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Original Research

Dynamics of salivary microbiota in pediatric cardiac patients: from the ward to the intensive care unit

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Background: Nosocomial infections pose a significant risk to pediatric cardiac patients, often linked to alterations in the oral microbiota during hospitalization. **Objective:** To identify microorganisms responsible for nosocomial infections in the saliva of children and adolescents with congenital heart disease admitted to a tertiary care hospital. **Methods:** This prospective study included patients aged 0–18 years who underwent cardiac surgery. Unstimulated saliva samples were collected at three time points: (1) within 24 hours of admission to the hospital ward; (2) within 24 hours after surgery in the Pediatric Intensive Care Unit (PICU); and (3) 48 hours postoperatively in the PICU. Oral hygiene status was assessed using the Visible Plaque Index (VPI). Samples were analyzed to identify the presence of microorganisms associated with nosocomial infections. **Results:** Among 50 participants, 44% exhibited alterations in their salivary microbiota, with *Klebsiella pneumoniae* (25%) being the most prevalent pathogen, followed by *Acinetobacter baumannii* (24%) and *Enterobacter cloacae* (20%). Nosocomial infections occurred in 10% of patients. Microbiota alterations were significantly associated with younger age (<12 months), systemic inflammatory response syndrome (SIRS), prolonged mechanical ventilation (≥72 hours), and extended cardiopulmonary bypass time (≥60 minutes) ($p<0.05$). No significant association was found between VPI and microbiota changes. **Conclusion:** Significant shifts in the oral microbiota, particularly involving pathogens with nosocomial potential, occur after 24 hours in the PICU. These findings underscore the importance of monitoring oral health and implementing preventative oral hygiene protocols to reduce systemic infections in pediatric cardiac patients.

Keywords: Saliva. Microbiota. Heart defects, congenital. Intensive care units, pediatric. Cross infection. Oral health.

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Introduction

Congenital heart diseases (CHDs) stand as the most prevalent birth malformations, affecting approximately 8 in every 1,000 live births globally. Recent advancements in medical diagnosis and cardiac surgical techniques have significantly improved survival rates and the quality of life for individuals with heart disease. However, children with CHDs require specialized care, especially when it comes to their oral health[1,2].

Studies have shown that pediatric patients with congenital heart diseases often face difficulties with oral hygiene, resulting in an increased prevalence of dental caries. These oral health challenges can be attributed to the nature of heart defects, extended hospital stays, and the neglect of routine dental appointments. Consequently, this population is at a higher risk of developing severe complications such as infective endocarditis (IE) and, when admitted to the Intensive Care Unit (ICU), nosocomial infections like ventilator-associated pneumonia (VAP). Sepsis resulting from oral infections in patients with cardiac compromise can be fatal; however, most parents of children with heart conditions are unaware that poor oral health increases the risk of developing IE[3].

Recognizing the importance of oral hygiene in healthcare, the Centers for Disease Control and Prevention (CDC) recommend implementing oral hygiene programs for ICU patients. Regular oral hygiene measures have been shown to improve patient health and mitigate the risk of infections and their associated complications. In this context, salivary microbiota emerges as an alternative for diagnosis and prognosis of systemic conditions and has been increasingly used to assess the presence of specific substances and analyze material characteristics, as it is clinically very informative, containing soluble biomarkers also found in blood and urine. Saliva sampling is simple, non-invasive, relatively inexpensive, and poses low risk to the patient[4-7].

Nosocomial infections after cardiac surgery in patients with congenital heart diseases are influenced by various factors. Prolonged surgeries, duration of cardiopulmonary bypass (CPB), continuation of antimicrobial prophylaxis for more than 48 hours, prolonged hospital stays, low body weight, younger age, and duration of mechanical ventilation increase the risk of infection[8-10].

This study seeks to investigate alterations in the salivary microbiota of children and adolescents with congenital heart diseases admitted to a specialized hospital. It aims to establish correlations between salivary microbiota changes and various factors, including the use of oral medications, protein-energy malnutrition (PEM), systemic inflammatory response syndrome (SIRS), cardiopulmonary bypass (CPB) duration during surgery, and mechanical ventilation (MV) duration.

This research holds the potential to shed light on the intricate relationship between oral health, the salivary microbiota, and systemic health outcomes in pediatric patients with congenital heart diseases. By better understanding these dynamics, we can develop more effective strategies for infection prevention and management, ultimately enhancing the overall well-being of this vulnerable population.

