heritable as judged by the top model identified in the model selection procedure (**Table 14**). At 42% (95% *CrI* 12%–67%), SSTI was more heritable than that observed for MRSA colonization. Interestingly, *H* plays an insignificant role in SSTI compared to MRSA colonization (**Figure 12**). The influence of combined common environment (*C*, *S*) and heritability of infection were nearly equal in magnitude, suggesting that close living proximity of household members was as important as shared additive genetic variation in explaining the incidence of SSTIs across households.

SSTI Heritability in the US Population

Due to the modest sample size of our observations collected in St. Louis and a lack of previous heritability estimates for SSTIs, we sought to validate our estimates in the much larger *Truven* Health MarketScan Commercial Claims and Encounters Database. This dataset describes over 150 million unique US residents, from newborns to 65 year-olds, subscribing to private health insurance during 2003-2011. These insurance records provide insurance dependency information along with longitudinal health observation. Here, we defined a 'family' as all individuals within a single insurance policy, with children as dependents and parents as primary and secondary beneficiaries. Typically, a person is present in the dataset for a period of 1-10 years, so we selected families with a relatively long-term (6 year) presence, and child age sufficient for observing a variety of phenotypes (≥6). The resulting prolonged-visibility family-

linked dataset described 2,348,070 of these patients (see Methods). Individuals were considered to have had an *S. aureus* infection when exhibiting at least one record of an *S. aureus* (MSSA or MRSA) infection (ICD9 codes '041.10', '041.11', and '041.12') at any body site. This large-scale longitudinal data complements our own data with its smaller sample size but verified pedigree information.

Staphylococcal infection was measured from these 608,729 family medical records over a 6-year period. Incidence of at least one staphylococcal infection was then modelled in a random subsample of these families (156,133 families of 602,500 individuals) using a threshold generalized mixed-effect model to assess heritability. As ~90% of staphylococcal infections are SSTIs, these estimates primarily measure the heritability and preventabilities of staphylococcal SSTI incidence (Loewen et al. 2017). As with the previous SSTI model, model selection was employed through DIC to determine the subset of genetic and environmental covariates that best capture the phenotypic variance of the population.

At 47% (95% *CrI* 42-52%), the staphylococcal infection heritability estimate from the *Truven* dataset closely recapitulated the estimate from the St. Louis study population and indicates a high heritability for this phenotype (**Table 14**). Environmental effects were also concordant with the St. Louis population, with the shared couple environment (*C*) also playing a significant role in SSTI phenotypic variation. Since the *Truven* dataset consists of a semi-random subset of American families, this indicates that the SSTI heritability estimate for the study population has not been significantly biased through study selection of families with a history of SSTI in children.

Discussion

Understanding and influencing the human microbiome is emerging as critical in promoting long-term health and wellbeing. While much work over the past decade has focused upon this new field, many of the foundational questions concerning the forces that shape composition and stability of the human microbiome in the built environment and how specific bacterial communities and even single genetic determinants within these communities directly impact health and disease are still being evaluated. Due to the difficulty in conducting controlled experiments in humans, researchers typically turn to gnotobiotic and germ-free mouse models to better elucidate how specific molecular mechanisms and environmental perturbations influence the microbiome. While these studies excel at answering questions regarding environmental influences upon the microbiome, due to the focus on inbred mouse lines in a homogenous environment, they are ill-suited to consider how host heritability may shape and stabilize a microbiome in the face of constant, new microbial exposure encountered in everyday life.

Here, we provide evidence that in a population exposed to a specific microbe, *S. aureus*, colonization and infection by this pathobiont is highly heritable, both across microbial strains and body sites; this result is robust with respect to variation of the temporal definition of microbial colonization. When accounting for experimental design, sex, and age, *S. aureus* colonization demonstrates 31–53% heritability, with secondary environmental contributions from household contacts, particularly within the household couple, 12–24% (**Figure 12**). MSSA colonization specifically seems to be much more heritable, while MRSA colonization is more the result of household and spouse-level preventability, indicating that hygiene and cleanliness practices may be more effective with this pathogen.

Vertical transmission is a well-studied mechanism by which the microbiome is passed from mother to child during childbirth and early life, and can muddle heritability estimates when not accounted for in model selection. *S. aureus* especially has been shown to colonize 42.6/100 newborn-colonized mother pairs at 72-100 hours after birth (Leshem et al. 2012). In our models, sibling preventability (*S*) captures this mechanism by describing both minors currently present within the same household as well as adult siblings present within households, in nearly all cases born from the same mother. Since *S* was excluded by model selection from the majority of colonization models and was very low even in models where present, vertical transmission may not be considered a prominent mechanism in explaining *S. aureus* colonization in these families, likely since only 1.5% (10/671) of the study population was <6 months old at enrollment.

By site, nares and inguinal folds show a high degree of heritability, while axilla colonization may be more the result of individual environmental practices (**Figure 12**). Since individuals tend to be mostly colonized in the nares and inguinal folds, this leads to overall colonization exhibiting a high heritability. Individual environment may have been high in axilla colonization due to the low overall incidence of colonization at this site (34% at least once, **Table 2**), leading to models with multiple covariates being too difficult to fit given the modest number of observations. Overall, our findings further the hypothesis that host genetic background shapes the long-term distribution and interactions of specific members of the microbiome in natural populations. Since our study examined colonization of community populations over time, we can conclude that *S. aureus* colonization is stable within the skin microbiomes of this population, undeterred by decolonization efforts (31% household members reporting trying ≥1 decolonization method at enrollment), transient antibiotic usage, and takeover by MRSA.

Staphylococcal SSTI demonstrates a consistent, strong heritability at 47% (95% *CrI* 42–52%) (**Table 14**). It is remarkable the two independent estimates agree as we observe, as our experimentally collected data from St. Louis households compared to the *Truven* dataset share marked dissimilarities. The St. Louis dataset consists of a small sample of households from a single urbanized area with known pedigree, whereas *Truven* comprises thousands of families from across the nation with inferred pedigrees. *Truven* records are further both in-patient and outpatient hospital records, and the self-reported SSTIs in the household members varied in severity, indicating that SSTI severity did not alter the heritability estimate. Overall, these data suggest that *S. aureus* SSTIs, both superficial and serious, are highly heritable when compared with shared environmental preventabilities.

Our study provides a robust heritability estimate by examining households with known *S. aureus* exposure and assessing the number of colonization occurrences several times across multiple body sites throughout the year. Previous, low heritability estimates (19% ± 21% SE and 19%, 95% *CI* 0-39%) for *S. aureus* colonization sampled the anterior nares once in all patients and twice only in patients that demonstrated colonization in the first sampling (Andersen et al. 2012; Roghmann et al. 2011). Subsampling the St. Louis household members in such a manner; however, does not alter the heritability estimate, with persistent (57%, 95% *CrI* 17-85%, 66%, 29%-89% for persistent colonization only sampled enrollment and 3mo or enrollment and 12mo, respectively). Further, the St. Louis dataset replicates previous measures of heritability for the well-studied phenotype of adult height (57%, 95% *CrI* 33%-77%) (Wells and Stock 2011; Wells 2017) and reports negligible heritability for colonization when the phenotypes are randomly sampled across the population (11%, 95% *CrI* 0%-31%). Our study finds a high heritability estimate for colonization since exposure is controlled for across the study population, which

allows the observation of colonization resistance in individuals, rather than a confounded stochastic exposure effect, differences in methodology, or demographics.

S. aureus colonization studies have demonstrated strong host selectivity in shaping the nasal microbiome and conditions associated with *S. aureus* colonization are highly heritable. A previous study investigating persistent *S. aureus* carriers and non-carriers found that after patients are decolonized and re-exposed to a mixture of different *S. aureus* strains, they return to their carriage state prior to intervention within two weeks, non-carriers losing all *S. aureus* strains and persistent carriers becoming re-colonized with the initial *S. aureus* strain (J. Nouwen et al. 2004). Skin disorders such as psoriasis and atopic dermatitis have all been shown to be highly heritable (Bataille, Lens, and Spector 2012), and are also significantly associated with increased staphylococcal colonization and infection (C. Y. Ng et al. 2017; Hill and Imai 2016; Totté, van der Feltz, Hennekam, et al. 2016; Totté, van der Feltz, Bode, et al. 2016). Another study in mouse lines investigating autoimmune skin blistering found several quantitative trait loci (QTLs) significantly associated with *Staphylococcus spp.* colonization (Srinivas et al. 2013). Our study furthers these observations in showing that there is a strong genetic underpinning to *S. aureus* colonization even amongst healthy members of the community.

Limitations and Future Directions

Accurate heritability estimates rely upon accurate pedigrees. The St. Louis families' pedigrees were assessed exclusively through interviews and were not independently verified via paternity tests, and thus it is possible that mis-assigned paternities exist. Mis-assigned paternity has been previously estimated in the United States at 3.7% (Bellis et al. 2005), and previous simulation experiments have shown that a mis-assigned paternity rate of 5% for 100 families yields an underestimation of social heritability by 3.4% (Charmantier and Réale 2005). Since the

Truven MarketScan families relied upon insurance dependency information, mis-assigned paternity and maternity is very likely present within the inferred pedigrees. However, even a 20% pedigree error in simulation studies yields only a 5% heritability underestimation (Charmantier and Réale 2005; K. Wang et al. 2017). Thus, if our heritability estimates are affected by mis-assigned paternity, they represent conservative estimates.

S. aureus is a polyclonal species, with numerous strains, both MRSA and MSSA, found across individuals. While our study shows high *S. aureus* heritability overall, these data do not capture such a strain-level understanding of colonization. In our models, an individual colonized with the same strain at all time points is identical to an individual with a different strain at all time points, and families with discordant colonizing strains appear identical to families sharing the same strain at all time points. The reservoir of such strains, whether within the household or from a common external source, is also unknown. While our models capture host susceptibility to *S. aureus* overall, they fail to capture to what extent an individual selects for a specific *S. aureus* strain temporally, critical knowledge for developing tailored probiotics and effective household decolonization strategies.

A critical difficulty in our study is the confounding of upbringing with heritability estimates. In controlled heritability experiments, it is typical to use foster parents to disentangle the influence of the common upbringing environment from additive genetic effects. Since our study was observational in nature, this was not possible, so that our heritability estimate is upwardly biased by the parent-offspring environmental effect. Further, since all families are contained in their own households, household-level environmental effects also slightly upwardly bias our heritability estimate, although our models consider household-level preventability in model selection. Our infection heritability estimates are also biased in that the etiology of the

SSTI reported is presumed to be *S. aureus*, although due to experimental constraints, it is unknown in nearly all cases. However, the impact of this effect is equally likely to downward or upward bias the heritability estimate.

While narrow-sense heritability is useful to understand the degree by which additive genetic effects influence a phenotype, the statistic does not pinpoint specific genetic loci for further investigation. Our analysis, however, hints how such a whole-genome association study or linkage analysis should be conducted: in households with a high degree of staphylococcal colonization and history of SSTI. This would allow for the stochastic effect of exposure to be controlled, and measuring multiple colonization events, rather than a binary phenotype of intermittent or persistent colonization, is much more amenable to statistical analysis. Thus, key future directions suggested by this study are first to examine additional pathobionts for heritability in exposed households, and second when high heritability is encountered, perform hypothesis-driven GWAS analysis to pinpoint significant contributors to species-level colonization susceptibility.

Conclusions

Here, we have shown that *S. aureus* colonization in households experiencing MRSA SSTIs exhibits a high degree of heritability far above that of common environmental effects. Our study is the first to examine the heritability of a prominent member of the skin microbiome at a high temporal resolution across hundreds of households. We further estimate *S. aureus* SSTI heritability at 42% (95% *CI* 12-67%) and 47% (42-52%) (Table 5) from two study populations, the first estimate published to date. These data provide strong evidence that the skin microbiome is critically shaped by additive genetic effects that stabilize the composition of this microbiome over time even in the presence of decolonization attempts and novel microbial exposure in

everyday life. Crucially, when considering previous studies that find low heritability for colonization, these data provide evidence that colonization by pathobionts is a resistance phenotype. Resistance cannot be demonstrated in the absence of challenge, and thus to observe such a phenotype, controlled exposure across the population is necessary.

Chapter 6: Discussion

A nuanced appreciation of the dynamics of *S. aureus* colonization and infection in the household is reaching maturity, driven by increasingly high-resolution molecular and epidemiological methods. The conception of the 'household' as a primary reservoir of *S. aureus* in the community has accordingly been reinforced: both due to the behavioral consequences of cohabitating with *S. aureus* colonized individuals and the resultant dissemination of *S. aureus* in the environment, but also critically due to the genetic relatedness of families. Individuals are predominately colonized with strains present within the household previously, either through persistence events or transmissions from other household members. Further, since both *S. aureus* colonization and SSTI are heritable, certain households are moreover significantly more susceptible to these events, and accordingly, such events are much more likely to occur over time. Considering *S. aureus* colonization holistically, SSTI prevention requires decolonization and hygiene regimens that are tailored to households and individuals.

Observed S. aureus Colonization as Describing a Complex Set of Underlying Acquisition Mechanisms and Multifactorial Risk Factors

Longitudinal screening at the household level combined with in-depth molecular typing has revealed that *S. aureus* colonization is a complex phenotype that may arise from one of several distinct mechanisms: introductions, transmissions, and/or persistence events. Re-conceptualizing colonization as the sum of these different mechanisms, rather than one 'exposure' mechanism, fundamentally changes how we understand *S. aureus* epidemiology and the intervention methods we propose to curtail *S. aureus* SSTI in homes. Critically, colonization risk must be considered as the combination of acquisition and persistence risk, which may be different for each individual and household due to differential hygiene practices, presence in the community, and degree of exposure to different reservoirs of *S. aureus*.

An understanding of colonization as the combination of acquisitions and persistence events highlights the need for multifactorial intervention efforts. Previously, individual behaviors, including hygiene and engagement in activities where *S. aureus* exposure is high, have been considered primary determinants of colonization. We have observed these same trends, and have noted that specific risk factors for colonization are uniquely associated with transmissions, introductions, and persistence events. Handwashing is specifically protective of introductions, while sharing towels and hygiene items is associated with transmissions, and nares decolonization and environmental cleanliness with persistence. Colonization risk, therefore, can be conceptualized as the combined risk of individual persistence, transmission, and persistence risk. This has critical clinical implications: if interventions are employed that solely reduce the risk for one of these methods, the individual may still experience a high burden of *S. aureus* exposure that may lead to recurrent infection.

Numerous studies and reviews have conceptualized *S. aureus* SSTI as a disease of households (Justin Knox, Uhlemann, and Lowy 2015a), and our observations reinforce such a hypothesis: the incidence of MRSA in both individuals and the environment as well as SSTI in individuals potentiates both further SSTI's in the household as well as the strain types associated with them. This could be the result of two mechanisms: one in which one or more household members are persistently colonized by a CA-MRSA strain even with decolonization regimens employed, and a second wherein one or more individuals is continually acquiring novel *S. aureus* strain types from an exogenous source that subsequently leads to infections in the household. Although the present studies indicate the former the dominant occurrence, the latter is possible, especially in households with a large burden of introductions. Transmissions and persistence events being significantly associated with SSTI's at the exclusion of introductions highlights that

the primary concern is indeed the household, and primarily because pathogenic strains can persist transmissions to other individuals and contamination of the environment, which makes decolonization often fail, even when the whole household participates.

