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Epidemiology and Risk Factors for Recurrent *Staphylococcus aureus* Colonization following Active surveillance and Decolonization in the NICU

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Abstract

Objectives: To examine neonatal risk factors associated with recurrent *Staphylococcus aureus* (*S. aureus*) colonization and to determine the genetic relatedness of *S. aureus* strains cultured from neonates before and after decolonization.

Study Design: Single-center retrospective cohort study of neonates admitted to the Neonatal Intensive Care Unit (NICU) from April 2013 to December 2015, during which weekly nasal cultures from hospitalized NICU patients were routinely obtained for *S. aureus* surveillance.

Setting: Johns Hopkins Hospital's 45-bed level IV NICU in Baltimore, Maryland.

Methods: Demographics and clinical data were collected on all neonates admitted to the NICU with *S. aureus* nasal colonization who underwent mupirocin-based decolonization during the study period. A decolonized neonate was defined as a neonate with 1 negative culture after intranasal mupirocin. Pulsed-field gel electrophoresis was used for strain typing.

Results: Of 2060 infants screened for *S. aureus*, 271(13%) were colonized, and 203(75%) of 271 received intranasal mupirocin. Of those treated, 162 (80%) had follow-up surveillance cultures and 63 (39%) of the 162 infants developed recurrent colonization after treatment. *S. aureus* strains were often genetically similar before and after decolonization. Presence of an endotracheal tube or

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Conflict of interest

AMM, AV, KCC received grant support from The Agency for Healthcare Research and Quality (AHRQ). AMM received prior grant support from Sage Products LLC outside the submitted work. KCC reports other support from GenePOC, Inc., GenMark, Inc and Curetis, Inc., outside the submitted work. Other authors report no conflict of interest

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nasal cannula/mask was associated with an increased risk of recurrent *S. aureus* colonization (HR 2.65 95%CI: 1.19 – 5.90 and HR 2.21 95%CI: 1.02 – 4.75, respectively).

Conclusion: Strains identified before and after decolonization were often genetically similar and the presence of invasive respiratory devices increased the risk of recurrent *S. aureus* nasal colonization in neonates. To improve decolonization efficacy, alternative strategies may be needed.

INTRODUCTION

Staphylococcus aureus (*S. aureus*) remains the leading cause of pediatric nosocomial infections and a significant threat to hospitalized neonates in the United States.¹

Staphylococcus species, which constitute early components of the neonatal skin, nares and gastrointestinal microbiomes, continue to be responsible for the majority of neonatal late-onset sepsis (LOS).^{2–5} *S. aureus* colonization often precedes invasive infections such as central line associated blood stream infections (CLABSI), bacteremia, pneumonia, and on rare occasions meningitis, putting premature infants at risk for long term neurological sequelae.^{4,6–8}

Historically, measures to decrease or eradicate *S. aureus* colonization, such as the use of intranasal mupirocin, have focused on methicillin resistant *S. aureus* (MRSA). However, rates of methicillin susceptible *S. aureus* (MSSA) infections in the neonatal intensive care unit (NICU) outnumber MRSA and infection-related mortality can be as high as 25% in very low birth weight infants (VLBW, weight <1500g).^{2,9} Active surveillance cultures (ASC) and decolonization of colonized neonates have led to reduced rates of *S. aureus* transmission and infections^{10–13}, an approach that benefits the vulnerable neonate and concurrently, the general NICU population.^{11,14}

Despite the success of decolonization as a strategy to reduce nosocomial infections, hospitalized neonates may develop recurrent *S. aureus* colonization which increases the risk for ongoing transmission and infection within the NICU.^{12,15,16} A previous study noted that up to half of the decolonized neonates who remain in the NICU for > 21 days may develop recurrent MRSA nasal colonization.¹⁷ Recurrent *S. aureus* colonization could be secondary to persistent endogenous reservoirs, environmental reservoirs (i.e. healthcare workers, contaminated surfaces, parents) or may be as a result of antimicrobial resistance.^{14,18,19} There are no available data describing which factors may impact the risk of recurrent *S. aureus* colonization in neonates. Our objectives were to identify neonatal risk factors associated with recurrent *S. aureus* colonization after decolonization and to examine the genetic relatedness of *S. aureus* strains before and after decolonization.

