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RESEARCH ARTICLE OPEN ACCESS

In vitro antimicrobial activity of selected mouthwashes on *Streptococcus* species isolated from adult patients with dental caries

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Abstract

Objective: Dental caries is a preventable oral disease with economic burdens and effects on the quality of life. The study aimed to isolate *Streptococcus* species implicated in dental caries and also evaluate the antimicrobial potential of three brands of mouthwashes.

Methodology: This was a cross-sectional study of patients with active dental caries attending the oral diagnosis dental clinic of a tertiary health facility in Lagos, Nigeria. Collected samples from carious lesions were cultured on selective Mitis Salivarius agar incubated anaerobically and characterized. Pure wild-type *Streptococcus sp.* isolated were subjected to antimicrobial assay against three commonly used mouthwashes obtained within the study area.

Results: The participants were forty consenting adults consisting of 27 males and 13 females. Sixty-six bacterial isolates were obtained and identified as *Streptococcus* sp (n = 49; 74.2%), *Enterococcus* (n = 14; 21.2%), and the unidentified (n=3; 4.6%). Of the 49 *Streptococcus* sp, 18 (36.7%) were *S. mutans*, 16 (32.7%) were *S. salivarius* and 15 (30.6%) were *S. mitis.* The antimicrobial assay revealed various zones that were concentrated and mouthwash type dependent. At full strength (100% commercial stock solution), mouthwash containing 0.2% chlorhexidine gave a more anti-streptococcal activity followed by mouthwash containing 0.03% Triclosan.

Conclusion: Commercial mouthwash may have antibacterial activities against oral *S. mutans*, *S. salivarius,* and *S. mitis*. The formulation containing 0.2% chlorhexidine showed more inhibitory activity against the wild-type *Streptococcus* sp isolated. It is critical to advocate for the use of mouthwashes with potent active ingredients such as chlorhexidine to promote better oral health.

Keywords: Dental caries, Mouthwash, Mitis Salivarius agar, *Streptococcus*, Chlorhexidine, Triclosan

Plain English Summary

The study evaluated the efficacies of three selected mouthwashes on Streptococcus isolates obtained from forty adult patients with dental caries. Our findings indicate that the Streptococcus isolates were Streptococcus sp, S. mutans, S. salivarius and S. mitis in decreasing order of abundance. The mouthwashes showed antimicrobial activities that varied even at full strength, that is. 100%. There is a

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need for routine checks by regulatory agencies to ensure that commercially available mouthwashes have the most effective active ingredients.

Background

Dental caries otherwise known as tooth decay is described as a biofilm-mediated, multifactorial, dynamic disease initiated by sugar, resulting in the phasic demineralization and remineralization of dental hard tissues (1) affecting all age groups (2). Dental caries is a prominent condition responsible for oral pain, halitosis (mouth odour), tooth decay, periodontal (gum) diseases such as gingivitis and periodontitis, and subsequent tooth loss if left untreated (3, 4, 5). The disease affects about 60 to 90% of children as well as a vast majority of adults (3, 4, 5). Over the years, the daily use of toothpaste and mouthwash has been described as the main reason for the overall decline of caries worldwide (1). This has been associated with the action of the active ingredients in the toothpaste and mouthwashes. The agents have the potential to suppress the growth or disrupt biofilm formation by microorganisms and supporting substances present in the mouth (6). Bacteria species majorly recognized as the etiological agents of dental caries include *Streptococcus* and *Lactobacillus sp.* (7, 8, 9). *Streptococcus mutans and S. salivarius* are facultatively anaerobic, gram-positive coccus commonly found in the human oral cavity where they are significantly associated with tooth decay. Similarly, *S. mitis is a* mesophilic alpha-hemolytic potential pathogen. These *Streptococcus* species are well-known oral microbiota initiating infections in the oral cavity when proper oral hygiene is not maintained and implicated in other systemic infections such as infective endocarditis, making them species of public health importance (10, 11).