Heritability of S. aureus Colonization in the Context of Acquisition and Persistence

Here, we have observed that heritability strongly influences both *S. aureus* colonization and SSTI; however, observed colonization is a phenomenon composed of acquisition and persistence events that are governed by a combination of individual behavior and exposure to novel *S. aureus* strain types. There are three possible hypotheses that can combine these observations: either mechanisms of acquisition or persistence are heritable, both phenotypes are heritable, or that they are both heritable, but through separate mechanisms. Since persistence events were particularly associated with nares colonization in our observations, and this was the most heritable colonization phenotype, these data suggest that being persistently colonized by *S. aureus* is indeed heritable. This reinforces previous observations of specific genetic loci associated with higher risk of *S. aureus* persistent nasal colonization (Ruimy et al. 2010; Panierakis et al. 2009). Certain individuals, due to their genetic background, are significantly more likely to remain colonized, or upon novel colonization, stay colonized longer than others.

The high degree of heritability partially explains the tenuous links found between hygiene behaviors and *S. aureus* colonization and contamination of the environment. There is significant evidence found previously that environmental contamination serves as a significant risk factor for colonization and recurrent SSTI (Justin Knox et al. 2016a; Fritz et al. 2014). We have shown here that the strongest indicator of transmission and persistence of a given strain type was the degree of environmental contamination present (beyond even that of individual CP). However, household cleanliness and hygiene measures were rarely significantly associated with acquisition

and persistence measures. This inconsistency is the result of the fact that, aside from MRSA colonization, household and individual preventability was very low: much of the variation in colonization was explained in heritability and close proximity (couple preventability). Those with a strong resistance to colonization may engage in highly unhygienic behaviors and still remain uncolonized, and for others, the most exacting hygiene behaviors are necessary to prevent colonization. This variation across the population leads to the difficulty in assessing how hygiene behaviors can be employed to prevent *S. aureus* colonization.

Persistent *S. aureus* carriers exhibit a combination of behavioral and biological factors that may predispose them to a higher degree of environmental *S. aureus* contamination. Previous studies have strongly associated individual colonization with environmental contamination (M. Davis et al. 2017; Fritz et al. 2014; Kurashige, Oie, and Furukawa 2016), although it is unclear whether all individuals equally contribute to such contamination, or specific individuals overwhelmingly contribute. Here, we have noted that the strongest indicator of transmission and persistence of a given strain type was not the CP of individuals within the household, but rather the degree of environmental contamination present. This may indicate that persistently colonized individuals more heavily contaminate their environment, indicating an enhanced capacity to shed their colonizing organism. This leads to persistent colonizers more likely to transmit to other individuals, either through direct contact or through household fomites. Whether this simply is the result of an increased duration of colonization of persistent carriers compared to transient carriers or indeed an increase in the degree of shedding at any given time is difficult to determine in the absence of high temporal resolution sampling.

S. aureus Strain Competition and the Household

In studying the household longitudinally, we have found that strain competition is a force that influences the incidence and diversity of *S. aureus* at the household level. Previous studies have highlighted the degree by which inter- and intra-species competition characterizes nasal colonization. Other common members of the nasal microbiota, including *Staphylococcus epidermidis*, have been negatively associated with colonization by *S. aureus* (Frank et al. 2010). There is also evidence that MRSA and MSSA strains exhibit competitive exclusion (Dall'Antonia et al. 2005). Here, we have shown that both transmission and persistence was strongly influenced by the distribution of strain types: the richer the diversity, the less likely any given strain type will persist or transmit to a new host longitudinally.

Developing intervention strategies capable of leveraging strain type competition is difficult without knowledge of the strain dynamics present in the household and the strain types an individual is exposed to in his or her daily life. These considerations are particularly salient when deciding between individual and household-level decolonization. For example, if only one individual is persistently colonized by CA-MRSA in the household, then individual colonization may allow other, possibly non-pathogenic strain types can then colonize and exclude the CA-MRSA strain type. However, if CA-MRSA is highly prevalent within the household, then complete decolonization may be necessary. Considering the present data, household decolonization may usually be the safer choice—the infecting strain was present on a non-index patient in ~30% of households at enrollment, and for interval SSTI's, when known, the infecting strain colonized another household member in ~40% of observed incidences.

The degree of heritability and high incidence of transmission and persistence of *S. aureus* strain types in households indicates that proposed methods to promote a healthy nasal

microbiome may prove insufficient. Observations of interspecies competition between members of the healthy nasal microbiome has led researchers to propose interventions to reduce *S. aureus* colonization in individuals through the introduction of non-pathogenic nasal 'probiotics' as well as small molecules derived from these commensal organisms (Brugger, Bomar, and Lemon 2016). The heritability of *S. aureus* nasal colonization implies that probiotics will likely fail in persistently colonized individuals, as these people are likely to harbor a microenvironment that inordinately benefits *S. aureus* colonization. Small molecule treatments may be insufficient when given as a sole intervention measure for SSTI's without continuous treatment, as it is likely that individuals can become exposed and infected by *S. aureus* from their fellow household members, household environment, or even from exogenous encounters in the community. It may be possible to engineer a non-pathogenic *S. aureus* strain capable of out-competing CA-MRSA; however, this poses its own dangers as this strain could acquire pathogenicity islands from bacteriophages and cause serious injury in hosts. Further research is needed in both how human genetics shape the nasal microbiome as well as the functional characteristics of pathobionts such as *S. aureus* is required to propose safe and efficacious microbiome-conscious therapies.

Adapting the Concepts of Precision Medicine to Household Treatment of SSTI's and Decolonization

Our studies indicate that a 'one-size-fits-all' intervention strategy for recurrent SSTI's may be insufficient due to each household exhibiting a varying underlying genetic susceptibility to *S. aureus* as well as the differing degree of risk between acquisitions and persistence. The significant heritability of *S. aureus* infections suggests that for some, strict decolonization may be necessary, paired with a hygiene overhaul of the household, and for others, none of these steps are required—it was an unlikely occurrence in the first place and is unlikely to occur again. The fact that some households experienced significant introduction events while rarely showing

persistent colonization indicates that a hygiene intervention, especially in handwashing, may be the only required intervention for these households. Prescribing mupirocin decolonization may simply promote mupirocin resistance, and may even disrupt the usually protective, or at least neutral, *S. aureus* strains present.

Each household in this study underwent comprehensive, longitudinal screening that, while capable of highlighting the underlying dynamics of each household, is far beyond the scope of the average clinician. In nearly all cases, even assessing the colonization status of the index patient, or identifying the etiological agent of infection, is unrealistic in a clinical context. Given this paucity of information, how can clinicians identify the underlying causes of SSTI's in the household and prescribe the correct degree of intervention? One crucial strategy is determining whether the parents have a history of skin infections, which could indicate a genetic component present. This could be paired with as assessing whether household contacts have exhibited more minor skin infections in the recent past (such as smaller abscesses or folliculitis), or even reaching out and asking whether community contacts (friends of the index patient, extended family members) have recently suffered from an SSTI. A family history of SSTI or recent history of SSTI in the household would demand a more comprehensive intervention strategy including both decolonization of household contacts as well as environmental decontamination. Conversely, if none of these are present, and the index patient or household contact was recently exposed to an infected individual or reservoir of *S. aureus*, such as a hospital setting, a hygiene intervention by stressing the importance of handwashing, frequent washing and decreased sharing of fomites, may be sufficient. One area of SSTI prevention research that currently demands more attention is the development and validation of intervention regimens given various amounts of colonization and family behavioral information.

Future Directions and Conclusions

While these studies have provided the groundwork for informing comprehensive decolonization strategies, especially for those targeting the environment, there are still significant open questions regarding the molecular basis for *S. aureus* persistence within both individuals and households and the role of the nasal microbiome in shaping these interactions. How the relative strength of the interaction between specific strain-host pairings is capable of influencing persistence is relatively unknown, and requires longitudinal sampling of persistent carriers coupled with knowledge of their genetic background. The role the nasal microbiome of household members protects or predisposes individuals to *S. aureus* colonization and infection is unknown, especially in terms of preventing transmission and contamination of the environment. While there is evidence that the nasal commensal state may shift *S. aureus* from neutral to pathogenic, whether this occurs in the community and the role this plays in recurrent SSTI's has not been investigated. These both demand longitudinal sampling of the nasal microbiome as well as the household environment as well as expression profiling of *S. aureus* strain types associated with infections in these households. Not only will these studies enhance our understanding of the role of the microbiome in shaping disease susceptibility in a real-world setting, it will also inform the efficacy and impact novel microbiome-targeted therapies may have. These therapies will be crucial to expand the repertoire of treatment options available for the treatment and decolonization of *S. aureus* in the community in light of the rise of antibiotic and mupirocin resistance.

Here, we have derived insights about longitudinal colonization and SSTI dynamics of *S. aureus* in a community setting, showing how the interplay of acquisitions and persistence events govern individual colonization risk, which varies between households due to varying behavioral, demographic, and biological factors. We have also developed statistical frameworks for

describing strain dynamics as well as longitudinal colonization in a household setting. These contributions not only inform the development of intervention strategies for the prevention of recurrent SSTI, but also provide a general framework for conceptualizing individual and household-level colonization as the sum of the semi-independent processes of introduction, transmission, and persistence. Combined, these are steps toward curtailing *S. aureus* SSTI's and invasive infections in the community and hospital settings, continuing the long tradition of scientific mastery over infectious diseases and human suffering.

Chapter 7: Microbiological and Statistical Methods

Participants, Data Collection, and Microbiological Methods

Participants

From 2012-2015, patients with culture-confirmed CA-MRSA infection (149 SSTI, 1 bacteremia/myositis/septic pulmonary emboli) were recruited for the HOME: Household Observation of MRSA in the Environment study from St. Louis Children's Hospital (SLCH), Cardinal Glennon Children's Hospital, and community pediatric practices affiliated with the Washington University Pediatric and Adolescent Ambulatory Research Consortium. Children with healthcare-associated MRSA infection (Klevens et al. 2006) (e.g., recent hospitalization, invasive medical device, residing in long-term care facility) were excluded. Within a median of 20 days (minimum 3, maximum 95) after infection, a baseline visit was conducted in the index patient's primary home. Household contacts (individuals sleeping in the home ≥4 nights per week) and indoor pet dogs and cats were also enrolled. The baseline visit included a detailed epidemiologic interview and sampling of people, pets, and environmental surfaces for *S. aureus* detection. Four longitudinal visits, conducted every 3 months, consisted of a follow-up interview and sampling people, pets, and environmental surfaces. The Washington University Institutional Review Board and Institutional Animal Care and Use Committee approved study procedures. Informed consent/assent was obtained for all participating household members and pets.

Data Collection

Enrollment surveys inquired about prior *S. aureus* infections, hygiene practices, activities, pet characteristics, household attributes, and cleaning practices. To ensure an objective assessment of household cleanliness, controlling for potential bias introduced by participants misrepresenting their cleaning habits, the research team assigned each household a "Home

Cleanliness Score" from 1 (above average) to 4 (very dirty), which considered odor, clutter, and grime, modified from the Environmental Cleanliness and Clutter Scale (Halliday and Snowdon 2009). Longitudinal surveys queried development of subsequent SSTIs, healthcare exposure, and use of systemic and topical antimicrobials. At each of the five visits, colonization cultures were collected from the anterior nares, axillae, and inguinal folds of all household members (Eswab, Becton Dickinson [BD], Franklin Lakes, NJ) and from the nares (minitip Eswab, BD) and dorsal fur (Eswab) of indoor pet dogs and cats (Fritz et al. 2014). Up to 21 environmental surfaces were sampled in the kitchen (n=5), bathroom (n=11), bedroom (n=1), and electronics (n=4) (See **Table 10** for exact sites sampled and colonization statistics) using Eswabs and Baird Parker Agar contact plates (Hardy, Santa Maria, CA) (Hogan et al. 2015).

Microbiological Methods

MRSA isolates and antibiotic susceptibility profiles of the enrollment SSTI were obtained from the clinical microbiology laboratory when available (n=91). *S. aureus* was recovered from Eswabs using broth-enrichment (BD); colonies were selected based on morphology from contact plates. *S. aureus* was identified and antibiotic susceptibility profiles were determined in accordance with established techniques according to the Clinical and Laboratory Standards Institute (Cockerill 2013; Del Vecchio et al. 1995) and coupled with staphylococcal cassette chromosome *mec* (SCC*mec*) typing using multiplex PCR (Boye et al. 2007). Molecular typing was performed by repetitive-sequence PCR (repPCR) using a 95% similarity cutoff to determine distinct *S. aureus* strains within each household (Marcela Rodriguez et al. 2015). Isolates recovered from pets and those with unusual repPCR patterns were confirmed as *S. aureus* by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (VITEK MS v2.0) (Rychert et al. 2013).

Definitions

Strain Type

A strain type is unique to each household and comprises the repPCR designation and methicillin resistance profile of a recovered S. aureus isolate. Accordingly, strain richness represents the number of unique strain types by this definition present among all individuals and/or environmental sites and pets sampled (personal strain richness and household strain richness, respectively).

Infecting Strain Type

The strain type of the isolate recovered from the index patient's enrollment infection. This was recovered in 91 of 150 households.

Colonization Pressure

Anatomical site colonization pressure (ACP) within each household was calculated via the following, adapted from (M. Rodriguez et al. 2013):

$$ACP \equiv \frac{(anatomical\ sites\ colonized\ (n))}{(sampled\ household\ members\ (n))*(anatomical\ sites\ (n))} \text{ (Equation 1)}$$

Three anatomical sites were sampled per individual (axilla, nares, and inguinal folds). In cases where ACP or household strain richness were considered in individual colonization models (i.e., models evaluating factors associated with personal colonization), colonization data for the individual being modeled was excluded from calculations.

Personal colonization pressure is the proportion of anatomic sites (3 total per person: axillae, nares, and groin), colonized by *S. aureus*, MRSA, or a given strain, divided by the total number of sites sampled (personal *S. aureus* CP, MRSA CP, or strain CP, respectively). Environmental contamination pressure is the number of environmental sites contaminated by *S.*

aureus, MRSA, or a given strain, divided by the total number of sites sampled (environmental *S. aureus* CP, MRSA CP, or strain CP, respectively).

Longitudinal CP refers to the proportion of sites over time colonized by *S. aureus*, MSSA, MRSA, or a given strain type (*S. aureus* CP, MSSA, MRSA, or strain type CP, respectively). For example, if an individual was sampled in three body sites for five time points, and was colonized in the nares in two time points, the *S. aureus* CP would be 2/15, or 13%.

Acquisition

An acquisition occurs when a strain type is found colonizing an individual who was not colonized with the given strain at the prior sampling. An acquisition could occur via either an Introduction or Transmission, described below.

