EAHRC
EAST AFRICAN HEALTH RESEARCH COMMISSION
Research for Health and Prosperity

ORIGINAL ARTICLE

Toothpastes Inhibitory Effects on Microorganisms Isolated from Dental Decay of Patients Attending Ruhengeri Referral Hospital, Musanze, Rwanda

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ABSTRACT

Background: Oral disease affects about 3.5 billion people worldwide annually, with 3 out 4 people affected are from middle income countries. Toothpastes have been used to improve and maintain oral health. This study was carried out to determine the inhibitory effects of selected toothpastes in Rwanda's market.

Materials and Methods: This was a cross sectional study carried out from September 2021 to January 2022. A total of 30 dental surface swab samples were collected from dental decay patients and cultured at INES-RUHENGERI clinical microbiology laboratory for microbial isolation and identification. The three types of toothpastes (white dent, ABC dent, Colgate) plus one mouthwash (sonatec) were selected as antimicrobials. Statistical analysis was done using SPSS version 24.0 (IBM Corporation, Armonk, NY, USA). ANOVA was used to test for mean difference of toothpastes inhibitory effects on isolated microorganisms.

Results: *Streptococcus mutans* 15 (29.4%) was the most isolated microorganism among dental decay patients. Colgate and ABC dent were the least effective while white dent and sonatec were the most effective, they showed inhibitory effects to all isolated microorganisms. *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, and *Candida albicans* exhibited resistance to both Colgate and ABC dent toothpastes. *Staphylococcus aureus* and *Lactobacillus spp* were resistant to Colgate, while only *Streptococcus mutans* demonstrated sensitivity to all types of toothpastes). The mean difference of inhibitory effect of toothpastes ($p = .0004$,) was statistically significant. The average mean values of toothpaste inhibitory effects (mm) were 15.166 for SONATEC, 5.666 for ABC dent, 24 for White dent, and 3.333 for Colgate.

Conclusion: *Streptococcus mutans* and *Lactobacillus spp* were the main colonizer of oral cavity in dental decay patients. white dent and sonatec were the most inhibitors of bacterial growth, dental decay patients should use the effective toothpaste such as SONATEC.

BACKGROUND

Dental decay is a significant global health concern, being the most prevalent oral diseases and the third most common illness globally, affecting over 3.48 billion individuals.[1] According to population research, 38% of adolescents and 73% of adults had untreated caries.[1] In East Africa, the combined prevalence of dental decay is 45.7.[2] The overpopulation of pathogenic microorganisms in the oral cavity is a primary cause of oral health problems. In sub-Saharan Africa, including Rwanda, dental decay poses a considerable burden on public health. The study found a 50% prevalence of dental decay in permanent dentition and 41.3% in mixed dentition in Eritrea, Sudan, and Tanzania, with average mean decayed, missing, and filled permanent tooth scores of 1.941 and 2.237 for primary teeth respectively, with female gender and poor teeth brushing behaviors as independent risk factors.[2] The prevalence of dental decay in Rwanda ranges from 42.4% to 71.5%, indicating a significant burden of this oral health condition in the country.[3] The oral microbiome, a complex collection of bacteria in the human mouth, is essential for oral health but may potentially contribute to oral illnesses if unbalanced. Pathogenic bacteria such as *Streptococcus mutans* and *Candida albicans* have been related to tooth caries and periodontal disorders, highlighting the significance of microbial dysbiosis in oral health.[4] The study investigates the antibacterial effects of toothpaste against oral microorganisms associated with dental decays. Several brands have showed antibacterial effectiveness against germs such as *P. gingivalis*, *E. coli*, *S. aureus*, and *C. albicans*.[5] Toothpastes containing compounds such as triclosan and chlorhexidine have significant antibacterial properties, controlling oral microorganisms that cause mouth odor and disease. Natural extracts, chlorhexidine, and triclosan also have

antibacterial properties against Gram-positive bacteria and yeasts, promoting oral health and reducing dental problems.[6] Therefore, investigating the effectiveness of various toothpaste products available in Rwanda on microorganisms isolated from dental decay patients at Musanze's Ruhengeri Referral Hospital was critical for moving oral health initiatives forward, preventing oral diseases, and improving overall health. This study sheds light on the effectiveness of oral hygiene products and informs evidence based strategies for promoting good oral health.