Evidenced-based current guidelines support the adjunctive use of mouthwashes alongside mechanical oral hygiene measures in managing tooth decay, and gum diseases (12, 13, 14, 15). Their added role in oral health practices is apparently because the liquid formulation might effectively penetrate areas that may missed during mechanical measures, making it an effective supportive oral hygiene aid for treating conditions such as bad breath, caries, gingivitis, plaque, dry mouth, yellow or discoloured teeth, and receding gums (6, 12, 13, 14). This agent is mostly patronized by adults. Evaluation of mouthwashes tends to show varying activities on microbes responsible for tooth decay (6, 12, 13, 14). Mouthwashes generally owe their antimicrobial activity to incorporated agents like fluorides, chlorhexidine, triclosan, or essential oils which aid in preventing the progression of oral diseases (6, 15, 16, 17, 18). Reports have shown that the activity of these agents is greatly enhanced when used in combination (6, 15, 16, 17, 18). Similarly, several studies have evaluated the antimicrobial efficacy of mouthwashes against several oral pathogens (13, 14, 19, 20). In addition, testing the antimicrobial efficacy of herbal formulations is well documented (13, 14, 19, 20). Furthermore, caries is seen as a childhood and adolescent disease and microbial studies tend to evaluate caries in children with little or no literature reporting dental caries in the adult population in Nigeria who may indeed be the major users of mouthwashes (2, 3, 4).

Several types of mouthwashes are commercially available in the Nigerian market, yet there is limited information on their effectiveness on local, wild strains of oral opportunistic pathogens (17, 18, 20). Moreover, most of these products may have not been formulated considering the phenotypic and genetic biodiversity among species and variants. In addition, available studies tend to culture the species aerobically using a conventional culture medium not ideal for growing micro-aerophilic or anaerobic *Streptococcus* oral species. Thus, evaluating the antimicrobial activities of oral species grown in a standard differential selective medium for a longer incubation period under micro-aerophilic conditions or anaerobiosis would enhance the expression of wild-type characteristics of these target strains. Providing information on the effectiveness of these agents on specific wild-type strains would create awareness and guide the selection of the best products for maintaining good oral hygiene in a Nigerian population. Nigeria is a resource-limited country with a high burden of untreated dental caries and a lack of good oral hygiene practices (8, 20). It is, therefore, important to investigate the effectiveness and adjunctive role of daily mouthwashes formulated for the prevention of dental caries. The aim of this study was therefore to evaluate the antimicrobial activity of three widely utilized mouthwashes in Nigeria, against wild-type *Streptococcus* species isolates from adult individuals with dental caries.

Materials and Methods

Study design

The study design is cross-sectional among consenting adults aged 18 years and above, with active dental caries attending the Oral diagnosis dental clinic of the Department of Preventive Dentistry, Lagos University Teaching Hospital, Idi-Araba. *Streptococcus* isolates were obtained from participants, and dental carious lesions and were tested for their susceptibility *in vitro* using three selected mouthwashes. The three different

commercial brands of mouthwashes were purchased from a supermarket located at Lagos University Teaching Hospital Idi-Araba. For experimental purposes, the branded commercial products were labelled as formula A containing chlorhexidine digluconate 0.2% w/v as the active ingredient, Formula B containing triclosan USP 0.03% w/v in combination with sodium fluoride 0.05%w/ and Formula C with cetylpyridinium chloride respectively.

Recruitment of participants
Consenting adults aged 18 years and above who volunteered were sampled after they were examined and confirmed by a consultant dentist to have dental caries. All the participants were recruited between 2016 and 2018, while they were accessing care at the Oral diagnosis dental clinic of the Department of Preventive Dentistry, Lagos University Teaching Hospital, Idi-Araba, Lagos State, Nigeria. A semi-structured questionnaire was used to collect demographic information and on the basic agents used for maintaining their oral health daily.

Collection of mouthwashes
A total of three (3) different mouthwashes were procured from a community supermarket situated at the Lagos University Teaching Hospital, Idi Araba. The collected mouthwashes were differentiated using their active ingredients as containing 0.2% chlorhexidine digluconate (Formula A); Triclosan USP 0.03% w/v in combination with sodium fluoride 0.05%w/v (Formula B) and sodium fluoride (226 ppm F) 0.05%, (Formula C).

Collection of dental caries samples
Using a dental scaler, carious dental plaque samples were collected from the carious lesion under the supervision of the dentist and transferred into a dental transport medium (AS – 920, ADTM - Anaerobe system, USA). This was then transported to the Medical Microbiology Laboratory, College of Medicine University of Lagos, Akoka within one hour of collection as previously reported (21).