Strain Type Introduction

An introduction event occurs when a strain type appears for the first time within a household, excluding enrollment sampling. The number of introductions at a time point is defined as the number of people simultaneously colonized by this novel strain at the first time point it appears (i.e., "personal introductions"), and one additional if the strain appears on at least one environmental surface (i.e., "environmental introduction"). For example, if a given introduction strain is recovered from 2 individuals and 2 environmental surfaces, this would constitute 3 household introductions (1 for each person and 1 for the environment). If a given strain appears exclusively in the environment at the time of first appearance, it is considered an "exclusively environmental introduction event."

Transmission

A transmission occurs when a person not previously colonized with a given strain at the prior time point becomes colonized (defined as a "transmission recipient") with a strain that was recovered from at least one person or environmental site in the household at the previous time

point (defined as a "putative transmission source"). The number of transmissions at a given point is equal to the number of "transmission recipients." Since there may be multiple individuals and environmental sites colonized with a given strain at a prior sampling, the number of putative "transmission paths" is the number of transmission sources multiplied by the number of transmission recipients of a given strain. A "successful transmission" occurs when the strain type colonizing a putative transmission source was recovered at the subsequent sampling on at least one other individual who had not been previously colonized by this strain type.

Normalized Transmission Risk

Normalized transmission risk (t_r) is the ratio of the mean proportion of personal or environmental sites colonized for a given putative source (S) when this strain type is transmitted at least once to zero times to a given recipient (R) across all households:

$$t_r(R,S) = \frac{\frac{\Sigma_{\substack{successful \\ transmission\ to\ R}} \frac{S_{sites\ colonized}}{S_{sites\ sampled}}}{n\ successful\ transmissions\ to\ R}}{\frac{\Sigma_{\substack{failed \\ transmission\ to\ R}} \frac{S_{sites\ colonized}}{S_{sites\ sampled}}}{n\ failed\ tranmsissions\ to\ R}} \quad \text{(Equation 2)}$$

For example, to calculate the transmission risk of the kitchen to female children, for every household and time point, the number of sites colonized is divided by the number of sites sampled (up to 5 sites in the kitchen) and grouped into whether this strain was recovered at the subsequent sampling (successful versus unsuccessful transmission) for each female child. The mean proportion of sites colonized across households is taken, and the ratio of these successful (at least one) versus unsuccessful transmissions (zero) is the normalized transmission risk. For individuals as the source, such as male children, this ratio is the number of male children colonized in at least one site over the total number of male children in the household.

A higher transmission risk indicates the degree by which a putative source is more commonly associated with a transmission event, with a transmission risk equal to one indicating no difference in risk, and higher than one increased risk. In order to assess significance, a Kruskall-Wallis test is used to compare the proportion of sites colonized across all households in successful versus unsuccessful transmissions for a given S, R pair.

Persistent Colonization Event

Colonization of an individual by an identical strain type for a consecutive sampling in at least one body site. The degree of persistent colonization is the number of consecutive samplings a strain is observed on an individual across body sites.

Person, Environmental, and Household Persistence

Colonization of at least one of a set of sampled person and/or environmental sites over two consecutive samplings by a given strain type. For example, in personal strain persistence, a strain type is observed colonizing one or more individuals for at least two consecutive samplings, while in environmental strain persistence, a strain type is found contaminating any set of environmental sites (within a household) over consecutive samplings, while household persistence is the same strain type persisting across personal and/or environmental sites between sampling

Statistical Methods

Univariate Analyses

The univariate analysis was conducted in the R statistical computing language (R Core Team 2016) or in Python version 2.7.15 using the scipy and numpy packages (version 1.1.0) (E. Jones, Oliphant, and Peterson 2001). For all 2x2 contingency table tests (e.g., calculating risk significance and odds ratios [*ORs*] for introductions, transmissions, and persistence events), Fisher's Exact test was employed. For pairwise comparisons between sets of continuous

observations (e.g., number of introductions in households with and without factors of interest), the Kruskal-Wallis nonparametric one-way analysis of variance was used. Spearman's nonparametric rank correlation was used when calculating correlation between two covariates (e.g., number of individuals per square foot by number of transmissions). Multiple test correction was performed using the Bonferroni method. Figures were developed through Python using the package 'Seaborn' (Waskom et al. 2017).

Generalized Linear Mixed Models using MCMCglmm

All presented multivariate models are Bayesian longitudinal, generalized linear logistic mixed models fitted using the R library 'MCMCglmm' (J. Hadfield 2016). The following general model formulation was employed:

$$y = link(X\beta + H + I) \text{ (Equation 3)}$$

where y is an n x 1 vector representing colonization liability, X the n x m design matrix of fixed effects, β the m x 1 coefficients for these fixed effects, *link* the link function, and H and I the household-level and individual-level random effect, used when appropriate to account for repeated sampling. In the case of logistic models, a binomial link was used, and for poisson models, a log link was employed.

All models were run for 10^4 iterations with a burnin of 10^3 and a thinning interval of 100 using the 'threshold' family with a chi-square prior for random effects (nu=1000, α.mu=0, α.V=1) and a normal prior for the fixed effects (mu=0, V=1) (Gelman 2006). Model diagnostics were conducted using the halfwidth test, stationarity test, and visual inspection of chain convergence (Gelman and Rubin 1992; Heidelberger and Welch 1983; Plummer et al. 2006).

A heuristic, two-stage model selection approach was employed to select the best set of covariates to describe a given outcome to mitigate the large covariate space and modest sample size of the dataset. In the first stage, the researchers selected 11 covariates previously demonstrated or *a priori* hypothesized to be associated with *S. aureus* colonization risk pertinent to the outcome of interest; all possible combinations of these models were fitted, with the best model selected via the lowest deviance information criterion (DIC), ties broken by the simpler model. The second stage consisted of stepwise addition of individual secondary covariates omitted in stage 1. All secondary covariates that reduced model DIC and displayed a pMCMC <0.05 were maintained. The final, presented models consisted of the primary model with these additional secondary covariates.

Enrollment Colonization Models: Colonization Pressure, Proximity, and Activity

Three different colonization model types were constructed to estimate how burden of household colonization, household factors, and personal behaviors influenced individual colonization. The 'Colonization Pressure' (CP) models considered the overall burden of *S. aureus* in the household as measured by colonization pressure and the number of unique *S. aureus* strains detected by repPCR as well as the individual's health history, hygiene practices, and activities. The 'Proximity' models were constructed by considering the relative proximity of an individual to other colonized individuals. Lastly, 'Activity' models were constructed by considering all covariates from the 'Proximity' models with the exception of any information concerning the burden and distribution of staphylococcal strains. The focus of this model was hygiene practices that could be modified to impact *S. aureus* colonization in the household, while controlling for socioeconomic and health status. All colonization models were constructed as generalized linear regression models and fit as described in Generalized Linear Mixed Models using MCMCglmm.

Enrollment Colonization Concordance Model

To model concordance of colonization strain types between household members, a generalized linear mixed-effect logistic regression model was employed, considering whether a given pair of individuals share ≥1 common strains. Eqn. 1 was employed as described above, with the modification that *y* represents whether a pair of individuals share at least one colonizing strain This concordance model was also fit and underwent model selection as described in Generalized Linear Mixed Models using MCMCglmm.

Enrollment Infecting Strain Prevalence Model

Prevalence of the MRSA isolate recovered from the index patient infection (i.e., "infecting strain") was evaluated at the household-level; thus, a negative binomial generalized linear regression was employed examining the number of sites colonized by the infecting strain type at the time of enrollment. The following general model formulation was employed:

$$c_B = link(X\beta + s) \text{ (Equation 4)}$$

where c_B is an n x 1 vector of the number of body sites across household members colonized by the infecting strain, X the n x m design matrix of fixed effects, β the m x 1 coefficients for these fixed effects, *link* the link function, and s an offset term representing the number of sampled individuals in the household. The Frequentist 'lme4' library was employed for model selection. In this case, due to the modest dispersion in the data, a negative binomial family was used with a log link function. Final models were evaluated using Bayesian 'MCMCglmm' with a Poisson link. A normal offset prior was employed to consider the number of sampled individuals (mu=1, V=1e-9 for number of individuals sampled while mu=0, V=10 for fixed effects).

Introduction, Transmission Recipient, and Transmission Source Multivariate Analysis

All models of *S. aureus* acquisition (Introduction, Transmission Source and Recipient models) were constructed as longitudinal, generalized linear logistic mixed models of individuals

sampled at consecutive samplings after enrollment. The modeled outcome was whether the individual experienced a given acquisition event as defined in the Definitions section. The 'Transmission Recipient' model specifically considers as observations all observed strain types at a given sampling, across all individuals. Thus, an individual in a household with two strain types, for example, appears twice for the given sampling. For the 'Transmission Source' model, a successful transmission was considered if at least one strain type colonizing the individual appears on another individual at a subsequent sampling. All models were constructed and underwent model selection as described in the section Generalized Linear Mixed Models using MCMCglmm, using the 'threshold' family and individual and household-level random effects.

Individual Persistence and SSTI Models

Individual longitudinal persistence and SSTI risk was assessed in multivariable Bayesian longitudinal, generalized linear logistic mixed models. The persistence model evaluated whether an individual colonized with a given strain type maintained colonization with the same strain type at the subsequent sampling versus clearing colonization of this strain type (albeit the individual could become colonized with a new strain type). For the SSTI model, reported interval SSTI risk was modeled for each patient at each follow-up visit.

Random effects of household and individual were included to control for repeated longitudinal sampling. Since these models were logistic in nature, the 'threshold' family was used with a chi-square prior for random effects and the built-in normal prior for the fixed effects. Model fitting and selection was conducted as described by Generalized Linear Mixed Models using MCMCglmm.

Strain Persistence and Infecting Strain Household Persistence Multivariable Models

Longitudinal strain persistence was modeled as a generalized linear logistic mixed model predicting whether a given strain type persists from a given sampling to the next on at least one

individual, environmental site, or pet. Model eligibility was all strain types encountered in households sampled twice consecutively across the study period. In the 91 households in which the infecting strain type was known, an additional 'Infecting Strain Household Persistence' generalized linear mixed model was constructed considering only whether this infecting strain persisted from one sampling to the next, when present. Both of these models were also fit and evaluated as described in Generalized Linear Mixed Models using MCMCglmm, with a household-level random effect to control for repeated sampling.

Markov Model of Household Strain Persistence

First-order Markov models were fitted using the R library package 'msm' (Jackson 2011). The input to these models included all pairs of timepoints with a sampled environment across every strain type with the following five states to describe the colonization status of a given strain type across a household: at least one child (<18y) colonized ("C"), at least one adult (>=18y) colonized ("A"), both a child and an adult colonized ("CA"), colonization exclusively in the environment ("E"), no colonization present ("X"). This model was run with stochastically generated initial parameters (gen.inits=TRUE) and fit using the BOBYQA optimization algorithm with 1M maximal iterations to ensure convergence. Transition probabilities were averaged across "C" and "CA", "A" and "CA" to present a simplified model consisting of states "C", "A", "E", and "X".

Heritability and Preventability Estimation of Colonization and SSTI

Variance component estimates for colonization and infection models were calculated using the Bayesian MCMC generalized linear mixed model approach (Kruuk 2004; Postma and Charmantier 2007). This method was chosen due to its capacity to handle complex, unbalanced pedigrees for non-Gaussian data while estimating non-transgenerational (sibling, parent) with genetic variance components. This Bayesian approach also has the advantage of directly

estimating variance components from a posterior distribution without relying upon approximation methods. See **Table 15** for a summary of all colonization (burden, fixed and variable probability, and autoregressive) and SSTI models along with the environments employed, link functions, priors, and fixed effects.

All models followed the following general form:

$$y = link(X\beta + G + e_S + e_C + e_H + e_I + \varepsilon) \text{ (Equation 5)}$$

Where y is an n x 1 vector representing either the liability of individuals for a given phenotype or the number of occurrences of a given phenotype, X the n x m design matrix of fixed effects, β the m x 1 coefficients for these fixed effects, G the additive genetic variance, and e_i the n x 1 common environmental vectors where i represents sibling (S), couple (C), household (H), or individual (I) environments. $g \sim N(0, \sigma_g^2)$, $e_i \sim N(0, \sigma_i^2)$, and ε, the n x 1 residual error, is $\sim N(0, \sigma_\varepsilon^2)$. Accordingly, V_y, the phenotypic variance matrix of phenotype y across the population, can be represented in the following:

$$V_y = M_g\sigma_G^2 + M_s\sigma_S^2 + M_H\sigma_H^2 + M_c\sigma_C^2 + M_I\sigma_I^2 + I\sigma_\varepsilon^2 \text{ (Equation 6)}$$

Where M_g represents the kinship matrix between any two individuals, M_s the sibling matrix, where each entry is 1 if the two individuals share a common environment during upbringing and 0 otherwise, M_H the household matrix, where each entry is 1 if the two individuals live in the same household and 0 otherwise, M_c the couple matrix, where each entry is 1 if the two individuals are in a long-term sexual partnership and 0 otherwise, and M_I the individual matrix, representing in time series data whether the individual is the same person.

All models were fit using the R statistical software language (R Core Team 2016) package 'MCMCglmm' (J. D. Hadfield 2010), with link functions informed by (J. Hadfield

2016), and priors informed by (Gelman 2006). MCMC chain convergence was evaluated through both visual inspection and standard MCMC diagnostic tests (stationarity test, halfwidth mean test) (Gelman and Rubin 1992; Heidelberger and Welch 1983; Plummer et al. 2006) and run for 1.8million iterations for burden models, 720,000 iterations for fixed probability models models (shorter runtime due to faster convergence), and 1.8million iterations for variable probability and SSTI models with identical burnins of 80,000 and thinning intervals of 100.

Model selection was conducted to determine the best subset of variance components to account for a given phenotype. This was conducted by running all linear combinations genetic and environmental components 20 times until convergence as measured by the above diagnostics. The model with the lowest minimum DIC was then considered the 'top' model, breaking ties by selecting the simpler model (model with fewer variance components). These models appear in **Figure 11**.

Heritability Estimation for SSTI

Although patients reported a continuous number of SSTIs over the course of the observation period, SSTI was considered using a threshold model contingent upon one SSTI (see Table 5 for statistical formulation). This method was chosen since the desire was to measure the first incidence of SSTI, rather than the influence of recurrent infections. Recurrent *S. aureus* SSTIs are typically common, with a 50% recurrence rate (Fritz et al. 2009). Recurrent infections also could have been the result of a number of stochastic events such as poor treatment of first infection or reinfection from the previous SSTI, rather than meaningful phenotypic differences.

Truven Healthcare Dataset and Heritability Estimates

The Truven Health MarketScan Commercial Claims and Encounters Database, consisting of 56,003,690 policies across 115,805,687 individuals, was formatted into family units by insurance policy. A 'family' was defined as a group of individuals under a single insurance

policy. Parents were considered those listed as primary and secondary beneficiaries, while dependents were considered children. These families were then constrained first by including only individuals with a minimum enrollment of six years to ensure a sufficient observational time, second by excluding families with children <6 years old, and third by selecting families where the difference between dependents and beneficiaries was 15 years to select for genetically related families. This led to a total of 608,729 nuclear families with 2,348,070 individuals with a median enrollment of 8.8 years. Although pedigree is not completely known, previous studies have shown this to only slightly downwardly bias heritability estimates, with a 20% error leading to 5% underestimation (Charmantier and Réale 2005; Bérénos et al. 2014).

