Acquired Oral Microflora of Newborns During the First 48 Hours of Life

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Objective: *To compare the oral microflora of a newborn during first hours after birth and after two days, and determine whether the newborn acquires his mother's microflora during this period.* ***Study design:*** *Saliva samples were taken from 50 newborns, on their first day of life, two days after, and from their mothers. Those samples were checked for total aerobic cultivated bacteria and mutans streptococci.* ***Results:*** *Soon after birth, most newborns lacked any of the tested microorganisms in their oral cavity. Two days later, oral microorganisms were detected. A significant correlation was found between the total aerobic cultivated bacteria counts of the mothers, and of their newborns.* ***Conclusions:*** *It can be assumed, that on the first 48 hours of life, the newborn gains a major part of his oral microflora from his mother. These results might shade light on a possible to control and change the acquired microflora, at the very beginning of a human's life, creating a new, but less cariogenic flora. An accurate protocol should be examine to avoid this initial transmission during these days, while the mother and her newborn are still in the hospital, and thus might be possible to reduce caries prevalence in the future.*

Keywords: oral microflora, newborn, mutans streptococci, total aerobic cultivated bacteria.

INTRODUCTION

Until the time of birth the human infant is usually considered germ-free. Immediately after birth the newborn is exposed to millions of microorganisms, which will turn into a small portion of his normal flora. During, and shortly after birth, the epithelial surfaces in the oral cavity of newborns become colonized by various bacterial species [1].

Most of the studies among infants regarding this issue were performed a few months or years after birth. However, bacterial colonization in the oral cavity probably begins even at the first hours or days after birth.

In an infant's mouth there are only mucosal surfaces exposed to salivary fluid flow which may acquire bacteria. *Mutans streptococci* (MS) could persist in such an environment by forming adherent colonies on mucosal surfaces or by existing free in saliva by proliferation and multiplying at a rate that exceeds the washout rate caused by salivary fluid flow[2]. Researchers have found in one month old edentulous babies Gram positive bacteria. Few months later Gram negative bacteria were observed [3].

Infants acquire the oral microflora from their surroundings, probably from the first people whom they have close contact with. Usually the first ones are the mothers. More advanced technology that utilized chromosomal DNA patterns or identical plasmids provided more compelling evidence to substantiate the concept of vertical transmission[4-5].

The aim of this study was to compare the oral microflora of a newborn during first hours after birth with the microflora after two days, and to determine whether the newborn acquires his mother's microflora during these two days.

MATERIALS AND METHOD

Fifty mothers, giving birth at Hadassah Ein-Kerem hospital in Jerusalem were chosen randomly. Their ages were between 22 and 40 (mean 31.14). Six mothers have left the hospital before all samples were taken. Premature babies were excluded, and only normal births were included.

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Antibiotics during delivery, blood type, amniotic fluids, body weight and gender were checked against medical records. The mothers completed a questionnaire on other possible confounders, such as age, number of deliveries prior to the present one, ethnic origin, occupation, education, socioeconomic status, feeding mode, behavior with the baby (kissing, hugging), and if someone else was in close contact with the newborn.

The protocol of this study was approved by the Hadassah Hospital human ethics ("Helsinki") IRB committee.

Bacterial Sample Collection and Cultivation

Total aerobic bacteria were cultivated on Brain Heart Infusion Agar (BHI).

Mutans streptococci (MS) were cultivated on mitis salivarius bacitracin agar (MSB).

Samples were taken between 1 to 18 hours after birth (mean 8.57±3.58 hours, and median of 8.23 hours). They were collected using sterile swabs from the mother and the newborn, by carefully swabbing the cheeks, tongue and vestibuli.

Samples were spread over the agar and incubated in 37◦C for 24 hours for BHI agar, and 48 hours for MSB agar in a 5% CO_2.

Mothers' and their babies' samples were then checked and compared. Each sample was graded between 0-4 (0- no growth at all, 4- massive growth) based on a commercial scale of CRT kits. Calibration was performed with an experienced investigator.

Two days later, a second sample was taken from the baby following the same protocol.

Statistical analysis

Differences in the bacterial count levels by independent variables were tested employing one way ANOVA; independent t-test; and Pearson Correlation when appropriate. In order to multi-valiantly identify independent influences on results, multiple logistic regression analysis was applied. Variables were included after reaching a maximal significance level of 20% ($p<0.2$). Data was entered into Excel software and then SPSS 19.0 for data analysis. The level of significance was set at 5%.