Culturing of dental caries samples
The samples were cultured on Mitis Salivarius agar plate selective for the growth of oral *Streptococcus* sp. (22). Streaked cultured plates were placed in an anaerobic jar (Product No. 1.13681 Millipore, 2.5Ltrs. Merck Germany). Then an Anaerocult™ C Gas Pak sachet (Product No. 1.32383 Millipore, Merck Germany) was immediately placed into the jar to create a low-oxygen, high-CO_2 atmosphere and an anaerobic indicator, Anaerotest™ Strips (Product No. 1.32371Millipore, Merck Germany) was also inserted into the jar to indicate an anaerobic atmosphere. The plates inside the anaerobic jar were placed and incubated for 48 hours at 35^{o}C. Following the incubation, the distinct colonies were sub-cultured onto a Fastidious anaerobe agar (FAA) (Lab M) plate supplemented with 5µg/ml hemin, 1µg/ml vitamin K and 5% of sheep blood and incubated for 48 hours under anaerobiosis to obtain pure isolates. In addition, the colony-forming units (cfu/ml) for each isolate were also determined by multiplying the number of colonies observed on each plate with the dilution factor and then dividing this by the volume of the sample inoculated in the culture plate (100µl).

Morphological and biochemical characterization of the isolates
The pure isolates were subjected to a myriad of morphological and biochemical tests. First, all the isolates that produced a blue or black pigmentation on Mitis Salivarius agar were selected and identified as oral *Streptococcus* species. Furthermore, all the isolates that produce gummy-like levan, sticky, mucoid, or gum-drop colonies on Mitis Salivarius agar were identified as *Streptococcus salivarius*. On the other hand, isolates that produced colonies that were small, flat, and light-blue were identified as *Streptococcus mitis*, while those that were undulate-shaped with a granular frosted-glass appearance were selected as *Streptococcus mutans*. Those that produced dark to blue-black colonies were identified as *Enterococcus* species. The morphological characteristics of the isolates were subjected to Gram reaction and catalase test. The identified and purified wild-type isolates primarily isolated from clinical samples were then utilized for the *in vitro* evaluation of the antimicrobial activity of the various mouthwashes.

Preparation of stock solution and serial dilution
The mouthwash solution in its commercial form (taken as 100% of the original solution solution) and two other different concentrations (1:5 and 1:95,) were prepared using a sterile dilution blank (AS – 907, Anaerobe Systems - USA) to form the solutions. The stock solutions were transferred into three sterile universal bottles labelled T_1, T_2, and T_3. T_1 contained 1:5 dilution (1ml of mouthwash to 4ml of dilution blank). Tube T_2 contained 1:95 dilution (62.5 µl of the mouthwash to 6ml of the dilution blank). T_3 contained 100% (10 ml of stock concentration mouthwash). All the prepared stocks (10 ml each) were sterilized by autoclaving at 121^{o}C for 15 minutes before they were then subjected to *in-vitro* antimicrobial activity against the isolates.

In vitro antimicrobial activity of the mouthwashes
This antibacterial activity of the mouthwash was performed using a modified Kirby-Bauer disk diffusion technique. First, round discs 6 mm in diameter from Whatman No.1 filter paper were produced using a puncher and sterilized by Ultraviolet light for 20 minutes. The sterilized disc was then impregnated with 0.1ml of the stock solutions and allowed to stand for 10 minutes to absorb the stock solution. The bacteria inocula were prepared by emulsifying a loop full of pure discrete colonies into sterile physiological saline and the suspension was compared with 0.5 MacFarland's standard (R20410, Latex - Oxoid, UK). The prepared inocula (one per plate) were then inoculated onto freshly prepared Muller Hinton Agar (CM 0337, Oxoid, UK) plates and using a sterile swab stick, an even distribution of each inoculum was achieved. Next, the impregnated discs (in triplicates per dilution and isolate) were then placed on the inoculated plates and transferred to an anaerobic jar (AG0025, 2.5Ltrs. Oxoid, UK) and incubated at 35^{o}C for 48 hours (22, 23). The results were taken by measuring the diameter of the zone size of inhibition in millimetres. *Streptococcus mutans* (ATCC 25175) was used as positive control.

Data analysis
Data obtained were analyzed using simple descriptive (pie charts, percentages, means, and standard deviations) and univariate statistics (Student t-test). All the data analyses were managed using Microsoft Excel (USA, version 2013). The student t-test was utilized to compare the mean zones of inhibitions between the test and control isolates at the different mouthwash dilutions. The level of significance was set at 0.05 (95%).

