Treating Parents to Reduce NICU Transmission Of *Staphylococcus aureus* (TREAT PARENTS Trial)

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1. Synopsis

More than 33,000 healthcare associated infections (HAI) occur in neonatal intensive care units (NICU) each year in the United States. HAIs are estimated to result in $28-45 billion in healthcare costs annually. In addition to the short-term costs of HAIs, neonatal infections contribute to devastating neurologic disabilities and poor growth outcomes. *Staphylococcus aureus* (*S. aureus*) is the second most common pathogen causing HAIs in neonates. Out of 57,000 very low birth weight (VLBW) infants, an estimated 3.7% develop bloodstream or central nervous system *S. aureus* infections annually with an attributable mortality of 25%. Despite aggressive measures to prevent *S. aureus* infections in neonates, the burden of *S. aureus* disease remains high in this population. Parents, rather than healthcare workers, may be a key reservoir from which neonates acquire *S. aureus* colonization in the NICU. This paradigm is consistent with a changing NICU environment where skin-to-skin contact between parents and neonates is encouraged and may promote *S. aureus* transmission, while at the same time, common hospital infection prevention measures have reduced healthcare worker transmission of *S. aureus*. The long term objective of the proposed study is to prevent HAIs in neonates, especially those caused by *S. aureus*. This protocol describes the TREAT PARENTS Trial (**Treat**ing **Pa**rents to **Re**duce **N**eonatal **T**ransmission of ***S****. aureus*), a randomized, masked, placebo-controlled trial that will measure the effect of treating parents with short course intranasal mupirocin and topical chlorhexidine antisepsis on acquisition of *S. aureus* colonization and infection in neonates. The findings of the proposed study could provide a new tool for HAI prevention in the NICU.

2. Background and Rationale

A. Background

The Centers for Disease Control and Prevention (CDC) estimate that approximately 1.7 million healthcare associated infections (HAIs) occur in U.S. hospitals, including more than 33,000 in the neonatal intensive care unit (NICU). *Staphylococcus aureus* is a leading cause of HAIs in NICUs. Despite appropriate therapy, neonatal *S. aureus* infections can have long-term sequelae including poor neurodevelopmental and growth outcomes. Preterm infants with infections are 30% more likely to have a lower mental developmental index, an important predictor of intelligence quotient. Immunization against *S. aureus*, either active or passive, is years or decades away from results that can impact clinical practice.

S. aureus is the second leading cause of catheter-associated bloodstream infections (CABSIs) in NICUs. Unlike other ICU populations such as adult intensive care units in which the *S. aureus* CABSIs decreased by 50% from 1997-2007, the incidence of *S. aureus* CABSIs in U.S. NICUs increased by 50% from 1998-2008. In a recent review of 8,444 very low birth weight infants in U.S. NICUs, 3.7% had *S. aureus* bacteremia or meningitis. Therefore, of the approximately 57,000 VLBW infants in the U.S. in 2011, an estimated 2,100 developed *S. aureus* bacteremia or meningitis, and more than 500 died from the infection. There are no national estimates of outcomes associated with other neonatal *S. aureus* infections, including pneumonia, so these projections likely underestimate the burden of actual disease in this population.

B. Rationale

Up to 40% of neonates acquire *S. aureus* in the first two months of life. Vertical transmission of *S. aureus* is rare, but postnatal transmission from mother to healthy infants is common in the first few months of life. Although healthcare workers have been implicated as a source of spreading *S. aureus* in NICUs, they are often not the source for transmission of *S. aureus* in NICUs. In addition to HCWs, parents have been identified as sources of *S. aureus* transmission.

Our preliminary data suggest that the majority of MRSA strains acquired by neonates in our NICU were not acquired from healthcare workers, so parents, rather than healthcare workers, may be the key reservoirs from which neonates acquire *S. aureus* colonization in the NICU. This finding is consistent with a changing NICU environment where skin-to-skin contact between parents and neonates is encouraged and may promote *S. aureus* transmission, while common hospital infection prevention measures have reduced healthcare worker transmission of *S. aureus*. We will test whether detection and treatment of *S. aureus* colonized parents with intranasal mupirocin and topical chlorhexidine bathing will decrease the risk of their infant acquiring *S. aureus* in the NICU. Reducing *S. aureus* burden has been shown to decrease infections. Rather than a patient-directed approach (screening and treating *S. aureus* colonized neonates) which has major limitations in the neonatal population, we propose a parent-directed approach that eliminates or delays a neonate's exposure. Similar to vaccinating pregnant mothers with influenza vaccine to prevent disease in newborn infants, we propose to engage parents in preventing HAIs in their children.

C. Significance of the Proposed Research

Among 30,000 patients who acquire HAIs in NICUs across the U.S. each year, more than 500 VLBW infants alone die from *S. aureus* infections. Novel strategies are needed to prevent HAIs and alleviate the billions of dollars in short-term healthcare costs and the long-term neurologic disabilities in children that survive neonatal infections. This proposal builds on our previous work, will test a strategy aimed at reducing HAIs, and uses a novel approach for this unique and vulnerable neonatal population. The findings of this proposal could change HAI prevention in the NICU from one that focuses on healthcare workers and the environment to one that recognizes and highlights parents and visitors as important sources of exposure to pathogens that contribute to HAIs.

3. Study Purpose, Objective and Hypotheses

A. Purpose: The goal of this project is to determine the effectiveness of decolonizing parents of neonates in reducing neonatal acquisition of *S. aureus* and neonatal *S. aureus* infections in the NICU.

B. Objectives: To compare the effect of treating parents with short course intranasal mupirocin and topical chlorhexidine bathing or placebo on acquisition of *S. aureus* colonization in neonates. To compare the relatedness of *S. aureus* strains colonizing parents and *S. aureus* strains acquired by their neonates in the NICU.

C. Primary Hypothesis

Treating parents of neonates requiring NICU care with intranasal mupirocin and topical chlorhexidine bathing will reduce the spread of *S. aureus* from parents to their neonates.

D. Secondary Hypotheses

Many neonates acquire *S. aureus* in the NICU from their parents

Treating *S. aureus* colonized parents of neonates requiring NICU care with intranasal mupirocin and topical chlorhexidine bathing will reduce neonatal *S. aureus* infections.

Treating *S. aureus* colonized parents of neonates requiring NICU care with intranasal mupirocin and topical chlorhexidine bathing will reduce parental *S. aureus* colonization.

4. Study Procedures

A. Summary

The TREAT PARENTS Trial, or Treating Parents to Reduce Neonatal Transmission of *S. aureus*, is a placebo-controlled, double-masked, randomized clinical trial to test the hypothesis that treatment of *S. aureus* colonized parents with intranasal

mupirocin and topical chlorhexidine gluconate antisepsis will decrease neonatal *S. aureus* acquisition. All neonates admitted to the Johns Hopkins Hospital NICU and the Johns Hopkins Bayview Medical Center NICU will be pre-screened and parents will be approached for enrollment in the study. After consent and baseline screening, approximately 400 neonate-parent pairs will be randomized. Parents will receive a 5 day treatment with intranasal mupirocin plus topical chlorhexidine gluconate antisepsis or placebo.

B. Intervention

Subjects (parents of neonates) will receive an ointment and cloths with standardized instructions for use. The instructions will be the same for all subjects. Approximately 1 cm ribbon of ointment will be self-administered by inserting a new cotton-tipped applicator into each anterior naris. A new cotton-tipped applicator will be used for each naris followed by gentle massaging of the naris to ensure distribution of the ointment. This procedure will be performed twice daily for five days. Verbal and printed instructions will be provided to parents. Study staff will demonstrate application of mupirocin ointment to the anterior nares. Parents will self-administer all doses of the intervention. Subjects will also be given pre-packaged cloths for daily skin cleaning. Each package contains 6 cloths. Each cloth will be used to wipe a designated body area (arms, legs, chest and neck, back and perineum) once a day for 5 days. Study staff will demonstrate how to apply CHG to the skin and instruct parents to not rinse after using the cloths. Participants will be instructed to perform the baths on the same 5 days they apply the intranasal ointment.

Compliance Monitoring

Verbal and printed instructions will be provided to parents. A member of the research team will contact each participant daily during treatment to review compliance. Contact will be made at the bedside or by text, email or phone call at the preference of the participant. Participants will be asked to return the medication tube (mupirocin or placebo) at the end of the treatment period and any unused cloths.

