Article

Sociocultural Aspects and Isolation of Streptococcus spp. from Dental Biofilm: A Comparison of Patients in Public Health and Private Dental Practices

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Original Research

Comparison of *Streptococcus* spp. Isolation from Dental Biofilm and Sociocultural Factors Between Public and Private Dental Patients

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Abstract: The different sociocultural context and lifestyle may influence oral health. To evaluate the interference of these factors, we conducted a quantitative-qualitative cross-sectional study. The research was carried out in a private dental office, in an urban area, and in the Family Health Unit (USF), in a rural area. A total of 100 patients, fifty of each location, participated on the research. A semi-structured questionnaire covering sociocultural profile, oral hygiene habits, drug consumption, use of medicinal plants, and antibiotics usage was applied. Also, laboratory tests to evaluate the tendency to have dental decay and *Streptococcus* sp colony counting was performed. There was no significant difference between patients in the two groups regarding gender, race, marital status, type of residence, number of residents in the same household, and type of employment. Private clinic participants were more prone to consuming alcohol and/or cigarettes and reported to brush the teeth more often. In general, participants from public health system showed less *Streptococcus* biofilm colonization. There was no association between salivary acidity and higher biofilm bacterial density. The usage of medicinal plants was more evident in the public service. In conclusion, the difference *Streptococcus* colonization was not in dental care system but lifestyle habits.

Keywords: Medicinal plants; tooth brushing, public health, oral dentistry.

1. Introduction

Since oral infections affect people's health and the country's economy, they are a cause for concern [1,2]. As per the World Health Organization (WHO) report 2023 [3], around 3.5 billion people worldwide are affected by oral diseases, with 3 out of 4 people affected living in middle-income countries [4]. In this context, Brazil, as an emerging upper-middle income economy, barriers to dental care access have been listed [5].

Biological and sociocultural factors, particularly economic deprivation and the sociosanitary conditions of the population, can influence the formation of dental biofilm and therefore the development of various oral diseases that impact the quality of life [6,7]. Difficulty accessing dental

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care [7] or exposure to high-sugar-content foods, tobacco smoke, inefficient and insufficient oral hygiene practices may affect the oral environment and can generate conditions that promote oral biofilm changes [8].

Moreover, the use of medicinal plants to treat oral diseases without scientific evidence [9] or extensively antibiotically therapy during the coronavirus disease (COVID-19) pandemia has been stated [10] and the influence on oral environment is still a question to answer. In dentistry, although plants for therapeutic purposes are still a topic considered little explored, there is a perspective of their usage regarding holistic treatment approach and its benefits as an adjunctive therapy since in vitro tests show their anti-*Streptococcus mutans* activity [11,12]. There are a lot of challenges to overcome to prove the usefulness and cost effectiveness of plant extracts, since there is a lack of good scientific evidence concerning the adequate statistical power, blinding, standardization of extracts or purified compounds, and quality control [13].

Among oral manifestations, biofilm carried on by the complexity of oral microbiome and community activities is considered a significant etiological factor for caries and other periodontal diseases [14]. The proliferation of microorganisms such as *Streptococcus* spp. and lactobacilli are the main reported agents related to progressive dental demineralization, carious lesions, and eventual tooth loss [15]. However, the relationship between lifestyle and the quality of oral health assistance are still to be explored. Knowing patients' habits which may contribute to imbalance oral microbiome homeostasis can assist in taking sanitary measures and the quality of dental patients.

Recognizing the great sociocultural and economic diversity of the Brazilian population, we designed this study assuming that there are aspects of this diversity that can influence the colonization of Streptococcus on the tooth surface. Thus, the aim of the present study was to compare the effect of sociocultural factors on biofilm colonization by *Streptococcus* spp of dental surfaces in subjects attending public and private dental clinics.