Results
Socio-demographic Distributions of Study Participants and Selection of Isolates for Study
A total of forty (n=40) participants were voluntarily enrolled in the study and their sociodemographic and oral health practices are presented in Table 1. The result indicates that 27 (67.5%) were males and 13 (32.5%) were females**,** representing a male-to-female ratio of 2.1:1. The age range of the participants was stratified into 18-28, 29-39, 40-49, and 50-59 years. The respective spread of the respondents in terms of age bracket was 18(45.0%), 14(35.0%), 5(12.5%), and 3 (7.5%). All the participants (100%) utilized fluoride-containing kinds of toothpaste and toothbrushes to maintain their oral hygiene, while a total of 7 participants admitted to the use of mouthwashes to maintain their oral health. On the other hand, only 5% (n = 2) of participants utilized chewing sticks, in maintaining their oral hygiene.

Table 1: Sociodemographic factors and oral health hygiene practices

Variables	Frequency (%) (n = 40)
Gender	
Male	27 (67.5)
Female	13 (32.5)
Age (years)	
18-28	18 (45.0)
29-39	14 (35.0)
40-49	5 (12.5)
50-59	3 (7.5)
Utilization of mouthwashes	
Yes	7 (17.5)
No	33 (82.5)
Utilization of chewing sticks	
Yes	2 (5.0)
No	38 (95.0)
Utilization of toothpaste and toothbrush	
Yes	40 (100.0)
No	0 (0.0)

Microbial isolates from the dental caries of participants.
The results of the microbiological analyses are presented in Table 2, and Figures 1 and 2. Figure 1 shows the various species of *Streptococcus* obtained in the study on an agar plate. Table 2 shows the distribution of the various isolates according to the samples. Figure 2 and Table 2 show the characteristic colonies of *Streptococcus* species on the Mitis Salivarius agar plate. Table 2 further reveals that a total of sixty-six (n=66) bacterial isolates were obtained from the 40 samples collected in this study. Furthermore, results indicate that the isolates were *Streptococcus* sp (n = 49; 74.2%), *Enterococcus* (n = 14; 21.2%) and unidentified

(n=3; 4.6%) (Figure 2). Of the 49 *Streptococcus* sp, 18 (36.7%) were *S. mutans*, 16 (32.7%) were *S. salivarius* and 15(30.6%) were *S. mitis* species. Twenty-two (44.9%) *Streptococcal* isolates: 8 (36.37%) *S. salivarius*, 7(31.8%) of *S. mitis* and 7(31.8%) *S. mutans* were randomly selected (one species per patient) for *in vitro* antimicrobial testing (Tables 2).

Table 2: Characterization of *Streptococcus* species isolated from adult patients with dental caries

S/N	Lab Id	Mitis Salivarius Agar	Gram Reaction	Catalase Test	Organism/Species
1	001	+	+	-	*Streptococcus salivarius* ***Streptococcus mutans*** *Enterococcus sp.*
2	002	+	+	-	*Streptococcus mitis* ***Streptococcus salivarius***
3	003	+	+	-	***Streptococcus mutans*** *Enterococcus sp.*
4	004	+	+	-	***Streptococcus mutans*** *Enterococcus sp.*
5	005	NG	-	-	**NG**
6	006	+	+	-	***Streptococcus mitis*** *Streptococcus salivarius* *Streptococcus mutans* *Enterococcus sp.*
7	007	+	+	-	***Streptococcus mitis*** *Streptococcus salivarius* *Streptococcus mutans* *Enterococcus sp.*
8	008	NG			**NG**
9	009	+	+	-	***Streptococcus mitis*** *Streptococcus salivarius* *Enterococcus sp.*
10	010	+	+	-	***Streptococcus mitis*** *Streptococcus salivarius* *Streptococcus mutans*
11	011	NG	-	-	**NG**
12	012	+	+	-	***Streptococcus mitis*** *Streptococcus mutans* *Enterococcus sp.*
13	013	+	+	-	*Streptococcus mitis* *Streptococcus salivarius* ***Streptococcus mutans*** *Enterococcus sp.*
14	014	NG			**NG**
15	015	-			**NG**
16	016	+	+	-	*Streptococcus salivarius* ***Streptococcus mutans***
17	017	-			**NG**
18	018	-			**NG**
19	019	+	+	-	***Streptococcus salivarius*** *Streptococcus mutans*
20	020	+	+	-	***Streptococcus mitis*** *Streptococcus salivarius* *Streptococcus mutans*