METHODS

Study design and population

We performed a retrospective cohort study of infants admitted to The Johns Hopkins Hospital's (JHH) 45-bed NICU from April 2013 to December 2015, a period of active surveillance and decolonization for both MSSA and MRSA colonized infants. The NICU has individual patient rooms and two separate medical teams with shared nursing staff

providing quaternary care to over 700 infants per year. The Johns Hopkins Institutional Review Board approved the study with a waiver of informed consent.

Case identification and definitions

Using administrative data, all neonates admitted to the NICU during the study period were identified and served as the source population. Microbiology data were collected from a computerized surveillance system (TheraDoc, Premier, Inc.) and merged with hospital billing data. The study cohort consisted of infants with *S. aureus* colonization, defined as an infant with one or more weekly nasal surveillance culture(s) that grew *S. aureus* during the initial NICU admission, and received intranasal mupirocin treatment after colonization. A retrospective chart review was conducted to identify clinical characteristics and demographic data at the time of *S. aureus* colonization. These data were collected and stored on a secure, online database hosted by REDCap.²⁰

Neonates with *S. aureus* nasal colonization underwent decolonization, per hospital protocol, which included a 5-day intranasal mupirocin treatment and topical chlorhexidine (CHG) antisepsis, when age appropriate.¹⁰ During the study period, CHG antisepsis was also part of other concurrent infection prevention bundles in the NICU, therefore, an infant that received intranasal mupirocin treatment and had at least one negative nares surveillance culture after mupirocin treatment was classified as decolonized. The primary study outcome was recurrent colonization after decolonization with mupirocin.

Recurrent colonization was defined as growth of *S. aureus* from a surveillance culture after decolonization. Microbiological data for *S. aureus* infections, defined as clinical culture growth from a sterile site, were also collected. Stored bacterial isolates from a subset of infants with recurrent colonization after prolonged nasal clearance (i.e. 2 negative surveillance cultures prior to recurrence of *S. aureus* colonization) were used for strain typing.

The following groups of infants were not eligible for the analytic cohort: 1) infants with no subsequent nares cultures after mupirocin treatment, 2) infants whose mupirocin treatments occurred before their first reported positive nares culture, 3) infants who had a subsequent negative surveillance culture prior to mupirocin treatment, and 4) infants with no negative surveillance cultures after decolonization (Figure 1).

Microbiology laboratory methods and bacterial isolate strain typing

All nasal surveillance *S. aureus* isolates were cultured and distinguished using selective chromogenic agar plates (SaSelect™; Bio-Rad, Hercules, CA).^{10,11,21} Isolates were archived in Tryptic Soy broth with 20% glycerol at -80°C. As previously described, isolates were characterized by pulsed-field gel electrophoresis (PFGE) using *Sma*I-digested bacterial DNA^{22,23}, and analyzed with a molecular analyst fingerprinting plus software (Fingerprinting II Version 3.0; BioRad Laboratories, Hercules, CA). They were deemed genetically similar if they had 3 band differences (85% comparison score).²² Mean comparison scores were measured for bacterial isolates. Gel images were maintained in a password protected file so that within and between run comparisons could be performed for each group of isolates.

Statistical analysis

Continuous variables were summarized by both their means and standard deviations as well as their medians and interquartile ranges [IQR]. Categorical variables were reported as absolute counts and percentages. Kaplan-Meier estimates were calculated from 1) NICU admission to acquisition of *S. aureus* colonization and 2) *S. aureus* nasal decolonization to recurrent *S. aureus* colonization for infants who remained at risk (Figure 2). Discrete-time Cox proportional hazards were used to explore relationships between clinical factors – methicillin resistance, systemic or topical use of anti-*Staphylococci* antibiotics (except mupirocin), presence of a peripherally inserted central catheter (PICC), nasogastric catheter, endotracheal tube, nasal respiratory cannula or mask - and time (in weeks) from decolonization to recurrent colonization. Discrete-Time logistic survival models were also estimated using LASSO (Least Angle Shrinkage and Selection Operator) with k -fold cross validation ($k = 10$) to perform shrinkage and variable selection due to the large number of coefficients relative to the sample size and number of events observed.²⁴ Analyses were performed on Stata 14 (StataCorp, College Station, TX) and R version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Acquisition of *S. aureus* nasal colonization within the NICU

Of the 2060 infants admitted to the NICU and screened for *S. aureus* during the study period, 271 (13%) were colonized with *S. aureus* (Figure 1). As shown in the Kaplan-Meier analysis (Figure 2), VLBW infants were significantly more likely to become colonized with *S. aureus* during NICU admission (Log rank test P -value = 0.003). Of the neonates with nasal *S. aureus* colonization, 203 received mupirocin intranasal treatment, and 167 of the treated infants had subsequent follow up surveillance cultures. Of the 167 infants, five infants with MRSA never cleared *S. aureus* from surveillance cultures, while 162 infants had at least one negative surveillance culture after mupirocin treatment and were included in the analytic cohort. Less than a third of the infants were colonized with MRSA (Table 1).

