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Staphylococcus aureus Colonization in Healthy Children during the First Year of the Severe Acute Respiratory Syndrome Coronavirus 2 Pandemic

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The early severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic was temporally associated with a reduction in many childhood infections, although the impact on bacterial colonization is unknown. We longitudinally assessed *Staphylococcus aureus* colonization prior to and through the first year of the pandemic. We observed a decline in methicillin-resistant *Staphylococcus aureus* colonization associated with SARS-CoV-2 prevention mandates. *(J Pediatr 2022;249:101-5).*

A number of investigators have noted a decline in common childhood respiratory infections as well as invasive streptococcal disease temporally associated with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic.[1-3] It is believed that interventions meant to limit transmission of SARS-CoV-2, such as physical distancing, mandatory masking, and school closures, were unintentionally responsible for these reductions in other pediatric infections.

Staphylococcus aureus is a common colonizer of the skin and mucus membranes in children, with the potential to cause serious disease.[4,5] It is conceivable that increased vigilance applied to handwashing, avoiding face touching, and limiting large group settings could reduce rates of asymptomatic transmission of *S aureus*. This is important, given that asymptomatic colonization is frequently a preceding step in invasive *S aureus* disease, as well as skin-and-soft-tissue infection (SSTI).

Methods

This study is part of an ongoing prospective longitudinal examination of *S aureus* colonization in healthy children compared with those with malignancy. Owing to the current public health impact of SARS-CoV-2 and its potential relationship with other pathogens, we present early outcomes from the healthy child cohort.

Between November 2019 and February 2020, healthy children from age 1 month to <18 years were recruited from 2 Houston area primary care practices affiliated with Texas Children's Hospital: clinic A, an urban practice, and clinic B, a suburban practice. Subjects with an active *S aureus* infection, a major medical comorbidity, ongoing use of mupirocin or chlorhexidine gluconate products, or a guardian unable to read/write English or Spanish were excluded. Only 1 child per household was eligible. Following informed consent procedures, subjects had anterior nares and axillary cultures obtained and completed a questionnaire (**Appendix**; available at www.jpeds.com). Additional questionnaires were administered and colonization cultures were collected every 3 months (±30 days) for a 12-month period. At the time of enrollment, guardians were instructed in culture collection.[6] Follow-up questionnaires, culture collection supplies, and written instructions were then shipped to the subjects, and questionnaires and culture swabs were returned promptly to study personnel. Enrolled subjects completed follow-up by March 31, 2021. Colonization culture swabs were inoculated into tryptic soy broth with 6.5% sodium chloride, followed by incubation on blood agar as described previously[7]; *S aureus* was identified using standard microbiologic techniques. Methicillin resistance was assessed by cefoxitin disc diffusion.[8] Methicillin-resistant *S aureus* (MRSA) isolates were characterized by pulsed field gel electrophoresis. This study was approved by the Institutional Review Board of Baylor College of Medicine.

Beginning on March 15, 2020, Houston area public schools (K-12) were closed to in-person activities. In Harris County, beginning on March 24, 2020, nonessential businesses were temporarily closed and/or had restricted access, residents were required to wear a face mask in public, and public gatherings were cancelled. Temporary restrictions on nonessential research activities were then enacted by our institution, and follow-up culture collections were not resumed until June 9, 2020. Subjects were queried on their compliance with local infection prevention mandates and

MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SSTI	Skin-and-soft-tissue infection

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behaviors at the 6-, 9- and 12-month follow-up encounters (**Appendix**). Beginning in August 2020, public schools were reopened with options for in-person and/or virtual learning at the discretion of individual school districts.

The frequency of *S aureus* colonization was compared at each observation period and presented as the proportion of subjects colonized with *S aureus* at either body site. The proportion of subjects colonized was compared with an interest in the temporal relationship between local infection prevention mandates and total *S aureus*/MRSA colonization. Categorical variables were compared between subjects using the Fisher exact test, and continuous variables were compared using the Wilcoxon rank-sum test or Kruskal-Wallis test.

Results

A total of 168 subjects were enrolled, with a median age of 6.1 years (IQR, 2.2-10.3 years) (**Table**). Three-quarters (75.6%) of the subjects provided ≥3 follow-up samples. Subjects recruited from clinic A were primarily African American (61.4% at clinic A vs 18.8% at clinic B; $P < .001$) and more often had public insurance (eg, Medicaid; 74.7% at clinic A vs 17.6% at clinic B; $P < .001$). There were no other differences in baseline characteristics between the clinics.