2. Materials and Methods

2.1. Study Design and Query

For this study, sample collection and sociodemographic surveys involved dental patients from both public health clinics and private clinics. The research was conducted from March 23 to August 17, 2022, in two study sites: the dental clinic of the USF Manoel Cipriano and a private dental clinic (PC), both located in the city of Ipiaú, state of Bahia, Brazil. Research participants were recruited in the waiting room of the public health unit or during consultation at the dental office. All subjects gave their informed consent before they participated in the study. The informed consent process was conducted in accordance with the guidelines approved by the Human Research Ethics Committee at Universidade Estadual de Santa Cruz under CAAE registry number 51682321.7.0000.5526.

Initially, the sample calculation based on the Epi Info™ statistical program and considering the research inclusion and exclusion criteria resulted in 200 participants. However, due to cost limitations of the techniques used and participants' adherence, a total of 100 participants were enrolled in the study, with 50 participants from each site. Inclusion criteria were individuals aged ≥ 18 years, of either sex, not undergoing dental treatment, no antibiotics for at least 15 days, and having at least three teeth in the mouth. Participants had also brushed their teeth at least two hours prior to sample collection. Women were not pregnant at the time of sample collection. Exclusion criteria included oral microbiological alterations, comorbidities, oral cancer, being edentulous, or having a positive COVID-19 diagnosis at the time of enrollment.

Participants were recruited by reviewing the USF medical and dental clinical records on the day and time of their consultation, either at the USF or at the private clinic. Research objectives were explained, and participants were given the opportunity to read the Informed Consent Form (ICF) and ask questions at this time. In addition, consenting patients were asked about symptoms such as colds, coughs, and shortness of breath, and when they last brushed their teeth. In cases where collection at USF was not feasible, researchers made appointments with community health workers (CHWs) and visited participants in the community. After obtaining consent, a semi-structured questionnaire was

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administered to assess clinical and socioepidemiological aspects (accessible from: https://figshare.com/s/330f0ce9e136ba85c5ea).

2.2. Dental Biofilm Collection and Analysis

Dental biofilm samples were collected by rubbing a sterile swab on the tooth surface near the gingival margin. The swab was then placed in a Stuart transport medium tube (Absorve, Cral). Immediately after biofilm collection, a sample of spontaneous saliva was collected in a sterile 15 mL polystyrene tube for the Snyder test. Both samples were transported to the Laboratório de Microbiologia Aplicada at Universidade Estadual de Santa Cruz, city of Ilhéus, Bahia within three hours of collection.

Each swab was transferred to a 1.5 mL polypropylene tube containing 600 µL of 0.9% NaCl solution. After homogenization, 100 µL of the solution were transferred onto Mitis-Salivarius agar (MS; HiMedia) and spread evenly across the plate using a sterile swab. Plates were then incubated for 24 hours at 37 ± 0.1 °C in a candle jar as per described in a previous study [16].

Colonies with the following characteristics were analyzed and counted: 0.5–1 mm in diameter, convex, wavy, opaque, light blue, granular with a "ground glass" appearance, and rough margins, sometimes exhibiting a shiny bubble on the colony surface or along its margin. After counting, one colony was selected and co-cultured on blood agar, then incubated for 24 hours at 37±0.1°C in a candle jar. Colony morphology, Gram staining, and catalase tests were then performed to confirm genus identity as Streptococcus. In addition, a salt tolerance test was performed to differentiate it from Enterococcus faecalis. Bacteria were inoculated into 6.5% NaCl medium and incubated for 48 hours at 37 ± 0.1 °C in a candle glass. Control samples included medium without bacteria and medium inoculated with Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC 5129), known for their tolerance to salt. Turbidity of the medium indicated the ability of bacterial isolates to tolerate salt concentrations and distinguished Streptococcus species (nonturbid) from *E. faecalis* (turbid/flocculation) [17]. After identification, one colony was selected and cultured in Brain and Heart Infusion (BHI) broth supplemented with 5% blood and a 20% glycerol solution (LGCBIO, Brazil) and incubated for 24 hours at 37 °C in a microaerophilic atmosphere. The colony was then stored at -20 °C.