					Enterococcus sp.
21	021	+	+	-	***Streptococcus salivarius*** *Streptococcus mutans* *Enterococcus sp.*
22	022	-			**NG**
23	023	-			**NG**
24	024	-			**NG**
25	025	-			**NG**
26	026	+			Unidentified
27	027	+			Unidentified
28	028	+			Unidentified
29	029	+	+	-	*Streptococcus mitis* ***Streptococcus salivarius*** *Streptococcus mutans*
30	030	+	+	-	*Streptococcus mitis* ***Streptococcus salivarius***
31	031	+	+	-	*Streptococcus mitis* *Streptococcus salivarius* *Enterococcus sp.*
32	032	+	+	-	***Streptococcus mitis*** *Streptococcus mutans*
33	033	+	+	-	*Streptococcus mitis* ***Streptococcus salivarius*** *Streptococcus mutans*
34	034	+	+	-	*Streptococcus mitis* ***Streptococcus mutans*** *Enterococcus sp.*
35	035	-	-	-	NG
36	036	-	-	-	NG
37	037	-	-	-	NG
38	038	+	+	-	***Streptococcus salivarius*** *Streptococcus mutans* *Enterococcus sp.*
39	039	-	-	-	NG
40	040	+	+	-	*Streptococcus mitis* ***Streptococcus mutans*** *Enterococcus sp.*

Key: NG= No growth; + = positive result; - = negative result; Species in bold letters were randomly selected (one species per patient) for *in vitro* antimicrobial sensitivity tests

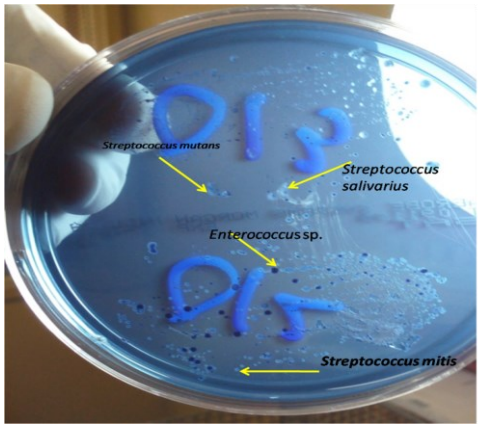

Figure 1: Characteristic colonies of *Streptococcus* species on selective Mitis Salivarius agar plate

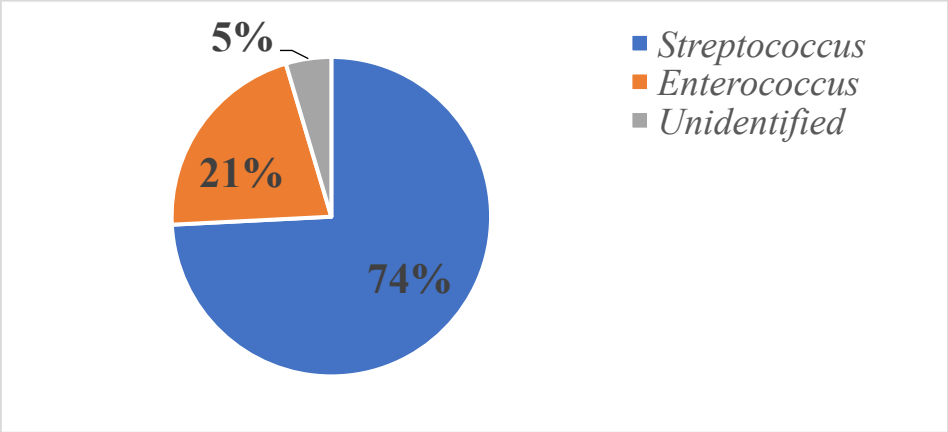

Figure 2: Distribution of the isolates identified from the participants

Table 3 shows the colony counts for the various *Streptococcus* species on the primary agar plate at various dilutions. At a dilution of 10^{-4}, the counts were 2.68 x 10^4, 2.75 x 10^4 and 2.85 x 10^4 cfu/ml respectively for a bacteria suspension of *S. salivarius, S. mutans, and S. mitis.* equivalent to 0.5 McFarland standard of 10^5 CFU/ml.

