

Research Article

Profile of the Nasopharyngeal Microbiota of Newborns Admitted to a Neonatology Unit at the Hospital Universitário Antônio Pedro

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Abstract

Objective: To characterize the nasopharyngeal microbiota of high- and low-risk newborns in the Hospital Universitário Antônio Pedro, Universidade Federal Fluminense (HUAP-UFF) neonatal unit.

Method: This prospective cross-sectional study included newborns from the Neonatal Unit of the HUAP-UFF divided into two groups: Intensive Care Units - Intermediate Unit ICU-IU (G1) and rooming-in (G2). After the newborn was breastfed and had approximately 24 hours of life, the material was collected from the nasopharynx.

Variables surveyed: type of delivery, gestational age, sex, birth weight, Apgar score, and use of antimicrobials. These data were statistically analyzed using the SPSS18.0[®] program.

Results: In total, 100 samples were collected, of which 20 were from G1 and 80 from G2. The mean birth weight in both groups was 3,025.75 g ± 757.45 g. The mean gestational age was 37.55 ± 2.77 weeks. The median 1-minute APGAR was 8, and the median 5-minute APGAR was 9. The culture grew coagulase-negative *Staphylococcus* in 91% of the samples, 2% grew coagulase-positive *Staphylococcus*, and in 7%, there and no growth of microorganisms. All cultures with coagulase-positive *Staphylococcus* grew from samples from group 2. Of the variables raised, only the use of antibiotics was relevant, and their use was related to the non-growth of microorganisms (p=0.021).

Conclusion: Coagulase-negative *Staphylococcus* was the predominant microorganism in the nasopharynx of newborns within 24 h of life, regardless of the place of admission in the neonatal unit. The only statistically significant variable was antibiotic use.

Keywords: Newborns; Nasopharyngeal; Microbiota; Neonatal unit

Introduction

The Ministry of Health defines a newborn at risk as one exposed to situations in which there is a greater probability of an unfavorable development, in addition to an above average chance of mortality and morbidity [1].

The term “high-risk newborns” identifies a group of newborns who are very likely to develop a serious acute illness or result in an adverse situation [2]. It represents the majority of patients hospitalized in Neonatal Intensive Care Units (NICU), where low weight and premature birth are the main determining factors [3]. Newborns with a gestational age below 37 completed weeks were considered premature. Low-birth-weight newborns are those born weighing less than 2500 g.

After birth, these children are usually hospitalized and monitored in the NICU until their conditions allow hospital discharge. They are particularly more prone to infections, mainly of the lower airway [4]. These infections can eventually evolve, causing the death [5] of these newborns or serious complications [6].

Investigations related to the diagnosis and treatment of diseases that develop in the NICU showed strong evidence that prematurity, in particular, potentiates the severity of infections, the etiological agent of which is often not identifiable. Diagnostic and therapeutic resources have been used for respiratory disorders in newborns, but their sensitivity and specificity have not yet reached the desired level in terms of understanding the problem. Thus, investigating in different ways, which is the most appropriate way to manage a patient in the NICU, is a task that leads researchers to employ new management proposals, including the identification of the nasopharyngeal microbiota of high-risk newborns, a subject with few published studies, both in Brazil and abroad.

Published studies with different objectives and proposals (analyzing the prevalence of bronchial asthma or infections during a viral episode) than what this research proposes or with other populations (different ages) suggest that high-risk newborns may have colonization of the nasopharynx by microorganisms different from those found in low-risk newborns and that this could contribute to the development of diseases that originate in the nasopharynx [7-9]. To date, no more detailed investigations have been carried out to

Citation: Heisler GHF, Barbosa ADM. Profile of the Nasopharyngeal Microbiota of Newborns Admitted to a Neonatology Unit at the Hospital Universitário Antônio Pedro. *J Pediatr Neonatol.* 2023;4(1):1025.

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Publisher Name: Medtext Publications LLC

Manuscript compiled: Jan 31st, 2023

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characterize the microbiota of these newborns and, thus, point out the most appropriate approach and conduct for an associated disease.

In a search on the SciELO and PubMed platforms about articles published in the last ten years on the nasopharyngeal microbiota in high-risk newborns, no results were found at the time of writing this research, using the keywords: Microbiota, Nasopharynx, Newborn, and Intensive Care Unit. However, in older age groups, there is a proliferation of scientific studies on nasopharyngeal microbiota, mainly related to asthma and recurrent otitis media [7-11].

