Decolonization of the oropharynx, an important and neglected reservoir of S. aureus colonization

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Scientific Abstract (Do not exceed one page)

Skin and soft tissue infections (SSTIs) are an extremely common reason for children to seek care. Four in 100 children will experience a medically attended SSTI annually, resulting in over 2 million visits to doctors' offices and emergency departments annually among children in the U.S. Among the general population, children are disproportionately affected by CA-*S. aureus* SSTIs. SSTIs can lead to severe disease that can cause hospitalization, bacteremia, disseminated disease, critical illness, and death.

The most commonly identified cause of SSTIs is *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA). *S. aureus'* success as a bacterial infection is related to its ability to asymptomatically colonize patients and later cause disease. Another aspect of its success is its ability to cause infection in both immunocompromised and non-immunocompromised hosts. Most SSTIs in children occur in otherwise healthy children. Of great concern is that, after an initial *S. aureus* SSTI, recurrence rates of *S. aureus* skin infections can exceed 50%.

Because of the large burden of *S. aureus* SSTIs in the community, there is a large interest in SSTI prevention. However, clinical trials of *S. aureus* SSTI prevention have been very disappointing. Fritz et al. found that skin decolonization with chlorhexidine (CHG) of affected children and their household members has been found superior to decolonizing just the affected child. However, the magnitude of decrease was minimal and 52% of children in the more aggressive decolonization regimen experienced a SSTI during the 12 month follow up period. Another recent clinical trial by Kaplan et al. randomized children with CA-*S. aureus* infections to bleach baths versus routine hygienic measures. Unfortunately, the skin decolonization afforded by bleach baths did not decrease the recurrent SSTI rate. Clearly, more effective means of decolonization are needed to meaningfully decrease rates of recurrent infections.

Traditionally eradication efforts of *S. aureus* colonization have focused on removing colonization in the anterior nares (nose) and skin. A recent investigation by our group found that among persons with an *S. aureus* SSTI and their household members, the most common site of colonization of *S. aureus* on the human body is not the anterior nares, but the oropharynx (26% vs. 24%), with overlap of colonization far from universal. In fact, 50% of oropharyngeally colonized persons were not nasally colonized. Other investigations show a similar magnitude of *S. aureus* orpharyngeal colonization. There are mounting data that decolonization regimens that ignore the pharynx are not effective at preventing recurrent *S. aureus* SSTIs. However, prospective data on oropharyngeal decolonization of *S. aureus* in children are lacking.

We hypothesize that suboptimal *S. aureus* prevention efforts stem from previous inattention to eradication of *S. aureus* oropharyngeal colonization. To this end, we will conduct a clinical trial on eradication of oropharyngeal *S. aureus* colonization. Data from our "proof of principle" study will form the foundation of more comprehensive efforts to prevent recurrent *S. aureus* infections in children.

We will perform a prospective, double blind, randomized controlled clinical trial of 0.12% chlorhexidine (CHG) oral rinse versus placebo oral rinse for children age 5-17 with *S. aureus* oropharyngeal colonization. We hypothesize that, compared to placebo, 0.12% CHG rinse will reduce oral *S. aureus* colonization. We choose CHG given its wide availability, excellent safety profile, and low cost. We will enroll 240 children who have a history of *S. aureus* infection or SSTI in the prior year. Consenting subjects will be screened for *S. aureus* oropharyngeal colonization using a rapid molecular test. Subjects will be eligible if they have *S. aureus* oropharyngeal colonization during a screening visit, are able to gargle, and can be followed for 28 days. CHG oral rinse will be used twice daily for 7 days total.

Our primary outcome is *S. aureus* eradication at 7 days (test of cure (TOC)). Secondary outcome is eradication at 1 month (long term follow up). Study staff will administer surveys to subjects and/or their parents about risk factors hypothesized to predict treatment success. Secondary outcomes include safety, tolerability, and adherence to oral CHG. We will also examine predictors of eradication, including pathogen level factors. Pathogen-level factors will be examined using *S. aureus* whole genome sequencing, which will allow us to quantify the presence or acquisition of the *qacA/B* gene, which is associated with CHG resistance. WGS will also allow us determine if stains associated with persistence after CHG use represent pre-existing colonizing strains or acquisition of new strains.

If CHG oral rinse is efficacious at eradication, our findings will form the foundation for a larger, more comprehensive intervention to prevent recurrent SSTIs in children. If CHG oral rinse cannot decolonize the oropharynx, then investigations may need to focus on other strategies to prevent *S. aureus* disease. Regardless of results, our trial will forward the field of prevention of this extremely common and potentially deadly infection.

Structured Abstract (Do not exceed one page)

Describe the medical problem as it relates to children

- Community-associated (CA) *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA), is the most commonly identified cause of skin and soft tissue infections (SSTIs). and affects children disproportionately.

State the incidence/prevalence of problem in children

- Four in 100 children in the U.S. will experience a SSTI annually, resulting in over one million infections annually, a problem further complicated in that over 50% of children who have a *S. aureus* skin infection will develop a recurrent infection.

Background that will lead to the research gap (*may use 2 sentences*)

- Our group found that, despite common belief, the most common site of colonization of *S. aureus* on the human body is not the anterior nares (nose), but the oropharynx. There are mounting data that decolonization regimens that do not eradicate oropharyngeal colonization are ineffective at preventing recurrent *S. aureus* SSTIs.

State the primary research gap this study will address

- Because trials on prevention of recurrent *S. aureus* infections have been disappointing, we will examine the efficacy of oropharyngeal *S. aureus* decolonization with 0.12% chlorhexidine gluconate (CHG) oral rinse, a widely available commercial product with an excellent safety profile.

Hypotheses

- Oral CHG rinse (0.12%) will be more effective than placebo at eradiating *S. aureus* oropharyngeal colonization after treatment completion.

Specific Aims

- To determine if CHG oral rinse can decolonize *S. aureus* oropharyngeal colonization better than placebo in children.

Study design

- Randomized, prospective, double-blind, placebo-controlled clinical trial of 0.12% CHG oral rinse vs. placebo oral rinse to decolonize the oropharynx among children.

Describe study population or sample material

- Subjects 5-17 years of age who have confirmed colonization of the oropharynx with *S. aureus* and who are able to gargle.

Sample size/power of primary endpoint

- The primary endpoint is eradication of *S. aureus* oropharyngeal colonization. To have 85% power to detect a 40% absolute difference in eradication between groups (10% for placebo and 50% for CHG), we will require 54 subjects total who complete the trial. We will need to screen potentially eligible children with rapid molecular tests for *S. aureus* oropharyngeal colonization to find eligible subjects. Estimating 25% of screened children will be eligible, and accounting for a 10% attrition rate, we will need to enroll 240 children for screening with the expectation that 60 will be eligible and participate and 54 will complete the clinical trial.

Assuming the project is successful, state the next step moving down the pathway to clinical applicability

- A randomized, prospective, multi-center, clinical trial of children of prevention of *S. aureus* skin infection that includes CHG oral rinses (in addition to body and nasal disinfectants) with a comparator group that will depend on standard of care at the time of development of the clinical trial protocol.

Hypothesis(es) and Aims (Do not exceed one page)

A. Hypothesis(es) to be Tested

Our primary hypothesis is 0.12% CHG oral rinse will be superior to placebo at eradicating *S. aureus* from the oropharynx of children age 5-17 colonized with *S. aureus* compared to placebo oral rinse.