MATERIALS AND METHODS

Ethical Consideration

In this study, ethical concerns were very important in order to respect participants' rights and privacy. The Ruhengeri Referral Hospital Ethical Review Board provided prior clearance (Ref/929/RRH/DG/2021).

Informed Consent

Before participating in the study, participants aged 15 to 50 years who were suspected or diagnosed with dental decay and gingivitis gave written informed permission. The permission process entailed explicitly presenting the study's aims, methods, possible risks and benefits. Participants were promised that they may resign from the research at any moment without penalty. Consent forms were created in language that the participants understood, and any questions or concerns were addressed before obtaining consent.

Confidentiality

Throughout the investigation, procedures were put in place to protect the anonymity of participants. Personal information and identification of participants were anonymized and kept private. Each participant received a unique identification to replace any personal information. Data gathered throughout the study were securely maintained and only authorized research workers had access to them.

Data Security

Data security mechanisms were used to ensure the integrity and confidentiality of study data. Electronic data was stored on password protected computers that could only be accessed by authorised persons. Hardcopy papers with sensitive information were kept in lockable file cabinets in a safe place.

Study Site and Study Design

A descriptive cross sectional study was carried out from September 2021 to January 2022 at Ruhengeri Referral Hospital in Muhoza, Musanze district, Northern Province of Rwanda. Ruhengeri Referral Hospital is both referral hospital and the only district hospital in Musanze District with 15 sectors, 68 cells, 432 villages and a population of 406, 557. The hospital which have a well-established department of stomatology, covers 16 health Centers, 1 Prison dispensary and 13 health posts. The hospital was chosen because of its reputation as a leading healthcare center in the region, providing specialist dentistry trea tments. Its location in Muhoza, Musanze District, Northern Province of Rwanda made it ideal for performing oral health studies. The hospital's broad patient group allowed for the collection of representative samples from people with dental decay, which increased the study's relevance and application. Overall, the hospital's reputation, specialized services, and diversified patient population made it an excellent candidate for studying toothpaste efficacy in treating oral illnesses.

Study Population and Sample Size

The study population consisted of patients with dental decay aged between 15-50 years old attending the Stomatology department of Ruhengeri Referral Hospital in Muhoza sector, Musanze district, Northern Province of Rwanda. patients who were diagnosed with dental decay were randomly selected where all patients fulfilling the inclusion criteria and volunteering to participate were given the equal chance to participate in the study, and 30 participants who consented for participation were recruited for this study.

Inclusion Criteria

The inclusion criteria for this study comprised patients aged between 15 to 50 years' old who were suspected or diagnosed with dental decay, and were attending the Stomatology department of Ruhengeri Referral Hospital. Additionally, individuals who consented to participate in the study by providing written informed consent were accepted to participate in the study.

Exclusion Criteria

Patients who did not provide consent to participate in this study, those from departments other than the stomatology department at Ruhengeri Referral Hospital, and those without oral illnesses were all excluded. Furthermore, patients below the age of 15 and above the age of 50 were dismissed.

Sample Collection and Bacterial Identification

Dental swabs of 30 patients with tooth decay aged 15 to 50 years were collected under aseptic conditions. A sterile cotton tip swab sticks were used to collect dental swabs. The entire tooth surface was covered with the swab stick, with an emphasis on the affected area. In a sample container, peptone water and the swab stick were swiftly combined. The tagged sample was delivered to INES-clinical Ruhengeri's microbiology laboratory for microbial isolation and identification. Three toothpastes (White Dent, ABC Dent, and Colgate) and one mouthwash (Sonatec) were chosen as antimicrobial agents.

Culture Media Preparation and Inoculation

Microorganisms were grown on a variety of culture media, including blood agar, MacConkey agar, Muller Hinton Agar, Potato Dextrose Agar, and Mannitol Salt Agar (made by HiMedia Laboratories Pvt. Ltd., India, Mumbai). Culture media were made by mixing a few grams of the appropriate quantity with distilled water, as described on the manufacturer's culture media container labels, then sterilizing in an autoclave at 121°C for 15 minutes. Plates were incubated at 37°C for 12-24 hours using the streak plate method for bacterial culture and the lawn plate method on Muller Hinton agar for bacterial susceptibility testing, respectively.