Studies suggest that high-risk newborns may colonize the nasopharynx by microorganisms different from those found in low-risk newborns, which could contribute to developing diseases that originate there. To date, no more comprehensive investigations have been carried out to characterize the microbiota of these newborns and, thus, point out the most appropriate approach and conduct for an associated disease. Thus, the general objective of the present study was to identify the predominant colonization of microorganisms present in the nasopharynx of newborns admitted to the Neonatal Unit of the Hospital Universitário Antônio Pedro, Universidade Federal Fluminense, with the specific objectives being to characterize the data of the participants of the sample groups and to describe the most frequent microorganisms that grew in samples collected from newborns.

Methods

Type and period of study

A prospective cross-sectional study was carried out in newborns born alive at the Neonatal Unit of Hospital Antônio Pedro, Universidade Federal Fluminense, HUAP-UFF, between January 6 and July 15, 2021, comparing the colonies of microorganisms in the nasopharynx identified in the two groups of newborns.

HUAP is a hospital at the Fluminense Federal University (UFF) managed by the Brazilian Hospital Services Company (EBSERH) and linked to the Brazilian Unified Health System (SUS). The HUAP Obstetrics service receives high-risk pregnant women, and the HUAP Neonatology service is divided into an ICU, an intermediate unit (IU), both allocated in group 1, and a rooming-in for mothers and low-risk newborns, group 2. Currently, approximately 390 newborns are born annually at this hospital.

Group formation and material collection

A high-risk new-born was defined as a child hospitalized in the NICU or the UI. Newborns accepted in the study were divided into two groups based on which neonatal service they were warded at the time of collecting material from the nasopharynx: (1) Originating from the ICU or IU (G1) and (2) originating from rooming-in (G2). Figure 1 shows the process of selecting participants and forming groups in this study.

Collection of material from the newborn's nasopharynx

Collecting material from the nasopharynx was performed approximately 24 h after birth. These collections were made using a No. 4 nasal probe, without suction pressure, functioning only as a less traumatic nasal swab. This probe was chosen instead of a traditional swab, considering the tiny dimensions of newborns, especially those of low birth weight, and the curved anatomical shape of the airways up to the nasopharynx.

After collection, the probe was rubbed in a solid culture medium

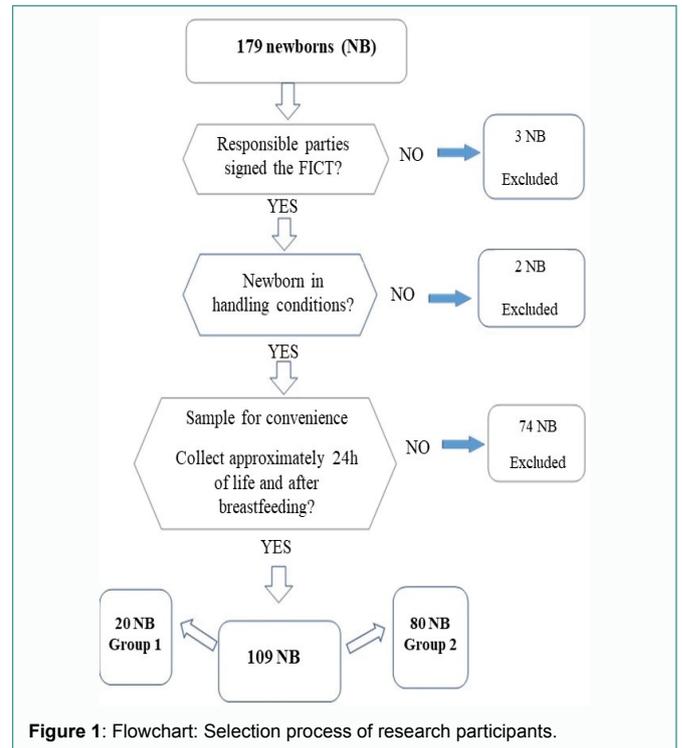


Figure 1: Flowchart: Selection process of research participants.

with Blood Agar, and the microorganisms collected were transferred to the culture medium. The friction mechanism on the plate was in a cross pattern, taking advantage of all four sides of the number 4 probe. The material was collected in the Neonatology Service by a single physician, assisted by the nursing staff to access the newborns. The nasal cavity that was more accessible without predilection was chosen. The solid medium plate remained close to the closed bed and opened for only a few seconds before sowing. Routine personal protective equipment, such as an N95 mask, 40-weight coat, cap, and gloves, were used during collection.