We have several secondary hypotheses: a) decolonization of oropharyngeal *S. aureus* colonization with 0.12% CHG will be sustained at 28 days; b) recolonization of the oropharynx will come from isolates that colonize patients nose and not from development of antimicrobial resistance, including the emergence of CHG-resistant *S. aureus* strains that contain the *qac A/B* genes; and c) predictors of failure to decolonize with CHG will include poor treatment adherence, nasal *S. aureus* colonization, and younger age.

If our primary hypothesis is correct, findings will form the foundation for a larger, randomized, prospective, multi-center, placebo-controlled clinical trial comparing skin, nasal, and oropharyngeal decolonization vs. standard of care decolonization regimen for the prevention of recurrent *S. aureus* skin infections. If oral CHG rinse is unable to decolonize the oropharynx, then other more aggressive oropharyngeal *S. aureus* colonization eradication strategies in children will need to be tested.

B. Specific Aim(s) of the Project

1) Determine the efficacy of oropharyngeal decolonization among children with a history of CA-MRSA, CA-*S. aureus* or skin and soft tissue infection (SSTI). To determine if oropharyngeal *S. aureus* colonization can be eradicated, we will perform a randomized, double blind placebo-controlled controlled clinical trial comparing 0.12% oral chlorhexidine (CHG) oral rinse to placebo among children age 5-17 with *S. aureus* oropharyngeal colonization. We hypothesize that 0.12% CHG rinse will reduce oral *S. aureus* colonization better than placebo treatment. Our findings will provide "proof of principle" data on the ability of oral antiseptics to eradicate oropharyngeal *S. aureus* colonization in children.

2) Assess the safety, tolerability, and compliance of oropharyngeal decolonization among children and their caregivers. Using a structured instrument, we will survey trial participants and their parents/guardians on side effects, tolerability, and adherence of the 0.12% oral CHG rinse and placebo. Findings will serve as a counterpoint to any benefits derived from oral decolonization.

3) Determine the *S. aureus* genetic backgrounds associated with breakthrough oropharyngeal colonization. We will molecularly characterize all baseline *S. aureus* isolates and "breakthrough" (failure) isolates using whole genome sequencing (WGS) for key *S. aureus* molecular markers, including emergence of *S. aureus* isolates with the *qacA/B* gene, which is associated with CHG resistance.

Background and Significance (See General Instructions for page limitations/recommendations)

Skin and soft tissue infections (SSTIs) are an extremely common cause of visits to healthcare practitioners. Among U.S. children, there are over 2 million visits to doctors' office and emergency departments annually for SSTIs.[1] SSTIs can results in significant morbidity, hospitalization, bacteremia, and death. Children are disproportionately affected by *S. aureus* SSTIs in the U.S., with a relative risk of 1.51 [1.19-1.92] higher relative risk compared to adults.[2] Furthermore, racial and ethnic minorities are disproportionally affected by SSTIs,[3] and African-Americans have CA-MRSA rates over 2 times higher than Caucasians.[2] SSTI incidence in the U.S. is increasing and the economic cost of hospitalization for SSTIs in children is estimated to be $184 million annually in the U.S.[4]

In the first decade of the 21st Century, there has been a dramatic rise methicillin-resistant *S. aureus* (MRSA) incidence. Between 2000 and 2006, SSTI hospitalization incidence in children has risen from 23.2 cases per 100,000 children to 62.7 cases per 100,000 children.[4] When including outpatient visits, approximately four in 100 children will experience a SSTI annually.[5]

The most common identified cause of SSTIs is *Staphylococcus aureus*[6] and the majority of CA-MRSA skin infections in the U.S. are caused by *S. aureus* isolates from the USA300 genetic background. This strain is particularly problematic as it is associated with high rates of household transmission and recurrent disease.[7] Approximately 20-70% of children with a *S. aureus* SSTI will develop a recurrent infection in the following year.[8] And approximately 13% of household contacts of persons with CA-MRSA will experience a similar skin infection in the subsequent 6 months.[9] Because of these high recurrence rates, there is tremendous interest in interventions to prevent recurrent *S. aureus* SSTIs.

Even after systemic antibiotic therapy, *S. aureus* colonization persists in nearly one-half of patients.[10] Understanding persistent colonization may be important for identifying patients at higher risk for recurrence. In hospitalized patients, *S. aureus* colonization is clearly associated with higher risk of subsequent *S. aureus* infection.[11] In the ambulatory care setting, there are data suggesting a relationship between colonization and invasive disease, although the data are less robust.[12] In one military study, 38% (9/24) of recruits who were nasally MRSA colonized suffered a subsequent SSTI in the next 2 months compared to 3% (8/229) with MSSA colonization ($P < 0.001$).[13] In a study of ambulatory children, Fritz et al. found that 32% (7/22) of children who were nasally MRSA colonized suffered a subsequent SSTI in the next 12 months compared to 10% (14/142) with MSSA colonization and 9% (33/370) in those without baseline *S. aureus* colonization ($P=0.03$).[14] Additionally, patients with MSSA colonization commonly suffer from recurrent infection.[15] These data strongly suggest that MRSA and *S. aureus* colonization is a risk factor for subsequent infection in ambulatory patients.

Studies on prevention of recurrent infections have been disappointing. Most SSTI prevention efforts have focused on body decolonization of *S. aureus*. Typical decolonization regimens have involved combined nasal and skin decontamination. For example, Fritz et al. randomized children with SSTIs to either skin decolonization with chlorhexidine (CHG) and nasal decolonization with mupirocin of the affected child or CHG skin decolonization + mupirocin given to the whole household.[8] While decolonization the whole household was superior (54% SSTI recurrence rate vs. 72% during the 12 month follow up period), the trial had disappointing results in that even in the more aggressively treated group, recurrence rates of SSTIs in the affected children exceeded 52%. The results suggest that even skin and nasal decolonization is insufficient to prevent recurrent SSTIs in children. Another recent study by Kaplan et al. randomized children with SSTIs, most caused by CA-*S. aureus* infections to bleach baths versus routine daily hygienic measures.[16] Unfortunately, the skin decolonization afforded by bleach baths did not result in a decrease rate of recurrent SSTIs compared with routine daily hygienic measures (17% vs. 21%, $P=0.15$).[16] These findings suggest that skin decolonization is ineffective at prevention of recurrent *S. aureus*-associated SSTIs. Clearly, more effective means of decolonization are needed to further decrease rates of recurrent infections.

Data from our group and others have demonstrated that extra-nasal *S. aureus* colonization is much more common than previously believed.[17,18] We found that among household contacts of children and adults with *S. aureus* SSTIs, the most common body site of *S. aureus* colonization on the human body is not the anterior nares (nose) but the oropharynx.[18] In this study, conducted in Los Angeles and Chicago, 30% of household contacts were colonized with *S. aureus* in the oropharynx, but on 25% in the nares.

Among this subset of 268 household contacts of persons with CA-MRSA skin infection, 31% were oropharyngeally colonized with *S. aureus*, but on 23% in the nares. Oropharyngeal *S. aureus* colonization has been found in other populations with a prevalence similar or higher than nasal colonization.[17-19] However, to date, virtually all interventions to prevent *S. aureus* SSTI have ignored oropharyngeal decolonization. And there are, to our knowledge, no data on eradication of *S. aureus* oropharyngeal colonization in children. Given the very high prevalence of oropharyngeal *S. aureus* colonization, we believe that the oropharynx may be an important unaddressed sanctuary site for *S. aureus* that, when not targeted, may lead to post-eradication re-colonization and recurrent *S. aureus* disease.