Table 3: Colony counts of the various *Streptococcus* species on primary agar plates (cfu/ml) and dilution factor

Organism/species	Number of colonies on on Mitis Salivarius Agar	Dilutions Tube 1	Dilutions Tube 3
	cfu/ml	10^{-2}	10^{-4}
Streptococcus salivarius	275	2.75 x 10^{-2}	2.75 x 10^{-4}
Streptococcus mutans	268	2.68 x 10^{-2}	2.68 x 10^{-4}
Streptococcus salivarius	285	2.85 x 10^{-2}	2.85 x 10^{-4}
Streptococcus mitis	-	10^{-2}	10^{-5}
Final sample dilution	-	10^2	10^5
Final dilution factor			

Inhibitory activities of different concentrations of mouthwashes on isolates

The antimicrobial activity of the various mouthwash formulations is presented in Table 4 for the representative *Streptococcus* isolates. Figure 3 shows a sample of the inhibition zone displayed by the test isolates on the Mitis Salivarius agar plate.

The zones of the inhibitions observed varied with the dilution used and the *Streptococcus* species utilized. At the concentration of 100%, all the mouthwashes showed the highest zones of inhibitions (mm). For formulas A, B and C at 100%, the zones observed for *S. mitis* ranged from 18 -25 mm, 12 -14 mm and 8 -10 mm, respectively. For *S. salivarius* at 100%, zones ranged from 16 -24 mm, 10 -16mm, and 8 -10 mm. However, the zones of inhibition for formula A were slightly higher than those of the control isolate. For *S. mutans* at 100%, the zones were

16-24 mm, 12 -16 mm and 8 -10 mm, respectively for formulas A, B and C. At an inhibition of 1:95 (mouthwash: diluent), no zones of inhibitions were observed except for the control isolates with formula A. At a dilution of 1:5, zones of inhibition that were generally lower than that of the highest concentration were observed. For *S. mitis* and *S. salivarius,* no significant differences were observed between the test zones of the various isolates and the control at the various concentrations. On the contrary, a comparison of the mean zones of the test *S. mutans* and the control isolates had significant differences ($p < 0.05$).

Table 4: Zones of inhibition (mm) produced by *Streptococcus sp.* and the control isolates against different concentrations of mouthwash

Sample NO	A_{T1} 1:5	A_{T2} 1:95	A_{T3} 100	B_{T1} 1:5	B_{T2} 1:95	B_{T3} 100	C_{T1} 1:5	C_{T2} 1:95	C_{T3} 100
S. mitis									
006	14	0	22	10	0	14	8	0	8
007	16	0	18	10	0	14	7	0	8
009	16	0	20	12	0	14	8	0	9
010	12	0	22	10	0	14	8	0	8
012	14	0	25	9	0	14	8	0	10
020	14	0	22	9	0	12	8	0	9
032	16	0	22	12	0	14	8	0	10
Mean±SD	*14.6±1.5*	*0.0±0.0*	*21.6±2.1*	*10.3±1.3*	*0.0±0.0*	*13.7±0.8*	*8.9±0.9*	*0.0±0.0*	*14.4±2.0*
S.mutans ATCC 25175	*14.0±.01*	*9.1±0.1*	*24.1±0.2*	*10.4±0.7*	*0.0±0.0*	*14.1±1.2*	*8.2±0.3*	*0.0±0.0*	*10.3±0.4*
S. salivarius									
002	16	0	20	10	0	12	8	0	10
019	17	0	24	9	0	12	8	0	10
021	16	0	16	10	0	10	8	0	9
029	12	0	18	10	0	14	8	0	9
030	14	0	20	8	0	16	10	0	10
033	12	0	18	12	0	16	8	0	9
038	14	0	24	10	0	16	8	0	8
Mean±SD	*14.3±2.0*	*0.0±0.0*	*20.0±3.1*	*9.9±1.21*	*0.0±0.0*	*13.7±2.4*	*8.3±0.8*	*0.0±0.0*	*9.3±0.8*
S.mutans ATCC 25175	*14.0±.01*	*9.1±0.1*	*24.1±0.2*	*10.4±0.7*	*0.0±0.0*	*14.1±1.2*	*8.2±0.3*	*0.0±0.0*	*10.3±0.4*
S. mutans									
001	12	0	18	8	0	14	8	0	9
003	12	0	16	8	0	14	8	0	10
004	14	0	24	8	0	16	7	0	9
013	16	0	16	12	0	16	8	0	10
016	13	0	18	10	0	14	8	0	10
034	15	0	20	8	0	12	8	0	8
040	12	0	18	10	0	14	8	0	9
Mean±SD	**13.4±1.6**	**0.0±0.0**	**18.6±2.8**	**9.1±1.6**	**0.0±0.0**	**14.3±1.3**	**7.9±0.4**	**0.0±0.0**	**9.3±0.8**
***S.mutans* ATCC 25175**	**14.0±.01**	**9.1±0.1**	**24.1±0.2**	**10.4±0.7**	**0.0±0.0**	**14.1±1.2**	**8.2±0.3**	**0.0±0.0**	**10.3±0.4**