Paper discs were created using a disc borer, sterilized via autoclave, and subsequently immersed in varying

concentrations of toothpaste, such as 1:1, 1:4, and 1:8 ratios. These paper discs, containing different toothpaste concentrations, were then placed onto Muller Hinton Agar plates and incubated at 37°C for 12–24 hours until inhibition zones were visible.

Gram Staining

The isolated colonies were Gram stained. Normal saline was placed on a labeled slide, a colony was taken from the petri dish, and a smear was made spreading bacterial colony on glass slide. The crystal violet solution was flooded and allowed to remain for one minute. Tap water was used to rinse off the smear. Iodine solution was applied for one minute. Tap water was used again to rinse off the smear. The decolorizer was applied for one to five seconds. Tap water again for the same purpose. The safranin stain was applied and remained for 30 seconds. The tap water was left to run slowly on the flooded area and finally, the slide was drained and dried in an upright position. Microscopy was done with x100 oil immersion to report bacteria and cells (Gram positives stain Purple and Gram negatives stain Red).

Biochemical Tests Preparation and Inoculation

Semisolid media such as Indole, Methyl Red, Urease test, Kligler iron agar, Simons citrate agar, Sulfide-Indole-Motility and Oxidase tests were prepared for the biochemical assays for the identification of bacterial species, and inoculated for the biochemical reactions depending upon bacterial characteristics. Stabbed and slanted tubes were inoculated and incubated for 12–24 hours 37^{0}C. To prepare the biochemical tests for the isolated microorganisms, we had to follow separate protocols for each test. Here are the general processes for preparing and carrying out each test:

Biochemical Tests for Gram Negative Bacteria *(Pseudomonas aeruginosa)*

Methyl Red Test: This test is used to determine if an organism can produce acids in a mixed sugar fermentation. To prepare the media, we added 0.5g of peptone, 0.5g of beef extract, 0.4g of dipotassium phosphate, 0.1g of monopotassium phosphate, 0.5g of glucose, 0.5g of lactose, 0.5g of sucrose, 0.2g of methyl red, and 15g of agar to 1L of distilled water. The pH was adjusted to 6.8 and the media was sterilized by autoclaving at 121°C for 15 minutes. Using organisms from an 18-24hour pure culture, the medium was gently inoculated and aerobically incubated at 37°C for 24 hours. After 24 hours of incubation, 1milliliter of the broth was transferred to a clean test tube. 2 to 3 drops of methyl red indicator were added to the aliquot, and the observed bright red immediately indicated a positive result.

The Urease Test: This test was used to assess if an organism could hydrolyze urea. To make the medium, we mixed 20g urea, 0.5g peptone, 1g glucose, 5g sodium chloride, 2g potassium chloride, and 20g agar into 1L of distilled water. We adjusted the pH to 6.8 and sterilized the medium by autoclaving it at 121°C for 15 minutes. To conduct the experiment, medium was inoculated with a pure fresh culture of the organism and incubated at 37°C for 24-48 hours. The appearance of pink signified a positive test result.

Simon Citrate Agar: This test was used to determine if an organism can utilize citrate as a sole carbon source. To prepare the media, we added 2g of citric acid, 2g of sodium citrate, 5g of sodium chloride, 0.5g of magnesium sulfate, 0.02g of bromothymol blue, and 20g of agar to 1L of distilled water. pH was adjusted to 6.8 and the medium was sterilized by autoclaving at 121°C for 15 minutes. To perform the test, we inoculated the media with a pure culture of the organism and incubated at 37°C for 24-48 hours. Growth with color change from green to intense blue along the slant, indicates a positive result.