Materials and Methods

This study was approved by the Ethics Committee of reference hospital, under Certificate of Ethical Approval number 40195620.0.0000.0045.

This prospective study followed the salivary microbiota of children and adolescents at three distinct time points: first, within 24 hours of admission to the ward; second, within 24 hours of admission to the ICU after cardiac surgery; and third, 48 hours after admission to the ICU. Children and adolescents up to the age of 18 with heart diseases, admitted to reference hospital and with indications for cardiac surgery were included. They were selected based on their medical diagnosis and reason for admission from June to December 2021. The selection was conducted using electronic medical records. Children and adolescents without indications for cardiac surgery, those directly admitted to the pediatric intensive care unit (PICU), those admitted to wards or ICUs of other hospitals, as well as individuals with tracheostomies and chronic obstructive pulmonary disease (COPD), were excluded from the study.

Statistical Power Analysis

A total of 50 pediatric patients with congenital heart disease were included in the study. To verify whether this sample size was sufficient to detect statistically significant associations between clinical variables and changes in salivary microbiota, a post hoc power analysis was performed. The analysis was conducted using Epi Info™ (version 7.2, developed by the Centers for Disease Control and Prevention – CDC).

The power calculation was based on comparisons of proportions between two independent groups (e.g., altered vs. non-altered microbiota) using a chi-square test with a two-tailed significance level of $\alpha = 0.05$. The analysis focused particularly on two clinical variables: mechanical ventilation duration (<72 hours vs. ≥72 hours) and presence of systemic inflammatory response syndrome (SIRS). Effect sizes were computed from the observed proportions in each group. Both platforms demonstrated a statistical power of 1.0 (100%) for these associations, confirming that the sample size of 50 patients was adequate to detect the differences observed in the study.

Oral Hygiene Assessment Using The Visible Plaque Index (VPI)

For the evaluation of dental biofilm, the Visible Plaque Index (VPI) was used, according to the methodology described by Ainamo and Bay[11].The index was calculated by dividing the number of tooth surfaces with visible biofilm by the total number of examined surfaces, with the result expressed as a percentage. Only tooth surfaces with visible plaque accumulation were considered, excluding teeth in the process of eruption or with crowns completely destroyed. The inspection was performed without prior drying of the tooth surfaces, using a portable flashlight, and the findings were recorded in a clinical form adapted from the Evaluation Form of the Federal University of Bahia and the National Oral Health Survey[12].

Collection Of Salivary Content And Tracheal Secretion Samples

Unstimulated salivary samples accumulated in the lower part of the buccal mucosa were collected bilaterally five times using sterile flexible swabs. These samples were then transferred to tubes with Stuart medium for transportation. Collection occurred at three time points: within the first 24 hours of admission to the hospital ward (C1), within the first 24 hours of admission to the PICU after heart surgery (C2), and 48 hours after admission to the PICU (C3) without prior mouth washing. In cases where the patient was on mechanical ventilation (MV), the possibility of suctioning tracheal secretions through a 12-mm tracheostomy tube using a sterile polypropylene bottle was discussed with the physiotherapist on duty at the unit. The salivary content and tracheal secretion samples collected were labeled with the participant's full name and the date of collection, and then sent to the hospital's clinical laboratory.

Laboratory Analysis

The identification of microorganisms responsible for nosocomial infections, such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli*, *Staphylococcus aureus*, and *Enterobacter cloacae*, was performed in the hospital's clinical laboratory. Buccal swab and tracheal secretion samples were initially seeded on two culture media: Blood Agar and MacConkey Agar. The material was deposited on the plates and spread with the aid of a sterile 10μL inoculation loop, making successive streaks in four quadrants until the material was completely spread. After the seeding process, the plates were incubated at a temperature of 35°C ± 2°C in an anaerobic jar with 5% CO2 for 24 hours. After this incubation period, the plates were examined for bacterial growth, and a screening was performed based on the colonial morphology and its macroscopic characteristics. Colonies with suggestive morphology were recorded as colony-forming units (CFU) before being reisolated in MacConkey Agar for Gram-negative bacteria and Blood Agar for Gram-positive bacteria. After reisolation, the materials were incubated and examined after 24 hours.

