Factors Influencing Oral Colonization in Premature Infants

Imad R. Makhoul MD DSc[1], Polo Sujov MD[1], Leon Ardekian DDS[2], Imad Kassis MD[3], Tatiana Smolkin MD[1], Imad Abu-Elnaa'j DMD[2], Ada Tamir DSc[4] and Dov Laufer DMD[2]

[1] Department of Neonatology, Rambam Medical Center, Haifa, Israel
[2] Department of Oral and Maxillofacial Surgery, Rambam Medical Center, Haifa, Israel
[3] Department of Infectious Diseases, Rambam Medical Center, Haifa, Israel
[4] Department of Community Medicine and Epidemiology, Rambam Medical Center, Haifa, Israel
All authors are affiliated with the Rappaport Faculty of Medicine, Technion-Israel Institute of Technology. Haifa

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Abstract

Background: Factors influencing the oral flora of premature infants have not been adequately investigated.

Objective: To investigate the effects of gestational age and of anti-bacterial therapy on the oral flora of premature infants.

Methods: Oral cultures were obtained at age 1 day and age 10 days from 65 premature infants, divided into three groups: a) 24 neonates of 30–34 weeks gestation who did not receive ABT, b) 23 neonates of 30–34 weeks gestation who received ABT, and c) 18 neonates < 30 weeks gestation who received ABT.

Results: Oral bacterial colonization increased from day 1 to day 10 of life. In 24–34 week neonates, gestational age did not affect early bacteremia or oral colonization at birth. Neither gestational age nor ABT affected late bacteremia or oral colonization at day 10. In 30–34 week neonates with ABT, the oral flora consisted mainly of non-*Escherichia coli* gram-negative bacteria, whereas those who did not receive ABT grew mainly alpha-hemolytic streptococci, *Klebsiella pneumoniae* and *E. coli*. In neonates < 30 weeks who received ABT the oral flora were mainly coagulase-negative staphylococci. Oral colonization with anaerobes was zero and colonization with fungi was minimal.

Conclusions: Acquisition of oral bacteria rose from day 1 to day 10 of life, regardless of gestational life or ABT. On day 10 of life, the spectrum of oral bacterial flora changed following ABT and consisted mainly of coagulase-negative *Staphylococcus* and non-*E. coli* gram-negative bacteria. Oral colonization showed few fungi but no anaerobes. These microbiologic observations merit attention when empirical anti-microbial therapy is considered in premature infants suspected of having late-onset sepsis.

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The gastrointestinal tract of the fetus is sterile except in cases of chorioamnionitis [1]. Rapid contamination of external and internal surfaces occurs when the fetus is exposed to the flora of the birth canal during vaginal birth and to the external environment immediately after birth, with the gastrointestinal tract becoming colonized within 24 hours of birth [2]. The rate and extent of neonatal gastrointestinal tract colonization depend on various perinatal and neonatal factors, such as gestational age, mode of delivery, place of hospitalization (nursery versus neonatal intensive care unit), type and mode of feeding, and anti-bacterial treatment. However, colonization of the gastrointestinal tract after birth could be delayed because of prematurity, cesarean delivery, or total parenteral nutrition [2,3].

Since the oral cavity of the neonate lacks teeth and only mucosal surfaces are available during the first months of life, organisms with ligands for the tooth are absent [4,5]. Epithelial binding sites for group A streptococci and their lipoteichoic acid in the oral cavity of term newborn infants are absent or minimal at birth, but reach adult levels between 48 and 72 hours after birth [4]. The oral colonization patterns differ among individuals already in infancy; variable bacterial load in saliva and other close contacts and the frequency of this bacterial exposure may partly account for individual differences [5].

Systemic and oral ABT could affect the oral flora of infants and adults [5–7]. Oral microbial acquisition by premature infants has not been adequately investigated. The aim of the present study was to investigate the effects of various perinatal and neonatal factors, mainly gestational age and administration of anti-bacterial therapy, on oral colonization of premature infants born at 24–34 weeks of gestation.