At the time of *S. aureus* colonization, infants in the cohort were often premature and had been admitted to the NICU for nearly 3 weeks (Table 1). One hundred and thirty three infants (81.5%) required respiratory support, and 132 (82%) had a nasally placed enteric tube. Nearly half of the infants (48.1%) had a central venous catheter inserted and 63 infants (38.9%) had received an antibiotic with *S. aureus* activity such as cefazolin, clindamycin, cefepime or vancomycin in the week prior to documented growth of *S. aureus* from surveillance cultures. Clinical characteristics and demographics have been summarized in Table 1.

Association between clinical characteristics and time to recurrent colonization

Among the 162 decolonized infants, 63 (39%) had recurrent *S. aureus* colonization after treatment with intranasal mupirocin (Supplemental Figure 3). Characteristics of the infants with recurrent colonization were similar to the study cohort such that they were mostly premature infants, required respiratory support and/or a nasogastric tube, and the majority were colonized with MSSA (Table 1). Using discrete-time Cox Proportional Hazards models

to explore the relationship between clinical risk factors and time to recurrent colonization, we found that the risk of recurrent colonization after mupirocin treatment was increased for neonates with an endotracheal tube or nasal cannula/mask (HR 2.65 95%CI: 1.19 – 5.90 and HR 2.21 95%CI: 1.02 – 4.75, respectively) (Table 2) (Supplemental Figure 4). Notably, methicillin resistance (HR 1.4 95%CI: 0.59 – 3.56), recent exposure to anti-*S. aureus* antibiotics (HR 0.5 95%CI: 0.31 – 1.07), and presence of a central venous catheter (HR 0.9 95%CI: 0.5 – 1.8) at the time of *S. aureus* colonization were not significantly associated with recurrent colonization after decolonization (Table 2).

Relatedness between *S. aureus* strains before and after decolonization

Of the 63 infants with recurrent colonization, 39 (62%) had stored isolates available for strain typing. Of the 39, twenty-five (64%) neonates had *S. aureus* strains that were genetically similar before and after decolonization (mean comparison score 97.8%, standard deviation (SD): 3.96). Fourteen neonates (36%) acquired a *S. aureus* strain that was genetically different from their pre-decolonization strain (mean comparison score 58.3%, SD: 9.17).

Association between clinical characteristics and recurrent colonization with genetically similar strains

Among the 39 infants with isolates available for strain typing, there was an insignificant increase in the odds of recurrent colonization with concordant strains for those with nasogastric tubes (OR 1.43 95%CI: 0.34 – 5.83), nasal cannulas (OR 1.5 95%CI: 0.4 – 5.73) or with exposure to antibiotics in the week prior to *S. aureus* colonization (OR 1.78 95%CI: 0.47 – 6.89) (Table 2).

DISCUSSION

Hospitalized neonates are at high risk for invasive *S. aureus* infections.¹ Decreasing neonatal nasal *S. aureus* burden, through decolonization, reduces risk of nosocomial infections.¹¹ In this retrospective study, we demonstrated that more than a third of hospitalized neonates developed recurrent *S. aureus* nasal colonization after a mupirocin-based decolonization treatment. These infants were mostly colonized with genetically similar strains before and after decolonization. Prematurity and respiratory support devices, known risk factors for nosocomial infections²⁵, were more common in infants with recurrent colonization. To our knowledge, this is the first study reporting NICU-patient level risk factors for *S. aureus* recurrent colonization and strain similarities before and after decolonization.