C. Enrollment

1. Patient Population and Setting

The Johns Hopkins Hospital (JHH) NICU is a 45 bed NICU in a quaternary care center that admits approximately 700 neonates per year. The Johns Hopkins Bayview Medical Center (Bayview) NICU is a 25-bed, level III unit with approximately 375 admissions per year. Neonates admitted to the JHH and Bayview NICUs and their parents or legal guardians will be screened for eligibility. **We will define parents as the biologic mother and the father. In the event that one of the parents is not available or does not visit the child in the NICU, we will ask the available parent to identify a primary visitor of the child in the NICU. Only 2 parents or one parent and one primary visitor will be enrolled for each neonate. In the protocol below, parents will refer to either a parent or a designated primary visitor.**

2. Eligibility

Inclusion criteria for parent-neonate pairs

i) Neonate has never had a prior clinical or surveillance culture grow *S. aureus*
ii) Neonate was transferred from another hospital or admitted from home and had admission screening cultures for *S. aureus* colonization that were negative (if admission cultures were not performed, they will be performed as part of the pre-randomization screening process)
iii) Parent(s) is(are) able to visit the child at the bedside
iv) Parent(s) test positive for *S. aureus* at screening
v) Neonate has anticipated stay longer than 5 days in the NICU (if estimated stay is unclear, parents can be screened for *S. aureus* colonization and decision to randomize can be delayed until hospital day 3 or 4 after reassessment of anticipated stay).
vi) Parents is(are) willing to be randomized
vii) No documented or reported allergies to any agent used in either treatment regimen
viii) Able to perform written informed consent

Exclusion criteria for parent-neonate pairs

i) Allergies to any agent used in either treatment regimen
ii) Neonate has had a prior clinical or surveillance culture grow *S. aureus*
iii) Neonate admitted to NICU from home and is greater than 7 days of age
iv) Neonate admitted to NICU from another hospital and is greater than 7 days of age
v) Neonate is a ward of the State
vi) Not able to provide written informed consent

3. Recruitment

We will pre-screen patients for eligibility (including anticipated length of stay, prior culture growing *S. aureus*). A member of the study team will approach the parent at the bedside and request participation as outlined below. If parents are not available at the bedside, a study team member will call the parents to arrange a time to meet and discuss participation in the study.

4. Consent

The consent process will be completed at the bedside in the neonate's private room or in a private area of the NICU or in the mother's private room in cases where the mother is still admitted to the labor and delivery ward. The consent form will be read aloud to any participant that has difficulty with reading. The consent has been developed on an 8th grade reading level. Each parent will be consented for participation. Only neonates who have a

parent colonized with *S. aureus* will be randomized, and a copy of the consent form will be placed in the child's chart; therefore, participants will be informed that people outside the study may become aware that one of the parents is colonized with *S. aureus*, but nowhere in the child's chart will it be documented which parent is colonized. A copy of the signed consent form will be made available to each participant, a copy will be placed in the neonate's bedside chart, and a copy will be kept in a regulatory binder. Parents who are not colonized will not be randomized; however, they will be asked to continue to participate in the study by completing a follow-up questionnaire approximately four weeks after their screening date. If parent does not allow long-term storage of biospecimen for future research they will be asked for consent to store bacteria collected from biospecimens.

Assessment of Protocol Comprehension Prior to Signing Consent

After the consent form is read, a member of the research team will evaluate comprehension with three protocol specific questions: 1) "What is the name of the bacteria or germ that we are trying to get rid of?"; 2) "How does this study plan to get rid of this bacteria or germ?"; 3) "How will you be assigned to receive either antibacterial cloths and ointment versus a plain cloth and ointment with no antibiotic?" We will deem the subject to have an acceptable level of comprehension based on their ability to answer those questions. We will review the corresponding section of the consent form with the participant if any of the responses are unsatisfactory and ask the questions again to ascertain comprehension.

5. Screening

The PI and/or a member of the research team will provide information regarding the study, answer any questions and obtain informed consent. The PI will be available in person or via telephone to answer any questions when a member of the research team is present for the consent. As part of the informed consent document, the subject will receive information on how to contact a study team member to discuss culture results or any noted treatments that may be related to *S. aureus*. Once consented, a member of the research team will assign a screening number (SN) to the subject. If the cultures are positive, the family unit will be enrolled and assigned a study identification number (SIN). This will be the primary mode of identification throughout the study. The SIN will identify the parent and the neonate (or neonates in the case of multiple gestations). The SIN will also identify both parents (if both consent to participate as both will be assigned to the treatment group). The SIN and/or SN will appear on the consent form, the data collection form, as well as all *S. aureus* cultures. If the cultures test negative, the family unit will not be randomized to treatment. However, parent participation in the study will not end. They will be asked to continue their participation in the study by completing a follow-up questionnaire approximately four weeks after their initial screening visit.

During the screening visit, a member of the research team will perform the necessary swabs to be sent for culture. The parent(s) who consent to undergo testing will be screened by obtaining a swab from the following anatomical sites: bilateral anterior nares, the throat, the groin/perineum, and the peri-anal area. A standardized check-list for obtaining and processing swabs will be used. For neonates transferred from an outside hospital or from home that did not have surveillance cultures obtained on admission, screening cultures will be performed by obtaining a swab from the following anatomical sites: bilateral anterior nares, umbilicus, groin, and peri-anal area.

All swabs will receive a study number pre-printed on a label. The SN will not identify the patient's name, medical record number or other identifying information. The SN will be recorded on the informed consent documents and the surveillance swabs. Before sending the swabs, a member of the research team will take a brief "time-out" to reassess that all paperwork is completed, that swabs are appropriately labeled with SN, and that all consent forms and swabs contain the same SN.

Laboratory methods for determining whether a patient has *S. aureus* colonization are described below in Section 7.B. *S. aureus* colonization will be defined as a culture from any anatomic site that grows *S. aureus*.

6. Allocation

Randomization and masking: This study has taken into consideration the guidelines for reporting of clinical trials as recommended by the CONSORT report to inform the study design. After recruitment and informed consent, parents will undergo pre-randomization screening. If both parents screen negative for *S. aureus* colonization, the neonate will be ineligible for the randomization and parents will be informed that they are not colonized at that time with *S. aureus*. If either parent screens positive for *S. aureus,* then both parents as a pair will be eligible for randomization to one of the two possible masked treatment arms. The neonate-parent "pair" will be the unit of randomization and each parent will be allocated to the same group if both consent. Because couples can re-expose each other after treatment (especially in households) we have chosen to treat both parents even if only one parent is colonized with *S. aureus*. To prevent potential feelings of guilt or one parent blaming another if only one parent is colonized, the parents will be told that one or both is colonized with *S. aureus* and they are eligible for randomization. Discordant parents will not be informed which is colonized and which is not colonized as we will treat both regardless.

Arm 1 will include assignment to masked intranasal mupirocin plus chlorhexidine gluconate cloths for topical antisepsis. Arm 2 will include assignment to masked placebo ointment and placebo cloths for skin antisepsis. Stratified permuted-block randomization will be performed using R statistical software to achieve balanced allocation of subjects within study site (Johns Hopkins Hospital vs. Bayview) and within strata of birth weight

(greater than or equal to or less than 1500g). Use of varying block sizes (4, 6 and 8) will decrease the risk of imbalance. Neonates of multiple gestations and their parents will be randomized as a single family unit. Investigators and participants will be masked to treatment assignment. The treating clinicians of the neonates will be masked to treatment assignment. The investigational drug pharmacy will use the randomization sequences to prepare supply kits. As subjects are enrolled, they will receive the next kit in sequence based on the study site and birth weight of the neonate. Each kit will contain either active drug or placebo. Active chlorhexidine-impregnated cloths, active mupirocin ointment and their respective placebos will be identified only by lot numbers, and the respective lot numbers will only be known to the investigational drug pharmacist.

7. Study initiation and baseline data collection

After results of the pre-randomization screening are completed, a member of the research team will contact the subject(s) to review the results and randomize the positive family units into either treatment or placebo group. After reviewing consent and the study with the parent(s), treatment will commence immediately upon assignment to treatment arm. At that time, the research team will perform the necessary baseline swabs to be sent for culture from the neonate. Neonates' screening cultures will be performed by obtaining a swab from the following anatomical sites: bilateral anterior nares, umbilicus, groin, and peri-anal area. Study team members will also gather residual samples from the clinical laboratory collected during routine clinical care. Parents will be asked about some demographic and medical history information for the researcher to complete on the case report form. Additional information will be obtained from the neonate's medical record (e.g. birth history, maternal prenatal labs).

Those neonates that test positive for *S. aureus* colonization at time of randomization will not be eligible for the primary outcome analysis and they will not contribute to the sample size required to power the primary outcome. Participants will be informed that their child is colonized with *S. aureus*, but participants will be asked to continue the assigned treatment, so secondary outcome data can be collected.