Patients and Methods

Study design and population

This prospective and controlled study was conducted in the NICU at the Rambam Medical Center between 1 September 1999 and 31 January 2000. The study was approved by the institutional Helsinki Committee and informed consent was obtained from parents of the infants. We enrolled 68 premature infants who were born prior to 34 weeks gestation and were

ABT = anti-bacterial therapy

NICU = neonatal intensive care unit

Table 1. Comparison of perinatal and neonatal data between study groups: group A vs. B, group C vs. B

	Group A 30–34 wk No ABT	Group B 30–34 wk ABT	Group C < 30 wk ABT
No. of neonates	24	23	18
Gestational age (wk)	33.5 ± 1.25	33.3 ± 1.23	27.8 ± 1.6**
Birth weight (g)	1838 ± 362	1730 ± 409	1072 ± 316**
Mode of delivery			
Vaginal/cesarean	9/15	10/13	7/11
Maternal fever during labor > 37.5°C	0	8.7%	11.1%
Premature rupture of membranes > 6 hr	12.5%*	43.5%	27.8%
Amnionitis	0	4.3%	11.1%
Antenatal non-reassuring fetal heart rate	0	0	16.7%
Maternal antenatal glucocorticosteroids	47.6%*	19%	31.2%
Maternal anti-bacterial therapy during labor	0*	17.4%	11.1%
Gender (female/male)	11/13	10/13	11/7
1 minute Apgar score	8.21 ± 1.4*	6.61 ± 2.7	5.9 ± 2.2
5 minute Apgar score	9.5 ± 0.59*	8.5 ± 2.1	8.9 ± 0.7
Parenteral nutrition	79.2%	100%	100%
Mechanical ventilation	8.3%*	34.8%	88.9%**

* Group A vs. group B: $P<0.05$

** Group C vs. group B: $P<0.0001$.

consecutively admitted to the NICU during the study period. These infants were divided into three groups [Table 1]: Group A comprising 24 premature infants born at 30–34 weeks gestation who did not receive ABT after birth, group B with 23 premature infants born at 30–34 weeks who received ABT, and group C with 18 premature infants < 30 weeks gestation who received ABT. This therapy, consisting of intravenous ampicillin and gentamicin for 5–7 days postnatally, was given to infants with suspected congenital infection or to those requiring invasive procedures, such as endotracheal intubation and mechanical ventilation or catheterization of umbilical vessels.

Microbiologic tests

Swabs for aerobic bacteria, anaerobic bacteria and fungi were obtained at days 1 and 10 of life from the alveolar-buccal mucosa of each infant, since this area is less manipulated by procedures such as insertion of orogastric and endotracheal tubes or suctioning of secretions. Swabs were immediately transferred to the laboratory. Aerobic swabs were inoculated on Trypticase Soy Agar (TSA, DIFCO, USA) + sheep blood plates, and microbial growth was checked after 24 and 48 hours.

Anaerobic swabs were inoculated on BH+sheep blood plates (Dehydrated Brain Heart infusion agar by Difco, Cat. 0418-17 fortified with sheep blood) in containers with an anaerobic environment (O_2 < 1%, CO_2 9–13%). We used an anaerobic plastic jar with a tightly fitting lid, and Anaero-Gen (Oxoid, UK) sachets placed in a sealed jar with no need for a catalyst. With this method, since the atmospheric oxygen in the jar is rapidly absorbed with simultaneous generation of CO_2, the reaction proceeds with no evolution of H_2 and therefore does not require a catalyst. The Anaero-Gen sachet can reduce the oxygen level in the jar to below 1% within 30 minutes. According to ISO guide 9002, QC strains (*Bacteroides fragilis* ATCC 23745 and *Clostridium perfringens* ATCC 13124) were inoculated and incubated in anaerobic jars as required.