For microorganism identification, the MicroScan Walkaway 96 Plus system was utilized. This system consists of microtiter panels containing substrates and dehydrated antibiotics, which automatically add reagents based on biochemical tests to read these panels. For this purpose, the identification panel was inoculated by positioning a PROMPT needle perpendicular to the plate surface, approaching it to an isolated colony larger than the tip of the needle. Then, this colony was placed in a flask, which was vigorously shaken to create a suspension of bacteria in 30mL of stabilized Pluronic-D water and poured into a seedling tray. After preparation, the RENOK rehydrating inoculator was positioned over the transfer lid to collect the inoculum, which was later positioned over the MicroScan panel to inoculate. This allowed the loading of all 96 wells of the panel while simultaneously transporting it to the equipment for incubation at 35°C. The equipment compared the panel reading with its database, enabling the identification of microorganisms and indicating the identification probability, with a percentage adopted by the device exceeding 85%. The readings were performed by this equipment within 18 to 24 hours or

even 48 hours, depending on the microorganism. The results were recorded on the computer using the MicroScan LabPro software to generate reports and were added to the patient's electronic medical record through a service order issued by the researcher.

Data Analysis

The collected data were entered into Excel, and the analysis was conducted using IBM SPSS Statistics version 25.0. Frequencies, measures of central tendency, and measures of dispersion were calculated. The Chi-square test was used for associations, with a 95% confidence interval and a p-value of <0.05.

Results

From June to December 2021, a total of 50 children and adolescents met the inclusion criteria and were evaluated. The length of stay in the ward before and after cardiac surgery and in the PICU was on average 11.94 days (SD = 7.01) and 4.64 days (SD = 3.66), respectively. The participants were mostly male (56%), with an average age of 3.63 years (ranging from 2 months to 15 years; SD = 3.93) and a mean VPI of 21.20 (SD = 16.61).

Clinical Data From Hospitalization And Microbiological Analysis

Clinical data from hospitalization are presented in Table 1. All of the 50 patients had CDH, of which 31 (62%) were receiving oral medications in the hospital ward. Regarding the use of antibiotics, only one patient with sickle cell anemia was using benzathine penicillin G (intramuscularly) every 21 days. In the PICU, as a standard protocol of the hospital the patients received prophylactic antibiotics (Cefuroxime) after cardiac surgery for a period of 48 hours. As for diet in the ward, 48 (96%) were prescribed an oral diet according to their age, while 2 (4%) made use of a nasoenteral tube.

Table 1. Clinical data and altered salivary microbiota of children and adolescents with heart disease admitted to a reference hospital from June to December 2021

Variable	n	%
Use of oral medications		
Yes	31	62
No	19	38
Use of antibiotics in the ward		
Yes	1	2
No	49	98
Diet in the ward		
Oral	48	96
Nasoenteral	2	4

Continue

Continuation		
Protein-energy malnutrition		
Yes	19	38
No	31	62
Cardiopulmonary bypass		
< 60 minutes	23	46
≥ 60 minutes	27	54
Altered salivary microbiota during hospitalization		
Yes	22	44
No	28	56

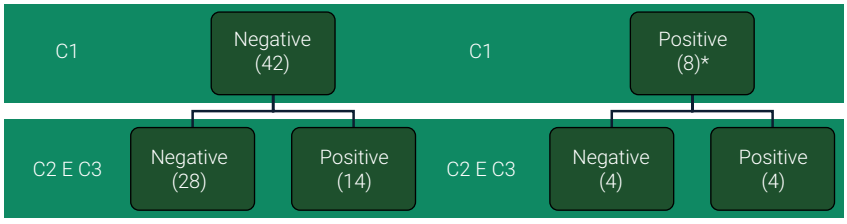
With respect to PEM, 19 (38%) patients had this deficit and 27 (54%) remained with CPB during cardiac surgery for 60 minutes or more.

A total of 128 salivary content and five tracheal secretion samples were used in this study. Twenty-two (44%) patients had their salivary microbiota altered during the time of hospitalization in the ward and the PICU.

Concerning the type of ventilation in the PICU, in C2 34 (68%) patients were on ambient air ventilation and 16 (32%) were on MV, while in C3 20 (40%) were on ambient air ventilation, 8 (16%) were on MV and 22 (44%) did not have their material collected because they had not completed 48 hours in the unit.