At time of enrollment, 25.6% of subjects had *S aureus* colonization, including 5.4% with MRSA colonization (2.9% with USA300 MRSA). Subjects with MRSA colonization were older than those without colonization (median age, 10.4 years [IQR, 4.1-13.1 years] vs 5.7 years [IQR, 1.8-9.5 years] for methicillin-susceptible *S aureus* [MSSA] and 5.9 years [IQR, 2.3-10.3 years] for no colonization; $P = .09$). There were no additional differences in enrollment demographics or baseline characteristics between subjects with and those without *S aureus*/MRSA colonization at baseline.

Throughout the course of the study, 201 *S aureus* isolates were recovered, including 17 MRSA isolates (8.5%) and 13 clindamycin-resistant isolates (6.5%). The MRSA isolates included 9 USA300 strains (52.9%). Eighty-six subjects (51.2%) were colonized during at least 1 encounter; 83 (49.4%) had nasal colonization and 16 (9.5%) had axillary colonization at least once. Fourteen subjects (8.3%) were colonized at least once with MRSA. Six subjects (3.6%) were colonized with clindamycin-resistant *S aureus*.

Rates of total *S aureus* colonization at any given time during the study period varied from 25.6% to 38.1%, without displaying any clear trend ($P = .8$); a nonsignificant decline in total *S aureus* colonization occurred between the 3- and 6-month encounters ($P = .1$) followed by an increase at the 9-month follow-up period ($P = .04$) (**Figure 1**, A). By contrast, MRSA colonization specifically was highest at the time of enrollment and the 3-month follow-up and declined thereafter during the postmandate period ($P = .04$); there were no statistically significant changes in USA300 MRSA colonization (**Figure 1**, B). There was a relative increase in MSSA colonization in the postmandate period ($P = .005$) (**Figure 1**, C). Two subjects were newly colonized with MRSA at the 3-month follow-up (2 of 37; 5.8%) and 3 additional subjects had new MRSA colonization at the 6- to d 12-month follow-ups (3 of 148; 2.1%). All 3 subjects with new MRSA colonization at the 6- to 12-month follow-up had a USA300 strain, and USA300 was detected in multiple samples in 2 of these subjects. Of the 3 subjects with new MRSA colonization in the postmandate period, 1 had MSSA colonization at baseline and 2 did not have colonization. Only 1 subject with MRSA colonization at baseline had persistent MRSA colonization at any of the 6- to 12-month follow-up encounters (1 of 9; 11%) (**Figure 2**; available at www.jpeds.com). All subjects with USA300 MRSA colonization at the enrollment and/or 3-month assessment lost colonization in the postmandate period. Among subjects with baseline MSSA colonization, 58.3% retained MSSA colonization in the postmandate period. There were no significant trends with respect to the site of colonization or clindamycin-resistant *S aureus* colonization.

Eight subjects reported not engaging in any form of SARS-CoV-2 mitigation measures (4.7%). These subjects exhibited higher rates of total *S aureus* colonization at the 9- and 12-month follow-up evaluations ($P < .05$) (**Figure 1**, D). Four of these 8 subjects had new *S aureus* colonization at the 9- and 12-month evaluations, compared with 23 of 125 subjects who contributed 9- and 12-month samples and were participating in prevention ($P = .05$). MRSA colonization was present in 1 of the 8 subjects without mitigation measures (12.5%) at both the 9- and 12-month evaluations, compared with 2 of 125 subjects with mitigation (1.6%) at the 9-month follow-up and 2 of 121 (1.7%) at the 12-month follow-up. *S aureus* colonization occurred at baseline and at least once during the postmandate period (6-12 months) in 2 of 8 subjects without mitigation (25%), compared with 21 of 130 subjects with mitigation (16.1%; $P = .62$). There were no significant differences in baseline characteristics, SSTI history, or colonization rates at enrollment or at 3 months between subjects with and without colonization at 6-12 months.

Discussion

Previous investigators have noted a decline in the frequency of common pediatric illnesses during the first year of the SARS-CoV-2 pandemic.[1,3,9] The potential direct and indirect impacts of SARS-CoV-2 on bacterial colonization in children are largely unknown. This report provides data on *S aureus* colonization in healthy children during the SARS-CoV-2 pandemic. The strengths of this study include the timing of longitudinal data collection immediately before and through the first year of the pandemic and the fact that cultures were obtained in community-dwelling children rather than through hospital-based surveillance.

