

## Influential Determinants On Oral Colonization In Term Newborns

Américo Munayco<sup>1,2</sup>, María Cortez<sup>1,2</sup>, Nadia Meneses<sup>1,2</sup>, Hugo Pérez<sup>1</sup>, Rosmery Rubina<sup>2,3</sup>, Diana Yataco<sup>2</sup>, Oscar Sotomayor<sup>1,2</sup>, Franco Mauricio<sup>1,2</sup>, Paúl Mendoza<sup>1</sup>

<sup>1</sup> Professor at the Faculty of Dentistry, Universidad Nacional Federico Villarreal, Lima, Peru

<sup>2</sup> Community of Knowledge Sustainable Innovation in Dentistry, Universidad Nacional Federico Villarreal, Lima, Peru

<sup>3</sup> Undergraduate Student Faculty of Dentistry, Universidad Nacional Federico Villarreal, Lima, Peru

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### Abstract:

**Background:** To assess the influential determinants of microbial colonization of oral cavity in term infants

**Materials and Methods:** This study was approved by the ethics committee of the Faculty of Dentistry-Universidad Nacional Federico Villarreal and was conducted at the Instituto Nacional Materno Perinatal (INMP), Lima-Peru in March 2022. The sample consisted of 30 full-term births and swabs on the palate and back of the tongue 4 and 8 hours after birth. Five culture media were used for reading and identifying microorganisms

**Results:** Eutocic births were female (36.7%). The mothers of the neonates did not receive dental prophylaxis in the last 6 months (76.6%). 46.7% of eutocic births received Exclusive Breastfeeding and were suitable for gestational age (AEG) in 83.4%. *Staphylococcus spp* was present 4 and 8 hours after birth ( $p=0.273$ ) and the rest of the microorganisms were absent ( $p=0.000$ ). Regardless of gestational age, *Streptococcus spp* and Non-Recoverable Anaerobes BGP are present at 8 and 4 hours respectively in the newborn, ( $p < 0,05$ ). Regarding birth weight regardless of whether AEG or large for gestational age (GEG) *Enterobacter agglomerans* and *Candida* are present at 4 and 8 hours respectively ( $p < 0,05$ ). Also, regardless of the number of prenatal controls (CPN), *Streptococcus spp* and *Klebsiella oxytoca* are present in 4 and 8 hours respectively ( $p < 0,05$ ). *Staphylococcus spp* are present in the first 4 hours in dystocic births ( $p=0,05$ ).

**Conclusion:** Term newborns present microbial colonization of the oral cavity in the first 4 and 8 hours of birth, with *Staphylococcus spp* being the most prevalent. Gestational age was the most influential determinant in oral cavity colonization for *Staphylococcus spp*. And, the determinants of eutocic delivery and exclusive breastfeeding show the presence of more microorganisms in the oral cavity.

**Key Word:** term births, microbial colonization, oral cavity

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Date of Submission: 22-05-2023

Date of Acceptance: 02-06-2023

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### I. Introduction

The first exposure to microorganisms in vaginal births occurs at the time of delivery and, exposure to bacteria in cesarean section is from the skin of parents and medical team. Therefore, different types of birth lead to differences in the gut microbiota of infants (Penders et al., 2006; Dominguez-Bello et al., 2010). A significant part of the oral microbiota in the early neonatal period originates in the mother and transient population of microorganisms consisting of intestinal bacteria (in naturally born newborns). The resident microbiota in this period depends mainly on external factors, such as gestational age, type of delivery, type of feeding, length of hospital stay after childbirth and general condition. (Machorowska-Pieniązek et al., 2017)

Mutans streptococci have been detected more frequently in the oral cavity and, at an earlier age, in children born by caesarean section than in those born by vaginal delivery (Thakur et al., 2011)