Sulfide Indole Motility Test: This test was used to determine if an organism can produce hydrogen sulfide, indole, and motility. To prepare the media, add 3g of peptone, 5g of sodium chloride, 0.3g of ferric ammonium citrate, 0.02g of phenol red, and 1.2 g of agar to 1L of distilled water. pH was adjusted to 7.4 and medium was sterilized by autoclaving at 121°C for 15 minutes. To perform the test, we inoculate the medium with a fresh pure culture of the organism and incubated at 37°C for 24-48 hours. Blackening of the medium along the stab line indicates hydrogen sulfide production. A red color in the alcohol layer after the addition of Kovac's reagent signifies a positive indole test, highlighting the ability of the organism to produce indole from tryptophan. Additionally, the presence of diffuse growth away from the stab line indicates motility.

Oxidase Test: This test was used to determine if an organism can produce cytochrome c oxidase. To perform the test, we use an oxidase test strip and pure and fresh colony of the organism was rubbed on it. Observation of a purple color within 5 to 10 seconds, indicated a positive test.

Biochemical Tests for Gram Positive Bacteria (*Staphylococcus spp* and *Streptococcus pyogenes*)

Catalase Test

Using an aseptic technique, one drop of hydrogen peroxide 3% was placed onto a clean glass microscope slide, a pure fresh colony from the petri dish was taken on a loop and added to the peroxide, for the presence of air bubbles or absence of bubbling. Catalase was done to differentiate *Staphylococcus spp*: catalase-positive and *Streptococcus spp*: for catalase negative.

Catalase test is used to differentiate bacteria based on their ability to produce the enzyme catalase, which catalyzes the decomposition of hydrogen peroxide into water and oxygen. A small amount of bacterial colony was transferred onto a glass slide, and a drop of hydrogen peroxide (3%) was added. If bubbles of oxygen gas form rapidly, the test was positive for catalase production.

Coagulase Test

A piece of the isolated colony was emulsified using a drop of physiological saline to create two thick suspensions on each end of a slide after gram staining. A drop of human plasma was gently incorporated into one of the suspensions. And for a successful outcome, the clumping of the organisms within 10 seconds was seen. To distinguish between any granular appearance of the organism and actual coagulase clumping, no plasma was

added to the second solution.

Coagulase test distinguishes between *Staphylococcus aureus* (coagulase-positive) and other *Staphylococcus species* (coagulase-negative). Coagulase promotes the formation of fibrin clot from fibrinogen in plasma. Two suspensions were prepared on a glass slide: one with the test organism and the other as control with uninoculated normal saline. A drop of plasma is added to each suspension, and the slide is observed for clot formation within a specified time (usually within 10 seconds). If clot formation occurs in the suspension with the test organism but not in the control suspension, the test is positive (+) for coagulase production.

Biochemical Tests for Fungal Species (*Candida albicans*)

Germ Tube Test

A confirmatory test for *Candida albicans* is the Germ Tube Test. This diagnostic procedure is used to distinguish *Candida albicans* from other fungal species. Human serum, approximately 0.5mL, was placed into a small tube. Using a Pasteur pipette, the yeast colony was gently emulsified in the serum, avoiding the use of an inoculum that was too large to prevent the development of the germ tube. The tube was then incubated at 37°C for 2 to 4 hours, and a drop of the serum was transferred to a slide for analysis. Under low and high power objectives, a coverslip was positioned and observed under a microscope.

Chemical Composition of Toothpastes

Table 1 shows the chemical composition of 3 toothpastes that were used and one mouthwash.

Antimicrobial Susceptibility Testing

Antibiotic sensitivity testing is the measurement of the susceptibility of bacteria to antibiotics but in this case, toothpastes were used. The disc agar diffusion method was used. An identified colony was suspended in normal saline and compared its turbidity with a 0.5 McFarland standard, the suspension was used to inoculate the prepared Mueller Hinton agar (made by HiMedia Laboratories Pvt. Ltd., India, Mumbai) plates using a sterile cotton swab, these plates were left for 15 minutes, the discs were aseptically put to the culture media using a sterile forceps, after a few moments the plates were inverted and incubated at 37oc for 18-24 hours, zone of inhibition were measured using a ruler in millimeters.