Forty-one (82%) patients were extubated in less than 72 hours, most of them in the immediate postoperative period, i.e., within the first 12 or 24 hours, whereas nine (18%) remained on MV in the PICU for 72 hours or more.

Figure 1 schematizes the dynamics of alteration in the results of saliva collections. It can be observed that of the 42 patients who did not have their microbiota altered, 28 remained negative and 14 became positive after admission to the ICU, whereas of the eight patients who had their microbiota altered, four remained positive and four became negative after cardiac surgery.



* Five had attended a recent outpatient visit or had been recently hospitalized
(C1= Collection 1; C2= Collection 2; C3= Collection 3)
Figure 1. Schematic representation of the dynamics of alteration in the results of saliva collection

Table 2 shows the microorganisms that were identified in C1, C2, C3 and in the tracheal secretion suction.

Table 2. Results of C1, C2, C3 and tracheal secretion suction performed in children and adolescents with heart disease admitted to the ward and the PICU of a reference hospital from June to December 2021

RESULT/COLLECTIONS	1st collection (WARD)		2nd collection (PICU)		3rd collection (PICU)		Tracheal secretion	
	n	%	n	%	n	%	n	%
Collection not performed	-	-	-	-	21	42	3	37.5
Negative	42	84	42	84	15	30	3	37.5
Klebsiella pneumoniae	2	4	4	8	3	6	1	12.5
Staphylococcus aureus	3	6	2	4	-	-	-	-
Acinetobacter baumannii	2	4	1	2	3	6	-	-
Escherichia coli	1	2	1	2	2	4	-	-
Pseudomonas aeruginosa	-	-	1	2	3	6	-	-
Enterobacter cloacae	-	-	2	4	4	8	1	12.5
Other species								
Klebsiella oxytoca	-	-	-	-	1	2	-	-
Enterobacter aerogenes	-	-	-	-	1	2	-	-
Burkholderia cepacia	-	-	-	-	1	2	-	-

In C1, whereas 42 (84%) patients were negative for the epidemiologically important microorganisms responsible for nosocomial infections, eight were positive for *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia Coli*, and *Acinetobacter baumanni.* Among these eight patients, five had attended a recent outpatient visit or had been recently hospitalized. After cardiac surgery, in C2 42 (84%) patients tested negative, of which ten had all species of interest present in their saliva/tracheal secretion samples.

In C3, 21 (42%) samples were not collected because the individuals were discharged before 48 hours of admission to the PICU. Fifteen patients (30%) had negative results, whilst five (10%) had the following species identified in C3: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter baumanni* and *Enterobacter cloacae.* Uncommon species to the oral microbiota and with potential risks of nosocomial infections were evidenced, namely, *Klebsiella oxytoca*, *Enterobacter aerogenes* and *Burkholderia cepacia*. Eight patients (16%) were on MV in C3, of which three (37.5%) could not have their tracheal secretion collected because the content was insufficient for aspiration, three (37.5%) were negative for the species studied, one (12.5%) was positive for *Klebsiella pneumoniae* and one (12.5%) was positive for *Enterobacter cloacae.* Of the eight patients intubated at the time of C3, seven (87.5%) were positive in one of the three buccal swab collections.

According to Table 2, it can be observed that *Klebsiella pneumoniae* was the most commonly found species (25%), followed by *Acinetobacter baumannii* (24%) and *Enterobacter cloacae* (20%).

Table 3 shows the alteration in the patients' salivary microbiota. As it can be seen, the salivary microbiota was more altered in children aged 12 months or younger (70.0%), with a statistically significant difference (p<0.05). Most patients (51.6%) that made use of oral medications and 52.6% with PEM had their salivary microbiota altered during hospitalization, but without any statistically significant difference was found (p>0.05). In addition, no positive association was found between VPI and microbiota alteration (p>0.05). The four patients (100%) with SIRS had their microbiota altered (p<0.05). Likewise, all patients on MV for 72 hours or more and 66.7% of those with CPB for 60 minutes or more during surgery had significant colonization of pathogenic microorganisms (p<0.05).