The first microorganisms to colonize approximately 8 hours after delivery collectively constitute the so-called pioneer microbial community or flora, the Streptococci (*S. salivarius*, *S. mitis* y *S. oralis*) They are the first to settle and the most numerous, but they are also present the Lactobacillus, Haemophilus, Actinomyces and some non-pathogenic species of Neisseria (Negróni, 1999). At birth by vaginal delivery, exposure is direct from the newborn to maternal vaginal and rectal microbes, while cesarean delivery allows microbes from maternal skin and hospital environment to be the first to seed the newborn. (Kaan et al., 2021) Neonates born vaginally tend to have a more diverse oral and intestinal flora, while neonates born by cesarean section have less diversity and a greater number of pathogenic bacteria such as *Clostridium difficile* by the late acquisition of bifidobacteria and *Escherichia coli*. (Li et al., 2005). Currently, there are few articles on the variables to be studied, both nationally and

internationally, so this study aims to answer the following question: What will be the determinants that influence microbial colonization of the oral cavity of term newborns?

## II. Material And Methods

**Spatial and Temporal Scope of the Study.** The data collection of this study began in March 2022, in the center of immediate attention of the newborn of the Delivery Unit of the Department of Neonatology of the INMP, the swabs were performed from the oral cavity of neonates born during the day shift.

Type of research is correlational and inferential by application of hypothesis tests. Design of this study is prospective, longitudinal by the measurement of the variables in two moments; 4 and 8 hours. The Universe: are newborns in the Department of Neonatology of the INMP. Sample: Neonates newborns in the Immediate Care Center of the Newborn Unit. The type of sampling was non-probabilistic for convenience due to the heterogeneity of the study population.

Sterile swabs, disposable latex gloves and sterile specimens with lids were used for sample collection. Each neonate had 2 swab samples recorded on the back of the tongue and hard palate: the first record (test A) was performed in the first 4 hours of life, the second record (test B) was performed during the 4 hours after the first collection, that is; during the 8 hours of life. The swabs used in the registries were placed in sterile specimens with saline solution without preservatives, then transferred to the microbiology laboratory of the INMP during the First 20 minutes after taking each sample. All microbiological processes were performed in the Microbiology Laboratory of the Clinical Laboratory service of the National Maternal Perinatal Institute (INMP). For the identification of the microorganisms, each sample obtained was added thioglycolate broth, then it was taken to incubator at 35°C for 24 hours together with the direct seedings of blood agar. After the incubation time, GRAM staining was applied, then the seedings were carried out in four types of culture media: MacConkey agar, Sangre Azida agar, salted Mannitol agar and Sabouraud agar, being taken back to the incubator with the same parameters. After 24 hours the cultures were removed, then biochemical identification was applied in the positive cultures by means of the catalase test, coagulase test, germination test, and subcultures were performed in TSI, LIA, Bilis esculina and other means for better identification, in addition Api 20 E tests were applied, Api Staph and Api 20C, again all the crops were taken to incubator for 35 ° C from 18 to 24 hours more. Luego tipificación de los microorganismos.

## III. Result

**Table 1: Oral colonization in neonates born at term at 4 and 8 hours after birth.**

Microorganismos aislados en cavidad bucal	4 horas después del nacimiento			8 horas después del nacimiento			
	ausente	presente	*sig.	ausente	presente	*sig.	
	n(%)	n(%)		n(%)	n(%)		
<i>Staphylococcus spp</i>	18(60)	12(40)	.273	14(46,7)	16(53,3)	.71	
<b>CGP</b>	<i>Staphylococcus epidermidis</i>	28(93,3)	2(6,7)	.000	25(83,3)	5(16,7)	.000
	<i>Streptococcus spp</i>	27(90)	3(10)	.000	22(73,3)	8(26,7)	.01
	<i>Enterococcus spp</i>	28(93,3)	2(6,7)	.000	26(86,7)	4(13,3)	.000
<b>BGP</b>	<i>Lactobacillus spp</i>	23(76,7)	7(23,3)	.003	19(63,3)	11(36,7)	.14
	<i>Anaerobios/No Recuperables</i>	27(90)	3(10)	.000	26(86,7)	4(13,3)	.000

*Staphylococcus spp* is present with (40%) at 4 hours after birth, but is not statistically significant (p=0,273) Meanwhile, the rest of the microorganisms isolated in the oral cavity are mostly absent (p=0,000). In addition, at 8 hours the microorganisms *Staphylococcus spp* and *Lactobacillus spp* are mostly present and absent respectively, but it does not represent statistical significance (p= 0,715 and 0,144) and, the rest of the microorganisms are mostly absent (p=0,000) (Table 1).