Preparation of Disks and Dilution of Toothpaste

Disks of 6mm were bored using a disc borer. The disks were then wrapped in foil paper and sterilized in a hot air oven at 121°C for 15 minutes. Later, they were soaked in different hand sanitizers for a period of one hour to ensure full saturation with the toothpaste. Subsequently, the disks were aseptically removed from the toothpaste solution and allowed to dry in an oven at 25°C. They were then packed into sterile bottles, corked, and stored in the refrigerator for future use in susceptibility testing. The toothpastes were diluted to ratios of 1:1, 1:2, 1:4, and 1:8, where 1 gram of toothpaste corresponded to 1000 microliters of distilled water. Dilution of the toothpaste helps identify the minimum inhibitory concentration, which is the lowest concentration of the toothpaste that stops the growth of bacteria, and is expressed in millimeters.

Statistical Analysis

SPSS (version 24.0 (IBM Corporation, Armonk, NY, USA) was used for data analysis. Two-way ANOVA was used to test for mean difference of inhibitory effects of toothpastes to the isolated microorganisms. The results were presented in tables and figure and tables.

RESULTS

Oral microbial profiles isolated from dental decay patients

Figure 1 shows identified microorganisms where *Streptococcus mutans* 15(29.4%) was the most predominant, followed by *Lactobacilli spp* 12 (23.5%), *Pseudomonas aeruginosa* 8 (15.7%), *Staphylococcus aureus* 7(13.7%), *Candida albicans* 6 (11.8%), and *Streptococcus pyogenes* 3 (5.9%)

Antimicrobial Activity of Toothpaste Against Isolated Microorganisms

Figures 2 shows the effect of different brands of toothpaste against isolated microorganisms. *Pseudomonas aeruginosa* was sensitive to sonatec (25mm) and white dent (18mm). The bacterium was resistant to both Colgate and ABC dent. *Streptococcus pyogenes* were sensitive to white dent (12mm) and sonatec (9mm) but was resistant to Colgate and ABC dent. *Staphylococcus aureus* were sensitive to white dent (15mm), ABC dent (10mm), and sonatec (7mm) but it was resistant to Colgate. *Streptococcus mutans* was sensitive to all toothpastes used in this study; White dent (30mm) followed sonatec (25mm), Colgate (20mn), and ABC dent (15mm). *Lactobacilli spp* were sensitive to three toothpastes, white dent (30mm) was the most effective followed by sonatec (15mm), and ABC dent (9mm). However, *Lactobacilli spp* was resistant to Colgate**.** *Candida albicans* was sensitive to white dent (39mm) and Sonatec (10mm). It was resistant to Colgate and ABC dent.

Figure 2. Antimicrobial Activity of Toothpaste Against Isolated Microorganisms

Mean Difference of Inhibitory Effects of Toothpastes on Microorganisms.

Table 2 shows the mean difference of inhibitory effect of toothpastes on isolated microorganisms. The mean difference was statistically significant {$p=.0004$, $F_{tabulated}$ (2.90059) < F_{crit} (2.901295)}. The average mean values of toothpaste inhibitory effects (mm) were 15.166 for SONATEC, 5.666 for ABC dent, 24 for White dent, and 3.333 for Colgate.

TABLE 1: Chemical Composition of Investigated Toothpastes and Mouthwash

Toothpaste	Composition
COLGATE	calcium carbonate, aqua, sorbitol, sodium lauryl sulphate, sodium monofluorophosphate, aroma, cellulose gum, sodium bicarbonate, tetrasodium pyrophosphate, benzyl alcohol, saccharin, sodium hydroxide, limonene
WHITE DENT	sorbitol, hydrated silica, aqua, polyethylene glycol, sodium lauryl sulphate, cellulose gum, flavor blend of herbal extracts, sodium benzoate, titanium dioxide, sodium saccharin active ingredients: 0.76% sodium monofluorophosphate and 0.1% triclosan
ABC DENT	calcium carbonate, sorbitol, silica, sodium carboxymethyl cellulose, saccharine, sodium lauryl sulphate, flavor, water. active ingredient: 0.8% sodium monofluorophosphate.
SONATEC	cetylpyridinium chloride 0.05% w/v

FIGURE 1: Frequency and Percentage of Microorganisms Identified from Isolates of Patients' Dental Decay