Chlorhexidine gluconate (CHG) is an ideal treatment for eradication of *S. aureus* on the skin and the oropharynx. CHG has been safely used for bathing, showering and dental hygiene for over 50 years. CHG is available over-the-counter product as a 4% topical solution to be directly applied to the skin as an antimicrobial skin cleanser. CHG is associated with marked reductions in skin bacteria following serial CHG bathing or showering,[20-26] and it is widely used as a pre-operative showering agent based upon CDC guidelines that recommend its use.[27] CHG is also the gold standard in periodontal hygiene, including oral care in ventilated patients.[28,29] However, CHG is poorly studied as means to eradicate *S. aureus* colonization in patients, especially children, suffering from recurrent *S. aureus* infections.

Data on CHG oral rinse for eradication of oropharyngeal *S. aureus* colonization are extremely sparse. One recent investigation in adults examined CHG oral rinses in adults, most of who were elderly, with *S. aureus* infection or colonization after hospital discharge for *S. aureus*. In this investigation, oral CHG rinses eradicated over 50% of *S. aureus* oropharyngeal colonization compared to no oral CHG rinses (no placebo was used in this study).[19] No data on CHG oral rinses for *S. aureus* colonization are available in children and examination of recolonization after a course of oral CHG rinses are lacking.

One concern with antibiotic use is the emergence of antibiotic resistance. Decreased susceptibility to CHG in *S. aureus* has been recently associated with a plasmid-mediated biocide resistance gene *qac A/B*, which encodes an energy-dependent export system that pumps chlorhexidine from the bacterial cell.[30] Although the clinical significance is not fully elucidated with large studies, *qac A/B* has been identified in a small percent of *S. aureus* isolates in the US, Europe, and Asia and there is interest in correlating the presence of the *qac A/B* gene with clinical outcomes, such as failure to eradicate colonization.[30-34] In a recent investigation among adults and children with SSTI, 1% of adults and children with SSTIs harbor the *qac A/B* gene.[35] Another study of 281 MRSA isolates from a children's hospital, 18.5% harbored the *qac A/B* gene,[36] suggesting that prevalence of this resistance gene among *S. aureus* may be high in selected populations. There are no data about the emergence *qac A/B* containing *S. aureus* after exposure to CHG products. Because of the concern of emerging resistance in trials of antibiotic treatment[37] studies using CHG products for decolonization purposes should to perform careful monitoring for the development of resistance.[33,38,39]

In summary, *S. aureus* SSTIs are extremely common, often recur, and efforts to prevent recurrent infections have been extremely disappointing. Because decolonization efforts almost always ignore oropharyngeal *S. aureus* colonization, there is a need to quantify a safe, well-tolerated oral rinse that can eradicate *S. aureus* oropharyngeal colonization. This trial will be the first in children to test such an approach and form the foundation for further more comprehensive studies to prevent this common and potentially deadly infection.

Supportive Preliminary Data (See General Instructions for page limitations/recommendations)

Oropharyngeal colonization is common but its role as a predictor of recurrent SSTIs is unexplored

The body site of greatest *S. aureus* colonization has traditionally been believed to be the anterior nares.[40,41] *S. aureus* has been known to colonize the axilla, perineum, rectum, vagina, and the throat (oropharynx).[42-45] However, only recently have data regarding the importance and scale of non-nasal colonization been appreciated.[42-45] An investigation conducted by our group in an area of high MRSA endemicity found that among persons with *S. aureus* SSTIs cultured at the anterior nares, oropharynx, and inguinal region, *S. aureus* colonized 40% (137/350) and 50% (405/812) of their household contacts.[18] A nares-only survey would have missed 48% of *S. aureus* and 51% of MRSA colonized persons.[18] Among the subset of patients with CA-MRSA infection, the proportion of oropharyngeal-only colonization was 12%, suggesting nasal-only screening for *S. aureus* colonization misses a sizable proportion of colonized patients with community onset SSTI. In fact oropharyngeal colonization was more prevalent than nasal colonization (26.0% vs. 24.0%) in our cohort of >1000 persons.[18] Investigations of patients with acute skin infections and other populations at high risk for *S. aureus* infection by our group and others have demonstrated the prevalence of *S. aureus* oropharyngeal colonization are similar or exceed that of nasal colonization.[17,43,44,46-49]

The failure of nares plus body decolonization at decolonizing patients and preventing recurrent *S. aureus* infections is disappointing. However, as outlined above, major efforts to prevent infection have employed mupirocin plus CHG (or bleach).[8,16] Both nasal and body decolonization are well-established methods to decrease *S. aureus* in hospitalized patients.[50-53] However, data on the efficacy of oral CHG rinses to eliminate oropharyngeal *S. aureus* colonization are extremely limited. Those few data that exist are from adults, largely elderly adults;[19] thus the applicability of these limited data to children is unclear. **To truly perform studies of comprehensive *S. aureus* decolonization, oropharyngeal *S. aureus* colonization must be addressed therapeutically. However, there are few data on the efficacy of CHG at oropharyngeal *S. aureus* eradication and none in children**. If CHG is found to be effective in children, strategies that incorporate oropharyngeal decolonization as part of a more global eradication strategy may be critical for *S. aureus* SSTI prevention.

Few data exist on the efficacy of oropharyngeal decolonization of *S. aureus*

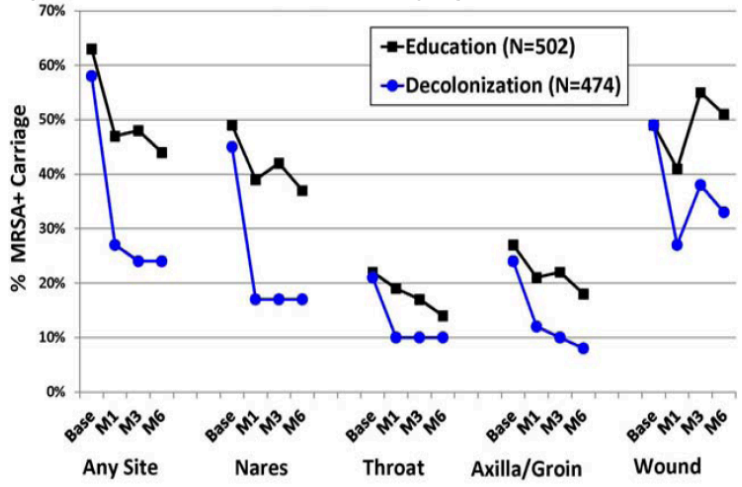
Virtually all decolonization efforts to prevent *S. aureus* infections have focused on nasal and body decolonization.[10] However, these efforts ignore *S. aureus* oropharyngeal colonization. In children with cystic fibrosis, a population at high risk for *S. aureus* infections, it has been noted that *S. aureus* persistence is more common in the oropharynx than the nares.[54] A similar observation of higher *S. aureus* persistence in the pharynx compared to the nares was seen in adults and staff in an orthopedic ward.[55] These data suggest that the oropharynx can be reservoir of *S. aureus* colonization.