From this population, *S. aureus* infection was measured in each individual as at least one report of ICD9 codes '041.10', '041.11', and '041.12', which refer to an infection caused by any *S. aureus*, MSSA, or MRSA, respectively throughout their health history. Variance components were estimated on these families as described above using the link, prior and effects listed in Table 5 with a random subset of nuclear families (156,133 families with 602,500 individuals) due to limitations in the MCMCglmm software. Due to the larger data set, models were run in 'MCMCglmm' for 600,000 iterations with a burn-in period of 150,000−330,000 iterations with a thinning interval of 500.

Heritability and Preventability Estimation

To determine h^2, the estimated narrow-sense heritability of the observed phenotypes, the following equation was employed from the variance components for each phenotype as eqns. 1-2:

$$h^2 \equiv \frac{\sigma_g^2}{\sigma_g^2+\sum_i \sigma_i^2+c} \text{ (Equation 7)}$$

c is a constant to account for the variance introduced by the link function (1 probit and $\pi^2/3$ for logit links) (Day and Bonduriansky 2011). Since the number and choice of fixed effects alters the heritability estimate by shifting the phenotypic variance, thus leading to a difficulty in comparing between different heritability estimates from other sources, fixed effects for age and sex were chosen as they represent common factors accounted for when considering narrow-sense heritability.

In a similar fashion as heritability, preventability, defined as the fraction of total variance a given environment contributes, was calculated as follows:

$$p_i^2 \equiv \frac{\sigma_i^2}{\sigma_g^2+\sum_i \sigma_i^2+c} \text{ (Equation 8)}$$

Where p_i^2 represents the estimated proportion of variance contributed by environment *i*.

Household-level preventability (p^2_H) was estimated as the fraction of total variance in the model explained by the household-level random effect from the models fitted in 'MCMCglmm' (K. Wang et al. 2017):

$$\mathrm{p}_H^2 \equiv \frac{V_H}{V_T} \text{ (Equation 9)}$$

where V_H represents the household-level variance estimate and V_T the total measured variation.

Household-level preventability measures the extent to which colonization patterns can be attributed to individuals living in the same household, and accordingly indicates how altering individual versus household behaviors may modify *S. aureus* colonization. A household preventability of 90%, for example, indicates that interventions targeting household-level behaviors, such as frequency of washing bedding or towels, may be more effective than interventions targeted to specific individuals, such as hand washing or bathing frequency.

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Appendix A: Figures

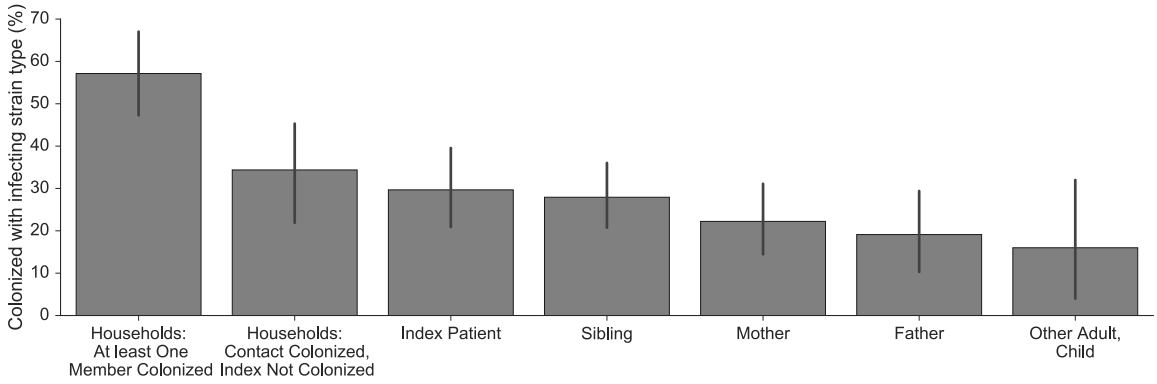

Figure 1: Occurrence of colonization with a strain concordant with the index patient infecting strain across households. Error bars indicate 95% confidence interval via the Clopper Pearson method. All pairwise comparisons between groups were not significant by Pearson's χ^2 test.

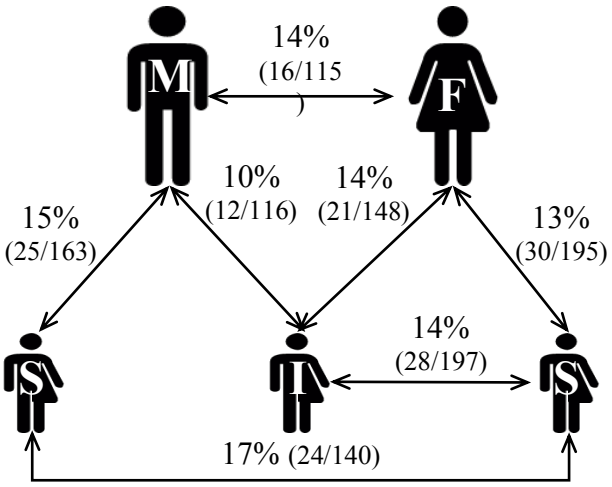

Figure 2: Colonization concordance between immediate family members. Concordance defined as two individuals both colonized with at least one of the same *S. aureus* strains by repPCR. Listed is the percentage concordance (number of concordant pairs over total observed pairs). Overall concordance across all family members was 15% (156/1074). Symbols clockwise from top left: male parent (M), female parent (F), sibling (S), index patient (I), sibling (S). Sex of index patients and siblings could be either male or female. Parents and siblings could be both biologically related or step-parents and step-siblings.

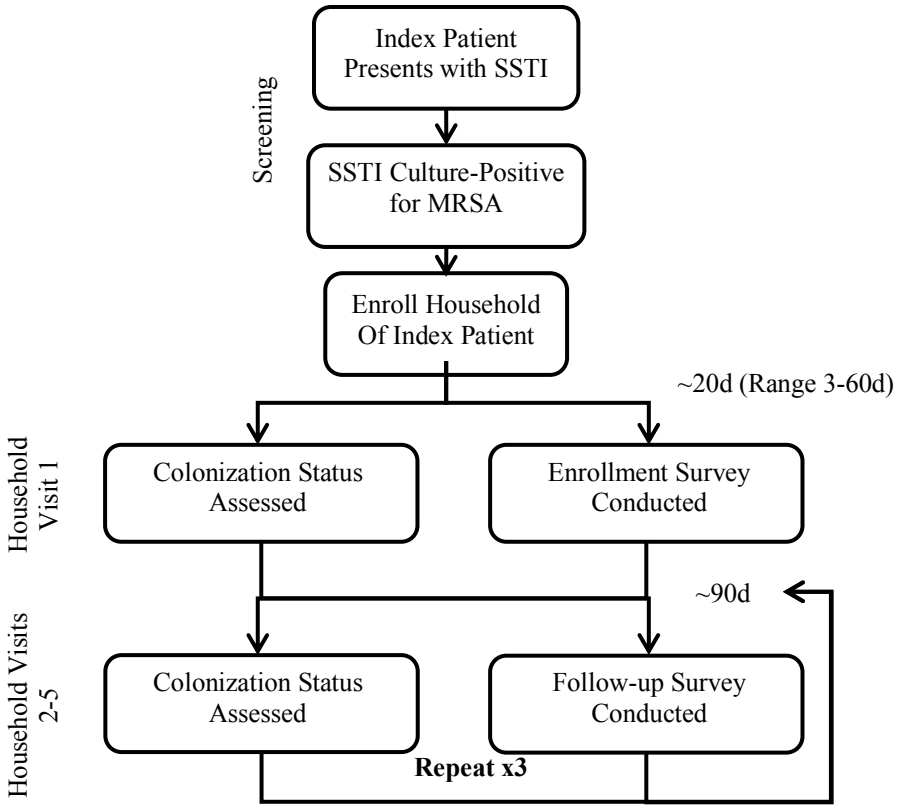

Figure 3: Study Population Colonization Assessment Workflow. Households with a minor, called the 'index patient,' presenting with a culture-positive MRSA SSTI were recruited from St. Louis Children's Hospital, Cardinal Glennon Children's Hospital, or a community pediatric practice in metropolitan St. Louis. 150 households with 692 individuals were enrolled from 2012-2015.

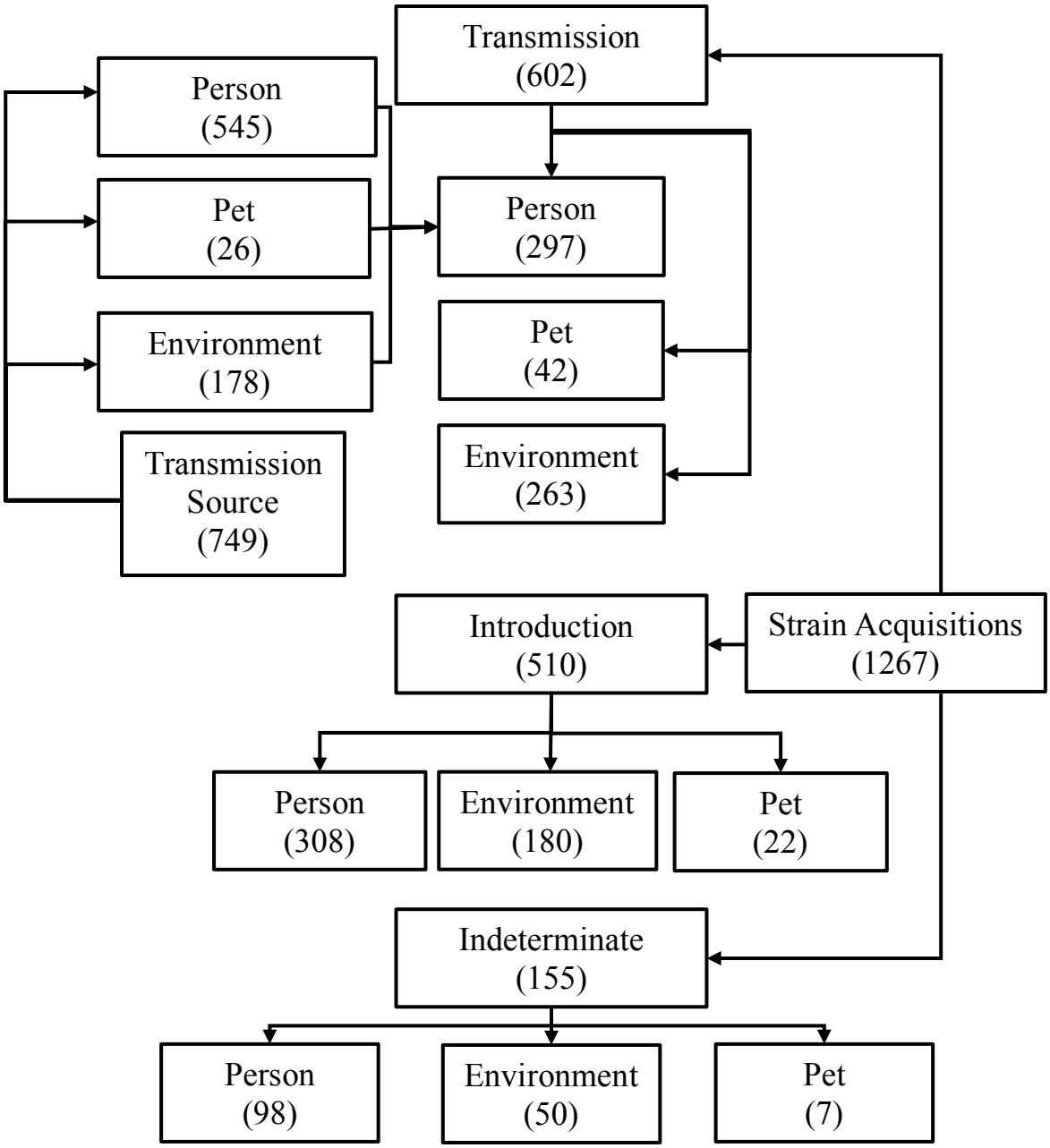

Figure 4: Flow Diagram of Strain Acquisitions. Across household members, pets, and the environment, a total of 1267 strain acquisitions were observed. Of these, 602 were transmissions, 510 were novel introductions, and 155 were indeterminate. For these 602 transmissions, there were 749 putative transmission sources to household members. Each individual or pet becoming colonized with a strain previously not present at the prior sampling counted for one acquisition, while one acquisition for the environment was counted when a strain appears on ≥1 environmental sites previously not found on at least one of those sites.

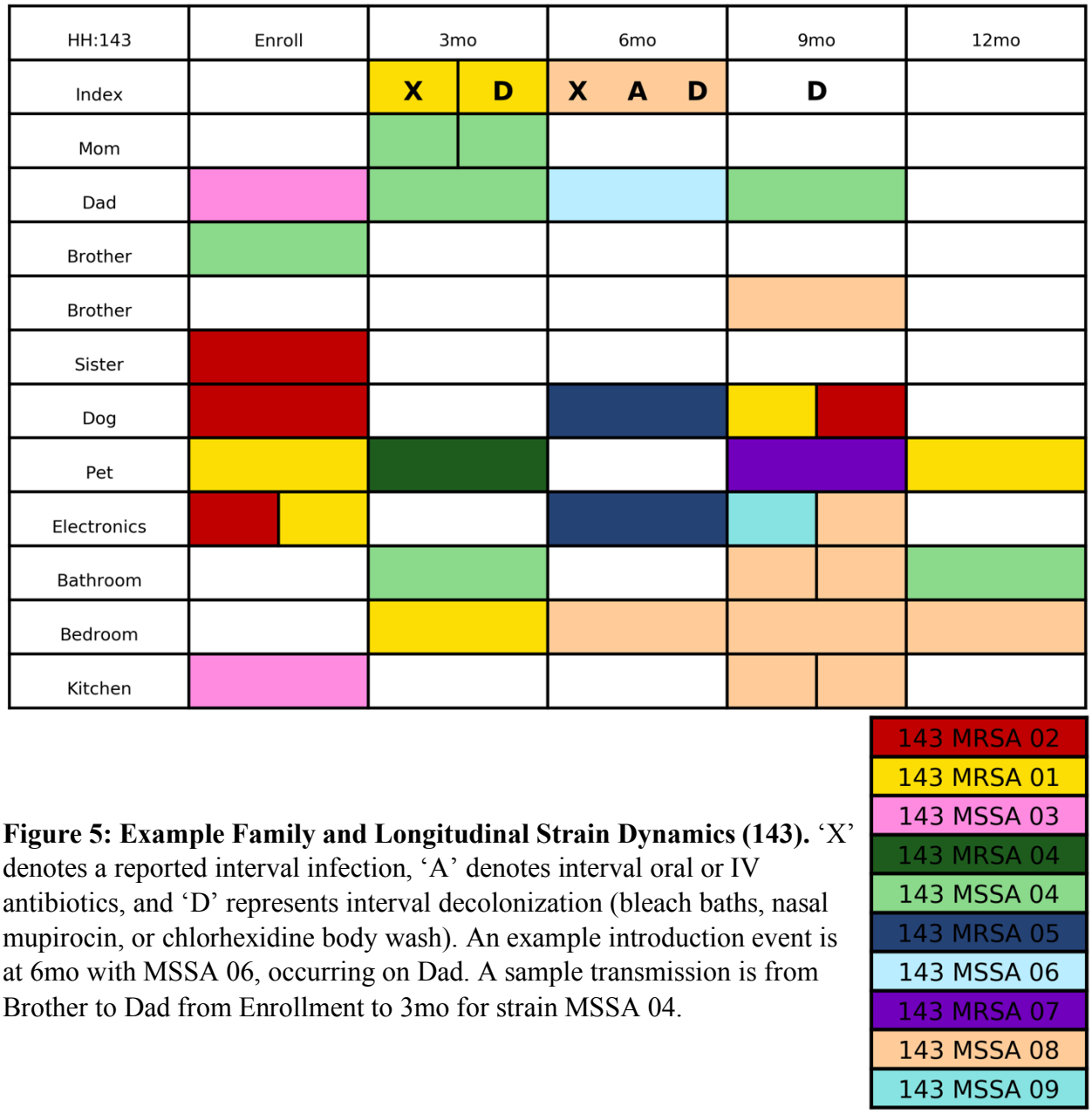

Figure 5: Example Family and Longitudinal Strain Dynamics (143). 'X' denotes a reported interval infection, 'A' denotes interval oral or IV antibiotics, and 'D' represents interval decolonization (bleach baths, nasal mupirocin, or chlorhexidine body wash). An example introduction event is at 6mo with MSSA 06, occurring on Dad. A sample transmission is from Brother to Dad from Enrollment to 3mo for strain MSSA 04.