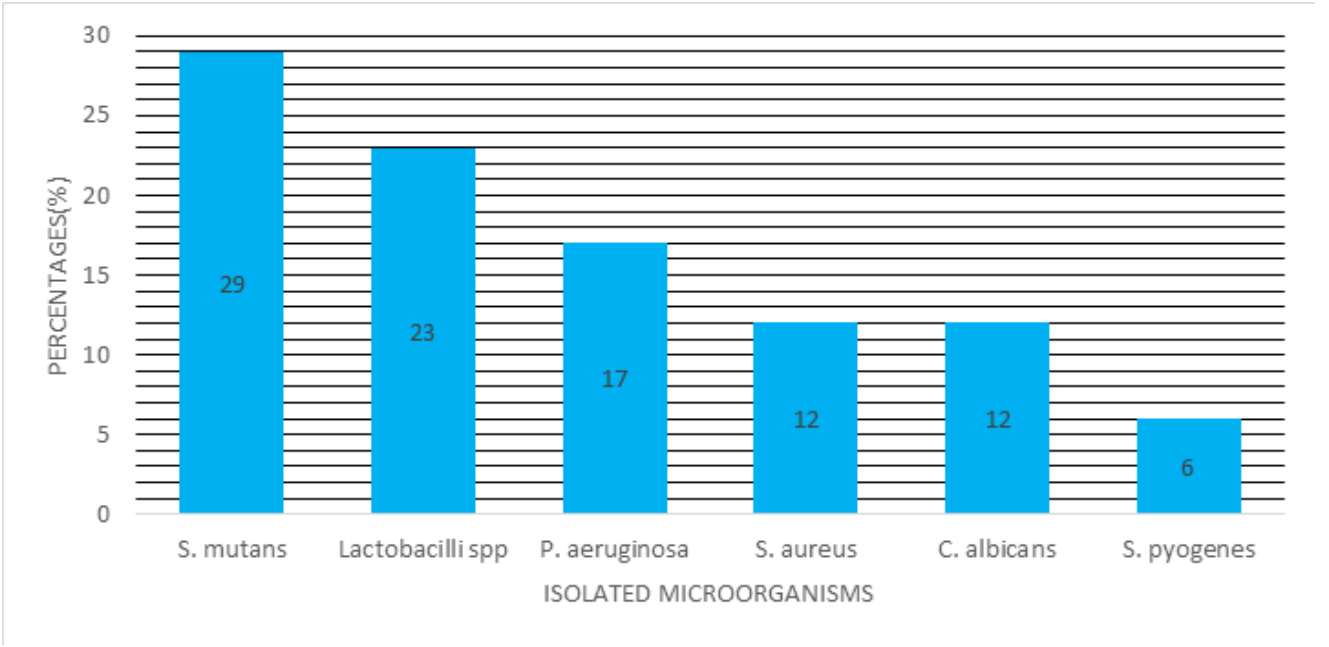


TABLE 2: Toothpastes' Inhibitory Mean Differences on Microorganisms

ANOVA: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
SONATEC	6	91	15.166	64.966
ABC dent	6	34	5.666	42.666
White dent	6	144	24	111.6
Colgate	6	20	3.333	66.666