Identification of these colonies was performed by the Microbiology Laboratory of the Pathology sector of HUAP-UFF from the culture of the plate with blood agar.

Exclusion criteria

1. Newborns born outside the Antônio Pedro University Hospital.
2. Newborns admitted to the Neonatal Unit of the Hospital Universitário Antônio Pedro during the study period who presented contraindications to the handling assistant doctor due to their clinical status.
3. Newborns who had not yet been breastfed.

Raised variables

The following variables were raised:

1. Type of delivery
2. Gestational age
3. Sex
4. Birth weight
5. Apgar score of 1 and 5 minutes
6. Use of antibiotics

Statistical treatment of data

The obtained data were entered into an Excel® spreadsheet and presented in tables. Next, the data were statistically analyzed using the SPSS18 program. 0°, using the chi-square test (or Fisher's exact test) to verify the association between variables, and Student's t-test (or Mann-Whitney) to compare means after verifying the data distribution, according to its normality, using the Kolmogorov-Smirnov test. A probabilistic error of 5% was obtained.

Research ethics committee and free and informed consent form

This project was evaluated and approved by the CEP/UFF on August 31, 2021 (Research Protocol No. 23636719.1.0000.5243). Those responsible for the newborns were instructed on the objectives and methods of the present study and asked to give their written consent for inclusion in the study, according to the Free and Informed Consent Terms (FICT).

Results

Cultures were obtained from a convenience sample collected from newborns with approximately 24 h of life from one hundred (100) research participants. Twenty samples comprised G1 (the ICU-IU Group), and 80 samples comprised G2 (the AC Group). During the study period, 179 newborns were born alive at our hospital.

Observing the profile of the newborns who made up the study, it is noted that most deliveries were cesarean (83%), that the predominant sex was male (55%), with an average weight of $3025.75 \text{ g} \pm 757.45 \text{ g}$ and mean gestational age of 37.55 ± 2.77 weeks. In addition, the median APGAR at 1 and 5 min was 8 and 9, respectively. As for the culture, it was observed that coagulase-negative *Staphylococcus* grew in 91% of the cases, and it was isolated in 88%. Most patients (90%) did not use antibiotics.

Table 1 shows the findings in relation to the place of accommodation for newborns. Of the 20 cultures performed in G1, colonies of coagulase-negative *Staphylococcus* grew in 17 (85%), and no bacterial colonies were observed in three (15%). Of the 80 cultures performed in G2, 74 (92.5%) showed coagulase-negative *Staphylococcus* growth. One of these was Gram-positive rod contamination. A second with concomitant growth of alpha-hemolytic Streptococcus, and a third with concomitant growth of *Proteus mirabilis* (representing 1.25% each). Four (4) cultures from this group did not grow (5%). Two (2) other cultures from this group resulted in the growth of coagulase-positive *Staphylococcus* (*Staphylococcus aureus*), representing 2.5% of this group.

No significant association was observed between the growth of colonies of microorganisms and the type of delivery. There was no association between colony growth and the newborn's place of accommodation.

Regarding the growth of the coagulase-positive or-negative *Staphylococcus* type, no significant difference was observed between the variables and the type of *Staphylococcus* that developed in the cultures.

Finally, Table 2 clarifies the relationship between the newborn's place of stay and the use of antibiotics, with a strong link between G1 and the use of antibiotics ($p < 0.0001$), and Table 3 shows the relationship between the use of antibiotics and the non-growth of colonies of microorganisms ($p = 0.021$).

Table 1: Association between new-borns according to the place of hospitalization in the Neonatology Service of the HUAP and the different variables and the growth of microorganisms

Variables	Group 1 (n=20)	Group 2 (n=80)	p-value
Type of delivery			
Caesarean	17 (85%)	66 (82.5%)	0.79
Vaginal	3 (15%)	14 (17.5%)	
Sex			
Female	11 (55%)	34 (42.5%)	0.315
Male	9 (45%)	46 (57.5%)	
Birth weight, mean \pm SD	2201.75 \pm 993.30	3231.75 \pm 516.72	<0.0001*
Gestational age, mean \pm SD	33.65 \pm 2.92	38.58 \pm 1.65	<0.0001*
APGAR 1min, median (p25-p75)	8 (7-8)	8 (8-9)	0.007**
APGAR 5min, median (p25-p75)	9 (8-9)	9 (9-9)	<0.0001**
Culture			
S.coagulase neg.	17	71	0.614
S.coagulaseneg.+ Rod	0	1	
S.coagulaseneg+P.mirabilis	0	1	
S.coagulaseneg+E.alpha Hemolytic	0	1	
S.coagulase pos.	0	2	
No colony growth	3	4	

SD: Standard Deviation

*Represents a significant difference ($p < 0.05$; t-student test); **represents a significant difference ($p < 0.05$; Mann-Whitney test); ***represents a significant difference ($p < 0.05$; Fisher's Exact Test).