RESULTS

Twenty five female babies and twenty five male babies were checked. They were from the 1st to the 11th baby who were born in their families, and weighed between 2,475-4,530 grams (mean 3,271.6±448.6 grams). Seventy two percent of the mothers had studied in a higher education institute, and home density rate was of 1.4 people / room. Eighty percent of the mothers did not take antibiotics before or during birth, and 82% had clean amniotic fluids. Contact with the baby was checked and 96% of the mothers reported they had hugged their babies, 86% have kissed them and in 96% of the cases a relative also had some contact with the baby. Most of the babies were breast fed (73%), some had prepared milk in bottles (6%), some had both (20%) and the rest didn't get the chance to eat yet (Table 1).

On a non selective agar (BHI) a significant correlation between the mother's total aerobic cultivated bacterial count and the 1st newborn sample was found (p-value=0.012; r=0.350) and also between the first and second samples which were taken from the baby (p-value=0.003; r=0.459). A statistical significant correlation was found between the 1st aerobic bacterial samples of the newborn and the 1st and 2nd MS samples of the newborn (p-value= 0.006; r=0.381, p-value= 0.022; r=0.341 respectively) (Table 2).

Multiple linear logistic regression model was applied to the data of the 2nd aerobic bacterial samples of the newborn in order to determine the independent effect of all the independent variables controlling for the others (Table 3). The variables which reached statistical significance as predictors of the baby's 2nd aerobic bacterial samples his mother's sample (p-value= 0.042), breast feeding (p-value= 0.005) and home density (p-value= 0.041).

DISCUSSION

Dental caries is a transmissible disease that still represents a significant public health problem in many countries [6]. The advent of this disease appears to be fairly recent in the history of man. Virtually unknown in ancient times, the prevalence of caries has increased with time until it has become almost ubiquitous in modern man [7]. The notion that dental caries is an infectious, transmissible disease was first demonstrated by Keyes (1960).

The oral cavity of the neonate lacks teeth and only mucosal surfaces are available during the first months of life in 5% CO2. Epithelial binding sites in the oral cavity of newborn infants are absent or minimal at birth, but reach adult levels between 48 and 72 hours after birth [8].

MS seems to be an important part of the total cultivated bacteria counts as the results show, though other studies suggest that MS acquisition happens later and other kinds of streptococci are dominant[8].

Earlier studies have demonstrated that newborns acquire their oral microflora during their first years, and even during their first days. Oral microbial acquisition by premature infants has not been adequately investigated.

Our study demonstrates a correlation between mother's and her newborn's bacteria, in accordance to other studies which have showed a commonality of MS genotypes between mothers and their infants at the time of initial acquisition [9].

Although, there were many studies regarding the correlation between the mother's bacteria and the bacteria that her baby acquired, our study is probably unique by examining that correlation immediately after birth, and by focusing in this pilot study on the specific behavioral aspects . A systematic review that had published shows that there was strong evidence established that mothers are the main source of MS colonization of their children [10], yet the ages of the children that were examined were between 0 to 5 years at the earliest study. The same review had emphasized that the role of other factors influencing transmission, are unclear, and thus that part of information was so essential to be studied in our research.

MS seems to be an important part of the total bacteria counts as the results show, though other studies suggest that MS acquisition happens later and other kinds of streptococci are dominant [11-13].

The results of this study, might lead to a better prevention plan among newborn and children. A longitudinal follow-up study at 19 years of age concludes that it is possible to be negative for MS into adulthood if the children were not colonized at an early age. The study suggested early schemes, including parental involvement, for long-term caries prevention among children by using behavioral and antimicrobial approaches [14]. Our study emphasized that the age of intervention should be from the first day of the newborn, which is with accordance to a review that targeted the 3 main factors that affect the colonization of MS: bacterial virulence, host-related and environmental factors. According to that review, complex interactions among these factors determine the success and timing of MS colonization. Thus, the conclusion is that the strategies for the prevention of dental caries should include timely control of colonization of the cariogenic bacteria in the mouths of young children [15].