Prior studies have associated prolonged inpatient stays, prematurity and vascular catheterization with *S. aureus* colonization.^{8,26–28} Since premature infants are frequently exposed to endogenous and exogenous reservoirs of *S. aureus* during extended NICU stays²⁹, we postulated that factors associated with initial colonization may likely affect recurrent colonization. Our study suggests that infants with recurrent colonization were mostly premature and the risk of recurrence was increased by presence of an endotracheal tube or nasal cannula/mask at the time of *S. aureus* colonization (Supplemental Figure 4). These data support the hypothesis that the frequent manipulation of respiratory equipment

around the anterior nares or the creation of biofilms may increase the neonatal exposure to *S. aureus* reservoirs.³⁰ However, presence of a vascular catheter and recent systemic exposure to anti-staphylococcal antibiotics, at the time of *S. aureus* colonization, did not affect time to recurrent colonization.

Although many studies have documented the risk of recurrent colonization after treatment, our study compared the relatedness of strains identified from neonates before and after mupirocin-based decolonization. Our findings suggest that recurrent colonization was largely due to genetically similar strains. This supports prior suggestions that decolonization regimens may attenuate nasal bioburden rather eradicate *S. aureus*.^{14,17,31} Additionally, extra-nasal sites of endogenous colonization may contribute to recurrent colonization. However, many hospitals perform nasal surveillance without testing other anatomic sites, as the nares remain the principal reservoirs for *S. aureus* and elimination of nasal carriage has been associated with decreased incidence of infections.^{11,32} Other factors may explain strain similarities before and after decolonization, such as ongoing exposure to a circulating strain within the NICU environment i.e. from HCWs, or re-exposure to a colonized parent.²⁹ We did not find clonal *S. aureus* strains among infants included in the study suggesting no common reservoir to which neonates were exposed.

We demonstrated that nasally placed respiratory equipment was associated with a greater risk of recurrent colonization after decolonization. If these findings are replicated, clinicians could consider alternate decolonization strategies or attempt newer methods to eliminate *S. aureus* reservoirs, such as the use of antibiotic coated respiratory equipment to reduce transmission. Universal decolonization, contact isolation and systemic antibiotics have been evaluated as alternative decolonization strategies with varying results.^{33–35} Targeted decolonization for both MSSA and MRSA have not been extensively evaluated in neonates, but in adult patients with high risk of *S. aureus* infections, targeted decolonization and at times mupirocin prophylaxis have proven to be effective strategies.^{10,12,15,36} Future research could identify which of these strategies would have the most benefit for neonates. After identifying infants at high risk for recurrent colonization, a systematic approach to rescreening and retreatment with mupirocin may also be beneficial. Questions regarding safety or antimicrobial resistance of mupirocin and chlorhexidine are beyond the scope of this study and could be included in future research. Moreover, numerous studies have reported minimal adverse effects or mupirocin resistance after implementation of ASC and decolonization.^{11,12,14}

There are several limitations to this study. Retrospective data collection and hospital-wide changes in electronic medical record software limited available clinical data. Small sample sizes, weekly *S. aureus* surveillance and single-center study design may have limited the ability to detect differences between groups, the precision of findings or their generalizability. Prospective assessment of risk factors, daily *S. aureus* surveillance or multi-center study design may provide findings that are more precise; however, feasibility and costs may be prohibitive. *S. aureus* isolates from infants with recurrent colonization were stored at random and available on convenience. Nevertheless, a good proportion (62%) of recolonized infants had available isolates for strain typing. Data regarding chlorhexidine use in colonized infants could not be reliably obtained from the medical records. Exposure to

CHG may have influenced rates of recurrent colonization and this could be assessed in future studies. Finally, although PFGE remains the current gold standard for bacteria strain typing, PFGE can have some limitations in assessing genetic relatedness.²³ To further discriminate between isolates, other techniques such as whole genome sequencing, could be used in future studies.^{26,37}

S. aureus colonization is common in the NICU. Neonates with recurrent colonization after mupirocin-based decolonization often have genetically similar strains of *S. aureus* before and after decolonization. To target neonates at greatest risk for recurrent colonization, clinicians may consider alternate decolonization strategies for premature infants requiring respiratory support equipment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1:
Flow chart used to identify neonates admitted to the Neonatal Intensive Care Unit (NICU) during the study period with *Staphylococcus aureus* colonization

Figure 2:

Probability of *Staphylococcus aureus* nasal colonization by birth weight using Kaplan-Meier survival estimates and time from admission to the Neonatal Intensive Care Unit (NICU).