Traditional methods to eradicate *S. aureus* colonization in the skin and nose do not appear to eradicate oropharyngeal colonization. In a Dutch study, two hospital staff found to be MRSA colonized were given traditional topical (skin) and nasal decolonization regimens. However, this traditional regimen, similar to regimens used for eradication of children with *S. aureus* infections, did not eradicate pharyngeal *S. aureus* colonization.[56] Only after systemic eradication with oral antibiotics was performed, were these persons' throats decolonized. The authors suggested that MRSA throat (oropharyngeal) colonization might be a reason why MRSA eradication fails in otherwise healthy persons.[56] Of note, systemic antibiotic use for *S. aureus* decolonization is limited by toxicity, tolerability, and collateral damage to healthy normal flora, which are disrupted by systemic antimicrobial therapy.[57,58] Another study looked at predictors of failure of MRSA decolonization. In their multivariate they found that oropharyngeal MRSA carriage was an independent risk factor for decolonization failure.[59] **These findings further reinforce that oropharyngeal *S. aureus* carriage is associated with failure of decolonization if just skin and nares are decolonization targets.**

Our group is one of the few to conduct studies of oropharyngeal decolonization in *S. aureus*. The trial, called Project CLEAR,[60] is an ongoing trial of decolonization of recently hospitalized adults with *S. aureus* infection and/or colonization. In this ongoing clinical trial of over 2,000 patients, we randomized patients to either standard of care (education) or decolonization with nasal mupirocin, topical CHG, and

0.12% oropharyngeal CHG oral rinse. Data from the first 976 subjects demonstrated that oropharyngeal colonization decreased over 50% (from a baseline of 21% to 10%) by 1 month with only a 22% to 19% decrease in the control group (P<0.05). (Figure 1) This data provides proof of principle that oropharyngeal *S. aureus* can be eradicated with 0.12% chlorhexidine. However data in other populations, especially children, are lacking. It is important to investigate *S. aureus* oropharyngeal eradication in children given results of studies in adults cannot simply be generalized or extrapolated to children, and adolescents.[61]

Figure 1. Figure 1: Efficacy of nasal mupirocin, topical (skin) chlorhexidine, and orophayngeal chlorhexidine



Baseline differences NS; all other comparisons significant p<0.05 except wound

Rapid diagnostic testing can identify extra-nasal *S. aureus* colonization

To identify children and patients with oropharyngeal colonization who are to be target for decolonization, a simple and rapid identification system is required. Several rapid molecular diagnostic technologies to directly identify *S. aureus*, including MRSA, for the detection of *S. aureus* in oropharyngeal and nares specimens. Rapid molecular *S. aureus* tests employ real-time polymerase chain reaction (RT-PCR) technology that targets *spa*, *mecA*, and SCC*mec* genes found in MRSA. They have a turn-around-time of ~ 1 hour,[62-65] as opposed to 48-72 hours for culture-based techniques commonly used in clinical microbiology laboratories. Test characteristics suggest these rapid molecular tests are of high clinical utility. Our group has used rapid molecular tests to identify patients with extra-nasal (inguinal) colonization.[66] Others have used rapid molecular tests for identifying *S. aureus* oropharyngeal colonization.[49,64]

Whole genome sequencing has high level discriminatory power to distinguish small changes among *S. aureus* isolates

With advances in sequencing technologies and the availability of large computing power and bioinformatics, whole genome sequencing (WGS) is becoming a method of choice to characterize emerging phenotypes and virulent subtypes of microbial pathogens. A comparative WGS can identify single nucleotide polymorphisms (SNPs), indels, small genetic rearrangements, and the acquisition and loss of mobile genetic elements in *S. aureus* strains associated with disease phenotypes.[67] WGS can distinguish differences in strains that cannot be appreciated by commonly used methods such as pulse field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and *spa* typing,[68-70] PFGE, MLST, and *spa* typing have been used for decades as the standard for *S. aureus* molecular epidemiologic investigations.[71] However, these methods suffer from poor discrimination of *S. aureus* isolates, especially among community isolates found in the U.S.

The discriminatory power of WGS is illustrated in studies of community-associated MRSA spread, in which the vast majority of isolates come from the USA300 background. These USA300 isolates, which form the vast majority of CA-MRSA strains in the U.S.[6,40] are indistinguishable via traditional typing methods even among isolates that come from very distinct sources and differences in geography of hundreds or thousands of miles.[6] WGS, however, is an extremely powerful tool that demonstrates inter-person spread and distinguishes differences in USA300 strains that cannot be appreciated by traditional methods.[72-74] The high discriminatory power of WGS is increasingly used to investigate outbreaks of *S. aureus* and is rapidly becoming the method of choice for molecular epidemiologic investigations of *S. aureus* spread. There are no data however, investigating strain relatedness within patients who have recurrence of colonization or failures of decolonization regimens. Given the very high discriminatory power of WGS for *S. aureus*, and the observation that colonizing strains within a given individual even with low discriminatory ability are frequently different,[18] we hypothesize that in patients using CHG oral rinses, isolates that are associated with recolonization after initial decolonization success will represent new strains or be related to nasal

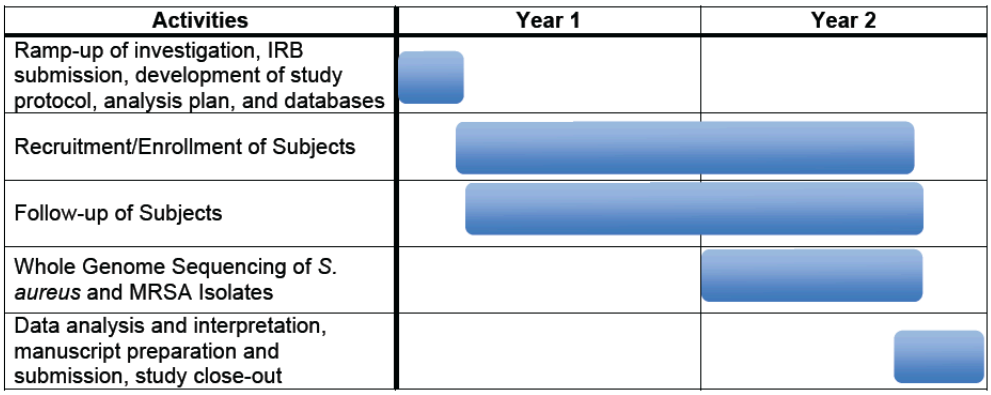
isolates from the same patient. No investigation has characterized isolates associated with treatment failure of decolonization regimens.

In summary, oropharyngeal colonization is extremely common among patients with *S. aureus* infections and there are mounting data suggesting that decolonization regimens that fail to decolonize the oropharynx leave behind a critical reservoir of *S. aureus*. This reservoir can then serve as a persistent source of *S. aureus* colonization that can be the nidus for subsequent infections. Because recurrence rates of *S. aureus* skin infection are so high, there is an urgent need to study the efficacy of oropharyngeal decolonization regimens for *S. aureus* in children before they can be used in more comprehensive decolonization regimens.

Experimental Design and Methodology (See General Instructions for page limitations/recommendations)

This investigation is a randomized, prospective, multi-center, placebo-controlled clinical trial to examine eradication of *S. aureus* from the oropharynx of children age 5-17 who recently experienced an SSTI and have proven oropharyngeal *S. aureus* colonization. We will compare eradication of oropharyngeal *S. aureus* colonization among those randomized to oral chlorhexidine (CHG) oral rinse compared to those randomized to placebo. Our investigative team has experience in *S. aureus* treatment and prevention, Pediatrics, Infectious Diseases, Emergency Medicine, Microbiology, Molecular Biology, Infectious Diseases Epidemiology, and Health Services Research. We have ample experience enrolling subjects in the Pediatric Emergency Department (PED) setting. Over the last 6 years, Dr. Miller's group has enrolled over 3,500 subjects into epidemiologic studies and clinical trials focused on infectious diseases, a third of which were children.[9,17,18,75-85] Drs. Miller and Young have collaborated on a National Institutes of Health (NIH) multi-center clinical trial comparing TMP-SMX and clindamycin for the treatment of skin and soft tissue infections in children and adults in the ambulatory care setting.[86] Ms. Eells has worked with Dr. Miller for over seven years on multiple federally-sponsored investigations including clinical trials sponsored by the NIH, Centers for Disease Control and Prevention (CDC), and the Agency for Healthcare Research and Quality (AHRQ).[18,75,77-82] Starting early 2015, Dr. Young will lead a team of research assistants whose mission is to help identify patients who are eligible for clinical trials and connect them with the appropriate recruitment team. The study activities and milestones are outlined in Figure 2.