8. Discontinuation of Treatment and Subject Withdrawal

Discontinuation of Intranasal Treatment
Study treatment may be discontinued for any of the following reasons:

- If participant(s) exhibits severe or life threatening side effects (Grade 3 or higher) that require subject to seek medical care. Side effects include pain, blistering, urticarial rash or hypersensitivity, trouble breathing, loss of consciousness, swelling of the tongue and airways, or other life threatening reactions judged by the treating provider. If the treating

provider and/or PI determines that the reaction is not related to study treatment, the subject will restart the treatment.
- Subject(s) request discontinuation of treatment.

Discontinuation of Topical Bathing Treatment
Study treatment may be discontinued for any of the following reasons:

- If participant(s) exhibits severe, or life threatening side effects (Grade 3 or higher) that require subjects to seek medical care. Side effects include pain, blistering, urticarial rash or hypersensitivity, trouble breathing, loss of consciousness, swelling of the tongue and airways, or other life threatening reactions judged by the treating provider. Mild skin irritation such as itching, dryness, and transient redness will not be reasons to stop treatment. If the treating provider and/or PI determines that the reaction is not related to study treatment, the subject will restart the treatment
- Subject(s) request discontinuation of treatment

If reaction occurs to topical bathing, but not the ointment, then the participant may discontinue the bathing and continue the ointment application after discussion with the study team.

In the case that a subject discontinues study nasal and/or topical bathing treatment(s), the parent(s) and their neonate(s) will be followed for swab collection, bacterial cultures, and medical history data collection as outlined in the protocol until they meet the criteria for study completion, unless the parent withdraws from the study and/or withdraws consent from disclosure of future information. In this case, the investigator may retain and continue to use any data collected before the subject withdrew their consent.

Subject Withdrawal

Participants may withdraw from study for any of the following reasons:
- Subject requests withdrawal of consent for further participation in study
- Subject meets criteria for withdrawal based upon investigator discretion (e.g. safety, behavioral, or administrative reasons)

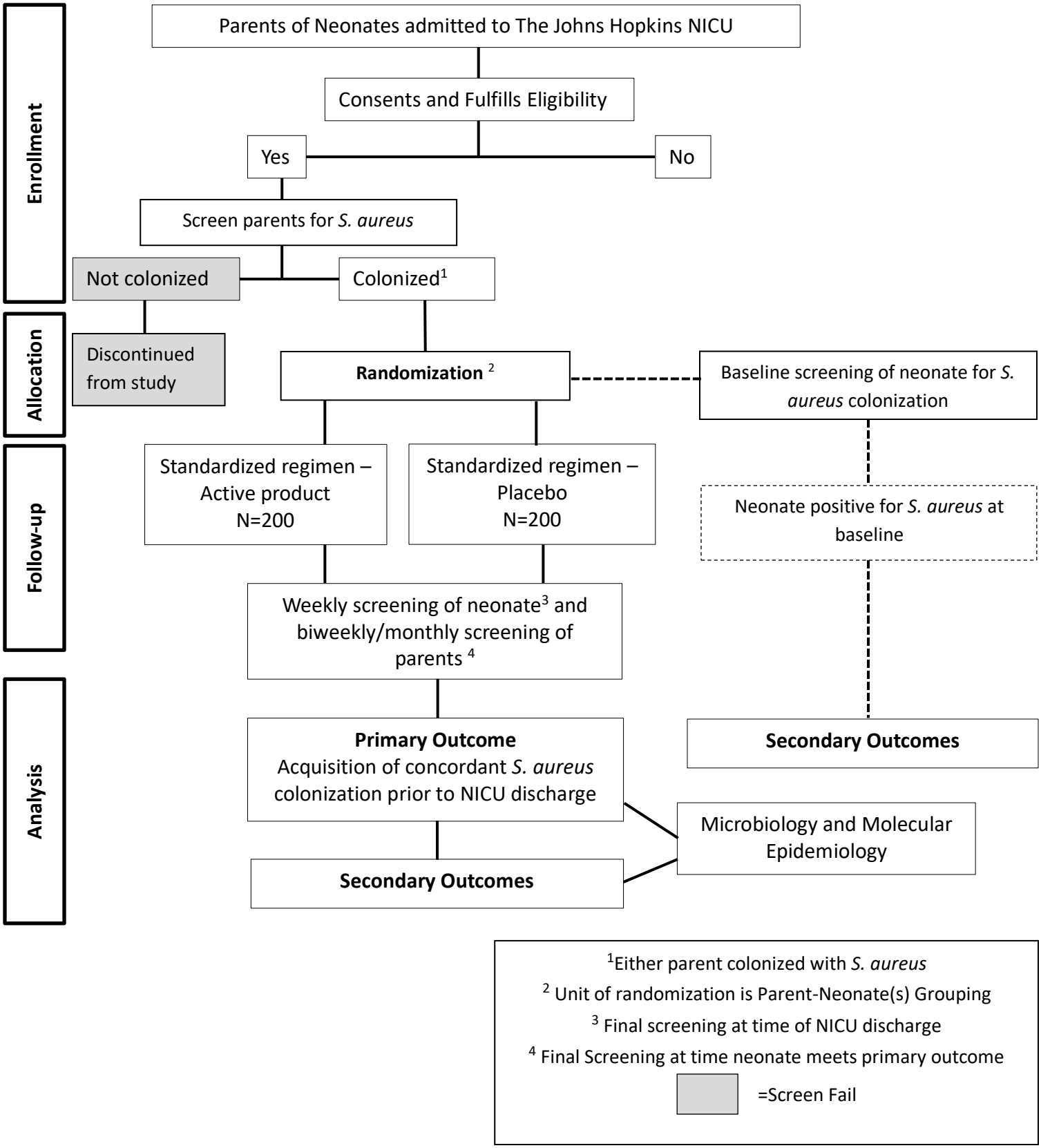
If patients discontinue treatment or withdraw from the study the primary reason for discontinuation/withdrawal must be provided and documented in the CRF. Discontinuation of treatment and/or withdrawal due to adverse event will be distinguished from withdrawal due to other causes and recorded on the appropriate adverse event CRF page.

9. Unblinding

In the event of a medical emergency where knowledge of the participant's blinded treatment is critical to their medical management, the blind may be broken by the investigator. Before breaking the blind, the investigator must determine whether or not knowledge of the blinded information will alter the immediate care of the participant. In some instances, it may be possible to

treat the participant by assuming they are receiving active product. The investigator will discuss the decision to unblind with the DSMB.
Blinding will only be broken for appropriate medical reasons such as managing treatment of a patient in an emergency or if required for reporting to regulatory authorities. Any event for which the study blind is broken will be reported as an SAE. The accompanying SAE report should include a comment about breaking the blinding.

Figure 1. Overview of Study Design



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D. Follow-up

Parent Evaluation Time Points: After randomization, the study team will provide the study drugs, study drug application instruction sheets and informational material. All participants will begin the 5-day treatment course at that time. During the 5-day treatment period, a study team member will contact the study participant daily at the bedside, on the phone, by email or by text message to assess compliance with treatment and to answer any questions. When available, parent(s) will be re-tested for colonization at 2-week intervals from randomization for the first 8 weeks and then every four weeks until discharge. Follow up swabs may be taken within a week of scheduled time points, based on participant availability. Anatomic sites to be tested and frequency of testing are detailed in Table 1. Peri-anal swabs will only be collected at enrollment and final screening. The final visit and testing will be performed at the time of a neonate's discharge from the NICU or at the time the child is identified to have acquired *S. aureus*.

Neonate Evaluation Time Points: After informed consent and randomization of parents, the neonate will undergo baseline testing to determine baseline *S. aureus* colonization status. This testing will occur on study day 1, the same day that parents begin treatment. Screening cultures will be performed by obtaining a swab from the following anatomical sites: anterior nares, the umbilicus, groin, and peri-anal area. If the nares site is not available to swab, the throat will be swabbed instead. Those neonates who test positive for *S. aureus* colonization at time of randomization will not be included in the primary analysis. After baseline testing, repeat testing will be performed every 7 days. Study samples will be saved and processed in the lab. The final visit and testing will be performed at the time of a neonate's discharge from the NICU.