Continue

TABLE 2: Continued

ANOVA: Two-Factor Without Replication

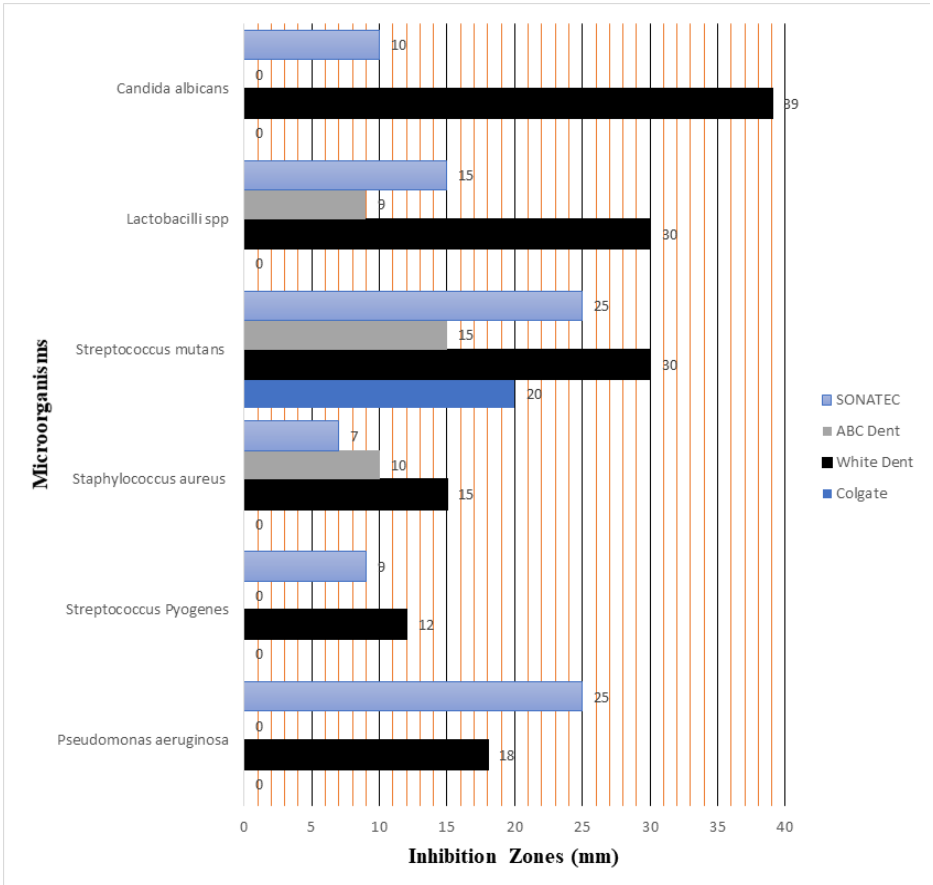
SUMMARY	Count	Sum	Average	Variance
P auregenosa	4	43	10.75	162.25
S.pyogene	4	21	5.25	38.25
S.aurues	4	32	8	39.333
S.mutans	4	90	22.5	41.666
Lactobacillus	4	54	13.5	159
C.albicans	4	49	12.25	340.25

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	1615.458	3	538.486	11.11363	0.0004	3.287
Columns	702.708	5	140.541	2.90059	0.050	2.901
Error	726.791	15	48.452			
Total	3044.958	23				

SS: Sum squares, MS: Mean Squares, df: degree of freedom, F: Fisher, P-value: Probability value