Multiple investigators have described the epidemiology of pediatric *S aureus* colonization. At any given time, 27%-36% of children are colonized with *S aureus*, with 60%-84%

Table. Baseline characteristics of enrolled subjects and colonization status at enrollment

Characteristics	All subjects (N = 168)	Colonization status at enrollment			*P* value
		MRSA colonization at enrollment (N = 9)	MSSA colonization at enrollment (N = 34)	No *S aureus* colonization at enrollment (N = 125)	
Age, y, median (IQR)	6 (2.2-10.3)	10.4 (4.1-13.1)	5.7 (1.8-9.5)	5.9 (2.3-10.3)	.09
Female sex, n (%)	81 (48.2)	4 (44.4)	18 (52.9)	60 (48)	.95
Race, n (%)					.68
White	86 (52.2)	5 (55.5)	16 (47.1)	65 (52)	
African American	67 (39.9)	4 (44.4)	12 (35.3)	51 (40.8)	
Asian	11 (6.5)	0	4 (11.8)	7 (5.6)	
Native American	2 (1.2)	0	1 (2.9)	1 (0.8)	
Pacific Islander	1 (0.6)	0	1 (2.9)	0	
Other race	12 (7.1)	0	2 (5.9)	10 (8)	
Race not disclosed	2 (1.2)	0	0	2 (1.6)	
Hispanic ethnicity, n (%)	52 (30.9)	4 (44.4)	9 (23.5)	40 (32)	.53
Insurance status, n (%)					.57
Private	82 (48.8)	5 (55.5)	20 (58.8)	57 (45.6)	
Public	77 (45.8)	4 (44.4)	12 (35.3)	61 (48.8)	
Both private and public	3 (1.8)	0	1 (2.9)	2 (1.6)	
Uninsured	5 (2.9)	0	1 (2.9)	4 (3.2)	
Number of people in household, median (IQR)	4 (3-5)	5 (4-5)	4.5 (4-5)	4 (3-5)	.51
Personal history of SSTI in the preceding 90 d, n (%)	9 (5.4)	0	3 (8.8)	6 (4.8)	.61
History of SSTI in household members in preceding 90 d, n (%)	6 (3.6)	0	0	6 (4.8)	.49
Personal history of known *S aureus* infection, n (%)	4 (2.4)	0	1 (2.9)	3 (2.4)	1
Site of enrollment, n (%)					.26
Clinic A	83 (49.4)	2 (22.2)	15 (44.1)	66 (52.8)	
Clinic B	85 (50.6)	7 (77.8)	19 (55.9)	59 (47.2)	