Table 1

Frequency of Parent Swab Collection			
Site	Screening	Weeks 2, 4, 6, 8, 12, 16, 20, 24, 28,….	Time Neonate Acquires *S. aureus*
Nares	X	X	X
Throat	X	X	X
Groin	X	X	X
Peri-anal	X		X

Table 2

Frequency of Neonate Swab Collection			
Site	Parent Randomization	Weeks 1, 2, 3, 4,….	NICU Discharge
Nares	X	X	X
Umbilicus	X*	X*	X*
Groin	X	X	X
Peri-anal	X	X	X

*Umbilicus will only be tested if the neonate has attached umbilical cord.

5. Primary and Secondary Endpoints and Outcomes

A. Primary: Primary outcome is neonatal acquisition of *S. aureus* strain that is concordant to parental *S. aureus* strain as determined by periodic surveillance cultures or a culture collected during routine clinical care that grows *S. aureus*.

Acquisition will be defined as meeting two criteria:

1) A neonate who had baseline surveillance cultures that were negative for *S. aureus*
2) A neonate who has a subsequent surveillance culture or culture collected during routine clinical care that grows *S. aureus*.

Concordant strains must meet the following criteria:

1) Strains that are related using pulsed-field gel electrophoresis (PFGE) analysis, as described below. Isolates will be considered related if their patterns have ≤3 band differences. Isolates with >3 band differences will be considered epidemiologically different strain types. A neonate who has a subsequent surveillance culture or culture collected during routine clinical care that grows *S. aureus*.
2) Alternative typing methods will be used to further discriminate highly prevalent strains or those that are not typable by PFGE.
3) The same strain from the initial parent screening is identified from the neonate.

Primary outcome ascertainment – We will measure each neonate's baseline *S. aureus* colonization status at the time of study enrollment. As described above, neonates will then have swabs collected weekly after enrollment and at time of discharge from multiple anatomic sites by a study team member. We also will use data from cultures collecting during routine clinical care in the NICU. These cultures include cultures of nasal swabs collected every Tuesday as part of an active *S. aureus* surveillance program and other cultures collected during routine clinical care (e.g. blood cultures, respiratory cultures, wound cultures, etc.). Cultures collected by the study team and cultures collected during routine clinical care with both be used to identify *S. aureus* acquisition in neonates. Of note, nasal surveillance swabs collected as part of the hospital surveillance program are plated on selective media plates to detect *S. aureus* colonization, as previously reported [1].

Reducing the risk of bias in outcome ascertainment: Clinicians will continue to send cultures to evaluate neonates for possible infection as part of routine clinical management. The primary care providers in the NICU will not participate in the study and will be masked to the parent-neonate pair assignment. Maintaining masking of the clinicians will reduce the chance that practices for obtaining clinical cultures will differ between the two groups.

The **observation period, or survival time,** is the time that a neonate is at-risk of concordant *S. aureus* acquisition. We will calculate survival time as 1) the interval

between randomization and the date of final culture (either at time of final surveillance culture or at time of discharge culture) for neonates who did not acquire concordant *S. aureus* and 2) the interval between randomization and date of first positive culture for neonates that acquire concordant *S. aureus.*

B. Secondary outcomes of interest include:

1. Neonatal acquisition of *S. aureus* as determined by periodic surveillance cultures or a culture collected during routine clinical care that grows *S. aureus*.

Acquisition will be defined as meeting two criteria:

1) A neonate who had baseline surveillance cultures that were negative for *S. aureus*
2) A neonate who has a subsequent surveillance culture or culture collected during routine clinical care that grows *S. aureus*.

Outcome ascertainment – same as for primary outcome

Observation period or survival time – same as for primary outcome but does not require the *S. aureus* strain to match that of the parent.

2. Neonatal *S. aureus* infection as determined by cultures collected during routine clinical care

A *S. aureus* infection will be defined using criteria established by the Centers for Diseases Control and Prevention National Healthcare Safety Network and will be determined using results of cultures sent during routine clinical care (other than surveillance cultures).

Secondary outcome ascertainment - Two study team members that are blinded to treatment assignment will review all clinical cultures for *S. aureus* during the treatment and control period and determine if the culture represents an infection due to *S. aureus*. A third study team member will review the case in the event of discordant results.

The **observation period**, **or survival time, is the** time a neonate is at-risk of *S. aureus* infection. We will calculate survival time either by 1) the interval between randomization and the date the neonate is discharged from the NICU or death for neonates who did not develop a *S. aureus* infection or 2) the interval between randomization and date of positive culture for neonates that develop *S. aureus* infection. Neonates will not be included if they have an infection after meeting the primary outcome of concordant *S aureus* colonization or the secondary outcome of neonatal acquisition of *S aureus*.

3. Eradication of *S. aureus* colonization in parents following treatment

Outcome ascertainment – Parents will be monitored as described above in section 4.D to monitor *S. aureus* colonization over time. Parents will be tested at baseline, and then every two weeks for the first two months, if available, while neonate remains in the NICU, monthly starting on third month their neonate remains in the NICU, at the time the infant acquires *S. aureus* colonization in the NICU, and at time of discharge from the NICU for a neonate that did not acquire *S. aureus* colonization in the NICU.

4. **Natural history of *S. aureus* colonization in parents receiving placebo**

   Outcome ascertainment – same as above for parents receiving placebo

5. **Adverse reactions to treatment**

   Outcome ascertainment – Participants will be instructed to report any rash, blister, itching, redness, swelling or other skin irritation, swelling of the face or limbs, difficulty breathing or swallowing, and any significant discomfort or illness that occurs during treatment. Study staff will specifically ask for these symptoms during the 5-day treatment period and after the treatment period at Day 6, and will report any adverse event that is thought to be related to the study on the Adverse Event Log as well as any other perceived adverse events.

6. **Feasibility and Feedback**

   Outcome ascertainment - Using compliance data and parent reporting, we will also determine if the intervention is feasible for this population. Compliance with treatment is already being measured. In addition, after completing the 5 day intervention at Day 6, parents will be asked several questions about the ease of the cloth and ointment use as well as their comfort level and other feelings about the treatment. During the follow-up testing of parents, we will ask for any comments, suggestions or concerns from the parents about their general experience as a participant in the study. This data will be both quantitative and qualitative and can help determine if the intervention is feasible for future use. The study team may on occasion audio record responses to these questions.

7. **Attitudes and Behavior**

   Outcome ascertainment – In order to examine the impact of *S. aureus* education and knowledge of *S. aureus* colonization, all consented parents will be asked to complete a follow-up questionnaire. The questionnaire will assess differences in behavior between ineligible parents (negative for *S. aureus*) and eligible parents (at least one parent positive for *S. aureus* and enrolled in study). In the questionnaire, parents will be asked about their interactions and behaviors with their newborns in the NICU, whether their hand hygiene and/or bathing routines have changed, and about modifications made to medication use over the course of the four weeks since their initial screening visit.

6. Risks and Benefits

A. Potential Risks

The risks for this study are minimal. There is a very small risk of bleeding associated with swabbing mucosal membranes; however, our institution has a long standing history of obtaining nares surveillance cultures for *S. aureus* and peri-anal surveillance cultures to detect vancomycin resistant enterococci without any reported adverse events. Throat swabs can make people gag but are routinely performed during clinical evaluations of pharyngitis without adverse events. Standard precautions will be applied for protection of the research team member during specimen collection. All parent swabs will be collected in a private room. Groin and peri-anal swabs will be self-collected by parents following instructions from the study team. If the parents prefer, the groin and peri-anal swabs can be collected by a study team member. A gown will be provided for the parent to protect modesty and privacy during collection if applicable.

This decolonization procedure is widely used in individuals with *S. aureus* colonization in certain settings such as pre-operative, recurrent infections, and those in the intensive care unit. This treatment has been well tolerated in all populations, with rare adverse events. Allergic reactions to mupirocin and/or chlorhexidine are uncommon. Patients with known allergy to any product ingredient will be excluded from the study. All agents are FDA approved for the target population and we have received an FDA exemption from IND. A number of recent studies have applied decolonization to households rather than just individuals, as close contacts can serve as reservoirs for recurrent colonization after treatment. Based on reports that treating households versus just individuals may have a greater impact on *S. aureus*, we have decided to treat both parents even if one is not colonized. To protect parent's privacy and prevent potential feelings of guilt or one parent blaming the other, we will not disclose discordant results to parents. Therefore, care providers will not know if a particular parent is colonized simply based on enrollment in the study, as it might be "the other parent" that is colonized.