Figure 2. Timeline of Study Activities

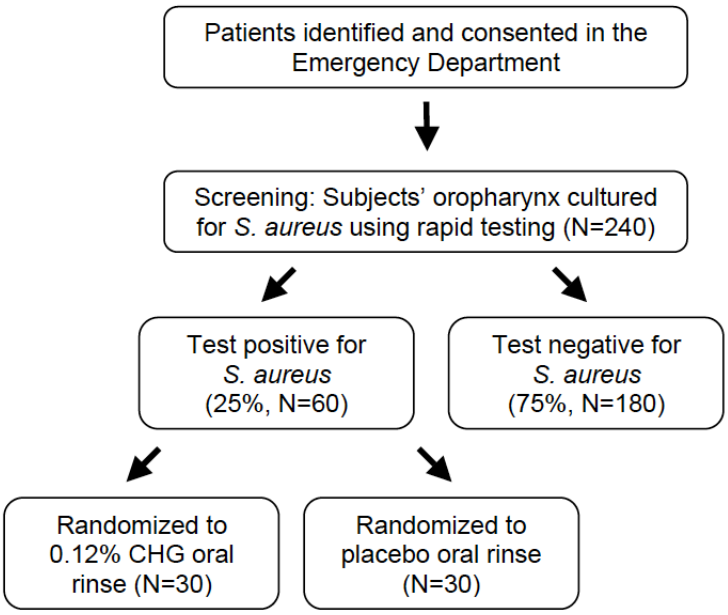


Enrollment of a cohort of pediatric patients with *S. aureus* skin and soft tissue infection

Enrollment

We will enroll 240 subjects with a history of SSTI in the Pediatric Emergency Department (PED) at Harbor-UCLA Medical Center. Subjects who meet the inclusion and exclusion criteria outlined below will undergo rapid screening for oropharyngeal *S. aureus* colonization. Assuming a 25% oropharyngeal colonization prevalence,[18] 60 subjects will have oropharyngeal colonization with a *S. aureus* and be eligible for the trial's decolonization regimen (Figure 3).

Figure 3. Screening of Subjects for Enrollment



All patients who participate in the clinical trial will be followed for 28 days for the purpose of determining which subjects achieve and maintain eradication. Participants will be enrolled regardless of race, ethnicity, or gender. While younger children commonly suffer from SSTIs, for this investigation we will only enroll children 5 years of age and older, as younger children may not be able to gargle. In a pilot study of 64 children from a general dentistry practice asked to gargle, among the 12 children ages 3-4, only 2 (17%) could complete a 30 second gargle, whereas 51/52 (98%) of those ages 5-8 could gargle successfully (Mitra Evans, DDS, personal correspondence).

To recruit subjects, study coordinators will screen the PED three times daily. During screening, they will query PED physicians, and nursing staff about potentially interested patients (e.g., those with a history of SSTIs) whose parents/guardians are willing to discuss possible participation in a study. PED staff will also be in-serviced about the study and encouraged by our PED physician Co-Investigator to call study coordinators as needed. To complement these efforts, flyers will be placed in the PED advertising to medical staff to call or page study coordinators about potentially eligible patients. These strategies have been highly successfully at each of the sites for enrolling subjects with SSTIs in the PED for our previous investigations. Once an interested patient is identified, the study coordinator will complete the informed consent process in a private setting in the patient's parent/guardian preferred language (English or Spanish).

Inclusion and Exclusion criteria

For this investigation, we will use strict inclusion and exclusion criteria. Inclusion criteria will include:

a) History of skin or soft tissue infection, as per patient report
b) Age $\geq$ 5 years and < 18 years.
c) Able to gargle (if needed, ability to gargle can be assessed at screening using water).
d) Willing and able to undergo nares and oropharyngeal swabbing.
e) Able to come to the research clinic for study follow-up visits for the study period.

Exclusion criteria for subjects will include:

a) Suspected or confirmed infection requiring systemic antibiotics.
b) Receipt of systemic antibiotics in the prior 28 days.
c) Plans for administration or likely receipt of systemic antibiotics in the next 28 days (e.g., if the patient suffers from recurrent infections such as otitis media or has a planned surgery that requires prophylactic antibiotics).
d) Plans for hospitalization or likely hospitalization in the next 28 days (e.g., if the patient suffers from recurrent infections).
e) Any of the following in the prior 6 months: hemodialysis, peritoneal dialysis, central venous catheter placement, and systemic chemotherapy for cancer, any immunecompromising condition. These criteria ensure that subjects enrolled with have CA-*S. aureus* infections and are likely colonized with USA300 *S. aureus*, the most common cause of SSTIs in the U.S.
f) Previous participation in the study.

Baseline visit

Once a patient consents to study participation, study coordinators will obtain a specimen from the subject's nares, and oropharynx for both rapid testing and standard culture based testing. A research assistant will then process the swab for rapid molecular testing (GeneXpert MRSA/SA platform) to determine if the purulent fluid has MRSA, MSSA, or neither. The sensitivity and specificity of this FDA approved assay in the nares are 97 and 96%, respectively;[65] sensitivity for throat specimens is 75%-81% with a very high sensitivity of 98%.[87,88] The specimen will be processed in the Miller Laboratory on the Harbor-UCLA Medical Center campus, which is a <5 minute walk from the PED. Rapid molecular testing results will be available within one hour and conveyed to the subject. Participants who are negative for *S. aureus* oropharyngeal colonization will be told they are not eligible for the intervention and doscontinued from the study.

Participants who are positive for *S. aureus* oropharyngeal colonization will be allowed to participate in the trial and data collection will commence. Of note, participants who are rapid test positive for *S. aureus* and later using clinical microbiology lab testing with culture-based methods are found to be negative for *S. aureus* will not be included in the analysis, although given the very high sensitivity of the GeneXpert assay in throat specimens (98%), we think this is an unlikely event[87] and these events are built into our expected attrition rate (see below). For ethical purposes, all subjects, regardless of rapid testing results, will receive educational materials regarding optimal hygienic practices to reduce risk of transmission, basic education about *S. aureus*, SSTI, and recognition of warning signs of serious skin infections. These materials were developed for previous investigations and available in English and Spanish.

Based on previous investigations of patients with a history of skin infection from our group,[18] we estimate 33% of children with a history of SSTIs will be positive for *S. aureus* (n=80, Figure 2). Assuming a 75% sensitivity of the GeneXpert assay, and 75% of those participants (n=60) will be positive at the oropharynx (see section on Power and Sample size, below). Study coordinators will complete the baseline visit for participants who are *S. aureus* positive on rapid molecular testing.

Subjects will also be swabbed for *S. aureus* and MRSA colonization at the anterior nares and oropharynx. Testing will be performed using two methods: direct microbiology cultures and rapid molecular testing. Microbiologic cultures will be performed by culturing swabs from sites taken to identify *S. aureus* and MRSA carriage will be transported to the research microbiology laboratory at LA BioMed at Harbor-UCLA and plated within 24 hours of collection. Swabs will be processed for *S. aureus* using blood agar and CHROMagar plates for rapid identification of *S. aureus* and MRSA. All *S. aureus* and MRSA isolates will be banked for pulse-field typing, determination of SCC*mec* type, and presence of the PVL locus (*lukF-PV* and *lukS-PV*). Rapid molecular testing will be performed using the GeneXpert MRSA/SA platform, which is currently available at our site.