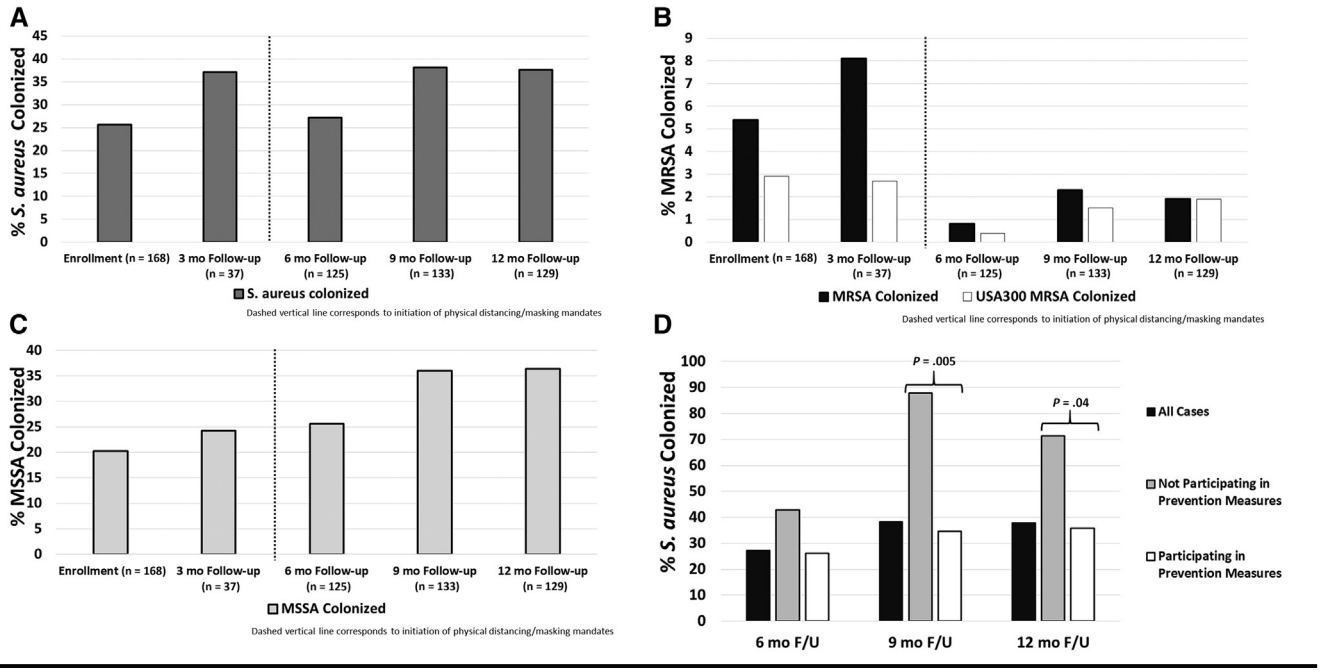

Figure 1. Colonization trends and relationship to SARS-CoV-2 prevention mandates. **A,** Proportion of subjects colonized with *S aureus* at any body site at each time point of observation. **B,** Proportion of subjects colonized with MRSA at any body site at each time point of observation. The dashed vertical line corresponds to the initiation of local SARS-CoV-2 prevention mandates. There was a statistically significant difference in MRSA colonization in the prepandemic and pandemic periods ($P = .04$). **C,** Proportion of subjects colonized with MSSA at any body site at each time point of observation. There was a statistically significant increase in MSSA colonization in the prepandemic and pandemic periods ($P = .005$). **D,** Relationship between participation in prevention mandates and *S aureus* colonization.

colonized at least transiently over the course of a year.[4,5,10] Our findings regarding total *S aureus* colonization are consistent with such previous data, and moreover, overall *S aureus* colonization remained relatively stable during the study period. The rates of MRSA colonization at enrollment and the 3-month follow-up in our study were higher than those in most of the published literature. In studies from the 2000s to the early 2010s, rates of MRSA colonization in healthy US children were typically in the range of 1%-5%, although frequency has been as high as 9%.[4,5,10,11] Of note, however, we observed a precipitous decline in frequency of MRSA colonization following the institution of SARS-CoV-2 prevention mandates, with a relative increase in MSSA.

The reasons for the temporal association between the SARS-CoV-2 outbreak and the consequent institution of prevention measures and a reduction in MRSA colonization are unclear. It could be hypothesized that mask wearing resulted in less touching of the face/nose, and that this, combined with increased vigilance surrounding handwashing and environmental cleaning, may have impacted MRSA colonization. The interactions between the hands, nose/mouth/face, and the environment are important in the transmission of *S aureus*. Reagan et al randomized adult subjects to receive intranasal mupirocin or placebo and ultimately found reduced rates of both nasal and hand *S aureus* colonization in the mupirocin group.[12] Likewise, aggressive handwashing has been associated with a reduction in new *S aureus* colonization events within households.[13] Our findings of a higher rate of overall and new colonization among subjects who did not participate in infection prevention measures support such a mechanism. Previous studies suggest that viral respiratory infections, such as influenza, impact the constituents of the nasal microbiome, including *S aureus*.[14] In studies of adults with nasal MRSA colonization, transmission was increased following experimental infection with rhinovirus; this effect was then partially ameliorated through masking.[15] Thus, it also is conceivable that the reduction in common respiratory viral illnesses that occurred in children during the first year of the pandemic[3,9] may have impacted new MRSA colonization or transmission events. The reasons for a reduction in specifically MRSA colonization, as opposed to all *S aureus*, are unclear. Other investigators have reported that MRSA colonization in healthy children may be more transient than MSSA colonization.[4] Moreover, the introduction of asymptomatic carriage into households with children may occur more often with MSSA than with MRSA.[13] This, combined with the increased proximity of household members during the early pandemic, might have contributed to our findings regarding MSSA colonization. The slight decline in total colonization that we observed at 6 months (stemming from decreases in MRSA), followed by a rebound in total colonization driven by MSSA, support these hypotheses. Conversely, given that the children with MRSA colonization at enrollment were older, it is conceivable that infection prevention measures (eg, masking, handwashing, distancing) might have been implemented more easily in this age group, contributing to the observed reduction in MRSA.