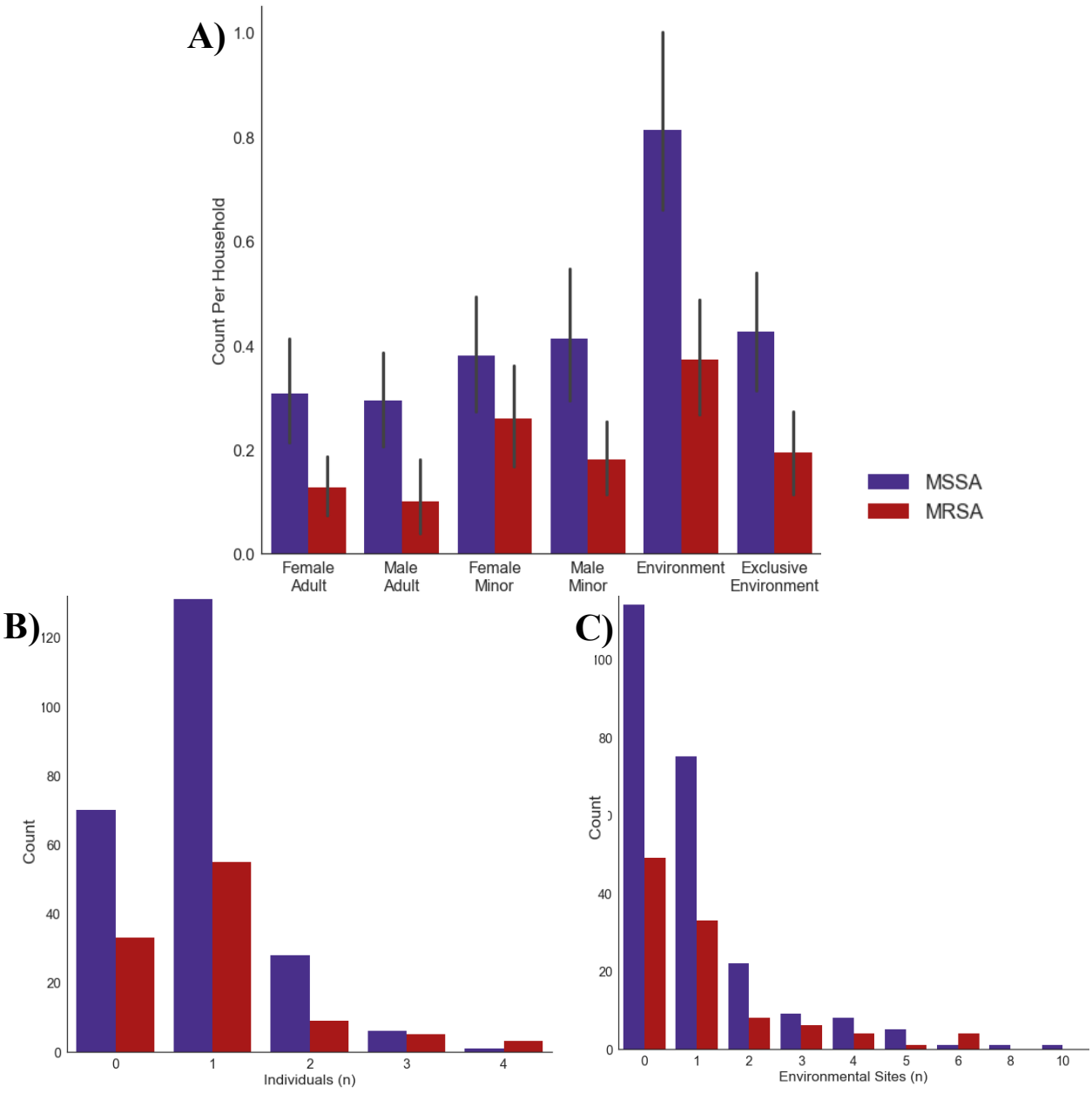

Figure 6: Introductions by Individual and Environment. A) Bar represents average across all households, uncorrected for number of possible individuals within households. **B)** Number of individuals colonized during a household introduction event (median 1, range 0-4). **C)** Number of environmental sites colonized during a household introduction event (median 1, range 0-10). For introduction events involving both individuals and the environment, the number of sites were significantly correlated (ρ=0.33, p=0.003, Spearman).

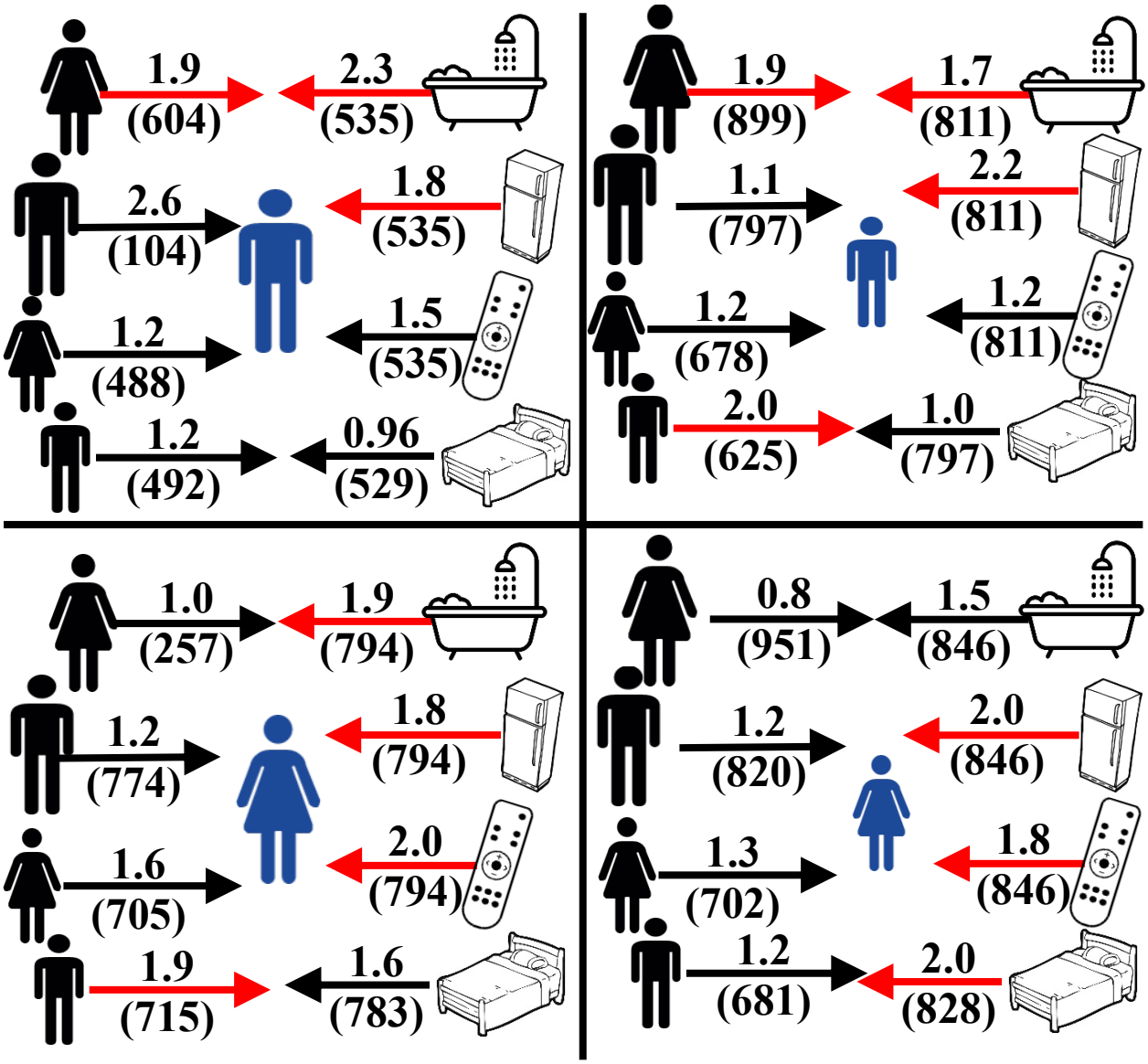

Figure 7: Normalized Transmission Risk of Household Members and Environment. For each recipient (blue), the normalized transmission risk for each putative person and environmental source is reported. Transmission risk is the ratio of the proportion of putative source sites colonized in successful versus unsuccessful transmissions. Red lines indicate a significant relationship by Kruskal Wallis, $p<0.05$. Pictographs: bathtub represents bathroom sites, fridge the kitchen sites, bed the index patient bed, computer the shared electronics, large sex icons the adults (≥18) and small icons the minors (<18). See Normalized Transmission Risk (p 98) for risk calculation.

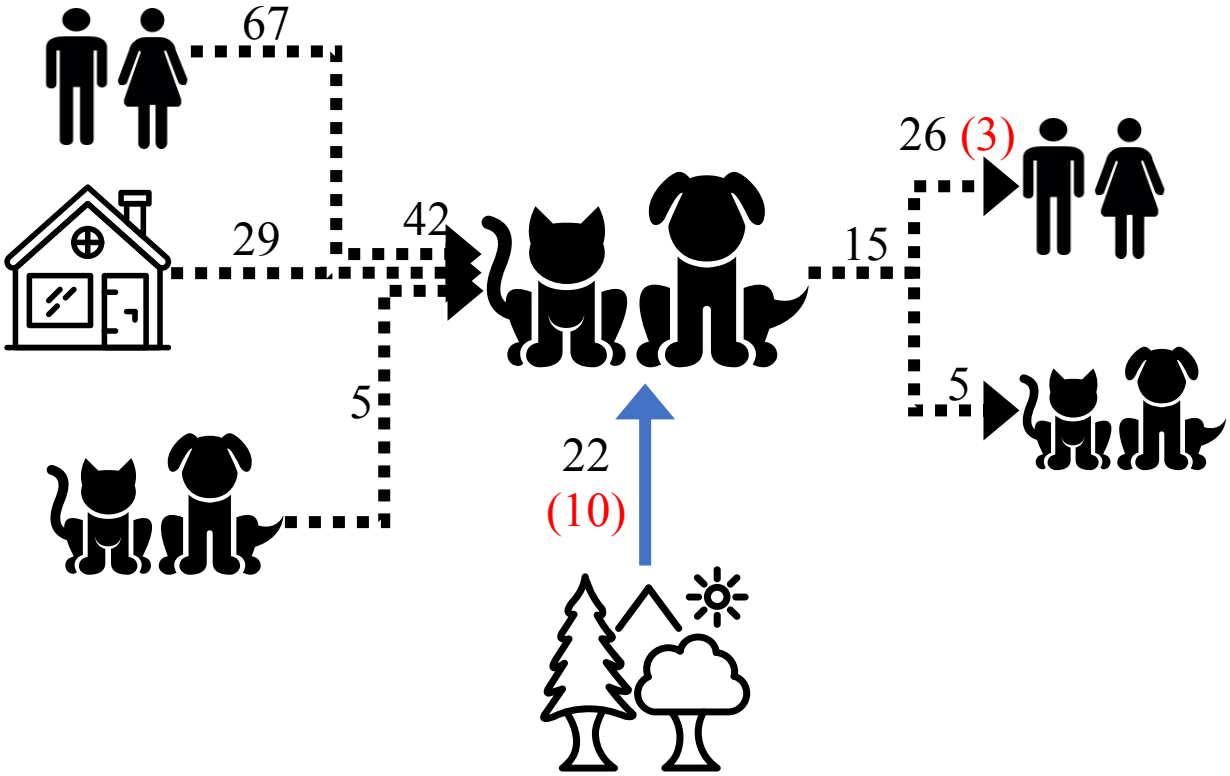

Figure 8: Putative Transmission Paths Among Pets. Incidence of transmissions amongst pets, household members, and the environment. 67 transmission paths from household members, 29 paths from the environment, and 5 paths from other pets lead to a total of 35 pets colonized as a result of a transmission across 42 events. 19 pets became colonized with *S. aureus* from an introduction in 22 instances. 15 pets served as putative transmission sources across 26 transmission paths to household members (3 with pets as the only source) and 5 other pets in the household. 'House' icon represents the household environment, 'cat/dog' icon represents pets, 'male/female' represents household members, and 'trees and mountains' represents the exogenous environment.