In a systematic review there were 12 reports of microbiological interventions to reduce transmission of MS, yet, these studies, according to the authors, were generally lack consistent findings regarding caries reduction, have a small sample size and inadequate control groups, and lack blindness of investigators and subjects. Our findings and this study highlighted that the efficacy of an accurate prevention to reduce caries risk in children still needs to be studied by well-designed clinical trials[10].

An example of such an intervention was recommended in few studies. A recent study found that a maternal xylitol consumption by gum provided better preventive outcomes on salivary MS levels among there children, as compared with fluoride varnish treatments [16]. Another study concluded that this intervention should be targeted to women with high levels of MS counts [17]. The maternal use of xylitol reduces also caries incidence and restorative treatment needs in children that were expected to be high caries risk to the average level [18].

The number of people living in the house seemed to affect the bacteria levels; other studies usually checked socioeconomic status when the babies were older. A correlation between MS and social deprivation was found to depend on the caries status of the infants [19].

Although a correlation between breast feeding and MS was found in this study, other studies indicate no correlation between them[1], and even present a preventive role in the colonization of MS [20]. A recent study did find that Lactobacilli colonized the oral cavity of breastfed infants significantly more frequently than formula-fed infants [21].

The aim of the present study was to investigate the oral flora acquired by a newborn during the first two days of his life. The assumption in the literature is that cariogenic bacteria as MS are inhabitants of the oral cavity only after eruption of the teeth. We have found that those bacteria colonize the oral cavity much earlier to teeth eruption, probably by contamination from the mothers' bacteria of the oral cavity. None of the mothers who were checked could avoid touching their child, most of them hugged them, kissed them and fed them. Correlations were found between the mother's bacteria and the bacteria that her baby had

Table 1: Associations between babies' 2nd sample of non-selective agar growth to ways of contact with them, their birth and their background.

		N (%)	Mean	Std. deviation	Confidence Interval		p- value
					lower bound	Upper bound	
Gender	Male	25 (50.0)	2.225	1.303	1.615	2.835	0.675
	Female	25 (50.0)	2.050	1.317	1.434	2.666	
Education	Elementary	3 (6.0)	2.500	.	.	.	0.940
	High school	11 (22.0)	2.050	1.189	1.199	2.901	
	High education	36 (72.0)	2.155	1.370	1.634	2.676	
Department	A	17 (33.3)	2.423	1.205	1.695	3.151	0.411
	B	21 (41.2)	2.167	1.414	1.463	2.87	
	C*	13 (25.5)	1.667	1.173	0.765	2.568	
Antibiotics	NO	40 (80.0)	2.250	1.276	1.790	2.71	0.278
	YES	10 (20.0)	1.688	1.361	0.550	2.825	
Amnion fluids	clean	41 (82.0)	2.203	1.337	1.721	2.685	0.739
	dirty	2 (4.0)	1.500	0.707	-4.853	7.853	
	unknown	7 (14.0)	2.000	1.304	0.632	3.368	
kiss	NO	7 (14.0)	2.375	0.479	1.613	3.137	0.705
	YES	43 (86.0)	2.111	1.358	1.652	2.571	
Breast feeding	NO	2 (10.0)	1.250	0.646	0.223	2.277	0.151
	YES	45 (90.0)	2.236	1.317	1.790	2.682	
Eating	Breast only	36 (73.5)	1.469	1.486	0.933	2.005	0.225
	Mother's milk in bottle	0	0				
	Prepared milk	3 (6.1)	0.333	0.577	-1.101	1.768	
	Both	10 (20.4)	2.056	1.286	1.067	3.044	
Someone else touched	NO	2 (4.0)	2.500	0.707	-3.853	8.853	0.690
	YES	48 (96.0)	2.118	1.323	1.684	2.553	

* In department C babies stay with their mother's during whole day and night, while in departments A and B they are sleeping in the nursery and treated there

acquired, proving that the mother is probably the main origin of the newborn's oral microflora.

Since a proof of a bacterial transmission was found within two days after birth, we can define them as the two critical days for oral bacterial contamination. A hypothesis may be put forward that it is possible to control and change the acquired microflora, at the very beginning of a human's life, creating a new, but less cariogenic flora. If we could find ways to avoid this initial transmission it might be possible to reduce caries in the future. One mean is to emphasize the importance of the mothers' oral hygiene during the last months of pregnancy and right after birth, before approaching the baby and touching him, another suggestion would be to clean the breast and nipple area before feeding the baby. This is just the beginning of the investigation into and understanding of, the establishment of oral microflora in newborns.

