Frequent Standardized Oral Care to Improve Health Outcomes in Premature Infants in the Neonatal Intensive Care Unit

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1. **Project Title**: Frequent Standardized Oral Care to Improve Health Outcomes in Premature Infants in the Neonatal Intensive Care Unit

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3. **Abstract:**

Premature very low birth weight (VLBW) infants are susceptible to complications related to infrequent and non-standardized oral care. Although the benefits of frequent standardized oral care are known to reduce oral dysbiosis (increased level of potentially pathogenic bacteria) and its associated complications in critically ill adults leading to established evidence-based guidelines, no such information exists for VLBW infants. Premature VLBW infants are highly susceptible to costly, life threatening and potentially preventable morbidities, such as ventilator associated pneumonia (VAP), bronchopulmonary dysplasia (BPD; oxygen requirement at 28 days of life), and need for prolonged respiratory support which require additional treatments, increase cost of care, and can lead to chronic illness, re-hospitalization, and developmental delay.[1,2] A dearth of information exists regarding oral care in VLBW infants and no such guidelines exist for infants admitted to the neonatal intensive care unit (NICU) which may negatively affect their health. Thus, research regarding the effect of frequent, standardized oral care on the health of VLBW infants is essential to develop guidelines thus potentially improving the health of this vulnerable population. If successful, this research could change practice in NICUs across the nation.

4. **Background:**

VLBW infants are at significant risk of adverse health outcomes including ventilator associated pneumonia, bronchopulmonary dysplasia, and prolonged respiratory support which increase costs and are associated with chronic illness, re-hospitalization, and developmental delay. The oral cavity of patients admitted to intensive care units contains high levels of pathogenic bacteria which can be aspirated, inhaled and invade the systemic, respiratory and gastrointestinal systems increasing the risk of adverse health outcomes. Extensive research regarding the benefits of oral care in critically ill adults has led to nationally recognized evidence-based guidelines.

In critically ill adults, evidence based guidelines for oral care are well established, known to reduce complications, and are included in the Institute for Healthcare Improvement's Five Million Lives Campaign.[3] However, in critically ill infants admitted to the neonatal intensive care unit (NICU), no such guidelines exist and there is a dearth of information regarding oral care for preterm VLBW infants who due to their suppressed immunity and physiologic immaturity are at increased risk of morbidity due to infrequent and non-standardized oral care. However, in critically ill infants admitted to the neonatal intensive care unit (NICU), no such guidelines exist and there is a dearth of information regarding oral care for preterm VLBW infants who due to their suppressed immunity and physiologic immaturity are at increased risk of morbidity due to infrequent and non-standardized oral care.

Lack of frequent, standardized oral care can result in abnormal levels of potentially pathogenic bacteria in the oral cavity including the cheeks, tongue, lips, and palate. When admitted to the intensive care unit, the oral cavity of adults become colonized with pathogenic bacteria within 24 hours[4] and oral flora changes from gram positive (normal) to gram negative (potentially pathogenic) within 48 hours of ventilation.[5] In premature infants admitted to the NICU, pathogenic gram-negative bacteria increase significantly in the oral cavity from day 1 to 10.[6] Premature VLBW infants may be particularly susceptible to abnormal oral cavity colonization due to the routine placement of oral devices such as feeding and endotracheal tubes which are known to acquire biofilm and to alter the oral flora.[7] In addition, the NICU environment contains abnormal and pathogenic

bacteria that can enter the infant's oral cavity during routine care. Finally, due to prematurity and physiologic instability, VLBW infants often have limited maternal contact and are unable to orally feed which interferes with healthy oral colonization that normally occurs following birth.