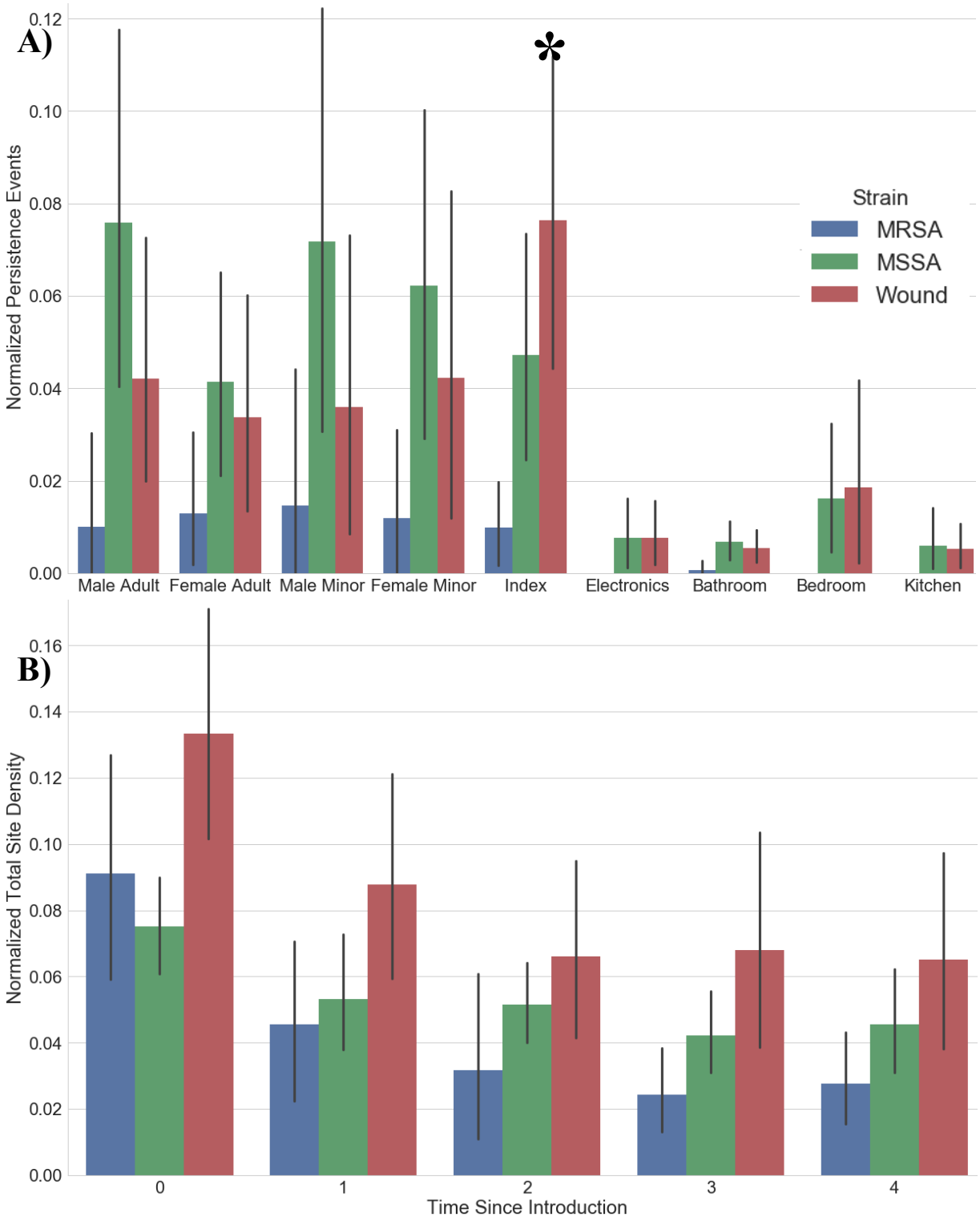

Figure 9: *S. aureus* strain persistence within exposed households over a year and wound strain known. A) Persistence by strain type across family role and environmental sites. *=p<0.05 comparing index wound persistence compared to household contacts, Kruskal-Wallis. HH=9. **B)** Strain density normalized by sampled environmental and person sites. Lines represents 95% *CI* estimate of bootstrapped mean. HH=91, wound known.

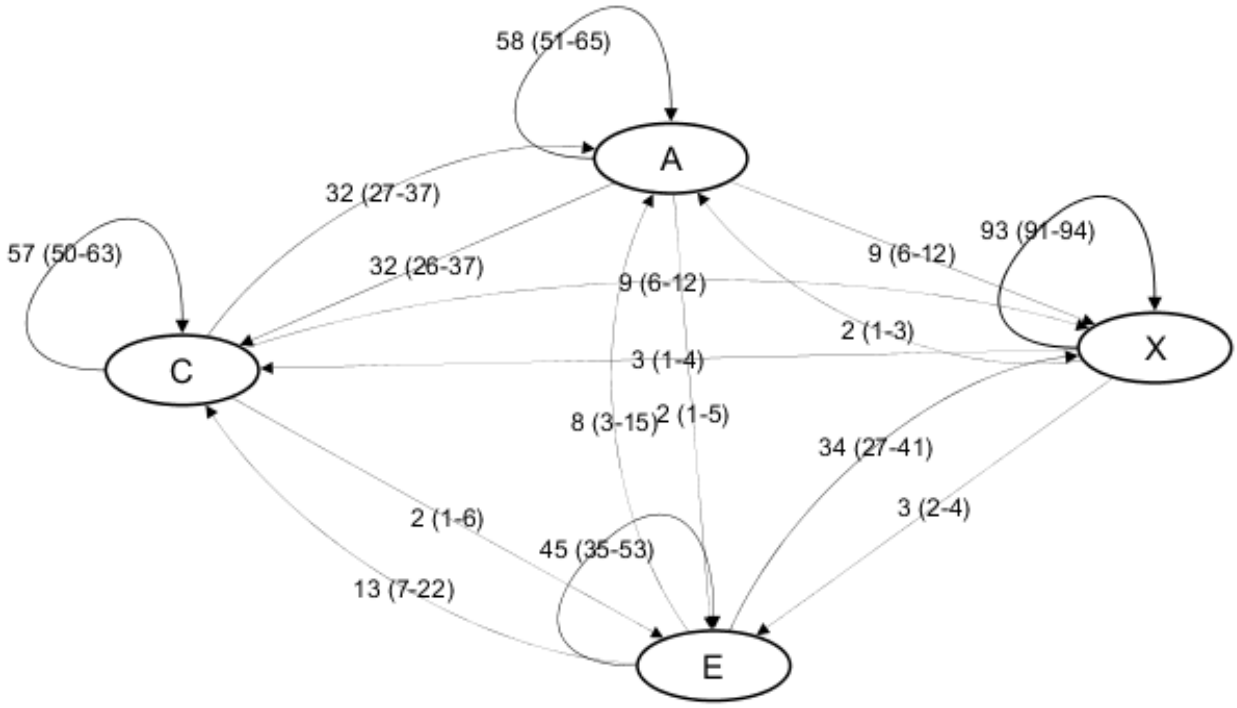

Figure 10: Markov Model of Strain Persistence Between Children, Adults, and the Environment. A 4-state Markov Model was fit across all households and strain types persisting at least twice consecutively, with the resultant transition probabilities displayed with the 95% *CI* interval estimates. State abbreviations: C; at least 1 minor colonized (<18), A; at least one adult colonized, E; at least one environmental site colonized (no household members), X; strain type not present in household.

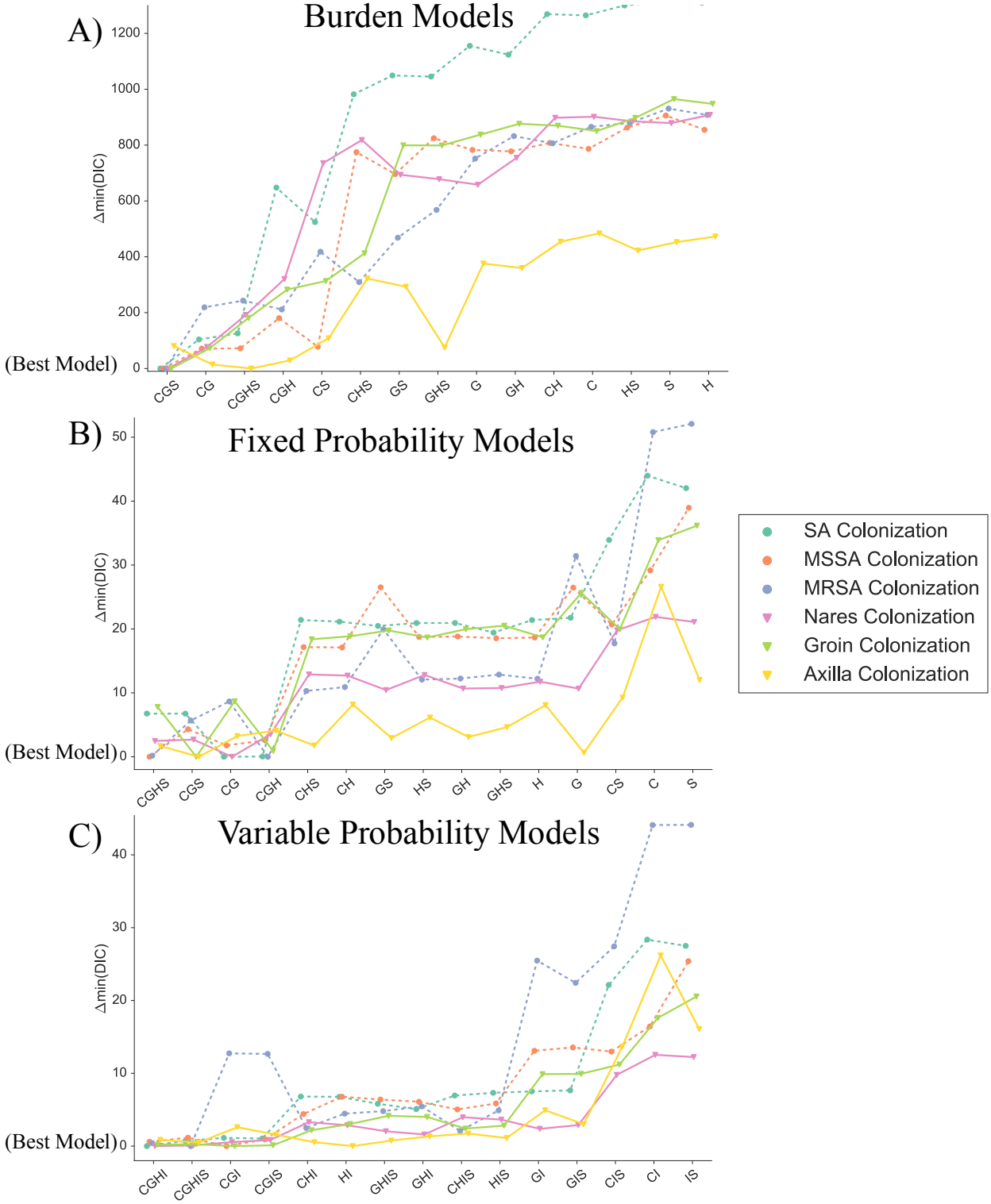

Figure 11: Model Minimum DIC Values for Colonization Phenotypes. A, B, C) Burden, Fixed, and Variable, respectively, model DIC values for the colonization phenotypes. See Methods for model convergence checks, model fitting, and selections procedures. Abbreviations: *G*, heritability, *H*, household preventability, *S*, sibling preventability, *C*, couple preventability, *I*, individual preventability.

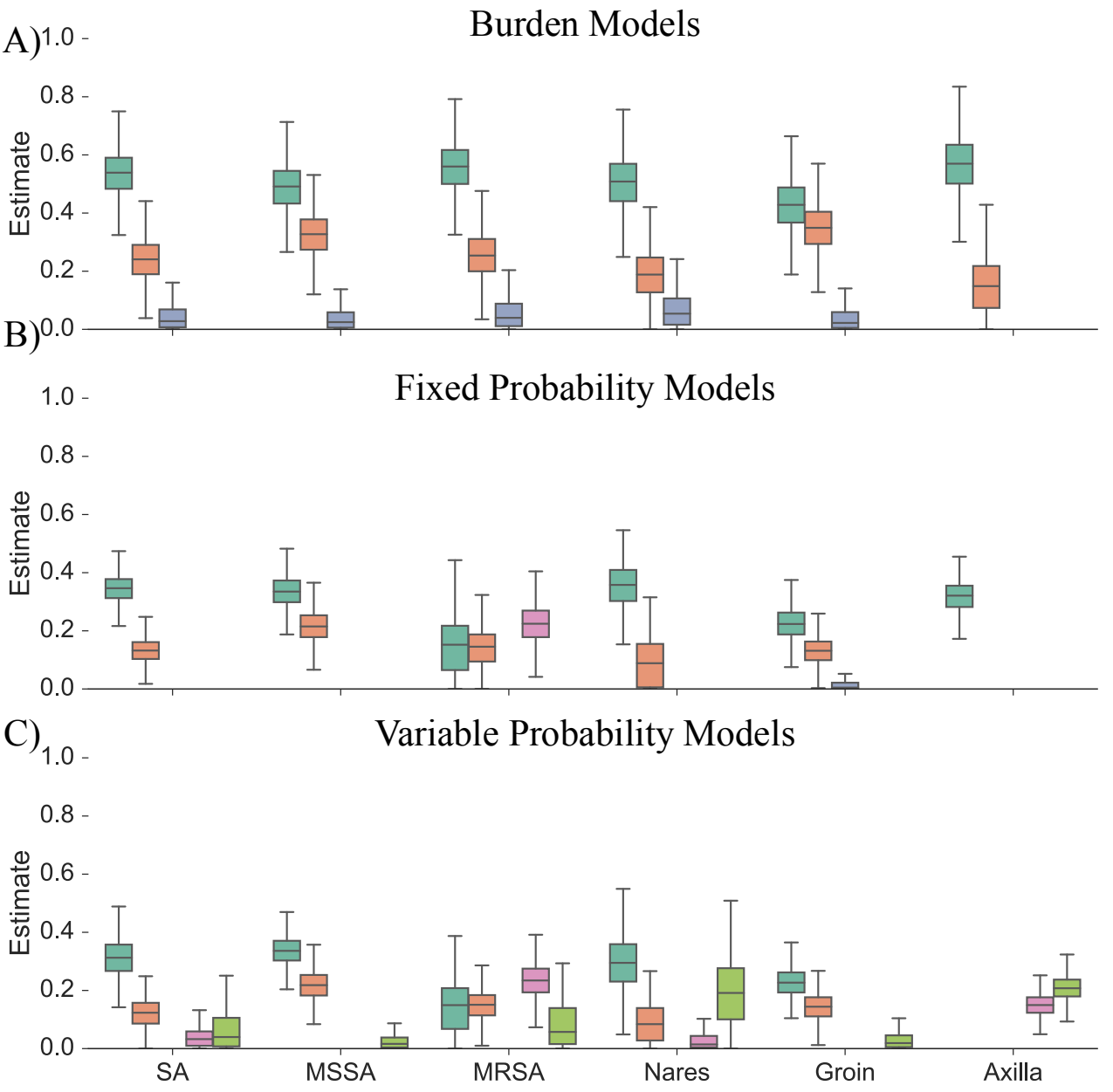

Figure 12: Variance Component Estimates for Colonization Phenotypes for Top Models. A, B,C) Box plots of variance component estimates for the top model as ranked by DIC across colonization phenotypes for the burden, fixed, and variable colonization definitions, respectively. All models were fit considering individual age, sex, and experimental design using MCMCglmm. See Methods for model convergence checks, model fitting, and selections procedures. Abbreviations: h^2, heritability.

Appendix B: Tables

Table 1. Population demographics of index patients and household contacts

Individual characteristics	All participants, N=671 (%)	Index patient, N=150 (%)	Household contacts, N=521 (%)
Age, median (range), years	15.0 (0.1-82.2)	2.98 (0.1-18.6)	26.6 (0.1-82.2)
Race			
Caucasian	464 (69)	102 (68)	362 (70)
African American	181 (27)	37 (25)	144 (28)
Multiracial	26 (4)	11 (7)	15 (3)
Latino/Hispanic ethnicity	32 (5)	9 (6)	23 (4)
Female sex	353 (53)	80 (53)	273 (63)
Health insurance			
Private	432 (64)	95 (63)	337 (65)
Medicaid	178 (27)	39 (26)	139 (27)
Medicare	5 (1)	0 (0)	5 (1)
Tricare	19 (3)	15 (10)	4 (1)
None	37 (6)	1 (1)	36 (7)
Health history			
Any chronic health condition[a]	333 (49)	58 (39)	275 (53)
Eczema	127 (19)	49 (33)	78 (15)
Asthma	111 (17)	26 (17)	85 (16)
Reported SSTI in year prior to study enrollment (excluding enrollment SSTI)	226 (34)	87 (58)	139 (27)
S. aureus infection ever (excluding enrollment SSTI)	149 (22)	62 (41)	87 (17)
Attempted decolonization in year prior to study enrollment[b]	209 (31)	73 (49)	136 (26)
Colonization status at enrollment visit			

Table 1, Continued.