In this study we have examined the prevalence of MS as one of the cariogenic bacteria and did not examine the numerous types of other bacteria that colonize the oral cavity, and affect the equilibrium with the human host. Therefore, future studies are needed.

CONCLUSION

Since a proof of a bacterial transmission was found within two days after birth with correlation to the mother's flora, we can define them as the two critical days for oral bacterial contamination. A hypothesis may be put forward that it is possible to control and change the acquired microflora, at the very beginning of a human's life, creating a new, but less cariogenic flora. These two days, while the mother and the newborn are still at the hospital, might be a great opportunity to control it. We should have an accurate protocol for the nurses, as well as for the mothers, to avoid this initial transmission during these days, and thus might be possible to reduce caries prevalence in the future. This is just the beginning of the investigation into and understanding of, the establishment of oral microflora in newborns.

Table 2: Pearson correlations of the 1st and 2nd samples, mother's and newborn's bacteria, background and time from birth.

		[a]BHI M	[b]BHI B	[c]BHI B2nd	[d]MSB M	[e]MSB B	[f]MSB B2nd	Mother's age	Weight	Birth #	Home density	Time from birth
[a]BHI M	r*	1	0.350	0.277	0.031	0.153	-0.072	-0.095	0.044	-0.019	-0.152	-0.136
	p**		0.012	0.083	0.830	0.284	0.641	0.512	0.762	0.896	0.290	0.340
[b]BHI B	r*		1	0.459	0.053	0.381	0.341	0.023	0.060	0.273	0.181	0.025
	p**			0.003	0.714	0.006	0.022	0.872	0.676	0.052	0.209	0.864
[c]BHI B2nd	r*			1	0.106	-0.015	0.154	0.012	-0.190	-0.036	-0.202	-0.310
	p**				0.515	0.927	0.342	0.944	0.241	0.825	0.211	0.051
[d]MSB M	r*				1	-0.245	0.043	0.091	0.121	0.267	0.185	-0.063
	p**					0.084	0.781	0.528	0.396	0.058	0.198	0.662
[e]MSB B	r*					1	0.247	-0.239	0.179	0.172	0.199	0.030
	p**						0.101	0.094	0.208	0.228	0.166	0.835
[f]MSB B2nd	r*						1	0.059	0.040	0.267	0.274	0.101
	p**							0.701	0.792	0.076	0.068	0.508
Mother's age	r*							1	-0.088	0.542	0.170	0.021
	p**								0.544	<0.001	0.238	0.882
Newborn's weight	r*								1	0.206	0.100	0.201
	p**									0.147	0.489	0.158
Birth # in family	r*									1	0.596	-0.002
	p**										<0.001	0.990
Home density	r*										1	0.102
	p**											0.482
Time from birth	r*											1
	p**											

*Pearson Correlation; **Sig. (2-tailed)

[a] Total aerobic cultivated bacteria Brain Heart Infusion Agar for the mother

[b] Total aerobic cultivated bacteria Brain Heart Infusion Agar for the newborn

[a] Total aerobic cultivated bacteria Brain Heart Infusion Agar for the newborn at the 2nd sample

[d] Mitis salivarius-bacitracin agar of Mutans streptococci for the mother

[e] Mitis salivarius-bacitracin agar of Mutans streptococci for the newborn

[f] Mitis salivarius-bacitracin agar of Mutans streptococci for the newborn at the 2nd sample

Table 3: Multiple linear regression model for independent variables' effect on 2nd BHI samples

	B	beta	sig.	Confidence Interval	
				lower bound	upper bound
Constant	3.407		0.058	-0.124	6.939
BHI B	0.628	0.392	0.014	0.136	1.119
BHI M	0.329	0.329	0.042	0.013	0.646
breast feeding	1.949	0.457	0.005	0.639	3.259
Home density	-0.797	-0.315	0.041	-1.559	-0.035