Table 2: Association between new-borns according to location and antibiotic use

Variáveis	ICU-IU (n=20)	Rooming-in (n=80)	p-value
Antibiotic use			
Yes	9	1	<0.0001*
No	11	79	

*Represents a significant difference ($p < 0.05$; Fisher's exact test).

Table 3: Association between the use of antibiotics in new-borns and the growth of colonies of microorganisms

Growth of colonies of microorganisms			
Variables	Yes (n=93)	No (n=7)	p-value
Use of antibiotics			
Yes	7	3	0.021*
No	86	4	

*Represents a significant difference ($p < 0.05$; Fisher's exact test).

Although this research was carried out during the SARS-COV 2 pandemic, only one (1) pregnant woman out of the 100 pregnant women, the result of the RT-PCR test for the virus was positive, and this fact had no relevance to the results obtained.

Discussion

Many neonatology services maintain identification through cultures of pathogenic microorganisms responsible for infections caused in newborns hospitalized in their accommodations, but this process can be time-consuming to perform efficiently.

Some services also routinely collect samples of the nasal microbiota, regardless of the infection present. However, these collected data are not generally tabulated, organized, statistically analyzed, and compared within a scientific work, ultimately becoming just a sample for guidance in a delimited space-time context.

The ICU is a hospital unit responsible for caring for patients who, due to their worse health status, are at greater risk and need uninterrupted medical assistance [12]. High-risk newborns are separated from their mothers and treated in the ICU. On the other hand, rooming-in is characterized by the permanence of the mother

and baby together in the postpartum period, which is the ideal condition to favor interaction between them [13]. In this case, the newborns are kept with the mother, and are considered to be at low risk of nosocomial infections [14].

In this study, variables were compared according to the newborn's place of stay. Lower birth weight and gestational age were observed in newborns in the ICU. These factors were expected, as these newborns had a mean gestational age of 33.65 weeks, associated with prematurity. This contributes to low birth weight and lower APGAR scores at 1 and 5 minutes, which are associated with higher morbidity and mortality among premature newborns, which is in agreement with the findings in the literature [14]. The findings regarding prematurity and low birth weight were also reported by Lee et al. [15], who evaluated bacterial colonization in hospitalized newborns with nasal cavity swabs in another context. In their research in California, swabs of the nasal cavities were initially performed during hospitalization in 90 newborns (regardless of their risk condition). Subsequently, at hospital discharge, 83 newborns returned. All the cells were seeded onto agar plates. The newborns were hospitalized for at least seven (7) days. Their results were 4%, with no culture growth after discharge, and their finding that *Staphylococcus* colonies can take up to three days to establish.

In our study the type of delivery, sex, and colony growth showed no significant association with the place of stay of the new-born. However, coagulase-positive *Staphylococcus* was observed in G1 (rooming-in), which was not observed in G2 (ICU). Although there was no significant association between these groups, some important points are worth mentioning.

Colonies of microorganisms present in different human body segments play an important role in maintaining host health. This can be explained by the fact that it prevents the installation of other colonies of potentially more pathogenic microorganisms or by improving the barrier function, immunomodulation, and possible production of neurotransmitters [16-18]. Coagulase-negative *Staphylococcus* is considered non-pathogenic with low virulence. Coagulase-positive *Staphylococcus* is associated with nosocomial infections and is a significant cause of infection in high-risk newborns [19-21]. Research by Lee et al. [15], investigated the factors that inhibit the formation of a biofilm by *Staphylococcus aureus* (coagulase-positive) from *Staphylococcus epidermidis* (coagulase-negative), especially Esp serine protease. They concluded that colonization by *Staphylococcus epidermidis* inhibits the growth of *Staphylococcus aureus* in newborns, probably by secreting proteases. However, there is no conclusive evidence regarding the Esp serine protease.