Mupirocin Ointment Possible Adverse Effects: Local adverse effects may be associated with mupirocin application. Mupirocin ointment may cause itching, pain, stinging, and burning. Local effects from the nasal formulation have included epistaxis, rhinitis, taste perversion, pharyngitis, burning, and cough. Gastrointestinal side effects have included nausea (1.1%), abdominal pain (<1%), and diarrhea (<1%). Dry mouth has been reported with mupirocin nasal (<1%). Nervous system side effects associated with intranasal mupirocin have included headache (9%). Dizziness has been reported with mupirocin ointment (<1%). Ocular side effects associated with mupirocin nasal ointment have included blepharitis (<1%).

Chlorhexidine Gluconate-impregnated Cloths Possible Adverse Effects: The soap may cause burning and dryness of the skin. It may cause burning of the eyes, but it's not recommended to be used above the neck. Skin erythema and roughness, dryness,

sensitization, allergic reactions are possible, but rare.

B. Protection against Risks

Protection against loss of information: We will institute strict procedures to maintain confidentiality. All patients will be assigned a study identification number. We will maintain a master list of patients with unique study identifiers. The master list will be maintained on a password protected computer or institutional network drive. Data will be entered into REDCap (http://www.project-redcap.org) which is a secure, web-based application designed exclusively to support data capture for research. The Johns Hopkins University is a member of the REDCap consortium and this application is freely available to consortium members. REDCap provides: 1) an intuitive interface for data entry (with data validation); 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages (SPSS, SAS, Stata, R); 4) procedures for importing data from external sources; and 5) advanced features, such as branching logic and calculated fields. To maximize quality control, staff will be trained by the PI in all data collection and entry procedures. The PI will have access to all data upon entry into REDCap. The research coordinator will monitor data collection by checking data for completeness. All research staff will be instructed regarding the security of data and will maintain the highest ethical standards in protocol adherence and data collection. After all data are collected, analyzed, and published, linkage between patients and their unique identifier will be destroyed in accordance with local regulations with the exception of data that have been collected for hospital surveillance activities. During the course of the study, information collected will not be disclosed to anyone other than the study personnel. No sensitive information will be collected. Following completion of each qualitative interview, all audiotapes (digital recordings) will be transcribed and marked with the session title and each subject's SID number. No identifying information will appear on the label of the media. All transcribed media will be stored on a secure Johns Hopkins server. Audio recordings with identifiers will be destroyed after being transcribed.

Protection against adverse events from treatment: As discussed above, treatment of *S. aureus* colonized individuals with intranasal mupirocin and chlorhexidine gluconate-impregnated cloths is routinely performed in many clinical settings. Very rare cases of anaphylaxis have been reported. Parents will be given written and verbal instructions for proper use. The research team will contact parents daily to review compliance with treatment, to answer questions, and to monitor for adverse events. Safety data including expected and unexpected events will be provided to the DSMB for review. Any unexpected events will be submitted immediately to the IRB.

The study team recognizes that subjects may seek advice about issues beyond the scope of this study and will refer all non-study related health issues to the patient's treating clinician.

C. Data Safety Monitoring Board

A Data Safety Monitoring Board (DSMB) will be assembled to oversee this study.

The DSMB will consist of the following members:

1. Anthony Harris M.D., M.P.H.: Dr. Harris is a Professor of Medicine and Epidemiology at the University of Maryland and will be a member of the DSMB for his expertise in *S. aureus*, epidemiologic methods, adult infectious diseases, and clinical trials.
2. Michael A. Rosenblum, Ph,D: Dr. Rosenblum is an Assistant Professor of Biostatistics in the Johns Hopkins Bloomberg School of Public Health. He is experienced in the design and analysis of clinical trials.
3. Mary Leppert, M.D.: Dr. Leppert is a member of the medical staff in the department of Neurodevelopmental Medicine at the Kennedy Krieger Institute in Baltimore, Maryland and an Assistant Professor of Pediatrics at Johns Hopkins University School of Medicine. Dr. Leppert is studying the parent experience in the NICU using the Parent Stress Index. She will serve as a member of the DSMB for her expertise in developmental pediatrics.
4. Neal Halsey, M.D: Dr. Halsey is a Professor of Pediatrics and International Health at the Johns Hopkins Bloomberg School of Public Health. Dr. Halsey will be a member of the DSMB for his extensive expertise in pediatric clinical trials and infectious diseases.

The DSMB will review safety data related to adverse events associated with treatment of parents and interim analysis of the primary outcome. The duties will include:

1. Meeting before study initiation to review the research protocol and plans for data safety monitoring.
2. Assessment of data quality and timeliness.
3. Monitor fidelity to study protocol
4. Review participant recruitment and retention
5. Protection of the confidentiality of the trial data and results of the monitoring.
6. Sharing recommendations and assessment with the investigative team.
7. Assessing any reported unexpected adverse events that may impact the safety of the trial.
8. Make recommendations to the IRB and investigative team concerning trial continuation or modification based on interim results as outlined below.

D. Disclosing results

Plan for Neonates Testing Positive for *S. aureus* and disclosure of results:
The Johns Hopkins Hospital has an active program to identify neonates that are colonized with *S. aureus*. A protocol exists such that colonized neonates are decolonized to reduce the spread of *S. aureus* in the NICU and the risk of infection in individual patients. If a culture collected during the research study grows *S. aureus*, this information will be provided to the clinical team.

Plan for Parents Testing Positive for *S. aureus* and disclosure of follow-up results:
Upon enrollment, all subjects will be provided a brief structured orientation and education session regarding *S. aureus* by a member of the research team. All patient questions will be answered and educational handouts will be provided with references for additional information. Part of the informed consent will include an understanding that parents will be made aware of their *S. aureus* colonization status. If either or both parents are colonized, parents will be informed that one or both parents are colonized,

but we will not share which parent to protect confidentiality. By participating in the trial, others may also become aware that one of both of the parents are colonized simply by their participation in the trial. We will explain that approximately 30% of healthy adults are colonized with *S. aureus*. We do not take special precautions or treat parents differently that are colonized with *S. aureus*. Additionally, we will enroll both parents even if only one parent is colonized, which will aid in protecting the privacy of participants as care providers will not know if a particular parent is colonized simply based on enrollment in the study, as it might be "the other parent" that is colonized.

Once parents are randomized to an assigned treatment, they will continue to be screened for *S. aureus* colonization throughout the study. The results of subsequent testing will not be made available to the parent. Disclosing subsequent testing results may lead to unmasking of the study. Parents will be informed that these results will not be available until the study is complete and the randomization code is un-blinded for data analysis. Parents will be provided with a means to contact the study team to learn their testing results and/or randomization group.

Parents with discordant culture results should not become aware of each other's colonization status. To decrease or eliminate guilt issues, the study team will not disclose which parent is colonized and will educate parents on the ubiquity of *S. aureus* and emphasize that parents are only one possible source of transmission.

E. Potential Benefits

There may be no direct benefit to the subjects in this study; however, information obtained in this research may lead to a better understanding of how neonates acquire *S. aureus* in the NICU and whether eradicating colonization in parents reduces neonatal *S. aureus* acquisition and infection.

7. Specimen Collection and Laboratory Methods

A. Specimen Collection

Swabs will be collected as outlined below:

Neonates

Using standardized methodology, four flocked swabs will be obtained from

1. Bilateral nares: by inserting a swab 0.5-1cm into each naris and rotating the swab 3 times (if nares is not available, study team will collect a throat swab instead by inserting a swab in the pharynx and swabbing the region of the tonsils with a quick rotating motion three times on each side).
2. Groin: The groin will be swabbed in a back and forth motion 3 times on each side.
3. Umbilicus: For children who still have an umbilical stump, the base of the umbilical cord will be swabbed in a circular motion 3 times (if there is no umbilical cord, there will be no umbilicus swab).

4. Peri-anal area: The peri-anal area will be swabbed in a circular motion 3 times

Parents

Using standardized methodology, four flocked swabs will be obtained from

1. Bilateral nares: Study staff will collect these by inserting a swab 0.5-1cm into each naris and rotating the swab three times
2. Throat: Study staff will collect this by inserting a swab in the pharynx and swabbing the region of the tonsils with a quick rotating motion three times on each side.
3. Groin: Parents will be instructed by study staff on how to collect these samples. Parents will be able to choose whether they prefer to self-collect the swab or have the study team member collect the swab.
4. Peri-anal: Parents will be instructed by study staff on how to collect these samples. Parents will be able to choose whether they prefer to self-collect the swab or have the study team member collect the swab.