Possible bacterial growth was tested after 48 hours and negative growth was determined only after 5 days. Whenever a growth was evident (one colony or more), identification of microbes was performed according to standard bacteriologic techniques, based on the Clinical Microbiology Procedures Handbook Isenberg, ASM 1998, and the Manual of Clinical Microbiology, ASM 1995. To test our laboratory's capability of identifying anaerobic bacteria, oral cultures were obtained from five physicians and all grew anaerobes.

Growth of fungi was determined by culturing the samples on Sabouroud Dextrose Agar by Difco Cat. 0109-17. We did not obtain oral cultures at day 10 from three neonates: one died at 2 days of age, one was discharged at 8 days, and one was transferred to another hospital. Oral flora beyond day 10 of life were not examined.

Data collection

The following data were collected for each infant:

- *Perinatal*: premature rupture of membranes, maternal intrapartum fever, clinical chorioamnionitis, and maternal ABT or corticosteroids during labor
- *Neonatal:* birth weight, gestational age at birth, mode of delivery, Apgar scores at 1 and 5 minutes of age, enteral versus parenteral nutrition, oral versus orogastric feeding, mechanical ventilation, and neonatal ABT
- *Microbiologic:* results of routine cultures at birth (external ear, gastric aspirate, bloodstream), and proven episodes of bacteremia or fungemia, and colonization of tips of central vascular catheters during the first 10 days of life.

Statistical analysis of data

Groups A and B were compared for the effect of ABT on oral flora, and Groups B and C were compared for the effect of gestational age on oral flora. Wilcoxon test was used for comparison of ordinal variables (birth weight, gestational age, Apgar scores), and chi-square test and Fisher's exact test were used for comparison of proportions between groups. McNemar test was used to assess the difference in oral colonization between day 1 and day 10 of life. A P value of less than 0.05 was considered statistically significant.

Results

In all study groups, the rate of oral colonization at day 1 of life was not significantly influenced by premature rupture of membranes, Apgar scores or maternal treatment with ABT and glucocorticosteroids. In addition, oral colonization at day 10 was not significantly influenced by mechanical ventilation. Oral colonization rates with aerobic bacteria increased significantly from day 1 to day 10 of age in all study groups ($P<0.01$) [Table 2].

Group B versus Group A: similar gestational age with or without ABT

- *Perinatal and neonatal data:* Compared with group A, group B neonates had significantly lower Apgar scores and less maternal glucocorticosteroid therapy, but higher rates of premature rupture of membranes, maternal ABT during labor, and mechanical ventilation. Other variables were not different between groups [Table 1].

Table 2. Comparison of positive cultures between study groups: group A vs. B, group C vs. B

	Group A 30–34 wk No ABT	Group B 30–34 wk ABT	Group C < 30 wk ABT
No. of cases	24	23	18
At birth			
External ear	8.3% (KP, EC)	17.4% (CONS, GBS, CB)	27.8% (KP, EC, EN)
Gastric aspirate	0 –	4.3% (GBS)	16.7% (EC, ACB, KP)
Bloodstream	8.3% (CONS)	0 –	0 –
At 2–10 days			
Blood	8.7% (CONS, HIB)	17.4% (CONS, KP)	16.7% (KP, CONS)
Central vascular catheter tip	1/5 (CONS)	3/7 (KP, ACB)	5/14 (CONS, KP,CB)
Oral flora (day 1)			
Anaerobic	0	0	0
Aerobic	12.5% (CONS, EN, AHS, KP)	8.7% (CONS, KP, GBS)	11.1% (EC, EB)
Oral flora (day 10)			
Anaerobic	0	0	0
Aerobic	65.2% (15/23)	72.7% (16/22)	70.6% (12/17)
Gram-positive bacteria*	11/15	8/16	7/12
Gram-negative bacteria*	23/15	21/16	10/12

* Number of isolates in neonates with positive oral cultures. Some patients grew more than one bacterial species.

KP = *Klebsiella pneumoniae*, EC = *E. coli*, CONS = coagulase-negative *Staphylococcus*, GBS = group B *Streptococcus*, CB = *Citrobacter* species, ACB = *Acinetobacter baumannii*, EN = *Enterococcus*, HIB = *Haemophilus influenzae* b, EB = *Enterobacter cloacae*, AHS = alpha-hemolytic *Streptococcus*.