Table 3. Associations between age, use of oral medications, PEM, VPI, SIRS, MV time and CPB time and alteration in the salivary microbiota of children and adolescents with heart disease admitted to a reference hospital from June to December 2021

Variables	ALTERATION IN SALIVARY MICROBIOTA				
	Yes	%	No	%	p-value*
Age					**0.003**
< 12 months	14	70.0	6	30.0	
≥ 12 months and ≤ 72 months	7	36.8	12	63.2	
≥ 84 months	1	9.1	10	90.9	
Use of oral medications					0.166
Yes	16	51.6	13	48.4	
No	6	31.6	28	68.4	
PEM					0.336
Yes	10	52.6	9	47.4	
No	12	38.7	19	61.3	
VPI					0.714
≤ 17.08	6	35.3	11	64.7	
> 17.08	5	29.4	12	70.6	
SIRS					**0.019**
Yes	4	100	-	-	
No	18	39.1	28	60.9	
MV time					**0.000**
< 72 hours	13	31.7	28	68.3	
≥ 72 hours	9	100	-	-	
CPB Time					**0.000**
< 60 minutes	4	17.4	19	82.6	
≥ 60 minutes	18	66.7	9	33.3	

*Chi-square teste (p<0.0)
PEM: protein-energy malnutrition; VPI: Visible Plaque Index; SIRS: systemic inflammatory response syndrome
MV: mechanical ventilation; CPB: cardiopulmonary bypass

The four patients (100%) with SIRS had their microbiota altered ($p<0.05$) Two patients (4%) had postoperative sepsis, but with no alteration in their salivary microbiota.

Nosocomial Infections

Figure 2 indicates the incidence of nosocomial infections and the associated risk factors and alterations in the salivary microbiota. Five patients (10%) had infections, such as urinary tract infection (UTI), respiratory tract infection (RTI) without specification, nosocomial pneumonia and VAP.

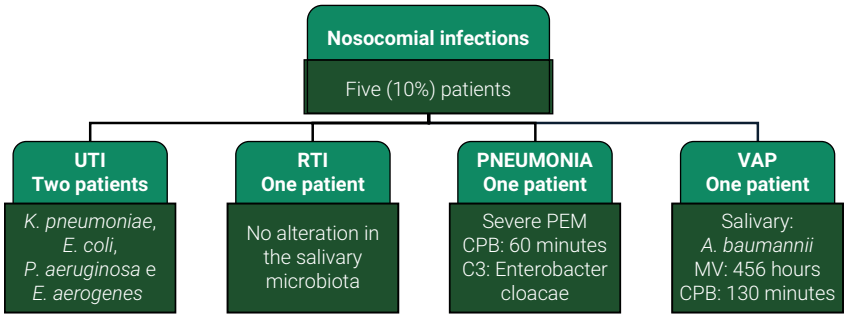


Figure 2. Nosocomial infections, associated risk factors and alteration in salivary microbiota

The two patients with UTI had their salivary microbiota altered after cardiac surgery, with the presence of the following pathogens: *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterobacter aerogenes*. There was no change in the salivary microbiota of the patient with RTI.

The patient with nosocomial pneumonia had important conditions and possible risk factors, namely, PEM, CPB time of 60 minutes, and contamination by *Enterobacter cloacae* in C3.

The patient diagnosed with VAP had pyosanguinolent tracheal secretion content, but negative results for the pathogens studied. During hospitalization, this patient had his salivary microbiota colonized by *Acinetobacter baumannii*. Moreover, some important parameters were observed in this patient: MV time of 456 hours (19 days) and CPB time of 130 minutes.

Discussion

Congenital heart disease (CHD) is the most common birth defect globally, resulting from abnormalities in the development of the heart and great vessels[13,14]. Over the past three decades, morbidity and mortality associated with congenital heart surgery have improved significantly due to advances in pediatric cardiac intensive care and increased expertise in managing children with heart disease[14,15].

Despite these improvements, changes in the composition of the oral microbiota occur within 48 hours of hospitalization, with a notable shift from Gram-positive cocci to Gram-negative bacilli – organisms commonly associated with nosocomial infections.

These changes may result from multiple factors, including physical and chemical interactions with microbial enzymes, decreased salivary flow, and reduced immunoglobulin production[16]. Since the oral cavity is susceptible to colonization by pathogenic microorganisms, patients with systemic conditions are particularly vulnerable to infection through the bloodstream and respiratory tract[17].