NOTE: NICU – Neonatal Intensive Care Unit; Bwt – Birth weight; *S. aureus* – *Staphylococcus aureus*

Administrative censoring occurred at 14 weeks and 122 neonates did not have birth weights documented

Table 1:

Demographics and clinical factors of Mupirocin treated Neonates with follow up nasal *Staphylococcus aureus* (SA) surveillance cultures

Demographic and Clinical Characteristics of <i>Staphylococcus aureus</i> (SA) Colonized Neonates Treated with Intranasal Mupirocin				
	Neonates with Recurrent SA Colonization N = 63 (%)	Neonates without SA recurrent Colonization N = 99 (%)	Sub-cohort of neonates with recurrent colonization and genetically related strains N = 25 (%)	Sub-cohort of neonates with recurrent colonization and genetically unrelated strains N = 14 (%)
Demographic characteristics				
MSSA (%)	55 (87.3)	86 (86.9)	23 (92)	12 (86)
Birth weight in grams, median [IQR]	990 [735, 1615]	1230 [865, 1860]	880 [720, 1280]	1225 [720, 1960]
Birth Gestational age in weeks, median [IQR]	28.4 [25.7, 30.4]	29.7 [26.3, 33.9]	27.2 [25.2, 29.6]	29.1 [26.5, 31.6]
Length of stay, days [IQR]	100 [67, 154.5]	40 [27, 73.5]	94 [67, 127]	142 [75, 206]
Median days to SA recurrent colonization, [IQR]	21 [14, 28]	NA	28 [21, 42]	49 [29, 56]
Median days to discharge or death, [IQR]	NA	13 [7, 28.5]	NA	NA
Death	4 (6.3)	6 (6.1)	2 (8)	1 (7)
Female	33 (52.4)	51 (51.5)	14 (56)	6 (43)
Method of delivery, CS	33 (52.4)	66 (66.7)	12 (52)	8 (73)
Method of delivery, vaginal	21 (33.3)	29 (29.3)	11 (48)	3 (27)
In-hospital birth	45 (71.4)	74 (74.7)	21 (84)	8 (57.1)
Clinical Characteristics				
Nasoenteric tube	41 (65.1)	91 (91.9)	18 (72)	9 (64.3)
Respiratory support	57 (90.5)	76 (76.8)	23 (92)	14 (100)
Nasal mask or cannula	39 (61.9)	58 (58.6)	15 (60)	7 (50)
Endotracheal tube	26 (41.3)	18 (18.2)	12 (48)	8 (57.1)
Use of systemic anti-SA antibiotics in 7 days prior to SA colonization	26 (41.3)	37 (37.4)	11 (44)	8 (57)
Documented SA Infection or growth from clinical culture	25 (39.7)	9 (9.1)	7 (28)	4 (29)

NOTE: SA – *S. aureus*; WGA – Weeks of Gestational age; IQR – Interquartile range; CS – Caesarean section

^a Infants had growth of SA from a nasal surveillance culture and at least one negative surveillance culture after mupirocin treatment

^b Infants had at least one SA isolate frozen and available for typing before and after nasal mupirocin treatment

Table 2:

Discrete-time Proportional Hazards and Univariate Logistic Regression of clinical risk factors associated with *Staphylococcus aureus* (SA) recurrent colonization after treatment

Discrete-time Proportional Hazards and Univariate Logistic Regression of clinical risk factors associated with <i>Staphylococcus aureus</i> (SA) recurrent colonization after treatment		
Clinical characteristics at time of initial SA colonization	Mupirocin treated infants with subsequent negative surveillance SA culture	Sub cohort of infants with recurrent colonization and genetically related SA strains before and after treatment
	Hazard Ratio (95% CI)	Odds Ratio (95%CI)
Methicillin resistant SA	1.45 (0.59 – 3.56)	0.52 (0.06, 4.78)
Use of systemic anti-SA antibiotics in 7 days prior to initial SA colonization	0.58 (0.31 – 1.07)	0.59 (0.15 – 2.19)
Peripherally Inserted Central Catheters	0.95 (0.50– 1.80)	1.78 (0.47 – 6.89)
Nasoenteric tube	0.41 (0.23 – 0.74)	1.43 (0.34 – 5.83)
Endotracheal tube	2.65 (1.19 – 5.9)	0.69 (0.18 – 2.58)
Nasal mask or cannula	2.21 (1.02 – 4.75)	1.5 (0.4 – 5.73)