S. aureus	275 (41)	57 (38)	218 (42)
MRSA exclusively	161 (24)	45 (30)	116 (22)
MRSA and MSSA at different body sites	19 (3)	5 (3)	14 (3)
Hygiene Practices			
Bathing ≥1/day	391 (58)	71 (47)	320 (61)
Brushing teeth ≥1/day	580 (86)	121 (81)	459 (88)
Always washes hands after handling wound[c]	264 (41)	35 (26)	229 (46)
Always washes hands after using bathroom	386 (63)	49 (43)	337 (68)
Launders bedding ≥1/week	264 (39)	60 (40)	204 (39)
Activities and Visitation			
Public pool usage, last 3 months	241 (36)	62 (41)	179 (34)
Sports participation, last year	195 (29)	38 (25)	157 (30)
Sports participation, contact sports (football, wrestling, hockey, lacrosse, rugby)	25 (4)	6 (4)	19 (4)
Used a public locker room, last 3 months	133 (20)	30 (20)	103 (20)
Attends or works at a daycare	151 (23)	72 (48)	79 (15)
Attends before/after school program (minors)[d]	32 (9)	8 (5)	26 (12)
Visited a patient in a hospital, last 6 months	315 (47)	48 (32)	267 (51)
Visited a patient in a nursing home, last 6 months	61 (9)	11 (7)	50 (10)
Visited a prison, last 6 months	28 (4)	5 (3)	23 (4)
Household factors (n=150)			
Distance from SLCH, median (range), miles		17.3 (0.9-76)	

Table 1, Continued.

Type of home	
House	122 (81)
Condominium	9 (6)
Apartment	19 (13)
Trailer	1 (<1)
Home ownership status	
Owns home	99 (66)
Rents home	51 (34)
Number of individuals, median (range)	
Per household	4 (2-13)
Per bedroom per household	1.3 (0.6-8)
Minors (<18 years)	2 (0-10)
Urban/Rural status[e]	
Urbanized area	130 (87)
Urban cluster	10 (7)
Rural	10 (7)
Household Colonization Pressure[f]	**% (SD)**
S. aureus colonization pressure	41% (±29%)
S. aureus anatomical site colonization pressure	19% (±18%)
MRSA colonization pressure	25% (±28%)
MRSA anatomical site colonization pressure	11% (±15%)

Table 1, Continued.

Abbreviations: SSTI, skin and soft tissue infection; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; SLCH, Saint Louis Children's Hospital.
Note: *S. aureus* encompasses both MSSA and MRSA.
a. Chronic health conditions include current diagnosis with asthma, seasonal allergies, seizures, heart disease, diabetes, cancer, kidney disease, liver disease, connective tissue disease, acid reflux, inflammatory bowel disease, human immunodeficiency virus (HIV), chronic granulomatous disease, depression, bipolar, attention deficit disorder, sickle cell disease, cystic fibrosis, or emphysema.
b. Decolonization measures include mupirocin ointment to the anterior nares, chlorhexidine body washes, or bleach water baths.
c. This information does not include infants and toddlers, so the total number of individuals surveyed was 637 household members; 136 index patients and 501 household contacts.
d. Only minors were included in the before/after school program variable; the total number of eligible household members was 360, while the number of eligible household contacts was 210.
e. Assignment based on 2010 U.S. Census Bureau TIGER/Line® Shapefiles. Urbanized areas and urban clusters are densely settled territory measured at the census tract and census block levels of geography that contain ≥50,000 people or between 2,500-49,999 people, respectively. All other areas are considered rural.
f. Colonization pressure was calculated as (number of colonized individuals in the household)/(total number of individuals in the household). Anatomical site colonization pressure was calculated across all household members as (number of colonized anatomic sites)/(total number of anatomic sites), where an anatomical site includes the nares, axillae, and inguinal fold for each individual.

Table 2: Univariate analysis of factors significantly associated with *S. aureus* and MRSA colonization across household members

	***S. aureus* Colonization**			**MRSA Colonization**		
Colonization Prevalence	**N=396 (59%)**	**N=275 (41%)**		**N=510 (76%)**	**N=161 (24%)**	
Colonization Pressure (CP) and Strain Richness	**Not colonized, mean ± SD**	**Colonized, mean ± SD**		**Not colonized, mean ± SD**	**Colonized, mean ± SD**	
S. aureus anatomical site CP[a]	0.15 ± 0.1	0.25 ± 0.2**		0.16 ± 0.2	0.28 ± 0.3**	
MRSA anatomical site CP[a]	0.08 ± 0.1	0.15 ± 0.2*		0.08 ± 0.1	0.22 ± 0.2**	
Strain richness per person[b]	0.26 ± 0.2	0.35 ± 0.3*		-	-	
Hygiene and Proximity to Colonized Individuals	**Not colonized, n (%)**	**Colonized, n (%)**	**OR (95% *CI*)**	**Not colonized, n (%)**	**Colonized, n (%)**	**OR (95% *CI*)**
Shares a bedroom with *S. aureus*-colonized individual	123 (31)	120 (44)	1.7 (1.2-2.4)*	167 (33)	76 (47)	1.8 (1.3-2.7)*
Shares a bedroom with MRSA-colonized individual	63 (16)	81 (29)	2.2 (1.5-3.3)**	79 (15)	65 (40)	3.7 (2.4-5.6)**
Shares a personal hygiene item[c] with *S. aureus*-colonized individual	209 (53)	182 (66)	1.7 (1.3-2.4)*	-	-	-
Shares a personal hygiene item[c] with MRSA-colonized individual	-	-	-	157 (31)	96 (60)	3.3 (2.3-4.9)**
Shares a face cloth with another individual	19 (5)	37 (13)	3.1 (1.7-5.8)*	-	-	-
Bathes or showers ≥ 1x/day	-	-	-	315 (62)	76 (47)	0.6 (0.4-0.8)*

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; SD, standard deviation; OR, odds ratio; *CI*, confidence interval.

Note: *S. aureus* encompasses both methicillin-susceptible *S. aureus* and MRSA. Significance: *=p (corrected) <0.05, **=p (corrected) <0.005. Dashed lines indicate that the covariate was not significant for the given colonization phenotype. All categorical variables underwent Fisher's exact test (all instances were 2x2 contingency tables for OR calculation) for significance calculation. For continuous covariates, the nonparametric Kruskal-Wallis one-way ANOVA on ranks was employed. All p-values were corrected with the Bonferroni method (tests=37), and only covariates that displayed a corrected p-value <0.05 are shown above.

a. Anatomical site colonization pressure, not including individual currently modeled (self), for a household; calculated as (number of positive anatomic sites)/(total possible anatomic sites).

b. Number of unique strains by repPCR present on all household members, not including the individual currently modeled (self); Calculated as (number of strain types colonizing any household member) / (total number of household members).

c. Item includes at least one personal hygiene product from the following list: facecloth, toothbrush, hand towel, deodorant, or razor.

Table 3: Generalized linear logistic regression analyses of factors significantly associated with *S. aureus* and MRSA colonization

	***S. aureus* colonization**	**MRSA colonization**
	OR (95% *CrI*)	**OR (95% *CrI*)**
Colonization Pressure model		
S. aureus colonization pressure[a]	1.4 (1.2-1.5)**	-
MRSA colonization pressure[a]	-	1.8 (1.6-2.1)**
Rents dwelling	1.2 (1.0-1.5)*	-
Strain-level richness[b]	-	0.5 (0.3-0.9)*
Age (years)	-	0.9 (0.8-0.98)*
Uses antibacterial soap	-	0.8 (0.6-0.97)*
Proximity model		
Shares bedroom with *S. aureus* colonized individual	1.1 (0.9-1.4)	-
Shares bedroom with MRSA colonized individual	-	1.5 (1.1-2.2)*
Age (years)	-	1.0 (0.8-1.1)
Rents dwelling	1.5 (1.1-1.9)**	1.4 (1.0-1.9)
Bathes or showers ≥ 1x/day	-	0.7 (0.6-0.98)*
Enrollment average monthly low temperature (°F)[c]	1.1 (0.96-1.2)	1.2 (1.01-1.4)*
Index patient[d]	-	1.2 (0.9-1.7)
Activity model		
Rents dwelling	1.5 (1.1-2.0)*	1.4 (1.01-2.1)*
Uses antibacterial soap	0.8 (0.6-0.95)*	0.7 (0.5-0.97)*
History of SSTI, past year	-	1.3 (0.99-1.6)
Enrollment average monthly low temperature (°F)[c]	-	1.2 (1.03-1.5)*
Bathes or showers ≥1x/day	-	0.7 (0.5-0.9)*

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; OR, odds ratio; *CrI*, credible interval; SSTI, skin and soft tissue infection.
Note: *S. aureus* encompasses both methicillin-susceptible *S. aureus* and MRSA. Significance: p≥0.05 unlabeled, *=p<0.05, **=p<0.005. Dashed lines indicate that the covariate was not employed in the given colonization model. Final model covariates were derived from a two-level variable selection methodology (see **Chapter 6: Methods**). Models were run as generalized linear mixed logistic regressions using both Frequentist (R package 'lme4') and Bayesian approaches (R package 'MCMCglmm'). Bayesian ORs and P-values were reported from the models.
a. Anatomical site colonization pressure, not including individual currently modeled (self), for a household; calculated as (number of positive anatomic sites)/(total possible anatomic sites).
b. Number of unique strains by repPCR present on all household members, not including the individual currently modeled (self); Calculated as (number of strain types colonizing any household member) / (total number of household members).
c. This is the reported average low temperature by month from NOAA's 1981 to 2010 Climate Normals.
d. The index patient is the household member with the MRSA infection, in comparison to a household contact.

Table 4: Colonization and infection incidence across the study population

	Mean Observed Frequency % (σ^2, Range)	At Least once % (n)	Mean times +/- σ^2
Individual	n=591-677	n=540 (Fully sampled)	n=540 (Fully sampled)
Colonized *S. aureus*	42 (2, 41-44)	78 (420)	2.1±1.7
Colonized MRSA	21 (2, 18-24)	49 (265)	1.1±1.4
Nares Colonized *S. aureus*	32 (2, 28-34)	63 (339)	1.6±1.7
Inguinal folds Colonized *S. aureus*	19 (2, 16-20)	51 (274)	0.9±1.2
Axilla Colonized *S. aureus*	10 (2, 8-13)	34 (186)	0.9±0.5
Report SSTI during Study[a]	11 (2, 9-13)	29 (152)	0.4±0.8
Reported ≥1 Topical Antibiotics Taken during Study (Follow Up)	5 (1, 4-6)	16 (86)	0.3±0.9
Reported ≥1 Oral Antibiotics Taken during Study (Follow Up)	5 (1, 4-6)	15 (83)	0.2±0.7
Reported Employing Decolonization Methods during Study[b] (Follow Up)	23 (9, 16-37)	49 (265)	1.0±1.2
	Mean Observed Frequency % (+/-σ^2, Range)	**At least one member in HH % (n)**	**Mean (Σ Colonized/members) +/- σ^2**
Household-level	n=135-150	n=129 (Fully Sampled)	n=129 (Fully Sampled)
HH Members Colonized *S. aureus*	42 (2, 40-44)	98 (126)	2.1±1.1
HH Members Colonized MRSA	21 (3, 18-25)	81 (104)	1.1±1.0
HH Members Reporting SSTI	12 (2, 10-15)	67 (87)	0.5±0.5

a. This includes a total of 250 reported skin abscesses and/or boils, 2 reported cases of impetigo, and 5 cases of cellulitis.
b. Decolonization methods include the following: Mupirocin or Bactroban to anterior nares or other body site, Chlorhexidine or Hibiclens body washes, or bleach water baths.

Table 5: Observed Transmission, Introductions Longitudinally

	Putative Total Paths, n (%)	≥2 Observations over Year (n)	Unique Sinks of Observations, n (%)
Person Transmissions	Paths: 545 (Unique: 297)	650 people	205 (31.5)
MSSA	150 (50.5)	650 people	107 (16.5)
MRSA	147 (49.5)	650 people	98 (15.1)
Sibling→Sibling	112 (20.6)	304 Siblings	67 (22.0)
Offspring→Parent	101 (18.5)	247 parents	56 (22.7)
Infant[a]→Father	4 (4.0)	20 fathers	7 (35.0)
Infant[a]→Mother	7 (6.9)	29 mothers	3 (10.3)
Parent→Offspring	79 (14.5)	338 offspring	58 (17.2)
Father→Infant	4 (5.1)	21 infants	4 (19.0)
Mother→Infant	7 (8.9)	30 infants	6 (20.0)
Cohabitating[b] Parents	25 (4.6)	210 Parents	25 (11.9)
	Total, n (%)	≥1 Observations over Year (n)	Unique Person Transmissions per Environment, n (%)
Environment Source	Paths: 178	650 people	147 (22.6)
Exclusively Present in Environment	62 (34.8)	650 people	57 (8.8)
	Total, n (%)	≥2 Observations over Year (n)	Unique of Observations, n (%)
Person Introductions	Total: 308 (237 events)	650	38.0 (247)
MRSA	99 (32.4)	650 people	87 (13.4)
MSSA	209 (67.6)	650 people	176 (27.1)
Child	188 (61.0)	373 children	152 (40.8)
Index Patient	84 (44.7)	144 Index patients	66 (45.8)
Non-Index Child	100 (53.9)	229 Non-index patients	86 (37.6)
Infant	18 (9.5)	30 infants	15 (50.0)
Adult	120 (38.8)	319 adults	94 (29.5)
Mother	57 (47.5)	141 mothers	45 (31.9)
Father	47 (39.2)	106 fathers	37 (34.9)

Table 5, Continued.