REFERENCES

1. Li Y, Caufield PW, Dasanayake AP, Wiener HW, Vermund SH. Mode of Delivery and Other Maternal Factors Influence the Acquisition of Streptococcus mutans in infants. J Dent Res 84: 806, 2005.
2. Könönen E.The oral gram-negative anaerobic microflora in young children: longitudinal changes from edentulous to dentate mouth. Oral microbial immunol 9: 136-141, 1994.
3. Gibbons RJ. Bacteriology of dental caries. J Dent Res 43:1021-1028, 1964.
4. Makhoul IR, Sujov P, Ardekian L, Kassis I, Smolkin T, Abu- Elnaa'j I, Tamir A, Laufer D. Factors Influencing Oral Colonization in Premature Infants. Isr Med Assoc J 4(2): 98-102, 2002.
5. Davey AL and Rogers AH. Multiple types of the bacterium Streptococcus mutans in the human mouth and their intra-family transmission. Arch Oral Biol 90: 453-460, 1984.
6. Caufield PW, Wannemuehler Y, Hensen J. Familial clustering of the Streptococcus mutans cryptic plasmid strain in a dental clinic population. Infect Immun 38:785-787, 1982.
7. Greene JC and Soumi JD. Epidemiology and Public Health Aspects of Caries and Periodontal Disease. J Dent Res 56: C20-C26, 1977.
8. Caufield PW, Childers NK, Allen DN, Hansen JB. Distinct bacteriocin correlate with different groups of Streptococcus mutans plasmids. Infect Immun 48: 51-56, 1985.
9. Li Y and Caufield PW (1995). The Fidelity of Initial Acquisition of Mutans Streptococci by Infants from Their Mothers. J Dent Res 74(2): 681-685.
10. Douglass JM, Li Y, Tinanoff N. Association of Mutans Streptococci Between Caregivers and Their Children. Pediatr Dent 30: 375-87, 2008.
11. Berkowitz RJ and Jordan HV. Similarity of bacteriocins of Streptococcus mutans from mother and infant. Arch Oral Biol 20: 725-730, 1975.
12. Berkowitz RJ, Jordan HV, White G. The early establishment of Streptococcus mutans in the mouths of infants. Arch Oral Biol 20: 171-174, 1975.
13. Plonka KA, Pukallus ML, Barnett AG, Walsh LJ, Holcombe TH, Seow WK . Mutans streptococci and lactobacilli colonization in predentate children from the neonatal period to seven months of age. Caries Res 46: 213-20, 2012.
14. Köhler B, Andréen I. Mutans Streptococci and Caries Prevalence in Children after Early Maternal Caries Prevention: A Follow-Up at 19 Years of Age. Caries Res 46: 474–480, 2012.
15. Law V1, Seow WK, Townsend G. Factors influencing oral colonization of mutans streptococci in young children. Aust Dent J 52(2): 93-100, 2007.
16. Alamoudi NM, Hanno AG, Sabbagh HJ, Masoud MI, Almushayt AS, El Derwi DA. Impact of maternal xylitol consumption on mutans streptococci, plaque and caries levels in children. J Clin Pediatr Dent 37(2): 163-6, 2012.
17. Nakai Y, Shinga-Ishihara C, Kaji M, Moriya K, Murakami-Yamanaka K, Takimura M. Xylitol gum and maternal transmission of mutans streptococci. J Dent Res 89(1): 56-60, 2010.
18. Laitala ML, Alanen P, Isokangas P, S€oderling E, Pienih€akkinen K. Long-term effects of maternal prevention on children's dental decay and need for restorative treatment. Community Dent Oral Epidemiol 41: 534–540, 2013.
19. Radford JR, Ballantyne HM, Nugent ZJ, Robertson M, Longbottom C, Pitts NB, Beighton D, Brailsford SR. Does social deprivation in 1, 2, 3 and 4-year-old Scottish infants influence the frequency isolation of caries-associated micro-organisms? J Dent 29: 325-332, 2001.
20. Thakur R, Singh MG, Chaudhary S, Manuja N. Effect of mode of delivery and feeding practices on acquisition of oral Streptococcus mutans in infants. International J Ped Dent 22: 197–202, 2012.
21. Vestman NR, Timby N, Holgerson PL, Kressirer CA, Claesson R, Domellöf M, Öhman C, Tanner AC, Hernell O, Johansson I. Characterization and in vitro properties of oral lactobacilli in breastfed infants. BMC Microbiol 13: 193, 2013.