If potentially pathogenic bacteria are not removed via frequent standardized oral care, it is possible that VLBW infants can aspirate or inhale saliva containing high levels of bacteria. In addition, these bacteria could invade their systemic circulation, respiratory and gastrointestinal tract through the fragile mucosal barrier thus increasing the risk of health problems such as VAP, BPD, and need for prolonged respiratory support. VAP is defined as bacterial lung invasion developing after > 48 hours of invasive ventilation.[8] VAP accounts for up to 32.2% of hospital acquired infections in neonates[9] and has been shown to prolong length of stay and increase cost of care.[10] Because the endotracheal tube bypasses the natural upper and lower respiratory track defense mechanisms against harmful microorganisms and bacteria laden oral secretions can leak around the tube into the airway, the risk of VAP is high. This is especially important in VLBW infants who use un-cuffed endotracheal tubes which are more likely to allow entry of oral secretions into the lungs. In adults, the same bacteria found in the oral cavity was present in the lungs of ~75% of VAP cases and VAP was preceded by an oral culture of the same bacterial species.[11] While minimal information exists regarding VAP and the oral microbiome in VLBW infants, VAP has been shown to be poly-microbial from primarily gram-negative organisms which significantly increase in the oral cavity over the first days after NICU admission.[9] Even when endotracheal intubation and invasive ventilation are not required, infants likely have micro-aspiration of oral secretions. When these secretions contain high levels of potentially pathogenic bacteria, prolonged respiratory support may be required. This is important because both invasive and non-invasive respiratory support can damage the VLBW infant's fragile lungs increasing the risk of BPD.[12]

To date, researchers do not know whether frequent standardized oral care can decrease the number of potentially pathogenic bacteria in the oral cavity of premature VLBW infants. Because frequent standardized oral care is known to reduce the amount of oral pathogenic bacteria in adult patients which are known to increase the risk of VAP, this is a vital piece of information that may allow NICU clinicians to: (a) reduce the amount of oral pathogenic bacteria, (b) reduce VAP, (c) decrease the incidence of BPD, and (d) decrease the need for respiratory support.

In a national survey of 129 neonatal nurses from 35 states, we found that while over 98% stated oral care was important for the health of VLBW infants, less than 37% felt their NICU adequately addressed oral care. In addition, tremendous variation existed in practices regarding oral care including the method, timing, equipment and whether or not oral care was even performed.

5. Specific Aims:

The long-term goal of this research is to improve short and long-term health outcomes for premature very low birth weight (VLBW; <1500 grams) infants by decreasing complications caused by lack of frequent standardized oral care.

Specific aims are as follows:

Aim 1: To compare the oral microbiome between groups including bacterial pathogenicity determinants.

Aim 2: To compare respiratory outcomes including the incidence of ventilator associated pneumonia, bronchopulmonary dysplasia and need for respiratory support between groups.

6. Research Plan:

Sample and Setting:

The proposed study will prospectively follow 217 VLBW infants and 217 mothers (dyad) for 4 weeks following birth (includes 69 previously enrolled mother/infant dyads). Infants will be randomized into 1 of 3 groups. Standardized oral care will be performed every 3-4 hours (Group 1) and every 12 hours (Group 2) using sterile water. Group 3 will have standardized oral care performed every 3-4 hours using human milk (mother's breast milk or donor breast milk). Mothers of multiples will also be potential subjects. UF NICU nursing staff will receive UF Qualtrics survey links to their UF email yearly. Survey responses are voluntary and anonymous and will include up to 225 RN responses.

Infants will be sampled by convenience from the NICU at UF Health Shands Children's Hospital. Approximately 150 premature VLBW infants are admitted to this NICU per year. Our consent rate in similar studies is > 60% which strongly suggests we will be able to recruit 217 infants and 217 mothers (dyad) over a 12-month period.

The proposed randomized controlled pilot study will follow a prospective cohort (n=217) of racially and economically diverse premature VLBW male and female infants following birth. Infants will be randomly assigned to one of three groups using a randomized blocks approach as implemented in SAS PROC PLAN. A randomized blocks approach will ensure comparable treatment group sizes, and a priori assignment will reduce possibility of bias in treatment group assignment. All groups will receive standardized oral care for 4 weeks. Infants in Group 1 will have standardized oral care performed every 3-4 hours with sterile water (dependent on scheduled care times), infants in Group 2 will have standardized oral care performed every 12 hours with sterile water, and Group 3 will have standardized oral care performed every 3-4 hours (dependent on scheduled care times) with human milk. Groups were chosen based upon recommendations for adults (every 3-4 hours) and data regarding common practices of neonatal nurses obtained from our national survey (every 12 hours) and the benefits of placing drops of human milk into the oral cavity of preterm infants.

Inclusion criteria: 1) mothers who are $\geq$ 18 years of age, 2) infant (s), including multiples, born at $\leq$ 30 weeks, 3) infant with birth weight at $\leq$ 1500 grams

Exclusion criteria: 1) infants with born congenital anomalies of the face, lungs, or gastrointestinal system 2) infant not expected to live > 7 days following delivery

Informed consent will be will be obtained from the mother or father within 72 hours after birth at the infant's bedside or in the mother's hospital room.