Keys: Formula A, B and C represent the various mouthwashes. Mean values in *italics* represent mean values of the test isolates of *S. mitis* and *S. salivarius* that did not differ significantly ($p > 0.05$) from the control while values in "**bold**" represent values of the test isolates of *S. mutans* that differ significantly ($p < 0.05$) from control.

Figure 3: Inhibitory zones produced by formula A (with 0.2% Chlorohexidine) T_3 on two of the test isolates on a Mitis Salivarius agar plate

Discussion

Dental caries is a preventable disease with economic burdens and effects on the quality of life. Intervention by the daily use of antimicrobial active ingredients in the form of toothpaste and mouthwashes has been linked to a decline in dental caries. Our study was aimed at isolating and characterizing *Streptococcus* species from dental caries and also evaluating the efficacy of mouthwashes against them *in vitro*. Poor oral hygiene provides the enabling environment needed to support the proliferation of the microorganisms involved in the initiation and progression of dental caries (22, 23, 24, 25). The study focused on patients with caries lessons to target the isolation of wild-type strains of *Streptococcus* species. We observed that all the participants utilized toothpaste and toothbrushes daily to maintain their oral hygiene and health and a very small fraction admitted to the use of mouthwash and chewing sticks, respectively.

The microbial species in our study were limited to *Streptococcus* a major agent of dental caries with public health importance. The species isolated and used were *S. mitis, S. mutans* and *S. salivariu*s. These species are known etiological agents of dental caries (9, 26, 27). These microbial communities were less diverse than that reported earlier by Oluremi et al. (21). In their study, in addition to *Streptococcus sp, Staphylococcus aureus, Klebsiella sp, Escherichia coli* and *Pseudomonas aeruginosa* were obtained and studied. Comparatively, our targeted isolates were those of *Streptococcus* species grown on a selective media and in anaerobiosis. This plate also supports the growth of *Enterococcus* species. These species were isolated but were not evaluated in this study. To the best of our knowledge, this is the first report of *Streptococcus* sp. grown in an anaerobic jar in Nigeria.

All the mouthwashes utilized showed antimicrobial activity against the *Streptococcus* species isolated from the patients with dental caries. The results obtained varied according to concentration and type of mouthwash used, as well as the species of *Streptococcus* evaluated. This variation is not unexpected as it is in line with previous findings (14, 19, 20, 28). The significant zones observed in our study against *S. mutans* are in line with the report of Thomas et al. (19) against *S. mutans* and *Lactobacillus* sp., against various mouthwashes that were either commercially obtained or made in their laboratory for that study. Oluremi et al. (20), reported the highest activity against *Streptococcal* species using 0.047% of thymol. This is in line with our report as brand A showed the highest antimicrobial activity at 100% and also at 20% dilution of the ready-to-use, 0.2% chlorohexidine digluconate-based mouthwash (Formula A). This is followed by the action of the triclosan-based formulation. A much lesser antimicrobial activity observed in Formula B and C is in line with a similar report by Teh et al. (13) and by Jesumirhewe and Ariyo (14). Although the study by Jesumirhewe and Ariyo (2023) (14) concluded that toothpastes are better than mouthwashes, their target organisms were the *Streptococcus aureus* species.

The variation in the mouthwash activities against the various *Streptococcus* species could be due to the various active ingredients which were chlorhexidine gluconate for Formula A, triclosan for B, and Sodium fluoride for C. Formula A which contains 0.2% chlorhexidine gluconate showed the highest inhibitory activity against selected isolates. This may be a function of the chemical compositions or diffusion rate exhibited by the mouthwashes. This study further supports the claim that mouthwashes containing 0.2% chlorhexidine as their active ingredient are effective in inhibiting *Streptococcus* sp. (17, 29). The result obtained in this study was also similar to that in Brazil (30) and India (31). In addition, Formula B with 0.03% triclosan showed a lesser mean zone of inhibition when compared to Formula A. These results did not align with a

similar study carried out using the same active ingredient in which their zone of inhibition was much wider (31, 32).