Upon confirmation of eligibility, the study coordinators will call the pharmacy to randomize the participant and obtain the oral rinse. The randomization assignment list will be generated in advance by pharmacy. Randomization procedures by an assignment list will be prepared by a computer method. To ensure adequate balance of subjects between the CHG and placebo groups, treatment allocation will be assigned using a random number generation with randomization of blocks of four subjects each. The randomization list will be kept by the pharmacy and treatment allocation randomization will not be shared with either the study staff of patients. The research pharmacists will dispense the appropriate oral rinse to the participant and their parent/guardian. Placebo will be created using the inert vehicles components of CHG, as has been done previously.[89] Participants and their parent/guardian will be given detailed instructions on the use of the oral rinse both verbally and in a handout in either English or Spanish as preferred by the participant and their parent/guardian.

Subjects randomized to oral CHG will be provided verbal and written instruction on use of CHG oral rinse. Subjects will be asked to use CHG oral rinse twice daily for 7 days. Subjects will be educated to rinse their mouth and gargle with CHG oral rinse for 60 seconds twice a day. Subjects will be instructed to use a watch or other timing device (e.g., smartphone) to ensure that at least 60 seconds have elapses. Research staff will demonstrate the technique for all subjects using drinking water. All subjects randomized to the CHG arm will be provided a new soft bristle toothbrush to prevent recontamination of the oropharynx with *S. aureus* from a contaminated toothbrush. Subjects randomized to usual care will not be provided with any of the above. Regardless of treatment allocation, study staff will educate all subjects' parent/guardian, and subject (if of sufficient age) with the clinical significance of the results of their positive test for oropharyngeal

S. aureus colonization. More specifically, they will be educated that currently testing for *S. aureus* oropharyngeal colonization is considered experimental and that if the test is positive, the clinical significance is unclear. They will also be educated that tens of millions, perhaps over 100 million Americans are colonized in the throat with *S. aureus*, and the vast majority do not suffer consequences of having this bacteria reside in the throat. Upon request, subjects will be provided with a written copy of their results as well as a written description of the clinical significance of oropharyngeal colonization that they can share with their provider, if they wish. All subjects, regardless of treatment allocation, will be provided written and verbal information about *S. aureus* and skin infection prevention and recognition of early disease. These materials were developed for previous studies done by our group.[18]

Of note, we debated whether the control group not receiving CHG should receive no therapy or a placebo oral rinse. Obviously, placebo controlled trials are of stronger study design compared to non-placebo controlled studies. More specifically, in non-placebo studies, both patients and investigators will know the treatment allocation, which can lead to bias by both subjects and the investigative team. We did have concern that oral rinses/gargling with placebo or an inert substance may affect *S. aureus* colonization. However, a 1970 investigation examined this issue using quantitative techniques. Investigators asked subjects to gargle with saline and took serial quantitative cultures. They found that gargling specimens taken at 5 minute intervals for 25 minutes showed no significant decrease in organisms concentration, with mean organism concentrations at baseline of 7.11, 6.85 at 5 minutes, and 6.92 at 25 minutes.[90] This suggests that placebo will have no ability to decolonize oropharyngeal *S. aureus* from study participants.

At the baseline visit, study coordinators will also administer a pediatric *S. aureus* risk factor survey similar to surveys used for previous *S. aureus* and MRSA epidemiologic investigations by our group.[9,17,18,76,80,84,85] Items from this survey have been based on careful reviews of the literature on *S. aureus* risk factors and have incorporated items used previously by the CDC, Health Departments, and other investigations examining MRSA risk.[80,84] The baseline survey will collect information about demographics, medical history, antibiotic use, and behavioral risk factors for *S. aureus* infection and colonization. Participants and their parent/guardian will be given phone numbers of the study coordinator for questions. Participants and their parent/guardian will be compensated for their time for this and all study visits.

Follow-up visits

Participants will be asked to come to the research clinic 7 days after initiation of oral CHG, i.e., at the end of their treatment (Visit 2). A subsequent visit will occur at 28 days (Visit 3). The purpose of these visits is to ascertain oropharyngeal eradication of *S. aureus* both short term (primary study outcome) and longer term. Based on previous investigations conducted at our center(s), we estimate a 10% attrition rate.[75,77,78,81,82] At Visit 2, participants will be swabbed the nares and oropharynx for the presence of *S. aureus* and to complete a follow-up survey about any recurrent infections, new medical history, or receipt of antibiotics. At Visit 3, participants will return to the research clinic to complete a follow-up survey. Study staff will also call all subjects remind them of their upcoming study appointments and at days 14 and 21 to address any questions the participant or their parent/guardian may have about the study. Adverse events will be measured at each scheduled and unscheduled (see below) follow-up visits. Adverse events will be measured using the Medical Dictionary for Regulatory Activities Terminology (MedDRA) version 14.0 or later terms.

Unscheduled visits

If participants or their parent/guardian experience potential adverse drug events that occur during the follow up period, subjects or their parent/guardian will be encouraged to contact study staff. If the event may be of clinical concern, the event will be referred to a study physician and the oral rinse discontinued, if applicable. If deemed necessary by the study physician or the subjects' primary physician, participants will be asked to return to the research clinic for evaluation by a study investigator.

Aim 1: Determine the efficacy of oropharyngeal decolonization among children suffering from CA-MRSA and other *S. aureus* skin infections

To determine if oropharyngeal *S. aureus* colonization can be eradicated, we will perform a prospective, double blind randomized controlled clinical trial of 0.12% oral chlorhexidine (CHG) oral rinse versus placebo for children age 5-17 with *S. aureus* oropharyngeal colonization. We hypothesize that 0.12% CHG rinse will

reduce oral *S. aureus* colonization better than placebo treatment. The findings from this Aim will provide "proof of principle" data on the ability of oral antiseptics to eradicate oropharyngeal *S. aureus* colonization in children.

Power and Sample Size

Using an alpha of 0.05 and expected cure rates of 50% and 10% in the CHG and placebo arms respectively, 54 subjects (27 per group) would be needed for 85% power to detect a 40% absolute difference in cure ($p<0.05$) between groups. Correcting for a 10% attrition rate, 60 enrolled subjects would be needed. To identify 60 subjects with oropharyngeal colonization, and assuming 33% of patients who are high risk for *S. aureus* colonization will have oropharyngeal colonization, 240 subjects will need to be screened to obtain 80 patients that are oropharyngeally colonized with *S. aureus*. Of these, 75% (n=60) will have *S. aureus* detected by the GeneXpert rapid test and will undergo randomization.

Data Analyses and Data and Safety Monitoring

The decolonization rate will be calculated as the number of subjects who remain decolonized divided by the number of subjects, and treatment group differences as differences in these rates. The precision of estimated rates and rate differences will be expressed with two-sided confidence intervals calculated as asymptotically normal approximations to binomial or differences in binomial random variables. The level of confidence for these intervals will be 95%. Statistical significance for differences between treatment groups in cure rates will be determined from Fisher's exact tests. The level of significance for these tests is 0.05. Factors associated with failure of decolonization in the CHG treatment group will also be examined. Based on prior investigations of *S. aureus* colonization from our group and others, these will include demographics (age, gender, race/ethnicity), co-morbidities, measures of hygiene, number of prior skin infections in the prior year, antibiotic use in the prior year, household history of skin infection, adverse effects and tolerability (see Aim 2, below), and treatment adherence. Additionally, we will examine pathogen level predictors of decolonization failure, based on molecular characterization of baseline isolates (see Aim 3). These factors will include baseline colonization with USA300 strain, presence of qac A/B gene, presence of nasal colonization, presence of nasal colonization with a strain identical to the oropharyngeal colonizing strain, and presence of nasal colonization with a strain differing from the oropharyngeal colonizing strain. Associations will be examined using Chi-squared, Fisher Exact test, Wilcoxon rank sum test, or T-test, as appropriate. Small sample size will preclude multivariable analyses of factors independently associated with failure to decolonize. This analysis will be key to identifying factors associated with decolonization failures. Identification of mutable factors associated with decolonization failure will be key towards improving the success of future efforts at oropharyngeal eradication of *S. aureus*. Identification of non-mutable factors will be key towards identifying groups that may need more intense intervention and or education.