Procedure:

Oral care will be performed in infants in Group 1 (every 3-4 hours) using sterile water, Group 2 (every 12 hours) using sterile water and Group 3 (every 3-4 hours) using human milk (if mother's breast milk, not available, donor breast milk will be used). The following procedure and a checklist to ensure treatment fidelity. Equipment will be easily accessible and provided in sterile pre-packaged kits at the infant's bedside. Study personnel involved with subjects will be unable to be blinded since information regarding oral care is visually obvious and recorded in the chart by nursing staff. However, those performing analysis of saliva and collecting data on respiratory outcomes will be blinded to the infant's designated group. Feeding tubes will be changed no less than every 7 days.

1. Using a sponge-tipped swab saturated with sterile water or human milk, the bedside nurse or research coordinator (with extensive NICU nursing experience) will clean all 4 quadrants of the gum surfaces and upper posterior part of the oropharynx for 15 seconds each.
2. Using a new sterile water or human milk saturated swab, they will clean the ventral and posterior surfaces of the tongue for 15 seconds.
3. Using a new sterile water or human milk saturated swab, they will gently clean the outer surface of the endotracheal tube (if present), feeding tube or other oral tubes.
4. The lips will be cleaned with a sterile gauze saturated with sterile water or human milk.
5. The oral cavity will be suctioned as needed with an oral suction devise to remove secretions (oral suction devises are changed every 24 hours per NICU protocol).
6. After the above oral care is complete, 0.1 mL of mom's breast milk will be placed into each buccal cavity (if available) until reaching day of life #7, if infant meets the NICU policy of a birth weight of $\leq$ 1250 grams. Orally administered breast milk is routine nursing care for premature infants in this NICU and may interact with the oral lymphoid tissue to help support immune system development.[15]

Aim 1 will determine the effect of standardized frequent oral care using human milk on the oral microbiome including bacterial pathogenicity determinants in premature VLBW infants. We hypothesize that standardized oral care performed every 3-4 hours using human milk will be associated with less dysbiosis than standardized oral care every 3-4 hours or 12 hours using sterile water. A sample of the infant's saliva will be collected weekly for 4 weeks by the research coordinator (a registered nurse experienced in the care of critically ill infants) by gently inserting a sterile brush into the infant's mouth and gently rotating the swab. Collection will occur immediately prior to the infant's scheduled oral care. Our team is skilled in the collection, processing and analysis of infant saliva.

DNA Extraction & 16S rRNA sequencing. DNA will be extracted from saliva using PowerFecal DNA isolation kit (MO BIO Laboratories) and quantified using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE). V4 universal primers will be used for 16S rRNA amplification and subsequent Illumina MiSeq sequencing. Samples will be amplified in 25 µl reactions containing 0.5 Units Phusion High-Fidelity Polymerase (New England Biolabs, Ipswich, MA), 1X Phusion HF Reaction Buffer, 0.75 µl DMSO, and 0.2 mM each dNTP. Triplicate PCR amplifications will be pooled for each sample, and cleaned with a QIAquick PCR Purification kit (Qiagen). 200 nanograms of each cleaned amplicon library will be submitted for sequencing at the ICBR core facility at UF. Sequencing will be performed on an Illumina MiSeq with a 300-bp paired-end protocol, using single indexing.

Microbiome 16S rRNA Gene Analysis. Sequencing reads will be parsed by Illumina index at the sequencing center. Paired reads will be merged, and primers and adaptors will be removed using a combination of tools in cutadapt, Galaxy, and eautils. Sample names will be added to the definition lines of sequencing reads using sed and concatenated into one fasta file, to make them compatible for analysis in QIIME v1.9.[16] Clustering of Operational Taxonomic Units (OTUs) at 97% similarity will be performed with the subsampled open-reference OTU picking method,[17] with no removal of singletons. The Greengenes reference dataset version 13.8 will be used as the reference for OTU picking and for taxonomy assignment with uclust.[18] Community structure will be analyzed in R with phyloseq and will be plotted with ggplot2. Analysis of similarities (ANOSIM) will be performed in R using VEGAN v2.0-8. Statistical significance of QIIME profiles will be assessed using STAMP for comparison of two independent groups. TIME will be utilized for analysis of the longitudinal microbiome data. These tests are appropriate for analysis between groups and over time.