Non-parent	16 (13.3)	72 non-parents	12 (16.7)
Present ≥ 1 Environmental Site	180 (52.8)	108 Household environments	87 (80.6)
Exclusively Present in Environment	94 (52.2)	108 Household environments	58 (53.7)

Notes:

A. Infant refers to a minor <1yrs of age.
B. Cohabiting parent refers to two parents which both share the same bed.

Table 6: Introductions, Transmissions by Environmental Site

Environmental Site	Aggregated Room	Colonized (n)	Colonized by Introduction n (%)	Colonized, Putative Transmission Source n (%)
Bed sheets and pillowcases	Bedroom	121	8.0 (30.8)	16.0 (61.5)
Refrigerator door handle	Kitchen	93	18.0 (29.0)	23.0 (37.1)
Bathroom sink	Bathroom	82	14.0 (34.1)	12.0 (29.3)
Bathroom sink faucet handle	Bathroom	65	16 (24.6)	27 (41.5)
Bathroom tub	Bathroom	77	22.0 (28.6)	39.0 (50.6)
Toilet seat	Bathroom	74	37.0 (30.6)	60.0 (49.6)
Kitchen table	Kitchen	72	22.0 (33.8)	43.0 (66.2)
Bathroom counter top	Bathroom	65	22.0 (44.9)	26.0 (53.1)
TV remote control	Electronics	64	10.0 (24.4)	18.0 (43.9)
Main telephone or index cell phone	Electronics	62	13.0 (28.3)	25.0 (54.3)
Toilet handle	Bathroom	54	16.0 (21.6)	39.0 (52.7)
Computer keyboard and mouse	Electronics	49	5.0 (31.3)	13.0 (81.3)
Bathroom light switch	Bathroom	47	15.0 (27.8)	27.0 (50.0)
Videogame controller	Electronics	46	11.0 (44.0)	11.0 (44.0)
Bathroom door handle	Bathroom	46	19.0 (50.0)	15.0 (39.5)
Index bathroom towel	Bathroom	41	12.0 (25.5)	28.0 (59.6)
Kitchen sink faucet handle	Kitchen	41	11.0 (23.9)	22.0 (47.8)
Kitchen sponge cloth	Kitchen	38	18.0 (22.0)	36.0 (43.9)
Kitchen hand towel	Kitchen	26	24.0 (25.8)	57.0 (61.3)
Bathroom hand towel	Bathroom	25	16.0 (25.0)	40.0 (62.5)
Soap dish and bar in bath shower	Bathroom	16	26.0 (36.1)	33.0 (45.8)

Table 7: Transmission Sink, Source, and Introduction Multivariable Models

Covariate	Estimate (95% *CrI*)	pMCMC
Transmission Source		
Household cleanliness (Clean/Dirty)	0.95 (0.71-1.27)	0.750
Shares bath towel	1.25 (1.01-1.57)	0.047
People per bathroom	1.10 (1.02-1.19)	0.016
Transmission Recipient		
Strain environmental contamination pressure	3.84 (1.92-7.91)	0.0004
Shares bedroom with strain colonized-individual	1.34 (1.12-1.59)	0.0008
Shares towel (bath, hand, or face) with strain colonized-individual	1.14 (0.97-1.34)	0.113
SSTI	1.36 (1.09-1.66)	0.006
Dwelling ownership	0.74 (0.61-0.90)	0.003
Nights spent in household	1.07 (0.96-1.18)	0.229
Sex (Male)	1.16 (1.01-1.34)	0.040
Environment contamination pressure of other strains in household	0.44 (0.24-0.78)	0.005
Introduction		
Handwashing score	0.86 (0.74-1.01)	0.064
Pet in household	0.89 (0.75-1.05)	0.190
Average monthly low temperature (^{o}F) at time of sampling	0.91 (0.85-0.98)	0.011

Table 8: Persistence Events by Individual and Relation to SSTI

Persistence Incidence			
Measure	All, median, IQR, (>1)	Index, median, IQR, (>1)	Household Contacts, median, IQR, (>1)
	n=540	n=128	n=412
SA, times colonized	2.0, 3.0, (306)	2.0, 3.0, (78)	2.0, 3.0, (228)
MRSA, times colonized	0.0, 2.0, (142)	1.0, 2.0, (39)	0.0, 1.25, (103)
MSSA, times colonized	1.0, 2.0, (161)	1.0, 2.0, (36)	1.0, 2.0, (125)
Strain-SA	1.0, 0.0, (127)	1.0, 0.25, (32)	1.0, 0.0, (95)
Strain-MRSA	0.0, 1.0, (109)	1.0, 2.0, (33)	0.0, 1.0, (76)
Strain-MSSA	1.0, 1.0, (112)	1.0, 1.0, (22)	1.0, 1.0, (90)
Relation to Reported SSTI			
Measure, >= 1 Colon SA, Correlation	All, p, rho	Index, p, rho	Household Contacts, p, rho
	n=420	n=100	n=320
SA, times colonized	0.03088, 0.11	0.06397, 0.19	0.10525, 0.09
MRSA, times colonized	0.0, 0.28	2e-05, 0.42	0.00024, 0.2
MSSA, times colonized	0.00564, -0.13	0.05663, -0.19	0.07985, -0.1
Strain-SA	0.14563, 0.07	0.47647, 0.07	0.25937, 0.06
Strain-MRSA	**0.0, 0.29**	**0.0, 0.44**	**0.00039, 0.2**
Strain-MSSA	**0.01724, -0.12**	**0.098, -0.17**	**0.14415, -0.08**
Measure, >=1 Colon SA, Kruskal Risk	All, p, risk	Index, p, risk	Household Contacts, p, risk
SA, times colonized	0.04993, 1.11	0.29436, 1.11	0.10712, 1.13
MRSA, times colonized	0.0, 1.81	0.00049, 1.8	0.00022, 1.77
MSSA, times colonized	0.00532, 0.67	0.04866, 0.61	0.07152, 0.73
Strain-SA	0.16867, 1.1	0.45069, 1.09	0.29497, 1.1
Strain-MRSA	**0.0, 1.8**	**0.00026, 1.8**	**0.00031, 1.72**
Strain-MSSA	**0.02001, 0.72**	**0.15588, 0.7**	**0.13003, 0.75**

Table 9: Strain Persistence Measures

Strains Present at Enroll, 3mo.	MRSA*, median, IQR, (>2)	MSSA, median, IQR, (>2)	Wound, median, IQR, (>2)
(fully swabbed households, n=129)	n=144	n=187	n=61
Person Persistence Degree	1.0, 2.25, (45)	1.0, 1.0, (40)	1.0, 3.0, (24)
Environment Persistence Degree	1.0, 2.0, (24)	1.0, 1.0, (10)	1.0, 2.0, (12)
Any Persistence	2.0, 3.0, (57)	1.0, 2.0, (50)	2.0, 4.0, (27)
Households with ≥ 1 Strain Types Persisting 3+ Samplings	MRSA* n (%)	MSSA n (%)	Infecting Strain n (%)
(fully swabbed households, n=129)	54 (41.9)	45 (34.9)	27 (20.9)
Colonized 1 Person Persistently	9 (16.7)	11 (24.4)	4 (14.8)
Colonized >=1 Environmental Site	51 (94.4)	41 (91.1)	26 (96.3)
Colonized 3+ Individuals	33 (61.1)	22 (48.9)	14 (51.9)

Table 10: Persistence and Longitudinal Strain Type CP of Sampled Environmental Sites

Environmental Site	Aggregated Site	Longitudinal *S. aureus* CP mean, SD (n>0)	*S. aureus* Persistence Degree mean, SD (n>0)
Bathroom sink faucet handle	Bathroom	14.4, 22.9 (58)	0.04, 0.2 (3)
Index bathroom towel	Bathroom	14.64, 25.4 (43)	0.04, 0.2 (3)
Bathroom tub	Bathroom	15.93, 22.9 (67)	0.07, 0.3 (8)
Toilet seat	Bathroom	14.07, 21.5 (59)	0.12, 0.4 (10)
Bathroom counter top	Bathroom	16.45, 24.4 (65)	0.07, 0.3 (6)
Bathroom sink	Bathroom	17.57, 25.2 (68)	0.12, 0.4 (10)
Soap dish and bar in bath shower	Bathroom	9.73, 24.3 (19)	0.1, 0.4 (4)
Bathroom door handle	Bathroom	9.37, 19.7 (44)	0.07, 0.4 (5)
Bathroom hand towel	Bathroom	12.12, 25.7 (33)	0.01, 0.1 (1)
Bathroom light switch	Bathroom	12.93, 23.9 (50)	0.04, 0.2 (3)
Toilet handle	Bathroom	10.43, 20.3 (48)	0.08, 0.5 (4)
Bed sheets and pillowcases	Bedroom	26.39, 32.5 (81)	0.2, 0.6 (14)
Main telephone or index cell phone	Electronics	14.0, 24.1 (53)	0.08, 0.4 (7)
Computer keyboard and mouse	Electronics	14.09, 25.3 (46)	0.1, 0.5 (5)
Videogame controller	Electronics	17.82, 28.8 (49)	0.07, 0.4 (3)
TV remote control	Electronics	17.59, 28.6 (61)	0.1, 0.5 (7)
Refrigerator door handle	Kitchen	19.2, 26.1 (70)	0.17, 0.5 (13)
Kitchen sponge cloth	Kitchen	10.03, 21.2 (40)	0.0, 0.0 (0)
Kitchen sink faucet handle	Kitchen	8.37, 16.8 (41)	0.04, 0.2 (4)
Kitchen hand towel	Kitchen	11.59, 26.6 (31)	0.05, 0.3 (2)
Kitchen table	Kitchen	14.93, 23.8 (55)	0.06, 0.3 (5)

Table 11: Interval Reported Infection Etiological Agent Statistics

Measure	n (%, total)
Follow-up Infections after Enrollment with Etiological Agent	19 infections, 17 households
MRSA	16 (84.2, 19)
Follow-up infection on Index	15 (78.9, 19)
Present in Household at Prior Time Point	14 (73.7, 19)
Individual colonized with strain at prior time point	8 (42.1, 19)
Individual Colonized with strain at current time point	5 (26.3, 19)
Household member (non-SSTI reporter) colonized with strain at prior time point	7 (36.8, 19)
Household member (non-SSTI reporter) colonized with strain at current time point	4 (21.1, 19)
Environment contaminated with strain at prior time point	9 (52.9, 17) (NA:2)
Environment contaminated with strain at current time point	3 (18.8, 16) (NA:3)
Interval infection is Index Wound Strain*	11 (78.6, 14) (NA:5)
Index persistently colonized with wound strain (>=2tp's)	7 (63.4, 11)
Infection on non-index	1 (9.1, 11)

*In some households, wound strain unknown. These were not included in the denominator.

Table 12: Multivariable Models of SSTI Risk and Persistence Measures

Covariate	Estimate (95% *CrI*)	pMCMC
Individual SSTI Risk		
Environmental MRSA CP	2.19 (1.1-2.3)	0.024898
Shares Room, MRSA-Colonized	1.22 (1.0-1.6)	0.127755
History of SSTI (Enrollment)	2.60 (1.9-3.5)	0.00020408
Bathes Daily	0.87 (0.7-1.1)	0.242857
Index	1.38 (1.1-18)	0.0220408
Individual Strain Type Persistence		
Environmental CP of Strain	2.74 (1.5-5.2)	0.00163265
Dwelling ownership	0.75 (0.6-1.0)	0.0228571
Handwashing Score	1.39 (1.1-1.7)	0.00612245
Nares Mupirocin Decolonization	0.44 (0.3-0.6)	0.00020408
Shares Brush/Comb	0.85 (0.7-1.1)	0.162857
Person CP of Other Strains in Household	0.38 (0.2-0.8)	0.00612245
Typically uses bar soap	0.77 (0.6-1.1)	0.102449
Towel Washed Frequently	0.82 (0.7-1.0)	0.0636735
Household Longitudinal Strain Type Persistence		
Decolonizations, Household (%)	0.9 (0.8-1.1)	0.191429
Had SSTI (%)	0.97 (0.8-1.1)	0.698776
Dwelling ownership	0.88 (0.6-1.3)	0.5
Household cleanliness (Clean/Dirty)	0.85 (0.6-1.2)	0.418776
MRSA Strain	1.49 (1.1-2.0)	0.00938776
Household crowding	0.52 (0.3-0.9)	0.00571429
Total Unique Strains (n)	1.89 (1.6-2.2)	0.00020408

Table 12, Continued.

Person CP of Other Strains in Household	0.8 (0.7-0.9)	0.00204082
Strain Person CP	39.71 (10.1-180.1)	0.00020408
Wound Strain Person Persistence		
Dwelling ownership	0.52 (0.2-1.5)	0.238776
People Per Bedroom	0.53 (0.2-1.4)	0.194694
Decolonizations, Household (%)	2.15 (0.3-25.1)	0.479592
Had SSTI (%)	1.77 (1.1-3.0)	0.0163265

Table 13: Influence of memory for staphylococcal colonization measured in an AR3 model

Phenotype	**AR1 OR (95%*CrI*)**	**AR2 OR (95%*CrI*)**	**AR3 OR (95%*CrI*)**
S. aureus Colonization	2.58 (2.1-3.1) (+)	2.06 (1.7-2.6) (+)	1.36 (1.1-1.6) (**)
MSSA Colonization	2.79 (2.2-3.6) (+)	2.42 (1.8-3.2) (+)	1.56 (1.2-2.1) (**)
MRSA Colonization	3.11 (2.3-4.1) (+)	2.15 (1.6-2.9) (+)	1.58 (1.2-2.1) (**)
Nares Colonization	2.74 (2.2-3.4) (+)	1.81 (1.4-2.3) (+)	1.54 (1.2-1.9) (+)
Groin Colonization	2.13 (1.6-2.8) (+)	1.87 (1.4-2.5) (+)	1.66 (1.2-2.2) (***)
Axilla Colonization	1.46 (0.9-2.3) (NS)	1.72 (1.1-2.7) (*)	2.07 (1.3-3.2) (***)

NS indicates the covariate pMCMC > 0.05. * pMCMC <0.05, ** <0.005, *** <0.005, and + < 6.00E-5.

Table 14: Summary of heritability and preventability estimates for *S. aureus* SSTI across datasets

Phenotype	Heritability (*G*) (95% *CrI*)	Sibling Environment (*S*) (95% *CrI*)	Couple Environment (*C*) (95% *CrI*)	Household Environment (*H*) (95% *CI*)
SSTI (Households)	0.422 (0.12 - 0.67)	0.089 (0.0 - 0.32)	0.332 (0.08 - 0.56)	---
S. aureus Infection (*Truven*)	0.472 (0.42-0.52)	0.039 (3.0E-6 - 0.08)	0.304 (0.27-0.34)	---

Table 15: Summary of Statistical Methods for Colonization and SSTI Models

Model Name	Data	Model Family	Link	Prior	Variance Components Considered	Fixed Effects Considered (*m*)
Burden Model: Colonization	St. Louis Families	Ordinal	Probit	χ^2, 1 D.F.	*G*, *C*, *S*, *H*	Sex, Age (4-level factor), Index Patient (T/F)
Fixed Probability Model: Colonization	St. Louis Families	Binomial	Logit	Inverse Wischart	*G*, *C*, *S*, *H*	Sex, Age (4-level factor), Index Patient (T/F)
Variable Probability Model: Colonization	St. Louis Families	Threshold	Probit	χ^2, 1 D.F.	*G*, *C*, *S*, *H*, *I*	Sex, Age (4-level factor), Index Patient (T/F), Season (4)
Autoregressive Model: Colonization	St. Louis Families	Threshold	Probit	χ^2, 1 D.F.	*G*, *C*, *S*, *H*	Sex, Age (4-level factor), Index Patient (T/F), AR1, AR2, AR3 colonization
SSTI Model	St. Louis Families	Threshold	Probit	χ^2, 1 D.F.	*G*, *C*, *S*, *H*	Sex, Age (4-level factor), Index Patient (T/F)
S. aureus Infection Model	*Truven* Marketscan	Threshold	Probit	χ^2, 1 D.F.	*G*, *C*, *S*, *H*	Sex, Age (Factor)