## CLINICAL RESEARCH ARTICLE

# Comprehensive modeling reveals proximity, seasonality, and hygiene practices as key determinants of MRSA colonization in exposed households

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**BACKGROUND:** *Staphylococcus aureus* is the leading cause of skin and soft tissue infections (SSTIs). To develop interventions to prevent recurrent infections, household attributes and individual practices influencing *S. aureus* colonization must be discerned.
**METHODS:** Households of healthy children with methicillin-resistant *S. aureus* (MRSA) SSTI ($n = 150$; 671 participants) were interviewed regarding health history, activities, and hygiene practices. *S. aureus* colonization was assessed in household members, and recovered isolates were typed by repetitive sequence-based PCR.
**RESULTS:** The number of unique strain types in a household (median 1, range 0–7) correlated with the number of colonized individuals ($p < 0.001$). The MRSA infecting strain type colonized a household member in 57% of 91 households with an available infecting strain, and was the most common strain type recovered in 45% of these households. In multivariable models, household MRSA colonization burden ($p < 0.001$), sharing a bedroom with MRSA-colonized individuals ($p = 0.03$), renting dwelling ($p = 0.048$), and warmer seasons ($p = 0.02$) were associated with increased MRSA colonization. Increasing age ($p = 0.02$), bathing at least daily ($p = 0.01$), and antibacterial soap use ($p = 0.03$) correlated with reduced MRSA colonization.
**CONCLUSIONS:** This study identified practices that correlate with MRSA colonization, which will inform physician counseling and multifaceted interventions among MRSA-affected households to mitigate MRSA in the community.

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## 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.[1–5] The high incidence of recurrent skin infections,[6] as well as increasing antibiotic resistance,[7] demands novel strategies to curtail the epidemic.

Asymptomatic human carriage is a critical reservoir for *S. aureus* infection.[4,8,9] Overall, half of all individuals are colonized at least intermittently with *S. aureus*.[10–12] Multiple members of the same household frequently experience MRSA infections, and a high proportion of household contacts of patients with MRSA infection are colonized.[13–16] 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"[17] such as mupirocin topical antibiotic or body washes with chlorhexidine or dilute bleach water), have yielded modest success.[6,18] To date, factors associated with MRSA SSTI and colonization have included prior skin infections, antibiotic history, and the burden of colonization among household members.[13,15,16,19,20] 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.

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## METHODS

### Participants

Index patients with a community-associated (CA)-MRSA infection (149 SSTI, 1 bacteremia/myositis/septic pulmonary emboli) were recruited from pediatric practices affiliated with the Washington University Pediatric and Adolescent Ambulatory Research Consortium, St. Louis Children's Hospital, and Cardinal Glennon Children's Hospital. Exclusion criteria included healthcare-associated infection, inability to confirm MRSA culture from SSTI, duration of >100 days from infection to enrollment, and home distance >80 miles from medical center. Of 247 eligible patients, 150 index patients and their household contacts (individuals sleeping at the household ≥4 nights/week) were enrolled in the HOME: Household Observation of MRSA in the Environment study. Enrollment visits were conducted between January 2012 and October 2015 within 20 days (median; range 3–95) of acute infection. The Washington University Human Research Protection Office approved study methods and written, informed consent was obtained for each participant.

### Data collection

At enrollment, the research team visited participants' homes to conduct an interview and assess the colonization status of household members. The interview consisted of questions concerning health history, S. aureus and other skin infection history, demographics, activities outside of the house, personal and environmental hygiene practices, daily proximity to other household members, and sharing of personal hygiene items. Study personnel assigned each home a cleanliness score accounting for odor, clutter, and grime, ranging from 1 to 4 (above average, average, below average, very dirty), modified from the established Environmental Cleanliness and Clutter Scale[21] to account for participant bias. To measure S. aureus colonization, swabs (Eswab, Becton Dickinson, Franklin Lakes, NJ) were collected[6] from the anterior nares, axillae, and inguinal folds of all household members.