Given this investigation is minimal risk, Data and Safety Monitoring (DSM) will be performed by the Investigative Team. DSM will be performed according to standards of The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Policy for Data & Safety Monitoring.[91] These will include reviewing monthly summaries of the number of patients enrolled, age, ethnic and gender makeup, Number of patients retained in the study, number of dropout and lost to follow up, adverse events (AEs), and serious adverse events (SAEs) (See Aim 2, below for further details). Subjects that report treatment related adverse events will discontinue the oral rinse and come to the research clinic for an unscheduled visit.

Aim 2: Assess the safety, tolerability, and compliance of oropharyngeal decolonization among children and their caregivers. We will administer a detailed and systematic survey of side effects, tolerability and compliance of the oropharyngeal decolonization regimen to determine the effects, concerns, and usability of this regimen in children for both the child participants and their parents/caregivers. Findings will identify adverse effects and barriers to use to be considered when possibly administering this regimen in the future.

Data Analyses

Adverse events will be coded to a Medical Dictionary for Regulatory Activities Terminology (MedDRA) version 14.0 or later terms. Verbatim description and the MedDRA System Organ Class and Preferred Term for all adverse events will be contained in the subject data listings. A separate listing sorted

by MedDRA System Organ Class and Preferred Term will include all verbatim descriptions associated with the Preferred Terms.
Data for adverse events will be analyzed using the treatment-emergent signs and symptoms philosophy.

Treatment emergent signs and symptoms are defined as adverse events where:
- onset occurs during exposure to study medication or within 7 days after the last dose of study medication, having been absent prior to receiving study medication, or
- onset reoccurs during exposure to study medication or within 7 days after the last dose of study medication, having been present but stopping prior to receiving study medication, or
- worsening in severity occurs during exposure to study medication relative to the pre-treatment state, when the adverse event is continuous.

All reported adverse events (regardless of treatment-emergent or not) will be included in a by-subject adverse event listing. Only treatment-emergent adverse events will be included in summary tables. The number of subjects (incidence) of treatment-emergent adverse events will be presented as well as the frequency of all adverse events reported.

Summary tables of adverse events will include the following:
- Summary of adverse events by severity and relationship
- Summary of related adverse events
- Summary of Serious adverse events
- A listing of any on-study pregnancies

Percentages of subjects who reported adverse events will each be compared separately among each group using Chi-Square or Fisher Exact tests, as appropriate.

Aim 3: Determine the *S. aureus* genetic backgrounds associated with breakthrough oropharyngeal colonization.
Advances in sequencing technologies and the availability of large computing power and bioinformatics have allowed WGS to become the method of choice to characterize emerging phenotypes.[72] WGS also allows examination of the relationship between presence of the *qac A/B* gene in *S. aureus* at baseline and treatment response. WGS allows detection of this plasmid-based resistance gene for comparative analysis among a subset of *S. aureus* isolates from our population, as described below.

We will molecularly characterize all baseline *S. aureus* isolates and "breakthrough" (failure) isolates using whole genome sequencing (WGS) for identification of breakthrough *S. aureus* isolates. We will compare breakthrough isolates with baseline nasal and orophangyeal isolates to determine if breakthrough isolates represent either: a) recolonization with the original oropharyngeal isolate, b) recolonization with the baseline nasal isolate, or c) acquisition of a new *S. aureus* strain. Additionally, we will investigate whether *S. aureus* associated with treatment failures have acquired or possess the *qac A/B* gene.

Sample Size
Data Analyses

WGS will be done in the laboratory of Barry Kreiswirth, PhD (see Letter of Support), using established techniques.[92,93] Dr. Kresiworh's lab has ample experience performing WGS on *S. aureus* and other bacterial pathogens.[92-94] Dr. Kreiswirth's lab will also supply analytic support to assist with interpretation of findings.

WGS sequencing will addresses several hypothesis in those subjects randomized to CHG oral rinses, specifically: a) strains that are found in the oropharynx at end of treatment (Day 7) will be identical genetically to those at baseline and not represent acquisition of new *S. aureus* strain; b) recolonizing *S. aureus* strains at Day 28 among subjects successfully eradicated at Day 7 will represent (re)colonization with the original isolate that colonized the nasopharynx from the same individual (if present at baseline) or a new *S. aureus* strain not present at baseline and; c) nasal isolates found at baseline and follow up will be identical to synchronously identified oropharyngeal *S. aureus* isolates.

Based on data in older adults,[19] we estimate that of the 30 subjects randomized to the CHG arm, 50% will be decolonized, leaving 15 subjects who will be *S. aureus* positive at the oropharynx at the end of treatment. Based on data of recolonization after decolonization in which recolonization can occur after the decolonization regimen is completed,[10] we estimate that a 10% increase in colonization will be seen at Day 28, i.e., 18 subjects will be oropharyngeally colonized with *S. aureus* at Day 28. Thus, we anticipate we will have results in 33 pairs of oropharynx isolates (15 between baseline and Day 7, and 18 between baseline and Day 28, for a total of 66 isolates total). Additionally we will conduct whole genome sequencing on baseline nasal *S. aureus* isolates associated with the subsequent oropharynx isolates identified at Day 7 and/or Day 28. Based on previous data suggesting that ~45% of those colonized at the oropharynx will be colonized at the anterior nares,[18,46,95] we estimate an additional 30 (45% x 66) nasal *S. aureus* isolates from baseline and follow-up will be present synchronously with oropharyngeal isolates.

In total 96 isolates will undergo whole genome analysis to address the hypotheses outlined above. Specifically, we will examine if decolonization failure of the oropharynx is due to any of the following: 1) a new and distinct *S. aureus* strain; 2) an isolate found at the nares at baseline; 3) an isolate found in the oropharynx at baseline; or 4) an identical strain from baseline but that has acquired with new resistance genes to CHG, specifically *qac A/B*. WGS typing of nasopharyngeal *S. aureus* isolates also allows us to determine if "breakthrough" oropharyngeal strains at Day 7 and/or 28 arise from endogenous or exogenous sources. Given that oropharyngeal and nasal isolates taken from the same person at the same time, are not always genetically identical,[18] one cannot assume that baseline strains will be concordant. Finally testing of nasal oropharyngeal and nasal *S. aureus* at baseline and the Day 7 and 28 follow up visits, can assist in determination if resistance genes to CHG, specifically *qac A/B*, emerged after treatment in non-oropharyngeal, specifically nares sites, as it is known that antimicrobial therapy is associated with collateral damage among strains not specifically targeted by that treatment.[37] I.e., oral CHG may result in the emergence of *qac A/B* containing *S. aureus* strain in the nares. Analytically, the above analyses will be descriptive thus there are no power calculations for this aim.