Sucrose, sorbitol, and mannitol tests were performed to confirm the Streptococcus species. For this, stored bacteria were cultured in 5% blood BHI broth under microaerophilic conditions for 24 hours, then cultured on blood agar and incubated for another 24 hours at 37 ± 0.1 °C in a candle jar. A negative control of sugar-free medium was included. Samples were then incubated for 72 hours at 37 ± 0.1 °C in a candle jar. Any utilization of sugar by bacteria was deemed indicative of *S. mutans* [18].

Colony counts were expressed as colony forming units per 100 µL (CFU/100 µL) and samples were classified into five categories: ≥500 CFU; 200-499 CFU; 100-199 CFU; 1-99 CFU; and zero CFU.

2.3. Susceptibility to Cavity Formation

To perform the Snyder test, a medium consisting of 1.4 g of peptone (Himedia), 1.4 g of dextrose (Dinâmica), 0.35 g of NaCl (Dinâmica), 1.4 g of agar base (Himedia) and 140 µL of 0.1% bromocresol green (Dinâmica) in 70 mL of distilled water was used. The medium was autoclaved at 121 °C for 15 minutes, and 5 mL was dispensed into sterile glass test tubes with screw caps. Immediately after sterilization, when the medium in each tube had cooled to approximately 45 °C, 100 µL of spontaneous saliva sample was added and homogenized by vortexing. Samples were then incubated at 37 °C for 24, 48, and 72 hours. A color change from bluish green to yellow (indicating acidification of the medium) signaled growth of acidogenic bacteria.

Salivary acidity and tendency to form caries were graded according to the criteria of Lilienthal and Reid [16]: NS (0) – no color change (little or no susceptibility to caries); Slight (+) – slight color change (slight susceptibility to caries); Moderate (++) – significant color change (moderate susceptibility to caries); High (+++) – complete color change (high susceptibility to caries).

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2.4. Statistical Analysis

Findings were analyzed descriptively and analytically. Graphs were generated using Prism, version 5 for Windows (GraphPad Prism Software Inc., 2007). Multiple Correspondence Analysis (MCA) of categorical variables (sociodemographic aspects and biofilm colonization) across different dental services was performed using R statistical software [19]. Pearson's test with significance set at $p < 0.05$ and 95% confidence interval was applied to correlate the use of medicinal plants, brushing habits, salivary acidity, and CFU density.

3. Results

In the present study, socioeconomic data (Table 1) showed a preponderance of women over men in both public and private dental services. Most participants were married or in stable relationships. The mean age of participants was 42.0 ± 11.9 years, with notable age groups being 25–39 years (39%; nT=39/100) and 40–59 years (44%; nT=44/100).

Both the public and private services reflected typical Brazilian miscegenation, with approximately half of the participants identifying as brown and more self-identified black individuals in the public service.

Level of education was similar between the two groups; however, private service participants appeared to have a slightly higher level of education than public service participants. Social status was similar between the two groups, as indicated by the number of people living in the same household.

Regarding employment status, participants living in rural areas were more likely to have informal work than those living in urban areas and attending private dental services. Unemployment rates were low in both study groups, with only 3% (nPC = 2/50; nUSF = 1/50) reporting unemployment.

Table 1. Socioeconomic profile of participants from private clinics and public health system assistance in Ipiaú, Bahia, Brazil from March 23rd to August 17th, 2022.