B. Laboratory Methods

Processing of cultures collected by the study team

Parents' Screening Cultures:

1. An aliquot (10µL) from each culture specimen will be plated on MSSA Select (Bio-Rad Laboratories, Redmond, WA) selective and differential medium to detect *S. aureus*. Presence of mauve colored colonies after 24 hours of incubation will be confirmed as *S. aureus* by Gram's stain and coagulase testing. In parallel, 10µL of each specimen will also be plated on a sheep blood agar plate. Residual specimen (Amies media) will be transferred to cryovials and stored at -70^{0}C. All cultures that grow *S. aureus* will be archived in Tryptic Soy broth with 20% glycerol at -70^{0}C
2. To enhance detection of *S. aureus,* an aliquot (100µL) of medium will be inoculated into Tryptic Soy broth with 6.5% NaCl and incubated overnight for 18-24 hours at 35^{0}C. 10µL of broth will be inoculated onto sheep blood agar and MSSA Select (Bio-Rad Laboratories, Redmond, WA) and processed as described above to detect *S. aureus*. Isolates recovered by this method will be archived in Tryptic Soy broth with 20% glycerol at -70^{0}C.
3. To distinguish methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA), isolates will be tested on the BD Phoenix Microbial identification system and with other phenotypic testing methods. Screening cultures obtained from parents will not be tested in real-time to distinguish MRSA and MSSA. Parents who are colonized with MRSA will not be identified. There is no standard of care in terms of how to manage parents or visitors that are colonized with MRSA. We do not screen visitors and there is no consensus that colonized visitors should be treated any differently other than following standard precautions (hand washing, covering wounds, etc.).

Neonates' Baseline Colonization Cultures:

Specimens from neonates will be processed as described in 7.B.2-7.B.3 above (no direct plating, i.e. all specimens will be inoculated into broth before plating).

Follow-up Cultures for both Neonates and Parents:

Follow-up cultures for both neonates and parents will be processed as described in 7.B.2-7.B.3 above (no direct plating).

Processing of cultures obtained during routine clinical care

1. As usual, all cultures sent to the Johns Hopkins Microbiology laboratory will be processed according to Clinical and Laboratory Standards Institute guidelines. All clinical cultures that grow *S. aureus* will be archived in Tryptic Soy broth with 20% glycerol at -70^{0}C.

***S. aureus* strain typing:**

1. Strains of *S. aureus* will be compared using pulsed-field gel electrophoresis (PFGE) analysis. DNA will be extracted by standard methods and the restriction enzyme digested using *Sma*1. A *Staphylococcus aureus* subspecies NCTC 8325 will be used as a control strain with a molecular weight ladder at the beginning and end of each gel. Control isolates, including all USA PFGE strain types, will be used for comparison. Using 1% pulsed-field certified agarose gel (BioRad Laboratories, Hercules, CA) electrophoresis will be performed on the CHEF-DR III (BioRad Laboratories, Hercules, CA) with 0.5X TBE buffer at 14^{o}C. The gels will be stained with ethidium bromide and scanned using a molecular analysis fingerprinting software (Fingerprinting II Version 3.0; BioRad Laboratories, Hercules, CA). If PFGE using standard enzymes was unable to yield an interpretable fingerprint, PFGE was then performed using an alternative method utilizing Achromopeptidase (Wako Bioproducts, Richmond, VA) as the lysis enzyme in order to obtain an interpretable fingerprint. Gel images will be stored and within and between run comparisons will be performed for each group of tested isolates. Isolates will be considered related if their patterns have ≤3 band differences. Isolates with >3 band differences will be considered epidemiologically different strain types.

2. Alternative *S. aureus* strain typing method: Because some *S. aureus* strains are genetically similar by PFGE, such as strain USA300, validation of highly prevalent and concordant strains will be carried out using a separate assay such as a PCR assay coupled with electrospray ionization-mass spectrometry (PCR/ESI-MS), whole genome or next generation sequencing, or multi-locus sequence typing. Isolates with no band differences will be considered identical, but those with 1-3 band differences will be considered for additional testing based on strain prevalence.
3. If strains could not be distinguished by PFGE analysis, multi-locus sequence typing (MLST) will be performed using whole genome sequencing. After DNA extraction, a sequence library will be generated using the Nextera DNA Library Preparation Kit and sequenced by Miseq (Illumina Inc, San Diego, CA). Sequenced fragments will be assembled using SPAdes and aligned to reference

S. aureus genome with Pavian (Johns Hopkins University, Baltimore, MD) for quality assessment. Strain type will be assigned by The Bacterial Analysis Pipeline (Illumina Inc, San Diego, CA) based on the alleles of seven housekeeping genes, including arcc, aroe, glpf, gmk, pta, tpi, and yqil. Two isolates will be considered concordant if both had identical alleles for all seven genes.

8. Data Collection and Data Monitoring

Prior to any work being performed on this research proposal it will be submitted for review and approval by the Institutional Review Board at JHU. We will maintain a master list of patients with unique study identifiers. The master list will be maintained on a password protected computer or institutional network drive. Data will be entered into a database that will be stored on a secure server at the Johns Hopkins University. All research staff will be instructed as to how to maintain the highest ethical standards in protocol adherence and data collection. Standard backup procedures are in place at the institution to prevent catastrophic loss of data. Validity and accuracy checks will be put in place as data are entered into the database using preset field choices where applicable. Edits of the data will be performed routinely to look for missing data, outliers, unusual or inconsistent values. We will collect information on all neonates and parents enrolled in the study.

For parents, using standard questions that will be incorporated into the case report form, we will collect their date of birth, race, ethnicity, gender, educational level, underlying medical conditions (especially skin diseases that predispose to *S. aureus* colonization), current medications, exposure to recent antibiotics, recent healthcare exposures, other household members, past use of mupirocin, and dates of, and treatments for any prior *S. aureus* infections.

For neonates, we will collect basic demographic information on all patients, including chronologic age (date of birth), birth weight, mode of delivery, gestational age, race, ethnicity, gender, antimicrobials (antibiotics, antifungals, antivirals) administered, other interventional or clinical medicines that may impact *S. aureus* colonization, presence of central venous catheter, respiratory support, feeding method (breastfeeding, orogastric tube, nasagastric tube), dates of NICU admission and discharge, primary reason for admission (primary condition), where neonate was admitted from (home, other hospital, inborn), procedures, number of trips to the operating room or radiology, on NP or resident service, number of consulting teams, APGAR scores, practice and timing of kangaroo care, days in isolette vs. open crib or bed, dates and results of all *S. aureus* surveillance cultures and other cultures sent during routine clinical care.

Compliance with Hand Hygiene

The JHH and JHBMC Departments of Hospital Epidemiology and Infection Control monitor compliance with hand hygiene on a monthly basis. We will have access to these data.

Adverse events collection and reporting

As described above, participants on treatment will be instructed to report any rash, blister, itching, redness, swelling or other skin irritation, swelling of the face or limbs, difficulty breathing or swallowing, and any significant discomfort or illness that occurs during treatment. Study staff will specifically ask for these symptoms every day while monitoring compliance with treatment and will report any adverse event that is thought to be related to the study on the Adverse Event Log as well as any other perceived adverse events. Neonates will be monitored for Serious Adverse Events.

Adverse Event Definitions -

Adverse Event (AE): An adverse event will be defined as an unusual and undesirable symptom or sign that occurs in parent participants during the clinical study. Adverse events include those clinically significant laboratory values and test results, concomitant illness, accident, medical occurrence or worsening of existing medical condition that emerge during study participation.

Serious Adverse Event (SAE): A Serious AE is any untoward medical occurrence that at any dose produces any of the following outcomes in neonate and parent participants:

- Results in death;
- Is life threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);
- Requires inpatient hospitalization or causes prolongation of existing hospitalization (see NOTE below for exceptions);
- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly/birth defect (note: reports of congenital anomalies/birth defects must also be reported on the Pregnancy Supplemental Form);
- Is an important medical event (defined as a medical event(s) that may not be immediately life threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [e.g., medical, surgical] to prevent one of the other serious outcomes listed in the definition above). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)

NOTE: For the purposes of this trial, the following hospitalizations will not be considered SAEs:

- Admissions as per protocol for a planned medical/surgical procedure;
- Routine health assessment requiring admission for baseline/trending of health status (e.g. post-partum complications, such as wound infection or bleeding);
- Medical/surgical admission for purposes other than remedying ill health state and was planned prior to entry into the study. Appropriate documentation is required in these cases;

- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, care-giver respite, family circumstances, administrative).

Non-Serious Adverse Event: A non-serious adverse event is any adverse event not classified as serious. All non-serious AEs will be reported to the IRB on an annual basis with the continuing review.