Microbiologic findings

- *Non-oral cultures up to 10 days of age:* Groups A and B did not differ in colonization of external ear and stomach at birth, or in bacteremia or colonization of central vascular catheter tip up to 10 days of age [Table 2].
- *Oral flora:* No anaerobic bacteria or fungi were found in groups A and B at days 1 and 10 of life. The rates and species of aerobic bacteria were not significantly different between groups [Tables 2 and 3]. The leading isolates on day 10 were alpha-hemolytic streptococci, coagulase-negative staphylococci, *Klebsiella pneumoniae* and *E. coli*.

Group B versus Group C: different gestational age + ABT

- *Perinatal and neonatal data:* The perinatal and neonatal variables did not differ between groups B and C, except for lower gestational age and birth weight and a higher rate of mechanical ventilation in group C [Table 1]. These differences were expected according to original group assignment.

Microbiologic findings

- *Non-oral cultures up to 10 days of age:* Groups B and C did not differ in colonization of external ear and stomach at birth, or in bacteremia or colonization of central vascular catheter tip up to 10 days of age [Table 2].
- *Oral flora:* Isolation of fungi was very low and no anaerobes were found in groups B and C at days 1 and 10 of life. One patient from group C grew *Candida tropicalis*. The rates and species of aerobic bacteria were not significantly different

Table 3. Comparison of the oral flora at day 10 of age* between study groups: group A vs. B, group C vs. B

	Group A 30–34 wk No ABT	Group B 30–34 wk ABT	Group C < 30 wk ABT
No. of patients with positive oral cultures	15/23	16/22	12/17
Gram-positive bacteria			
Alpha-hemolytic *Streptococcus***	7***	4	0
Coagulase-negative *Staphylococcus***	4	4	6
Enterococcus	0	0	1
Gram-negative bacteria			
*Klebsiella pneumoniae***	8	10	4
*E. coli***	9	3	4
Enterobacter cloacae	3	3	0
Pseudomonas aeruginosa	2	2	1
Serratia marcescens	1	2	1
Acinetobacter baumannii	0	1	0
Fungi			
Candida tropicalis	0	0	1

* Oral cultures in some patients grew more than one bacterial species.
** Not statistically significant when comparing group A vs. B or group C vs. B (Fisher exact test).
*** Number of isolates in neonates with positive oral cultures.

between groups [Tables 2 and 3]. The leading isolates were coagulase-negative *Staphylococcus*, *Klebsiella pneumonie* and *E. coli*.

Discussion

In premature infants born after 30–34 weeks gestation, ABT did not significantly affect the rates of bacteremia, colonization of central vascular catheter tip, or the microbiology of the oral cavity at day 10 of age. In all study groups, oral colonization rates with aerobic bacteria increased significantly from day 1 to day 10 of age, rising from 8.7–12.5% to 65.2–72.7%, regardless of gestational age, ABT or mechanical ventilation, as compared to published rates of 13%, 77% and 93% at 0–4 days, 6 days and 9–12 days of age, respectively [2,3]. Perhaps the lack of significant difference in oral bacterial colonization between groups in our study might reflect, in part, similar modes of feeding and nature of nutrition.

Despite the lack of influence of ABT on the overall rates of bacterial colonization of the oral cavity, there were some differences, albeit non-significant, in the spectrum of bacterial species colonizing the oral cavity at day 10 of age. In infants born at 30–34 weeks gestation who received ABT, the oral flora consisted mainly of non-*E. coli* gram-negative bacteria, whereas those who did not receive ABT grew mainly alpha-hemolytic streptococci, *Klebsiella pneumoniae* and *E. coli*. The oral flora that grew from infants < 30 weeks gestation who received ABT were mainly coagulase-negative staphylococci.