Limitations

This investigation is limited as it is a single center study. However, our center serves a ethnically and socioeconomically diverse population and findings from our investigations have been comparable to similar investigations in other populations.[18,96,97] Our intervention is also singular in decolonization efforts (oropharynx only). However, no investigation has looked at the effect of *S. aureus* eradication on the oropharynx in children, so the addition of other decolonizing agents would prevent us from determining the efficacy of CHG oral rinse. Additionally, our trial is a "proof of principle" study that is required prior to a more comprehensive trial on *S. aureus* decolonization as a means for SSTI prevention. Finally, we are not performing WGS typing on all colonizing *S. aureus* isolates at all study time points, as it is known that *S. aureus* can colonize not only the nares and oropharynx, but the axilla, inguinal fold, perirectal area, vagina, and areas of skin that have lost integrity.[42,47,98,99] However, the extra cost of testing these sites for possible sources of *S. aureus* that can later recolonize the oropharynx would be extremely expensive. Thus we chose just to perform testing in sites in which *S. aureus* is most commonly found (nares, oropharynx).

Future Directions

Investigation on prevention of recurrent *S. aureus* SSTI have been extremely disappointing, suggesting more comprehensive decolonization regimens need to be developed, especially those that focus on sites of colonization not previously targeted in clinical trials. Our investigation will be the first study to examine the efficacy of CHG to eradicate *S. aureus* from the oropharynx of children. Dr. Miller and his team provide ample experience in the area of MRSA infection and colonization, infectious disease expertise, clinical trials, and pediatrics. Besides the aims addressed for this investigation, our team is poised to address future aims related to *S. aureus* decolonization efforts and SSTI recurrences by generating information on the efficacy of CHG. In summary, the results of this investigation will provide the critical foundation for the development of future clinical trials. Data generated from our investigation will further investigative work on prevention of recurrent *S. aureus* SSTIs, which remain an extremely common and vexing clinical problem for clinicians, and more importantly, patients.

For this investigation, Harbor-UCLA Medical Center pediatric emergency department providers (MD, NPs) will refer patients agreeable to being approached by research coordinators about the study. If patient and their guardian are agreeable, research staff will approach patients and initiate the informed consent process. If the patient and/or their parent or guardian consent and the patient is eligible, the patient will undergo the first part of the study (oropharyngeal screening for *S. aureus,* which will be evaluated using the GeneXpert platform). Study staff will explain the study to the potential subject's parent or guardian. The informed consent process will be conducted by a study team member in their preferred language (English or Spanish). Written informed consent will be sought, obtained, and documented by a research coordinator and the site's PI using a form approved by the institution's IRB. Subject's who are capable will participate in the informed consent process and those 7-12 will be asked to sign an assent form. Subject's older than 12 will co-sign the consent form with their parent/legal guardian. The informed consent process will explicitly state that if the oropharyngeal screening for *S. aureus* is positive, they agree to participate in the clinical trial and that if the oropharyngeal screening for *S. aureus* is negative, they are not eligible for the clinical trial.

All subjects who have consented will have the rapid *S. aureus* test performed with results shared with the subject's parent/guardian, the subject, and the clinician, if approved by the parent/guardian. Study staff will educate and the subject's parent/guardian, and subject (if of sufficient age), the clinical significance of the results, either positive or negative. More specifically, they will be educated that currently testing for *S. aureus* oropharyngeal colonization is considered experimental and that if the test is positive, the clinical significance is unclear. They will also be educated that tens of millions, perhaps over 100 million Americans are colonized in the throat with *S. aureus*, and the vast majority do not suffer consequences of having this bacteria live in the throat. Upon request, subjects will be provided with a written copy of their results as well as a written description of the clinical significance of oropharyngeal colonization that they can share with their provider, if they wish.

Subjects with a positive screening test will undergo 3 study visits total: baseline/enrollment visit (Visit 1), Day 7 (Visit 2), and Day 28 (Visit 3). Consenting subjects will have their anterior nares and oropharynx swabbed at Visits 1, 2, and 3. A survey will be administered at the initial visit and a follow-up survey will be administered at Visits 2, and 3. The survey administered at Visit 1 will collect information about demographics, medical history, and behavioral risk factors for skin and soft tissue infection and colonization. Subjects will be randomized to 0.12% CHG oral rinse or placebo use for 7 days. All subjects will receive educational materials regarding *S. aureus* and skin infections and their prevention. Those randomized to the intervention will receive additional verbal and written information on use of CHG oral rinse. The survey administered at Visit 2 will collect information about the adherence, tolerability, and adverse events during the intervention period. The survey administered at Visit 3 will collect information about (late) adverse events, changes in household dynamics and individual behavior (e.g., hygiene) that may affect results of Visit 3 nasal and oropharyngeal colonization studies.

Patients at Harbor-UCLA Medical Center come from a diverse racial and ethnic background, with approximately 51% Hispanic, 26% Caucasian, 18% African-American, and 6% "other" (largely Asian and to a lesser extent, mixed race). Two hundred and forty patients are anticipated to be enrolled over 18 months to randomize 60 subjects. Subjects constitute a cross-section of pediatric patients either presenting to our health care facilities that had a history of a skin and soft tissue infection, and will thus consist of pediatric patients age 5-17 and both genders.

There are risks to subject confidentiality, which will be mitigated by all PHI information being kept at the individual site with secure locked filing cabinets and/or on a password-protected computer file. This likelihood of subject confidentiality being broken is minimal. Risks to confidentiality will be protected by keeping all study data in a locked office with access restricted to study personnel. All identifiers will be removed and destroyed when data is logged into an Access database. We will protect against psychological risk to the patient by including an educational discussion with the patient including the nature of bacterial colonization, the risks, current treatments, the lack of acceptance of routine treatments, and the ability to attempt treatment in the case of recurrent infections. If a subject suffers psychological harm, they may contact the Principal Investigator and they will discuss further options. Subjects will be told that their information will not be shared with any persons except for study personnel unless the subject provides

permission. Finally, subjects will be told they can choose not to participate in the study or end their participation at any time without any effect on the care they will receive from their treating physicians or at their treating institutions.

Risks associated with study medication is believed to be minimal. Chlorhexidine-gluconate (CHG) oral rinse is an FDA-approved medication for the treatment of gingivitis. The known side effects of oral CHG oral rinse include staining of teeth and other oral surfaces, an increase in calculus formation; and an alteration in taste perception.[100] All of these potential side effects are transient, reversible, or preventable with education and standard oral hygienic practices (e.g., tooth brushing). All subjects will be educated on the proper use of the oral rinse. Subjects will be asked to report any adverse events to study personnel and to stop the use of the oral rinse if any severe side effects develop (swelling, pain, etc.). Staff will also be able to counsel subjects about the risks and benefits of the oral rinse to decolonize the oropharynx. Study personnel will also have cell phones, which subjects can call to ask questions about the study, use of the intervention oral rinse, and report adverse events. Subjects that report serious adverse events or other medical needs will be referred to the Harbor-UCLA Medical Centers' Pediatric Emergency Department.

An Investigational New Drug (IND) application will not be required, as a clinical investigation of a marketed drug is exempt from IND requirements if all of the criteria for an exemption in § 312.2(b) are met:

- The drug product is lawfully marketed in the United States.
- The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication and there is no intent to use it to support any other significant change in the labeling of the drug.
- In the case of a prescription drug, the investigation is not intended to support a significant change in the advertising for the drug.
- The investigation does not involve a route of administration, dose, patient population, or other factor that significantly increases the risk (or decreases the acceptability of the risk) associated with the use of the drug product (21 CFR 312.2(b)(1)(iii)).
- The investigation is conducted in compliance with the requirements for review by an IRB (21 CFR part 56) and with the requirements for informed consent (21 CFR part 50).
- The investigation is conducted in compliance with the requirements of § 312.7 (i.e., the investigation is not intended to promote or commercialize the drug product).

Because the use of 0.12% CHG oral rinse in this investigation meets all of the above criteria for required for an IND exemption.

Data and safety monitoring will be conducted as described in Aim 2.

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