**Table 2**  
**Oral colonization in term newborns in sociodemographic determinants, gynecobstetricians.**

Determinantes sociodemográficos										
ausencia	Grado de instrucción		*OR	Sexo		*OR	Edad de la madre		*O	
	Básico n(%)	superior n(%)		masculino n(%)	femenino n(%)		Menor 35 n(%)	Mayor igual 35 n(%)		
<i>Staphylococcus spp</i>	4 horas	17(56,7)	1(3,3)				17(56,7)	1(3,3)	8,5(8-8)	
	8 horas	13(43,3)	1(3,3)	5,66(0,51-62)	7(23,3)	11(36,7)	6,36(1,4-2,7)	13(43,3)	1(3,3)	4,3(4-4)
<i>Escherichia coli</i>	4 horas	25(83,3)	4(13,3)	,8(,7-9)	13(43,3)	16(53,3)	,5(,3-7)	24(80)	5(16,7)	8,(7-9)
	8 horas	22(73,3)	4(13,3)	,8(,7-9)	12(40)	14(46,7)	2,5(2-28,0)	22(73,3)	4(13,3)	1,8(1-2)

  

Determinantes ginecoobstétricas										
ausencia	Edad gestacional			Peso al nacer			Control prenatal			
	37-38 n(%)	39-40 n(%)	*sig	AEG n(%)	GEG n(%)	*sig	-5 n(%)	+≥5 n(%)	*sig	
<i>Staphylococcus spp</i>	4 horas	0(0)	2(6,7)	,41	2(6,7)	0(0)	,56	2(6,7)	0(0)	,01
	8 horas	4(13,3)	4(13,3)	,03	8(26,7)	0(0)	,19	2(6,7)	6(20)	,90
<i>Anaerobios no recuperables BGP</i>	4 horas	4(13,3)	3(10)	,01	7(23,3)	0(0)	,23	3(10)	4(13,3)	,26
	8 horas	3(10)	8(26,7)	,69	9(30)	2(6,7)	,55	5(16,7)	6(20)	,07
<i>Enterobacter agglomerans</i>	4 horas	0(0)	3(10)	,31	1(3,3)	2(6,7)	,00	0(0)	3(10)	,27
	8 horas	1(3,3)	3(10)	,93	4(13,3)	0(0)	,39	0(0)	4(13,3)	,19
<i>Candida no albicans</i>	4 horas	0(0)	0(0)	---	0(0)	0(0)	---	0(0)	0(0)	---
	8 horas	0(0)	1(3,3)	,57	0(0)	1(3,3)	,01	0(0)	1(3,3)	,54
<i>Klebsiella oxytoca</i>	4 horas	26(86,7)	4(13,3)	---	13(43,3)	17(56,7)	---	25(83,3)	5(16,7)	---
	8 horas	25(83,3)	4(13,3)	,8(,7-9)	13(43,3)	16(53,3)	,5(,3-7)	24(80)	5(16,7)	,8(,7-9)