Stool samples will be collected at the end of week 1 and 4 by the research nurse. Following collection, all samples will be placed in a sterile specimen container and immediately frozen at -80 degrees Celsius for later analysis for inflammatory markers and 16S rRNA sequencing.

To determine bacteria in the infant's mouth which may originate from the mother's breast milk or donor milk, a small sample of breast milk (3-5 mL) will be obtained from the infant's mother (after her consent) at the end of week 1 and 4 and 16S rRNA sequencing will be performed. Samples will be obtained from the NICU milk room. If mother is pumping < 5mL/, no sample will be obtained but no deviation reported. If donor breast milk is used for oral care (mother is not providing breast milk to her infant), 3-5 mL of donor breast milk will be obtained and 16s rRNA sequencing performed

Aim 2: will compare respiratory outcomes including evidence of VAP (positive ETT culture, incidence of ventilator associated events), days on invasive and non-invasive respiratory support, and incidence of BPD between groups. We hypothesize that infants who undergo standardized oral care every 3-4 hours will have less VAP, require fewer days of respiratory support, and have less BPD than Group 2. Descriptive statistics, including confidence intervals, will be generated to describe the distribution of days of respiratory support in each group, and measures of effect size (Cohen's ***f***) will be calculated from the between-subjects (Treatment Group, with 3 levels) general linear model (GLM). Incidence of occurrence for VAP and BPD will be described for each group using frequencies and percentages along with confidence intervals for the proportions. Effect sizes (Cohen's ***w***) for each of those two outcome variables will be calculated from a 3 (Treatment) by 2 (Occurrence) contingency table.[19]

Information concerning infant demographics and health outcomes will be collected from the infant's electronic health records. Traditionally, VAP has been diagnosed using radiographic evidence of pneumonia. However, radiographs have not been shown to be a valid or reliable method of diagnosis especially in neonates who often have underlying lung disease making radiographic diagnosis even more difficult. Therefore, in accordance with the National Healthcare Safety Network 2021 recommendations for neonatal and pediatric patients, VAP will be defined as the incidence of ventilator associated events (VAE).[1] VAEs are objectively defined events consisting of deterioration in respiratory status after a period of stability or improvement in ventilated infants. VAEs occur when a ventilated infant has either an increase in daily minimum oxygen requirement of $\geq$ 0.25 or mean airway pressure of $\geq$ 4 cm sustained for more than 2 days after more than 2 days of stable or decreasing daily minimum oxygen level or mean airway pressure.

If the infant is ventilated with an endotracheal tube, a weekly (for 4-weeks) culture and gram stains will be obtained from the endotracheal tube and sent to the laboratory for analysis to assess for bacteria which may be in the infant's airway. This ETT culture will be obtained with assistance by the infant's nurse or respiratory therapist when suctioning was to occur as part of routine care. The culture and gram stain will be sent to UF Shands Laboratory. Furthermore, days on respiratory support (ventilation, continuous positive airway pressure, high flow nasal cannula or low flow nasal cannula), incidence of BPD (oxygen requirement at 28 days of life), and length of hospital stay will be collected during the infant's NICU stay. Other clinical information which could potentially affect the risk of oral secretion aspiration will be collected including days requiring sedation, days of antibiotic therapy, and number of intubations.[6]

All de-identified saliva, stool and breast milk samples will be conducted at a UF lab outside of Shands. No EPIC charges apply, as these are being directly paid to the lab by research funds. ETT culture samples will also be de-identified before sending to Shands CoreLab. There will be no EPIC orders and no results of culture will be placed in EPIC. Core lab services are being set up as a direct pay.

A yearly anonymous and voluntary survey will be administered to the NICU nursing staff using a UF Qualtrics link sent to their email regarding staff concerns and questions. An IRB approved waiver of consent, will be sent with each survey, with an expected response of 225 staff. Yearly Q & A sessions will be scheduled as needed for staff questions and/or concerns. To encourage protocol compliance, nurses will receive quarterly emails or flyers will be posted with study updates or information important to study compliance. New nurses will be

formally oriented to the study during their orientation period. We have previously successfully used these strategies.