### Microbiological methods

S. aureus was detected using broth-enrichment culture-based methods; identification and antibiotic susceptibility testing were conducted in accordance with established techniques.[22,23] All S. aureus colonizing isolates ($n = 389$) and the index patients' MRSA infection isolates prompting enrollment ($n = 150$) were subjected to antibiotic susceptibility testing. Ninety-one infection isolates were available for molecular typing. Thus, 480 isolates were subjected to staphylococcal cassette chromosome mec (SCCmec) typing using multiplex PCR.[24] Strain-level uniqueness was assessed through repetitive sequence-based PCR (repPCR).[25,26] Strain relatedness was assigned within each household; strains with ≥95% similarity were considered identical (Supplementary Figure S1).

### Statistical summaries

Colonization proportions across individuals and households were compared using Fisher's exact test in R.[27] The number of unique strains between observed groups (e.g., frequent vs. infrequent bathers) was calculated with the nonparametric Mann–Whitney U and median Kruskal–Wallis tests for two and more than two groups, respectively. Correlation between variables was quantified via Spearman's ρ in R. For univariate analyses, categorical and continuous covariates were tested for significance through Fisher's exact test and Kruskal–Wallis one-way ANOVA, respectively. Multiple test correction was performed using the Bonferroni method. Figures were developed through Python using the package 'Seaborn'.[28]

Anatomical site colonization pressure (ACP) within each household was calculated via the following, adapted from:[20]

$$ACP \equiv \frac{(\text{number of anatomical sites colonized})}{(\text{number of sampled household members}) \times (\text{number of anatomical sites})}$$

Three anatomical sites were sampled per individual (axillae, nares, and inguinal folds). Household strain richness was also measured as the number of unique strain types by repPCR present among all household members across these sites. 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.

### Models to identify factors associated with colonization, strain concordance, and infecting strain prevalence

To explore factors associated with various colonization phenotypes, the following multivariable models were constructed: "Colonization Pressure" to define the burden of S. aureus in households, "Colonization Proximity" to determine how relative proximity to colonized individuals modulates colonization risk, "Colonization Activity" to understand how hygiene behaviors influence colonization, "Colonization Concordance" to observe how proximity leads to pairs of individuals sharing a specific S. aureus strain, and "Infecting Strain Prevalence" to measure which factors led to the prevalence of the strain isolated from the index patient's infection prompting study enrollment (infecting strain). All colonization models were constructed as generalized linear regression models and fit using the R libraries "lme4" and "MCMCglmm." See Supplementary Methods for detailed descriptions of each colonization model, model selection, and model references. See Supplementary Tables S1–S4 for the specific list of covariates used in model selection for each model type. See Supplementary R Code for Model Selection and Parameter Estimation for further details.

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 vs. household behaviors may modify S. aureus colonization. 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':[29]

$$p_H^2 \equiv \frac{V_H}{V_T}$$

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

## 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 to 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).

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**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 | | | |
|   *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) | |
| 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) | |

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**Table 1** continued

| Individual characteristics | All participants, N = 671 (%) | Index patient, N = 150 (%) | Household contacts, N = 521 (%) |
|---|---|---|---|
| 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%) | |

Note: *S. aureus* encompasses both MSSA and MRSA.

*SSTI* skin and soft tissue infection, *MRSA* methicillin-resistant *Staphylococcus aureus*, *MSSA* methicillin-susceptible *Staphylococcus aureus*, *SLCH* Saint Louis Children's Hospital

[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 US 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 2500 and 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 folds for each individual

### 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, Supplementary Table S5). Over half (57%, 124/218) of children 6 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, Table 1). 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 (Supplementary Table S6). At the household level, 125 (83%) and 89 (59%) households had at least one household member colonized with *S. aureus* and MRSA, respectively (Supplementary Table S7).

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 US epidemic CA-MRSA (Supplementary Table S8). 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 I 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, and ceftaroline, while 93%, 15%, and 46% 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 (Supplementary Table S9). 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 $\rho = 0.35$, $p < 0.001$), and was even more significant when considering only colonized individuals (Spearman's $\rho = 0.77$, $p < 0.001$).