Women at 8 hours of birth have a higher risk of the presence of *Escherichia coli* OR 2,5(0,2-28,0) But it is not statistically significant. With respect to mothers with a basic education and under 35 years of age, they are at greater risk of presenting *Staphylococcus spp* at 4 o'clock in your full-term newborns, but, which is not statistically significant OR 5,66(0,51-62) and OR 8,5(0,8-88,8) respectively. For gynecobstetrician determinants, regardless of gestational age, isolated microorganisms *Streptococcus spp* and *Non-Recoverable Anaerobes BGP* are more likely to be present at 8 and 4 hours respectively in the newborn, (p=0,03; p=0,01 respectively). Regarding birth weight regardless of whether AEG o GEG microorganisms *Enterobacter agglomerans* and *Candida no albicans* are more likely to be present at 4 and 8 hours respectively in the neonate (p=0,00; p=0,01 respectively). Also, regardless of the number of prenatal check-ups, microorganisms *Streptococcus spp* and *Klebsiella oxytoca* are more likely to be present in the first 4 and 8 hours respectively in the newborn (p=0,01; p=0,01 respectively) (Table 2)

**Table 3**  
**Oral colonization in term newborns in behavioral determinants: LME-Formula and dental prophylaxis of mothers.**

Isolated microorganisms present	hours	LACTATION (neonate)			DENTAL PROFILAXIS (mothers)		
		Fórmula n(%)	LME n(%)	*sig	yes n(%)	no n(%)	*sig
<i>Lactobacillus spp</i>	4	3(10)	4(13,3)	,666	2(6,7)	5(16,7)	,708
	8	5(16,7)	6(20)	,705	2(6,7)	9(30)	,612
<i>Anaerobios no Recuperables BGP</i>	4	2(6,7)	1(3,3)	,543	0(0)	3(10)	,314
	8	0(0)	4(13,3)	,032	0(0)	4(13,3)	,236
<i>Klebsiella oxytoca</i>	4	0(0)	0(0)	---	0(0)	0(0)	---
	8	0(0)	1(3,3)	,309	0(0)	1(3,3)	,575
<i>Klebsiella Peumoniae</i>	4	0(0)	0(0)	---	0(0)	0(0)	---
	8	2(6,7)	0(0)	,143	2(6,7)	0(0)	,008

It is observed that *non-recoverable anaerobic microorganisms isolated BGP* y *Klebsiella Peumoniae* are more likely to be present at 8 hours in the newborn regardless of the type of formula breastfeeding or exclusive breastfeeding and, whether or not mothers have undergone dental prophylaxis in the past six months (p=0,032; p=0,008 respectively). (Table 3)

**Table 4. Oral colonization in full-term newborns according to the presence of microorganisms from the mother**

Microorganisms of the mother			Type of delivery					
			4 hours			8 hours		
			Eutocic	Dystocic	*sig	Eutocic	Dystocic	*sig
Staphylococcus spp	absent	n(%)	13(21,7)	8(13,3)	,050	11(18,3)	12(20)	,671
	present	n(%)	2(3,3)	7(11,7)		4(6,7)	3(5)	
Enterococcus spp	absent	n(%)	14(23,3)	15(25)	,317	14(23,3)	15(25)	,317
	present	n(%)	1(1,7)	0(,0)		1(1,7)	0(,0)	
Lactobacillus spp	absent	n(%)	15(25)	15(25)	1,000	15(25)	15(25)	1,000
	present	n(%)	0(,0)	0(,0)		0(,0)	0(,0)	
Escherichia coli	absent	n(%)	10(16,7)	15(25)	,016	14(23,3)	11(18,3)	,148
	present	n(%)	5(8,3)	0(,0)		1(1,7)	4(6,7)	

*Enterococcus spp* is present in eutocic births, but not statistically significant (p= 0,317) and, *Lactobacillus spp* It is not present in any type of delivery. However, the microorganism *Staphylococcus spp* is more likely to be present in the first 4 hours in dystocic births. (p=0,050), the microorganism *Escherichia coli* is likely to be present in the first 4 hours in eutocic births (p=0,016). (Table 4)

#### IV. Discussion

This study conducted at the National Maternal Perinatal Institute (INMP) was approved by the Ethics Committee of the Faculty of Dentistry of the Federico Villareal National University (UNFV), aimed to evaluate the influential determinants of microbial colonization of the oral cavity in term newborns, during the month of March 2022. Microbiological processing was performed in the Microbiological Laboratory of the INMP using microbiology methodology and protocol for the identification of microorganisms in the oral cavity for term newborns.