Data Entry and Management

All data will be entered into REDCap by a research assistant and verified by a Research Coordinator. A unique identifier will be assigned to each enrolled participant that will serve as the unique subject ID. Personal Health Information will not be stored in the study database. The study case report forms (CRFs) will be maintained in study specific folders. The CRFs are considered the primary data collection instruments for this study. When CRFs are not actively being processed, they will be secured in locked file cabinets. In addition to the use of passwords and other security measures, all documents containing identifying information are considered confidential materials and are safeguarded to the greatest possible extent. No individually identifying information will be released or discussed with anyone other than study staff.

Table 1. Instruments

Variable	Measurement	Timing
Descriptive (1-2) below		
1. Maternal, prenatal and perinatal demographics	Data extracted from medical record: Maternal history, prenatal and perinatal complications and medications, mode of delivery, whether mother received antibiotics and/or antenatal steroids	Collected upon entry into study.
2. Infant demographics	Data extracted from medical record: Race/ethnicity, sex, gestational age, birth weight, Apgar scores, resuscitation at birth and neonatal acuity (SNAP II) score	Collected upon entry into study.
3. Infant data	Type and amount of feeding, whether or not the infant is NPO, number of days and type of antibiotics, whether the infant was diagnosed with oral thrush and type of treatment. Length of skin-to-skin care	Collect upon entry into study and until infant discharge
Respiratory Factors (1-2)		
1. Presence of bacteria in the infant's breathing tube	Presence of positive tracheal aspirate on ventilated infants	Weekly for *4* weeks
2. Respiratory Support	Days of invasive and non-invasive respiratory support.	Day of birth until NICU discharge
3. Evidence of pneumonia	Episodes of radiologic evidence of pneumonia	Day of birth until NICU discharge
Analysis of stool (1-2)		
1. Microbial analysis	16S rRNA and metagenomics sequencing	End of week 1 & 4
2. Inflammatory markers	Analysis of inflammatory markers	End of week 1 & 4
Nutritional outcomes (1-6) below		
1. Enteral intake	24-hour enteral feeding intake in mL/kg and calories, episodes of emesis, # of oral colostrum buccal swabs received	Day of birth until discharge from the NICU.
2. Time to full feeds	First day infant received 120 mL/kg/d and 150 ml/kg/d of feedings	Recorded daily until 150 mL/kg/d reached
3. Hours of parenteral nutrition	Number of hours infant received some parenteral nutrition	Daily until NICU discharge

4. Parenteral nutrition associated liver disease	Liver function tests (level of direct bilirubin, alkaline phosphatase, AST and ALT)	Weekly or biweekly until NICU discharge (with clinically sent labs)
5. Hours of central line access	Number of hours infant has a central line	Daily until NICU discharge
6. Growth indices	Weight, length, and head circumference	Daily (weight) and weekly (head circumference and length) until NICU discharge.
7. Length of time feeding and endotracheal tubes were in place	Hours tubes remained in place.	Daily for 5 weeks.
Other health outcomes (1-3) below		
1. Episodes of late onset sepsis	CBC lab results, episodes of culture proven or presumed sepsis (treated with $\geq$5 days of antibiotics but with negative cultures) occurring $\geq$3 days after birth	Daily from birth until NICU discharge
2. Episodes of necrotizing enterocolitis	Episodes of radiologic or surgical necrotizing enterocolitis	All incidents until NICU discharge
3. Discharge summary	Days infant remains in hospital, hospital course, subject diagnosis	From birth until discharge
Covariates		
1. Analysis of maternal breast milk (if mom pumping) and donor breast milk	Microbiota and metagenomic sequencing (3-5mls)	At the end of week 1 and 4
Oral Conditions		
1. Bacteria in saliva	Amount and type of bacteria in the mouth	Sputum samples weekly for 4 weeks
2. Examination of mouth	Assessment of skin integrity and presence of erythema, exudate or thrush.	3x a week for 5 weeks.
3. Oral diseases and treatment	Oral medications and disease processes	From birth to NICU discharge.

7. Possible Discomforts and Risks:

There may be some mild discomfort for the infant when cleaning the mouth and lips.

8. Possible Benefits:

Frequent and standardized oral care may improve the health of VLBW infants by reducing oral dysbiosis and its associated complications in critically ill infants.

9. Conflict of Interest:

There are no conflicts of interest.

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