	Private assistance		Public health assistance		
	N	%	N	%	$\chi 2$
Gender					0,2945
Female	35	75	30	60	
Male	15	25	20	40	
Marital status					0,5632
Married/stable union	32	64	28	56	
Single	16	32	20	40	
Divorced	1	2	2	4	
Widow(er)	1	2	0	0	
Not informed	0	0	0	0	
Age group (years)					0,1948
18 - 24	1	2	6	12	
25 - 39	22	44	17	34	
40 - 59	23	46	21	42	
≥60	4	8	6	12	
Not informed	0	0	0	0	
Schooling (years)					0,1250
0	0	0	0	0	
1-3	1	2	2	4	
4-7	6	12	14	28	

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8-11	6	12	8	16	
>12	37	74	26	52	
Ethnicity					0,0754
White	8	16	1	2	
Black	13	26	17	34	
Yellow	0	0	1	2	
Brown	28	56	29	58	
Indigenous	1	2	0	0	
Not declared	0	0	2	4	
Type of residence					0,0000
Appartment					
House	50	100	50	100	
Tween house	0	0	0	0	
Hut	0	0	0	0	
Cottage	0	0	0	0	
Mansion	0	0	0	0	
Homeless	0	0	0	0	
Number of resident/habitation:					0,1209
0	1	2	5	10	
2	4	8	0	0	
3	24	48	22	44	
4	13	26	10	20	
5	6	12	8	16	
>6	2	4	5	10	
Not informed	0	0	0	0	
Profession					0,4935
Student	1	2	2	4	
Housewife	4	8	4	8	
Unemployed	2	4	1	2	
Retired	3	6	2	4	
Informal Professional	15	30	23	46	
Servant	15	30	14	28	
Businessperson	3	6	0	0	
Others/Not informed	7	14	4	8	

[1]p value<0,05.

Among the sociocultural aspects (Table 2), the habits of tooth brushing and alcohol consumption stand out. Overall, approximately half (52%) of the participants reported brushing their teeth an average of three times a day, but participants from private clinics (72%) reported a higher frequency (2 and 3 time/day) of tooth brushing than those from public services (40%) (Figure 2). Additionally, alcohol consumption was more prevalent among participants from private services.

Table 2. Sociocultural aspects of participants from private clinics and public health system assistance in Ipiaú from March 23 to August 17, 2022.

	Private assistance		Public health assistance		
	N	%	N	%	$\chi 2$
Daily brushing					**0,0036

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(time/day)									
1	2	4	3	6	
2	12	24	27	54	
3	32	64	20	40	
4	4	8	0	0	
Drug consumption					**0,004
No	14	28	28	56	
Alcohol	31	62	17	34	
Alcohol and cigarette	3	6	4	8	
Alcohol and marihuana	2	4	1	2	
Not informed	0	0	0	0	
Medicinal plants used (last 6 months)					0,080
Yes	31	62	39	78	
No	19	38	11	22	
Medicinal plants used to treat oral disease (last 6 months)					0,000
Yes	0	0	0	0	
No	50	100	50	100	

[1]** valor de p<0,05.

Regarding the use of medicinal plants in the last six months, there was no statistical difference between the two services through $\chi 2$ method, even if the frequency and MCA analysis (Figure 2) show a greater tendency to use medicinal plants among participants from the public service located in a rural area (78%; n = 31/50). Notably, none of the groups reported using medicinal plants specifically for oral conditions.

Concerning the use of antibiotics by the participants three months prior to the study, most of them reported not to have used any. Among those who reported use, amoxicillin, azithromycin, cephalexin and ciprofloxacin were reported as antimicrobials.

Observing medicinal plants used by the participants in the six months prior to the survey (Table 3), the responses were quantitatively equivalent between the two groups. However, the types of plants reported differed significantly between participants in private and public services.

Table 3. Medicinal plants consumed by patients from public and private clinics and health system assistance in Ipiaú interviewed from March 23 to August 17, 2023.