Some limitations to this work should be acknowledged. The results are from a single region and might not be generalizable. In addition, although this is a prospective cohort, the number of subjects in certain secondary analyses is relatively small and varied over time. The limited prepandemic data raise questions regarding the potential impact of seasonal variation; however, prior work demonstrated higher rates of *S aureus*/MRSA colonization and infection in late spring to early fall,[16] corresponding to the 6- and 9-month follow-up periods. Thus, the observed decline is contrary to the expected seasonal variation. Subjects also self-collected culture swabs, which conceivably could have affected organism recovery. Notably, previous studies have shown a high level of concordance between patient-collected and physician-collected colonization cultures.[6] In addition, a significant impact of collection technique would be expected to impact MSSA and MRSA equally. The study relied on the completion of questionnaires by subjects/guardians, which introduces the potential for recall bias, particularly with respect to preceding SSTI. Finally, our study provides a temporal association and a causal relationship cannot be inferred, strictly speaking. A number of investigators have reported declines in the incidence of MRSA SSTI and invasive disease in US children in the past decade following a peak in the mid-late 2000s[17,18]; however, longitudinal data are limited regarding trends in MRSA colonization in community-dwelling children in recent years. Thus, it is unclear whether the changing incidence of infection would have necessarily impacted our colonization data. Moreover, given that the MRSA colonization rates at enrollment and the 3-month follow-up in our study are comparable with those reported in the literature from the height of the community-acquired MRSA epidemic,[4,5] the impact of recent trends in invasive disease on our findings is likely minimal. Furthermore, a recent publication from our center found stable incidence rates of invasive community-acquired *S aureus*/MRSA infection during 2020 relative to the years 2017-2019.[2]

In summary, overall *S aureus* colonization in healthy children remained relatively constant in the pre- and post-SARS-CoV-2 pandemic periods; however, a temporal association with the SARS-CoV-2 pandemic and reduced MRSA colonization was observed. These findings highlight the potential impacts of aggressive infection control practices on community MRSA colonization. ■

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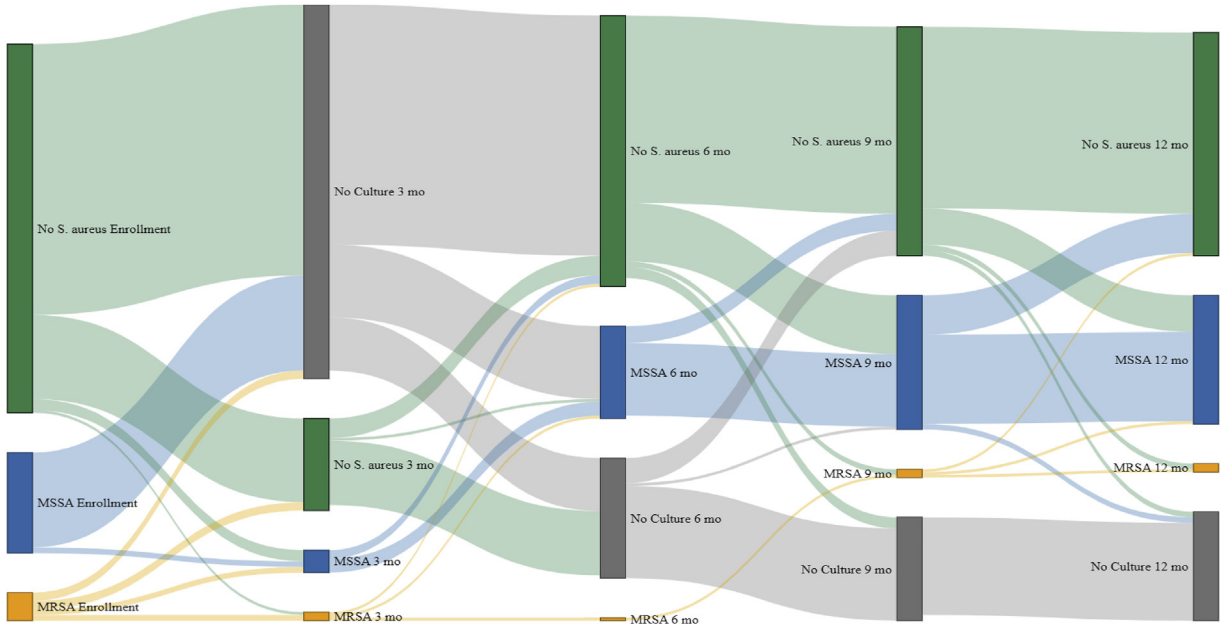

Figure 2. Sankey diagram of colonization dynamics showing shifts in colonization status by observation period.