Colonization with coagulase-negative *Staphylococcus* (35.3%) following ABT in our neonates < 30 weeks gestation conforms with published rates of 72.2% and 43% [2,3]. Coagulase-negative *Staphylococcus* is the leading pathogen in late-onset sepsis of neonates weighing < 1,500 g in the U.S. [8]. Colonization with coagulase-negative *Staphylococcus* might be explained by the need for extra handling and the low state of immunity of premature neonates as compared to term newborn infants, including lower immunoglobulin G and complement levels, lower opsonophagocytic activity, and abnormalities in neutrophil storage pools [9].

We found that gestational age did not significantly affect the rates of bacteremia or of colonization of external ear, stomach and mouth at birth, nor did it affect the rates of bacteremia, colonization of central vascular catheter tip or of mouth at day 10 of age. In all three study groups, oral colonization rates with bacteria at birth were not influenced by Apgar scores, premature rupture of membranes, or maternal treatment with ABT and glucocorticosteroids. In addition, oral colonization rates at day 10 of age were not affected by mechanical ventilation. We could not examine the effects on oral flora of the site of hospitalization, mode of nutrition, or route of enteral feeding, since all the neonates in this study were hospitalized in the NICU; 79–100% of the patients received total or partial parenteral nutrition, and all were fed via the orogastric route (breast milk + special milk formula for premature infants).

Oral anaerobes are rare before eruption of deciduous teeth [10]. Kononen et al. [11] demonstrated that in an infant aged 2 months, anaerobic bacteria could be detected in the oral cavity, with *Veillonella* being the most frequent anaerobic bacteria at this age. We did not find oral anaerobic bacteria at days 1 and 10 of age, regardless of gestational age, maternal ABT or premature rupture of membranes. Our results agree with those reported in a previous study [2], but are in conflict with those of another study, where *Bifidobacterium* colonized the mouth in 31% of premature infants at day 6 of age [3].

The rate of fungal colonization during the first 10 days of life was very low in all sites cultured, regardless of gestational age or ABT. This is contrast to previous reports, where higher rates of oral fungal colonization (77% and 71.4%) were found in premature infants at days 6 and 7 of life [3,12]. Nevertheless, our data are consistent with the findings of our recent work [13] demonstrating low rates of both early and acquired fungal sepsis in neonates weighing < 1,500 g in our NICU: 0.58% and 6.8%, respectively, as compared to 1% and 9% in the U.S. [8,14].

To the best of our knowledge, this is the first controlled study that reliably examines the effects of perinatal factors, gestational age and ABT on the oral flora of premature infants. Three previous studies in this field have been reported from Nigeria, Australia and India, but all have some drawbacks. In the study performed in Nigeria [3], only 23 neonates were included, the oral flora were examined only up to day 6 of life, and analysis of the effect of ABT (15/23) or of gestational age on the oral flora of premature infants could not be reliably accomplished. In the study performed in Australia [2], the small number of neonates (n=28) precluded reliable conclusions on the effect of ABT (21/28) or of gestational age on the oral flora of premature infants. In the Indian study [12], inordinately high rates of fungal colonization were found in premature infants during the first week of life, a finding that requires clarification.

We conclude that: a) in premature infants born at 24–34 weeks gestation, oral colonization with bacteria at day 1 of life is low, regardless of gestational age or perinatal risk factors for neonatal infection; b) oral bacterial colonization rose significantly from day 1 to day 10 of life, regardless of gestational age, ABT or mechanical ventilation; c) on day 10 of life, the spectrum of oral bacterial flora changed following ABT and consisted mainly of coagulase-negative staphylococci and non-*E. coli* gram-negative bacteria; d) oral colonization with fungi was very low and no anaerobes were isolated up to 10 days of life; and e) our results merit attention when empirical anti-microbial therapy is considered in premature infants suspected of having sepsis since most pathogens growing beyond the first week of life would be resistant to ampicillin and gentamicin.

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Correspondence: Dr. I.R Makhoul, Dept. of Neonatology, Rambam Medical Center, Haifa 31096, Israel.
Phone: (972-4) 854-2219
Fax: (972-4) 854-3430
email: Makhoul@rambam.health.gov.il