Common name	Scientific name	Private assistance	Public health assistance
Water cress	*Nasturtium officinale*	0	1
Rosemary	*Salvia rosmarinus*	1	4
Blackberry	*Rubus subg. Rubus*	1	1
Mastic	*Schinus terebinthifolia*	1	1
Ayahuasca	*Banisteriopsis caapi* *Psycotria viridis*	1	0
Aloe vera	*Aloe vera*	1	0
Boldo	*Peumus boldus*	2	0

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Chamomile	*Matricaria chamomilla*	4	1
Canabis	*Cannabis sativa*	1	1
'Pariparoba'/'Capeba'	*Piper regnellii*	0	1
Lemon grass	*Cymbopogon citratus*	1	**11**
Lemon balm	*Melissa officinalis*	8	5
Diuretic herbs	NI	1	0
Anise	*Pimpinella anisum*	**9**	5
Soursop (leaves)	*Annona muricata*	0	1
Orange (leaves)	*Citrus sinensis*	1	0
Pine cone (leaves)	*Annona squamosa*	0	1
Brazilian cherry (leaves)	*Eugenia uniflora*	2	0
'Espinheira-santa'	*Maytenus ilicifolia*	0	1
Ginger	*Zingiber officinale*	1	0
Hibiscus	*Hibiscus* sp	0	1
Spearmint	*Mentha spicata*	2	2
Princess vine	*Cissus sicyoides*	0	1
Jesuit's tea/ herba sanctæ Mariæ	*Dysphania ambrosioides*	1	1
Noni	*Morinda citrifolia*	0	1
Kalanchoe	*Kalanchoe* spp	1	0
Oregano	*Origanum vulgare*	1	0
Job's tears	*Coix lacryma-jobi*	0	1
Flame violet	*Episcia cupreata*	2	0
Waybread	*Plantago major*	1	0
Cat's claw	*Uncaria tomentosa*	1	0
Total		**44**	**41**

[1]NI – not informed.

Colony forming unit enumeration and isolate identification were performed by laboratory experiments and are detailed in the Supplementary Material. Regarding the quantification of typical Streptococcus colonies isolated from dental biofilm and their tendency to cause dental caries, the findings indicated a higher density of CFU/100 µL in participants from private clinics (34% participants with >100 CFU/100µL), while both groups had a similar tendency towards salivary acidification (~40% high salivary acidity) (Figure 1). However, when both variables were evaluated together, no significant association was observed (r = 0.15).

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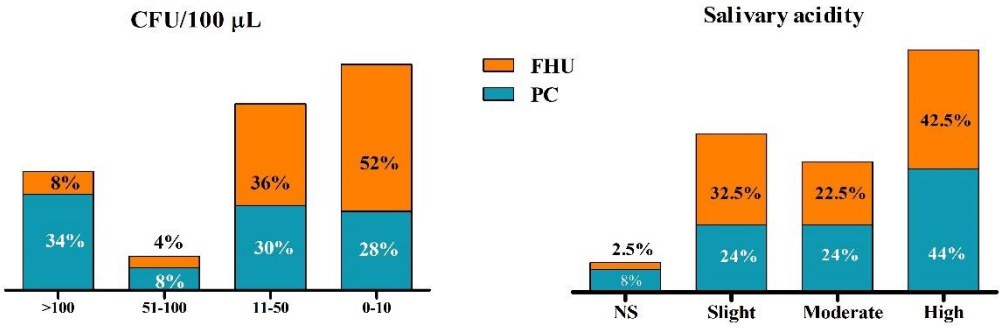

Figure 1. Percentage of participants from private clinic (PC) and family health unit (FHU) according to the number of Streptococcus colony forming units (CFU)/100 µL ranking and dental cavity formation tendency detected by salivary acidity level in Snyder test. NS: little or no susceptibility to caries; Slight: slight susceptibility to caries; Moderate: moderate susceptibility to caries, and High: high susceptibility to caries.

Of the selected colonies, presumptive identification of Streptococcus species by biochemical testing confirmed the presence of nine S. mutans colonies, 43 non-mutans Streptococcus colonies, and 10 inconclusive samples.