Adverse Event Grading: The Division of AIDS Table of Grading Severity of Adult and Pediatric Adverse Experiences (DAIDS) Version 2 will be used to assess severity and provide the grade for each AE that is reported. The investigator will evaluate the severity of any adverse event not identified within the below criteria using the following definitions:

- **Mild (Grade 1)**- Mild symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated
- **Moderate (Grade 2)**- Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated
- **Severe (Grade 3)** - Severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated
- **Potentially life threatening (Grade 4)** - symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability.
- **Death (Grade 5)**: Indicates death.

AEs graded as Grade 3 or higher which are possibly, probably, or definitely attributable to the use of the investigational drug will be recorded and monitored until the event has resolved to meet the definition of 'mild'.

All adverse events will be further evaluated for attribution as per the following:

- **Unrelated**: Adverse event is clearly not related to the investigational agent
- **Unlikely**: Adverse event is doubtfully related to the investigational agent
- **Possibly**: Adverse event is possibly related to the investigational agent
- **Probably**: Adverse event is probably related to the investigational agent
- **Definitely**: Adverse event is definitely related to the investigational agent

Participants who withdraw from the study, but who have AEs grade ≥ "moderate" which are possibly, probably, or definitely attributable to the investigational drug will have AEs recorded and monitored until the event has resolved.

9. Statistical Analysis Plan

A. Sample size and power estimates:

The primary outcome will be time to concordant *S. aureus*-colonization. Time to concordant *S. aureus* colonization will be expressed using an indicator variable for concordant colonization and time to concordant colonization. The a priori assumed

control group concordant colonization rate is 10% and power calculations are based on the unadjusted Cox proportional hazards model where the primary covariate is the treatment group indicator. In time to event analyses, power is driven by the number of events; i.e. number of concordant colonizations. Given our assumed control group concordant colonization rate of 10%, 38 events are required to detect a 60 percent reduction in the hazard of concordant colonization (Hazard Ratio: HR 0.4) in the intervention group with 80% power and Type I error rate of 5%. The 38 events includes a roughly 2% inflation for pre-planned interim analyses (see Section 9.B). Given that we expect 5% of the parent-neonate pairs will be non-singleton births, we increased the number of required events to 40 to account for the possible clustering of concordant colonization among non-singleton neonates. Some neonates may test positive at baseline for *S. aureus* after randomization and will not be eligible for the analysis of the primary outcome. Similarly, if one neonate from a multiple gestation birth is positive for *S. aureus* at baseline and another neonate is not, then the negative infant will be eligible. Eligible parent-neonate pairs will be assigned to either treatment or control as a unit until 40 events are confirmed. Prior to the start of the study, given our *a priori* assumption of a 10% concordant colonization rate in the control group and a HR of 0.40, we anticipated recruiting 400 parent-neonate pairs to achieve the required 40 concordant colonizations. With 400 parent-neonate pairs, we have roughly 80% power to detect an absolute risk difference of at least 7% for the secondary analysis of the indicator of concordant colonization within 90 days after randomization.

Table 3. Power estimates based on anticipated outcomes

Assumption about outcomes among twins	Time to concordant colonization		Power to detect a difference if concordant colonization rate in treated group is 2%
	HR detectable with 90% power	HR detectable with 80% power	
Independent (38 outcomes)	0.35	0.40	0.89
Dependent, 5% twins (40 outcomes)	0.35	0.40	0.87
Dependent, 10% twins (42 outcomes)	0.34	0.39	0.85

B. Interim Analyses

Several interim analyses will be performed. We will perform interim analyses for efficacy based on the number of accumulated primary outcomes. Specifically, we will perform an interim analysis after 20 and 30 neonates have been identified to have the primary outcome of concordant colonization. After accruing 20 neonates with the primary outcome, the standard Cox proportional hazards model with main effect of treatment will be fit to estimate the treatment effect, i.e. the hazard ratio comparing the hazard of concordant colonization comparing the intervention and control groups, and the test statistic for the treatment effect will be computed using the methods described in Section

9.C. The DSMB will review the results of the Cox proportional hazards model to determine if the trial will stop for efficacy. The **minimum** criteria for stopping for efficacy will be if the test statistic for the hazard ratio is less than $Z_r = -2.74$. If the trial continues, after accruing 30 neonates with the primary outcome, the Cox proportional hazards model will be fitted. The DSMB will review the results of these statistical analyses to determine if the trial will stop for efficacy. The **minimum** criteria for stopping for efficacy will be if the test statistic for the hazard ratio is less than $Z_r = -2.36$. If the trial continues, after accruing the 40 neonates with the primary outcome, the treatment effect will be deemed statistically significant if the test statistic for the primary analysis as described below falls within the rejection region defined by $Z_r = -2.03$.

To define the above rejection regions, we applied an approximation to the error spending function of O'Brien and Fleming ($f(t) = min\{\alpha t^3, \alpha\}$) such that we spend the overall Type I error rate of 0.05 as 0.0062, 0.0148 and 0.029 after accruing 20, 30 and 40 patients, respectively. The derived rejection regions at each interim analysis (i.e. Z_r) are based on the hazard ratio (equivalent to the log-rank test comparing the survival experience in the treatment groups) using an approximation to the information available at each interim analysis (i.e. number of accumulated events at the interim analysis divided by 4).

In addition to the interim analyses conducted to determine whether the trial should stop early for efficacy, the DSMB may request additional analyses to be performed to determine whether the trial should stop for futility after accruing 20 and 30 concordant colonizations.

C. Statistical analysis plan

Final analysis methods and changes in the analyses from those described in the protocol will be documented in the Statistical Analysis Plan prior to locking the database and unmasking the assignment. A CONSORT flow diagram will be created. In addition, descriptive statistics will be calculated to describe the feasibility of the study including number of parent-neonate units approached by a study team member, proportion of parents that consent, proportion of parents colonized among consented, proportion of parents colonized who subsequently do not complete the treatment, and the distribution of adherence, as defined below, to the randomized treatment.

Analyses will be conducted according to the modified intention to treat (mITT) principal. The same day parents are randomized, the neonate will undergo baseline testing to determine baseline *S. aureus* colonization status. Neonates who test positive for *S. aureus* colonization at the time of randomization do not satisfy the inclusion criteria and will not be included in the analysis; hence defining the modified intention to treat analysis. Exploratory analyses of baseline characteristics for the treatment groups will be evaluated by Student t test for continuous data and Pearson's χ^2 test or Fisher Exact test for categorical data. No missing data should occur for baseline parent and neonate variables. No missing primary or secondary outcomes are expected. However, it is possible that neonates have no subsequent cultures between the baseline culture and NICU discharge, e.g. a neonate enters the study on Friday and is discharged on Sunday prior to their scheduled surveillance culture (Friday in this example) or discharge culture. Based on our definition of observation time (see Sections 5.A and 5.B), such neonates

will contribute no observation time for the analysis of the primary and secondary outcomes. A sensitivity analyses will be conducted where the observation time for these neonates will be defined as the time from randomization to NICU discharge. All tests will be 2-sided with a type-1 error rate set at 0.05. Data will be managed and analyzed using Stata, version 13.0 (Stata Corp., College Station, TX) and R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria, 2014).

The primary analysis will evaluate our hypothesis that detection and treatment of *S. aureus* colonized parents with intranasal mupirocin and topical chlorhexidine gluconate bathing (Treatment Group) will decrease the risk of a child acquiring *S. aureus* with a concordant strain in the NICU within 90 days of randomization relative to the control parents (Control Group). The parent-neonate pair will be the unit of measure. For neonates that do not acquire concordant colonization within 90 days of randomization, their survival time will computed as the time from randomization to the date of final culture (surveillance or discharge) within 90 days of randomization. We will define the treatment effect as the relative hazard of concordant colonization comparing the Treatment to Control group and will estimate this hazard ratio using the standard Cox proportional hazards model that includes only a main term for treatment. To account for the possibility of clustering within multiple gestation parent/neonate dyads, standard error estimates and bias-corrected and accelerated (BCa) confidence intervals for the log-hazard ratio will be derived using a bootstrap procedure. To preserve the correlation structure within the dyads, the bootstrap procedure will generate 10,000 bootstrap samples by sampling with replacement the parent/neonate dyads, as opposed to resampling the neonates. The analysis described above will be conducted at each pre-planned interim analysis with the test statistic derived from the estimated log hazard ratio from the Cox model and corresponding bootstrap standard error estimate, and the confidence level for the BCa bootstrap confidence intervals will be determined by the rejection region definitions specified in the Interim Analyses section (see Section 9.B).