In the current study, there were two cases of coagulase-positive *Staphylococcus* in G1 (rooming-in patients), which was initially surprising since this type of growth was expected to occur in newborns from the ICU (G2) [22]. However, this fact may be associated with the safety behaviors adopted in the care of newborns by ICU health professionals compared to the safety care adopted in the rooming-in, which is much less rigorous. Additionally, joint accommodation allows the entry of companions and visitors, who are potential carriers of contaminants [23]. It is impossible to be sure of the real source of contamination, and currently, coagulase-positive *Staphylococcus* is already present in all hospital sectors without distinction.

The results showed that the predominant microorganism in all samples was coagulase-negative *Staphylococcus* (91% of the samples).

Evaluating the variables associated with the microorganism profile, it was observed that newborns with lower birth weights showed the presences of coagulase-positive *Staphylococcus* colonization (-12%). Low birth weight is associated with prematurity and infections [14]. Studies have shown that premature birth promotes a change in the initial environment of the nasopharynx and is an important risk factor for respiratory infections; however, in the present study, an association between gestational age and the growth of microorganisms was not observed [9,23,24]. In this study, the reduced number (two cases) of colonization by coagulase-positive *Staphylococcus*, both in rooming-in, limited the analysis and possible conclusions.

Thus, a suggestion for future work would be to increase the sample size to obtain a greater number of cultures with positive coagulase *Staphylococcus* and, consequently, a more significant analysis.

Teo et al. [9] studied 234 children in their first year of life, evaluating their viral and bacterial communities, and observed that most children were initially colonized by *Staphylococcus* or *Corynebacterium* until they stabilized *Alloicoccus* or *Moraxella* colonies. They highlighted that identifying the microbiome is crucial for assessing lower airway infections, the severity of the inflammatory symptoms that accompany them, and the risk of future development of asthma.

Dominguez-Bello et al. [23] compared newborns with vaginal delivery and those born via cesarean section. The microbiota was observed and reported that the type of delivery influenced the acquisition and structure of the initial microbiota of the newborn. Whereas the present study showed no significant association between type of delivery and growth of microorganisms. However, they did not observe an association with the sex of the newborns, which corroborates the results of the present study. A possible explanation for this disagreement could be the period of approximately 24 hours between the samples of the two surveys. In these 24 hours, the growth of *Staphylococcus* colonies could have already displaced other microorganisms from the nasopharynx, thus justifying the presence of coagulase-negative *Staphylococcus* in the nasopharynx of newborns born by transvaginal delivery after the first feeding at approximately 24 hours of life in the current study.

In this study, it was also observed that the use of antibiotics interfered with the growth of colonies of microorganisms in the nasopharynx of newborns and that these drugs are primarily used in high-risk G1.

These findings were expected and were found by other researchers [25-29]. It is likely that this mechanism may be responsible for future unfavorable nasopharyngeal recolonizations in newborns, with the replacement of coagulase-negative *Staphylococcus* by other more aggressive colonies, such as coagulase-positive *Staphylococcus*. Further studies with longer follow-up times are needed to answer these questions. Perhaps, in a longer follow-up period of high-risk newborns, the results would be different.

Finally, it is worth mentioning that we observed a very low percentage of deliveries via the transvaginal route, with only 17% versus 83% of cesarean sections performed. However, this study was carried out in a maternity hospital that receives high-risk pregnant women, which may explain this high rate. These numbers are left to contribute to future comparative research between similar institutions. No similar studies were found in the literature to discuss the results.

The limitations of this study include the convenience sample size, especially the ICU-UI sample size (20), which is considered small and unrepresentative; the follow-up time (24 hours of life) would probably be different in a longer follow-up period, especially considering the factor of antibiotic use with the sterilization of the original nasopharyngeal microbiota and possible replacement by a potentially more aggressive one. Perhaps with 72 hours or more of life, the results were different. However, there were no errors or any complications during the collection of material from the newborns, and the use of aspiration probe number 4 as a swab was the right approach, as it allowed the collection of material safely, without accidents, trauma, or greater discomfort in newborns who have a short airway and are prone to accidents.

Conclusions

It was concluded that the predominant microorganism in all cultures identified was coagulase-negative *Staphylococcus*. No difference was identified between cultures developed on blood agar plates collected from the nasopharyngeal microbiota of newborns from both groups examined at approximately 24 hours of life. No difference was identified between the colonization of the nasopharynx of newborns born by cesarean section and by transvaginal delivery, and the use of antibiotics was associated with the non-growth of microorganism colonies in newborn nasopharyngeal cultures.

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