When multiple correspondence analysis was performed, the association between sociodemographic aspects, biofilm colonization (Streptococcus CFU/100µL) and distinct dental services (Figure 2) in the community studied led to the inference that 1) participants served at private dentistry service are more prone to consume alcohol and cigarette; 2) the possibility of having dental problems (high number of UFC/100µL) was not associated with good oral hygiene practices; 3) the use of antimicrobials is more related to access to health care; and 4) the use of medicinal plants is highly associated with public health service.

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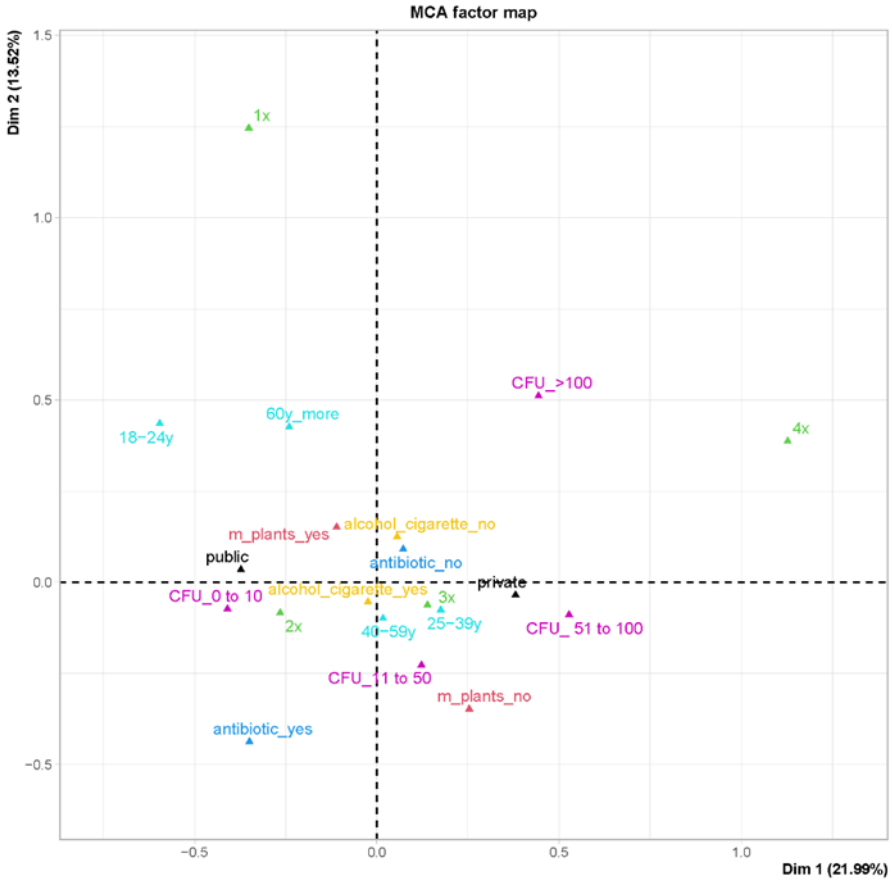

Figure 2. Multiple correspondence analysis (MCA) of sociodemographic aspects of categorical variables, antimicrobial and medicinal plant use, and biofilm colonization according to different dental services (public versus private). Legend: Age range (light blue) in years (y) - 18-24y, 25-39y, 40-59y, 60y_more; alcohol and cigarette consumption (yellow) (alcohol_cigarette_no/alcohol_cigarette_yes); antibiotic use in the last 3 months (cian blue) (antibiotic_yes/antibiotic_no); use of medicinal plants in the last 6 months (light red) (m_plants_no/m_planst_yes); and colony forming unit (CFU) rank (pink) (CFU_0 to 10/CFU_11 to 50/CFU50 to 100; CFU_>100); frequency of toothbrushing/day (green) 1x, 2x, 3x, and 4x.