Pediatric patients – particularly infants – are more prone to such microbial changes due to the immaturity and plasticity of their oral microbiota. The dynamic nature of the microbiome during early life, along with an underdeveloped immune system, may predispose younger patients to opportunistic infections. As children age, their oral microbiota becomes more diverse and stable, reducing their susceptibility to colonization by pathogenic species[18].

In this study, nearly half of the patients experienced significant changes in their salivary microbiota during hospitalization, with *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Acinetobacter baumannii* being the most frequently identified pathogens. *K. pneumoniae* is a Gram-negative bacillus commonly isolated in hospitalized patients and is associated with pneumonia, wound infections, bacteremia, and meningitis in pediatric populations[17]. *S. aureus* is a leading cause of hospital-acquired infections and is known to cause complications such as endocarditis and pneumonia[19]. *A. baumannii* has been implicated in 5–54% of nosocomial infections in both general wards and ICUs, often leading to VAP and bloodstream infections[20].

The pathogens detected, such as *K. pneumoniae*, *A. baumannii*, and *E. cloacae*, are not part of the resident oral microbiota and are considered opportunistic colonizers during hospitalization, especially in intensive care settings. Initially, they may be present as transient microorganisms, but under conditions such as antibiotic use, mechanical ventilation, and prolonged hospital stay, they can establish themselves as colonizers capable of persisting in the oral cavity and acting as reservoirs for systemic infections[4,16].

Although no association was found between the VPI and microbiota changes in this study, it is essential to monitor oral infection foci primarily dental caries and periodontal disease in daily clinical care. Previous studies have demonstrated that children with CHD often present with poor oral health and higher levels of dental biofilm compared to healthy peers[21-23]. During hospitalization, biofilm accumulation increases, elevating the risk of colonization by multidrug-resistant bacteria commonly linked to respiratory infections. Regular oral hygiene can help reduce microbial contamination in mechanically ventilated patients[16,24]. Periodontal diseases in children ranging from gingivitis to more severe forms are frequently associated with immune deficiencies that impair the host response to oral pathogens[25].

The nosocomial infection rate in this study was relatively low (10%); however, literature reports a higher prevalence in pediatric cardiac patients, particularly in low-income settings. Infections may account for up to 17% of postoperative deaths in this population[10,26,27]. Risk factors include prolonged surgeries, extended CPB duration, long hospital stays, young age, low body weight, and prolonged mechanical ventilation[8-10].

In our sample, 54% of the patients underwent prolonged CPB due to complex heart conditions. The statistically significant association between CPB duration and changes in the oral microbiota highlights CPB as a potential risk factor for intraoperative contamination and postoperative infections[9,10,26].

VAP remains the most critical ICU-acquired infection in pediatric patients. Early extubation, when hemodynamically feasible, is recommended to reduce complications and limit adverse effects on cardiac function[27]. The average MV duration post-surgery is approximately 72 hours[28]; exceeding this threshold increases the likelihood of nosocomial infections[8].

The presence of a dental professional in the hospital setting is essential for promoting oral hygiene, managing biofilm, moisturizing mucosal tissues, and detecting or eliminating infectious foci. Their integration into the multidisciplinary care team has been shown to lower rates of nosocomial pneumonia and VAP, reduce hospital costs and stay duration, and improve patient outcomes[29,30].

In conclusion, significant alterations in the oral microbiota of pediatric cardiac patients were observed after 24 hours in the ICU. The most frequently isolated pathogens – *K. pneumoniae*, *S. aureus*, and *A. baumannii* – pose a high risk for systemic infections. This study reinforces the need for further investigations into the oral microbiota of hospitalized children and emphasizes the importance of incorporating dental professionals into hospital care teams. Developing standardized oral hygiene protocols may significantly reduce contamination by potentially life-threatening pathogens and lower the incidence of nosocomial infections in intensive care settings.

Significant changes in the oral microbiota were observed in children and adolescents with congenital heart disease after 24 hours of admission to the Pediatric Intensive Care Unit. *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Acinetobacter baumannii* were the most frequently isolated pathogens – organisms with known associations to serious nosocomial infections.

These findings highlight the oral cavity as a critical reservoir for hospital-acquired pathogens and emphasize the need for preventative strategies within the ICU environment. Routine oral hygiene measures, along with the integration of dental professionals into multidisciplinary care teams, may play a key role in mitigating the risk of systemic infections. Furthermore, these results call attention to the importance of developing standardized protocols for oral care in pediatric critical care settings, particularly for high-risk populations such as those with congenital heart disease.