Planned analysis of secondary outcomes will include the following: a) Repeat the primary analysis where time to concordant colonization will not be censored at 90 days after randomization; time will be defined as the time from randomization to the first of concordant colonization or last available culture (clinical, surveillance or NICU discharge), b) Define the treatment effect as the difference in the proportion of neonates acquiring concordant *S. aureus* by 4 and 8 weeks after randomization comparing the Treatment to Control group; this treatment effect will be estimated by taking the difference in the observed proportions comparing the Treatment and Control groups with standard error estimates and confidence intervals derived from the bootstrap procedure described above, c) Using the methods described above, estimate the treatment effect on the acquisition of *S. aureus* (regardless of concordant status), treated as both time to acquisition and the binary indicator for any acquisition by 4 and 8 weeks, and d) Using the methods described above, estimate the treatment effect on neonatal *S. aureus* infection, treated as both time to infection and the binary indicator for infection by 4 and 8 weeks, For the analysis of the S. aureus infection, we will first consider only *S. aureus* infections that occur prior to a neonate being identified as acquiring *S. aureus* colonization. For example, if a neonate acquires *S. aureus* colonization and then has a subsequent infection 7 days later, this infection will not be included. Only clinical cultures that occur before or on the same day as the first positive surveillance culture will be

considered. The NICU has a policy to decolonize all *S. aureus* colonized neonates which will impact the risk of infection in those that are first identified as colonized. Despite the possible independent impact of neonate decolonization on *S. aureus* infection risk, we recognize the importance of reporting all *S. aureus* infections in study patients; therefore, we will repeat the analysis, including *S. aureus* infections that occur at any time during a neonate's admission in the NICU. Lastly, a descriptive analysis of neonate mortality and bloodstream infections will be conducted, estimating the rate of occurrence separately for each treatment group.

Adverse events, as defined in Section 8, will be reported overall and separately by treatment group. Comparisons across treatment groups will be made using Fisher's Exact tests.

In addition, a series of planned secondary analyses will be conducted including subgroup analyses for the primary outcome, baseline-covariate adjusted analyses for the primary and secondary outcomes, per-protocol (PP) analyses for the primary and secondary outcomes and evaluation of patterns of loss and reacquisition of *S. aureus* among the parents (both treated and control).

Planned subgroup analyses include repeating the analysis for the primary outcome within strata defined by i) neonates with MRSA vs. those with MSSA, ii) collection site of colonization for the parent/caregivers, categorized as nares alone (i.e. all cultured parent/caregivers colonized in the nares) vs. nares + other (i.e. at least one parent/caregiver colonized in the nares) vs. non-nares; iii) LGA (large for gestational age) vs. SGA (small for gestational age); and iv) birthweight (< 1500g vs. ≥ 1500g). The subgroup analyses will be based on Cox proportional hazards models that include a main term for treatment, a main term for subgroup (binary indicator) and the interaction between treatment and subgroup. Confidence intervals and the hypothesis test for no interaction will be derived based on the bootstrap procedure defined above.

We will explore the prognostic power of three pre-specified baseline covariates collected at the time of NICU admission which, if found to be prognostic, could be used in subsequent trials to improve the precision of the estimated treatment effect (Rubin et al 2008). Specifically, we will derive baseline-covariate adjusted estimates of the treatment effects defined for the primary and secondary outcomes. The baseline covariates include birth weight (treated as a continuous variable), an indicator for whether the neonate was born at the Johns Hopkins Hospital or the Johns Hopkins Bayview Medical Center (inborn) or admitted to the NICU from home or an outside hospital (outborn). For the survival outcomes, we will utilize the method developed by Lu and Tsiatis (2008) implemented in the R package "speff2trial". The method produces an estimate of the relative hazard of concordant colonization comparing the Treatment to Control group analogous to the hazard ratio parameter obtained from fitting a Cox proportional hazards model with a treatment indicator as the only covariate. In addition, the test for this relative hazard equates to applying the log-rank test in the case of proportional hazards. The method relies on constructing models for the treatment indicator and censoring indicator that account for chance imbalance in the selected baseline covariates. We will include all of the selected baseline covariates in the analysis as main effects. The large sample property of this estimator guarantees that if any of the selected baseline covariates are correlated with the outcome, the method yields an estimate of the relative

hazard that has statistical variance that is smaller or equal to the results of the Cox model. The estimate of the treatment effect will be constructed using the neonate as the unit of analysis. Similar to the methods proposed for the primary and secondary analyses, standard errors and confidence intervals for the covariate-adjusted estimate of the treatment effect will be based on a bootstrap approach to preserve the correlation structure within multiple gestation dyads. For the binary outcomes, e.g. acquiring concordant *S. aureus* by 4 weeks after randomization, we will utilize novel methods proposed by Rotnitzky et al in 2012 and described in further detail in Colantuoni and Rosenblum (2014) that account for the specified baseline covariates. Similar to the methods proposed by Lu and Tsiatis (2008), the goal is to leverage information in the baseline covariates to produce an estimate of the treatment effect (i.e. absolute risk difference) that is as precise as or more precise than the unadjusted estimator of the treatment effect. We will follow exactly the algorithm detailed in Colantuoni and Rosenblum (2014) where the model for the treatment indicator we will be a logistic model with main effects of the specified baseline covariates.

We will conduct a per protocol (PP) analysis. Compliance to treatment will be defined first for the individual parent and then for the family or parent/neonate dyad (Table 4). Individual parents will be classified as: confirmed compliant where the parent returns a treatment kit with ointment tubes with a broken seal; reported compliant where the parent gave responses to questions about feasibility and potential adverse effects (see Section 4.B compliance monitoring); and non-compliant where a) kits were returned unused, b) the parent reported non-use, or c) parent was lost to follow up without reporting use. We will define *compliance to treatment* for the individual parent as confirmed compliant, with a sensitivity analysis including reported compliant in the definition of *compliance to treatment*. Family compliance will be defined as both parents satisfying the *compliance to treatment* definition, with a sensitivity analysis where we include partially compliant families, i.e. if the parent identified to be colonized with *S. aureus* is compliant based on the individual parent definition and the non-colonized parent is non-compliant. Baseline characteristics of the parents, including age, race, education level, and recent antibiotic use, will be compared across the individual parent compliance definitions, overall and separately by treatment group. Baseline characteristics of the ITT and PP neonates will be compared, overall and separately for each treatment group. The analysis of the primary and secondary outcomes described above will be repeated among the subset of parent/neonate dyads who are observed to be compliant. Note, that we will assume that all placebo group patients are compliant. We will conduct the PP subset analysis using this assumption and then separately incorporating the compliance definitions above to the placebo group as well. Conditioning on compliance, a post-randomization variable, may introduce bias into any treatment comparisons. Therefore, we will also implement a causal inference approach using available baseline parent and neonate variables within an inverse probability weighted approach to estimate the treatment effect for the primary and secondary outcomes, where the treatment effect compares what would have happened if all patients receiving the treatment were compliant vs. if all patients receiving the placebo were compliant.

Table 4: Definition of individual parent and family compliance to treatment

Individual parent compliance assessment		
Category	Sub-category	Definition
Compliant	Confirmed compliant	seal broken on nasal ointment
	Reported compliant	participants reported use
Non-compliant	Confirmed non-compliant	kits were returned unused
	Reported non-compliant	parent reported non-use
	Presumed non-compliant	parent was lost to follow up without reporting use
Family- level compliance assessment		
Category	Individual 1 compliance	Individual 2 compliance
Compliant	Compliant	Compliant
Partially compliant[+]	Compliant (SA colonized)	Non-compliant (not SA colonized)
Non-compliant	Compliant (not SA colonized)	Non-compliant (SA colonized)
Non-compliant	Non-compliant	Non-compliant

[+]partially compliant if *S. aureus* colonized parent is compliant, even if the non-colonized parent was non-compliant

Lastly, within each treatment group, we will use standard survival analysis methods (e.g. Kaplan-Meier survival function curve) to explore the loss of and potential reacquisition of *S. aureus* among the parents, separately for each treatment group. Cox proportional hazards models will be used to describe the association between loss of and reacquisition of *S. aureus* with baseline parent characteristics and treatment.

10. Payment and Remuneration

Each study participant will receive gift cards worth $10 dollars at screening, when each kit is returned, if the participant completed treatment, and at Week 4, or at the final surveillance screening, whichever occurs first, up to a maximum of $30 total.

11. Costs

There are no direct costs to the patient in this study. All study related supplies will be provided.

References:

1. Akinboyo, I.C., et al., *Epidemiology and risk factors for recurrent Staphylococcus aureus colonization following active surveillance and decolonization in the NICU.* Infect Control Hosp Epidemiol, 2018. **39**(11): p. 1334-1339.