4. Discussion

The present study has focused on the influence of sociodemographic and cultural factors on *Streptococcus* species biofilm colonization in two different contexts of dental service: public health and private clinic.

First, contrary to expectations, there was no simple relationship between toothbrushing habits and biofilm colonization in either group. Although participants from the private clinics reported a higher frequency of tooth brushing, they had a higher density of *Streptococcus* colonization. According to the literature, higher brushing frequency is typically associated with less biofilm formation and fewer bacteria adhering to tooth surfaces [20]. In this study, the higher density of CFU/100µL in the private clinic samples may be attributed to the minimal time since last brushing. Participants reported brushing their teeth the night before sample collection, approximately eight hours prior to sample collection. While participants were instructed to avoid brushing within two hours of sample collection, the maximum allowable time since brushing was not clearly communicated to the first ten participants. This lack of clarity may have contributed to the uncountable CFU (20%) observed in the private clinic group.

Another point to consider is the analysis of CFU/100µL and the Snyder test together. Although no association was found between Snyder test findings and CFU counts, patients from private clinics showed a greater tendency to salivary acidification. Also, the lower density of *Streptococcus* in the

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samples from the public service participants may be related to the number of dental remnants observed during sampling. Of the 50 participants, 28 had partial and/or full dentures, indicating a significant number of edentulous individuals.

A third point to note is that the high reported rates of daily tooth brushing observed in this study may not accurately reflect the actual habits of the participants. Positive responses regarding the frequency of daily tooth brushing may be influenced by social norms and oral hygiene programs [21].

Alcohol consumption was also significant in this study, with notable differences between the two populations studied. Due to ethical restrictions and study design, neither the prevalence of dental caries nor the frequency of alcohol consumption was determined. However, it is well documented that excessive alcohol consumption may contribute to dental erosion [22]. Combined with cigarette and marijuana use and the high prevalence of dental caries among participants attending private clinics, these findings underscore the need for further study and more robust oral health promotion campaigns among this population.

Ultimately, the main point of this study was to show the different characteristics of the users of two Brazilian dental care systems reflect both social inequalities and the exchange of knowledge between neighboring social groups. Inequalities are more pronounced in terms of public health investment for populations with accessibility to dental health problems than in terms of cultural or educational diversity.

The fact that there are sociocultural aspects also negatively influencing oral health in private clinics patients, investments in oral health improvement campaigns should be implemented in both populations to address these challenges. The pathophysiology of the dental biofilm is important to implementing proper management strategies. Although dental biofilm cannot be eliminated, it can be reduced and controlled through daily oral care [23].

5. Conclusions

In conclusion, while exploring the *Streptococcus* spp. isolation from dental biofilm and sociocultural factors between public and private dental patients, in the community studied, living close to nature and having healthy habits seems to have advantages over oral health.

Supplementary Materials: The following supporting information can be downloaded at: (https://figshare.com/s/2bc04e55a69e8bf683e4).

Author Contributions: Conceptualization, Edmone Eça, Luciana Carvalho and Aline Conceição; Data curation, Aline Conceição; Formal analysis, Marcelo Ferraz, Luciana Carvalho and Aline Conceição; Investigation, Edmone Eça, Mariana Oliveira, Valdineia Oliveira and Aline Conceição; Methodology, Edmone Eça, Luciana Carvalho and Aline Conceição; Project administration, Aline Conceição; Supervision, Aline Conceição; Validation, Edmone Eça, Luciana Carvalho and Aline Conceição; Visualization, Mariana Oliveira, Valdineia Oliveira, Marcelo Ferraz, Luciana Carvalho and Aline Conceição; Writing – original draft, Edmone Eça, Luciana Carvalho and Aline Conceição; Writing – review & editing, Edmone Eça, Mariana Oliveira, Valdineia Oliveira, Marcelo Ferraz, Luciana Carvalho and Aline Conceição.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

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Conflicts of Interest: The authors declare no conflicts of interest.

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