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Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon request.

Author Contributions

Andressa Chang Rodrigues Fernandes da Silva: contributed to data acquisition, conception, design, analysis, and interpretation, drafted the manuscript, and gave final approval. **Luanderson Lopes Pereira:** contributed to the interpretation and critically revised the manuscript. **Felipe Barreto Lemos:** contributed to the interpretation and critically revised the manuscript. **Fernanda Conceição Machado:** contributed to conception, design, and interpretation, and critically revised the manuscript. **Fernanda Pereira Lima:** contributed to the interpretation and critically revised the manuscript. **Camila Conceição Lima:** contributed to laboratory analysis. **Vania Riccio Teixeira:** contributed to laboratory analysis. **Delson Arcanjo Silva:** contributed to conception, design, and interpretation, and critically revised the manuscript. **Andréia Cristina Leal Figueiredo:** contributed to conception, design, data acquisition, analysis, and interpretation, drafted the manuscript and graphics, and granted final approval. All authors actively revised and approved the final version of the manuscript.

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Conflict of Interest Statement

The authors declare no conflict of interest.

Ethics Statement

This study was approved by the Ethics Committee of Ana Nery Hospital, located in the municipality of Salvador, Bahia, Brazil, under Certificate of Ethical Approval number 40195620.0.0000.0045.

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APPENDIX A

Informed Consent Form (ICF)

"Dynamics of Salivary Microbiota in Pediatric Cardiac Patients:
From the Ward to the Intensive Care Unit"

Dear Guardian,

The purpose of this research is to characterize the salivary microbiota of children and adolescents with heart conditions during hospitalization in the pediatric cardiology ward and the Pediatric Intensive Care Unit (PICU). Specifically, we aim to assess whether the microorganisms present in the mouth change during hospitalization and to associate these changes with the length of stay, the presence of dental caries, and the likelihood of developing infections in other organs.

Heart conditions can be influenced by oral health, as the mouth is a gateway for disease, a source of contamination, and a means of bacterial spread to other parts of the body, such as the heart and lungs. Infective Endocarditis is one of the complications that can more easily affect these children/adolescents due to their heart condition. Poor oral hygiene during hospitalization, combined with medication use, may alter the types of microorganisms and the condition of the saliva, increasing the patient's risk of infection.

Initially, an interview will be conducted using a questionnaire to gather information on personal data, medical, and dental history. During the first phase, in the cardiology ward, a clinical oral examination will be performed to assess the presence of caries and dental plaque, and a saliva sample will be collected. In the second phase, in the PICU, after cardiac surgery, a second saliva sample will be collected, followed by another sample 48 hours later. If the patient is breathing with the aid of tubes, secretion will be aspirated using a plastic tube, which is a routine procedure in the PICU. All procedures will be conducted by the researcher using the necessary Personal Protective Equipment (PPE), such as caps, masks, protective glasses, face shields, gloves, surgical clothing, and disposable impermeable gowns, ensuring biosafety.

The direct benefits of the child's/adolescent's participation in this study include information about their oral health condition, which can impact overall health, as well as guidance on possible care measures during hospitalization and after discharge. If dental treatment is necessary, the patient will be referred to the Hospital Dentistry Service before cardiac surgery, as part of the hospital protocol. The results of this research will serve as valuable study material for many professionals.

The information obtained from the interview, examinations, and sample collection, as well as your and the child's/adolescent's identity, will remain confidential; however, the results of this research may be shared within the scientific community through journals and conferences.

If you agree to participate, please feel free to ask any questions before signing. The researcher will be available at any time to provide clarifications or if you wish to withdraw from the study. This study will not cause you any expenses or harm from participating, and if you choose not to participate, it will not affect the care, treatment, or services provided by the hospital team.

If the research causes any harm to the child or adolescent, you have the right to seek legal action to ensure your rights.

Finally, having understood everything that was explained about participation and being aware of our rights, I agree to voluntarily include the participant _____ _____ in this study. I will sign at the end of this document, which is in two copies, one for myself and the other for the responsible researcher.

____/____/____ _____

Signature of the patient's guardian

____/____/____ _____

Signature of the researcher