There were significant differences in zone sizes of inhibition among these selected mouthwashes as they showed varying degrees of antimicrobial activity which agrees with previous findings of Chaudary et al (33). It had also shown that the mean zone size of inhibition of mouthwashes, may not be compared directly with that of another because there may be other active constituents that might likely diffuse at different rates (34). This agrees with this study. The mouthwash with 0.2% chlorhexidine was shown to be the most effective. However, the antimicrobial activity of chlorhexidine has been extensively investigated and is the gold standard for inhibitory activities of mouthwashes (30, 31, 32, 33, 34, 35, 36, 37). Nevertheless, chlorhexidine sometimes causes brownish discolouration of teeth, altered taste sensation, and oral mucosal erosion (38, 39). But its antibacterial action lasts for longer periods possibly due to its high substantivity. It also has anti-plaque effects by ensuring a high level in the oral tissues (40). Although, none of these beneficial factors were analyzed in this study.

The mouthwash which contains 0.03% triclosan also displayed inhibitory activity against the isolates, thus, supporting previous claims that it plays a vital role in the prevention of dental caries associated with oral *Streptococcus mutans* (41). At this concentration, triclosan acts by blocking bacterial fatty acid biosynthesis. Cetylpyridinium chloride completely demonstrated the smallest zones of inhibition when compared with the mouthwash containing chlorhexidine. It is therefore important to note that, in the disc diffusion method, the diameter of inhibition is considered to be directly proportional to the antimicrobial activity. Moreover, it is also significant to note that, it can be influenced by the thickness and the composition of culture media, the concentration of the antimicrobial agent in the paper disc, and as such the degree of rate of diffusion of tested substances (42). These oral antimicrobial agents have shown *in vitro* activity, there is an underlying possibility that *in vivo* testing may be influenced by factors such as systemic conditions, the presence of oral biofilm and dental anatomy (37). Instead of these, further studies need to evaluate *in vivo* effects in a diverse population of Nigerians.

Conclusion

Streptococcus species are often implicated in dental caries. Our findings indicate that the commercial mouthwash has antibacterial activities against oral *S. mutans*, *S. salivarius* and *S. mitis*. The mouthwash with chlorhexidine, triclosan, and cetylpyridinium as active ingredients has efficient and varying degrees of antimicrobial activities on *Streptococcus* sp. The formulation containing 0.2% chlorhexidine showed a more susceptible inhibitory activity against the wild-type *Streptococcus* sp. found in Nigerian adult patients with dental caries. It is important to advocate for the use of mouthwashes with proven potent active ingredients to promote better oral health.

Limitations of the study

The study has some limitations. First, the study utilized only the three most popular commercially available mouthwashes used by the public in the study area. Second, identification of the *Streptococcus* isolates was done using cultural and molecular methods. Although a selective media was used, cultural identification relies heavily on cultural and biochemical characteristics in the identification of isolates, and it is not as definitive as molecular techniques.

List of Abbreviations

AS: Anaerobic system
ATCC: American Type Culture Collection
CO2: Carbon IV oxide
CFU/ml: Colony forming unit per millimetre
NG: No growth
SD: Standard deviation
USA: United States of America
UK: United Kingdom

Declaration section

Ethics and approval and informed consent

Study approval was obtained from the Health Research Ethics Committee, College of Medicine of the University of Lagos, (HRECCMUL) with approved number: M/HREC/MLS/05/16/033. All the participants in the study gave their informed consent after the aim and objectives of the study and the confidentiality of their data were adequately explained to them. Recruitment of participants into the study was purely voluntary.

Consent for publication

All the authors gave their consent for publication of the study under the Creative Commons Attribution-Non-commercial 4.0 license.

Competing interest

All the authors declare that there is no competing interest to declare.

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Not applicable.

Data availability

All the data obtained in this study are within the manuscript. However, raw data will be made available upon request from the corresponding author.

Authors' contributions

All the authors were involved in various aspects of the study and this is given as a breakdown below.

Conceptualization:	FON, KAU
Data acquisition:	CUN, OMO, FON
Data analysis:	FON, AO, UOE
Article writing:	FON, KAU, OMO, UOE
Manuscript review:	FON, UOE
Supervision:	FON, UCN
Final approval:	FON, UCN, OMO, UOE, AO, KAU

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