### 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 (Fig. 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-Poisson 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 (Supplementary Table S10). Seasonality (as measured by monthly low temperature at time of enrollment)[30], 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 vs. individual behaviors upon individual colonization, three models measuring "household preventability" (proportion of variance explained through a household-level random effect) were constructed.[29] 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; see Supplementary Table S11 for all measured covariates). 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 (≥1× per 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 (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,
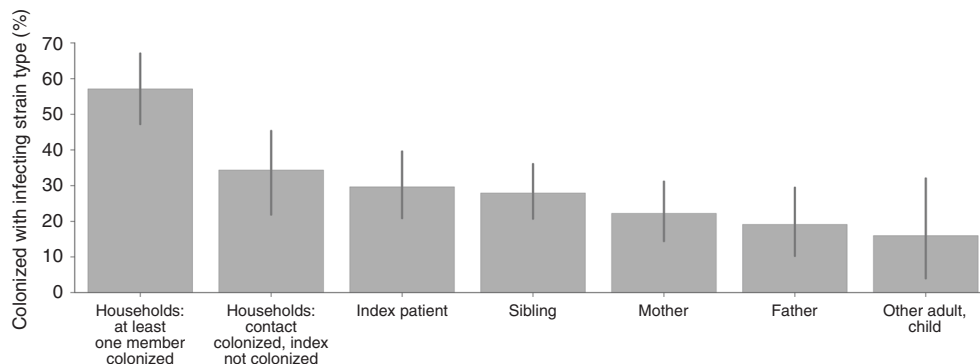


**Fig. 1** Occurrence of colonization with a strain concordant with the index patient infecting strain across households. Error bars indicate 95% confidence interval via the Clopper Pearson method. All pairwise comparisons between groups were not significant by Pearson's $\chi^2$ test

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

| Colonization prevalence | S. aureus colonization | | | MRSA colonization | | |
|---|---|---|---|---|---|---|
| | 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 ≥1× per day | – | – | – | 315 (62) | 76 (47) | 0.6 (0.4–0.8)[*] |

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 2 × 2 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. The list of all univariate tests performed as well as their significance can be found in Supplementary Table S11
[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

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)[30] 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 (Supplementary Table S12). 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 (Supplementary Table S13; see Supplementary Table S3 for covariates used). Strain concordance between immediate family members was significantly higher than pairs of unrelated individuals (OR

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

| | S. aureus colonization OR (95% CrI) | MRSA colonization 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 ≥1× per 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 ≥1× per day | – | 0.7 (0.5–0.9)* |

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: $p \geq 0.05$ 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 Supplementary 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[30]
[d]The index patient is the household member with the MRSA infection, in comparison to a household contact



**Fig. 2** Colonization concordance between immediate family members. Concordance defined as two individuals colonized with ≥1 of the same S. aureus strains by repPCR. Listed is percentage concordance (number of concordant pairs over total observed pairs). Overall concordance across all family members was 15% (156/1074). Symbols: (M)ale parent, (F)emale parent, (S)ibling, (I)ndex patient. Sex of index patients and siblings could be either male or female. Parents and siblings could be biologically related or step-parents/step-siblings

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 vs. interaction with common fomites contributes to such risk. Previously, S. aureus colonization pressure was shown to be associated with increased risk for colonization,[20] 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 United States, higher relative humidity, but not temperature, was significantly correlated with MRSA colonization of ICU patients.[31] However, two other studies

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%
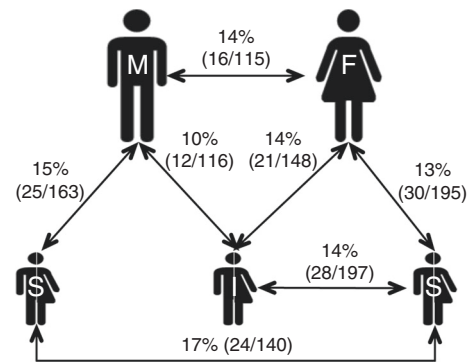
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evaluating the impact of meteorological factors upon MRSA SSTIs in outpatients in India and the southwest US found significant correlation between higher temperature and incidence of MRSA SSTIs.[32,33] Rather than climate directly increasing incidence 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).[13] 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.[16] 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.[34] 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 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[35]) 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.[36] 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.[37] 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.

## DISCLAIMER

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Agency for Healthcare Research and Quality.

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## ADDITIONAL INFORMATION

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