Dominguez-Bello It states that the development of the human fetus is in an environment free of microorganisms and, at birth, it is exposed to many of them that usually acquires them during and after passing through the vaginal route. This study reported microbial colonization of the oral cavity during the first 4 and 8 hours after birth, presumably due to extrinsic factors such as type of lactation or delivery.

The microorganism isolated in oral cavity reported as most prevalent in our study was the *Staphylococcus spp* at 4 and 8 hours after birth with 40% and 53.3% respectively. However, Negroni reported *Streptococcus spp.* with 30% prevalence. These differences are due to the presence of microorganisms not frequent in the oral cavity such as large negative bacilli. Among them we have *Enterobacter agglomerans*, *Escherichia coli* that we have found at 4 and 8 hours after birth and we relate it to the hospital environment. Plonka, et al. In his study on colonization of streptococci mutans (SM) and lactobacilli (LB) in pre-toothed children from the neonatal period to 7 months, he detected the presence of these microorganisms at 34 days after birth. In contrast, our study did not detect *Lactobacillus spp* during the first 8 hours, but *Streptococcus spp* was found during the first 4 hours (5%) and 8 hours (13.3%). And, this is due to transmission by the type of delivery and type of breastfeeding especially if it is with a bottle.

Our study found that gestational age from 37 to 38 weeks and 39 to 40 weeks was the most influential determinant in oral cavity colonization for *Staphylococcus spp* at the first 8 hours after birth (13.3%). Makhoul et al, In his study on evaluation of the effect of gestational age and antibiotic therapy on the microbial oral flora in preterms, he reported that after antibiotic therapy the oral microbial flora undergoes changes with predominance of *Staphylococcus spp*. These reports deserve important attention in the case of self-administration of antibiotic therapy triggering late-onset sepsis. These differences are due to the fact that our study was conducted in full-term babies without antibiotic therapy.

## V. Conclusion

Term newborns have microbial colonization of the oral cavity in the first 4 and 8 hours of birth, with *Staphylococcus* spp being the most prevalent. Gestational age was the most influential determinant in oral cavity colonization for *Staphylococcus* spp. And, the determinants eutocic delivery and exclusive breastfeeding show the presence of more microorganisms in the oral cavity.

It was evidenced that at 4 hours after birth *Staphylococcus* spp is present and the rest of the microorganisms isolated in the oral cavity are mostly absent. At 8 hours the microorganisms *Staphylococcus* spp are mostly present and the rest of the microorganisms are mostly absent.

In gestational age, the isolated microorganisms *Streptococcus* spp and Non-Recoverable Anaerobes BGP are more likely to be present at 8 and 4 hours respectively in the newborn. Regarding birth weight regardless of whether AEG or GEG microorganisms *Enterobacter agglomerans* and *Candida albicans* are more likely to be present at 4 and 8 hours respectively. Also, regardless of the number of prenatal controls, the microorganisms *Streptococcus* spp and *Klebsiella oxytoca* are more likely to be present in the first 4 and 8 hours respectively in the newborn.

The non-recoverable anaerobic microorganisms isolated BGP and *Klebsiella pneumoniae* are more likely to be present at 8 hours in the newborn regardless of the type of lactation and, whether or not the mothers had dental prophylaxis in the last six months.

The isolated microorganisms *Enterococcus* spp are present in eutocic births and *Lactobacillus* spp is not present in any type of delivery. However, the organism *Staphylococcus* spp is more likely to be present in the first 4 hours in dystocic births. The microorganism *Escherichia coli* is likely to be present in the first 4 hours in eutocic births.

**Conflicts of interest:** No conflict of interest is reported by the authors of this study or professors of the Faculty of Dentistry of the Federico Villarreal National University.

**Authors' contribution:** All members of this study contributed to the literature review and the writing and correction of the manuscript.

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