FIGURE 2: Antimicrobial Activity of Toothpaste Against Isolated Microorganisms



DISCUSSON

This study was carried out to determine the inhibitory effect of toothpastes on microorganisms isolated from patients with dental decay. *Streptococcus mutans* and *Lactobacillus* were the most predominant while *Streptococcus pyogenes* was the least isolated (Figure 1). This is in line with the high isolation of *Lactobacilli spp* and *Streptococcus mutans* in the oral cavity among children with dental decay was reported in other study[7] where *Lactobacillus* was implicated in the facilitation of dental decay progression, and *Streptococcus mutans* was reported as the initiator of dental decay.[8] Another study carried out in Ethiopia reported *Streptococcus mutans* as the predominant microorganism among dental decay patients.[9] *Streptococcus mutans* was observed as the main and threat colonizer of the dental surfaces and was reported as persecutor to tooth damage in serious conditions of dental decay.[10] In Korea, *Streptococcus mutans*, *Streptococcus sobrinus, and Lactobacillus spp* were reported as etiological agents of dental decay among children where the proportion of *Streptococcus mutans* and *Streptococcus sobrinus* were significantly correlated with the color of the dental plaque.[11] Our study identified different bacteria associated with dental decay. Despite being the prominent cariogenic bacteria, the absence of *S. mutans* does not mean the absence of dental decay, some other *streptococci* species such as *Streptococcus sobrinus* and other bacteria may cause the disease.[12] *Lactobacilli spp* was the second predominant bacteria observed in the current study. Evidence from other study whch demonstrated the presence of *Lactobacilli spp* in dental decay cases and absence of *Lactobacilli spp* in healthy oral cavity samples, implied that *Lactobacilli spp* play a role in dental decay progression.[13] Recently, *Lactobacilli* was isolated from samples collected from active carious sites presenting a threat with its presence in dental decay conditions.[14] Other studies reported *Lactobacilli* as the cause of some coronal caries at low percentage in world population.[15] *Streptococci mutans* and *Lactobacilli* are creators of acidic environment in the oral cavity risking to different oral diseases including dental decay and gingivitis.[10] The antimicrobial activity of selected toothpastes was performed to test for their inhibitory effects on the isolated bacteria. *Pseudomonas aeruginosa*, *streptococcus Pyogenes*, and *Candida albicans* were sensitive to white dent and SONATEC and resistant to Colgate and ABC dent (Figure 2). The findings of the study carried out to evaluate the antimicrobial effects of commercial toothpastes on isolated bacteria reported the resistance of *Pseudomonas aeruginosa* to all selected toothpastes in Brazil.[16] It was also reported that *Pseudomonas aeruginosa* showed the least sensitivity to commonly used toothpastes, and with its resistance capacity, it may cause chronic periodontal infections.[17] In Vitro study on antimicrobial efficacy of available herbal dentifrices against specific oral microflora showed the sensitivity of *streptococcus Pyogenes* to all selected herbal dentifrices.[18] *Candida albicans* was reported as a cause of root caries among dental decay patients.[19] The inhibitory effects of toothpastes on *Candida albicans* was reported based on the ingredients containing in the toothpaste, where Paradontax toothpaste showed the significant effect *Candida albicans*.[20] In our study *Staphylococcus aureus and Lactobacilli spp* were sensitive to white dent, ABC dent, and SONATEC but resistant to Colgate. In Nepal, all selected toothpastes showed the effectiveness to inhibit the growth *Staphylococcus aureus* isolated from oral cavity.[21] It was also observed that different types of tooth pastes inhibited the growth of Lactobacilli where 5 types of toothpastes were effective and one types did not show effect to this bacterium.[22] *Streptococcus mutans* which is the initiator of dental decay was sensitive to all selected toothpastes in our study. The fact that the selected toothpastes inhibited the growth of *streptococcus mutans* will reduce the chance of dental decay initiation among people with habit of using toothpastes for their dental hygiene. Another study reported the inhibitory effects of different types of toothpastes (*Mentha spicata*, *Curcuma longa*-TH1, *Sunthi*, *Allium sativum*, *Pudinasatva*, *Piper nigrum*-TH2, Triclosan-TR3, Sodium lauryl sulfate (TR4) etc.) on microorganims such as *P. aeruginosa*, *S.aureus*, *C.albicans*, *Lactobacillus spp* where all toothpastes showed the inhibitory effects at different levels and depending on the toothpaste and microorganisms type.[24] Dental plaque is a sticky film of bacteria that forms at the walls of the teeth, they produce an acid which destroys the teeth and cause serious condition of dental decay,[25] the application of antiplaque herbal toothpastes showed the significant reduction of dental plaque scores after brushing, an evidence of the effectiveness of these toothpastes to prevent dental decay associated pathogens.[26] Antimicrobial activity of toothpastes was associated with bioactive compounds containing toothpastes, the inhibitory effect of Fluoridated toothpastes on *Streptococcus mutans* remained effective at all concentrations compared to herbal made toothpastes.[27] Colgate was resisted by all bacteria isolated except *Streptococcus mutans* in our study, contrary, antimicrobial effect of herbal dentifrices showed the significant effectiveness of herbal made Colgate for both *Streptococcus mutans* and *Lactobacilli*.[28] The antimicrobial activity of SONATEC mouthwash is an alcohol which is used to maintain oral hygiene and prevent pathogen microorganisms in oral cavity.[29] In our study, SONATEC and white dent were effective to all isolated microorganisms, a study carried on antimicrobial activity of common mouthwash solution showed that only mouthwash solutions containing chlorohexidine or cetylpyridinum were effective to majority of pathogen of interest.[29] Our study tested the inhibitory effect difference of the used toothpastes on isolated bacteria, the mean difference (p=0.0004) was statistically significant (Table 2). A study on antimicrobial efficacy of different toothpastes and mouth rinses showed the high antimicrobial activity of toothpastes on *Escherichia coli* compared to *Streptococcus mutans* and *Candida albicans*, for mouth rinses, at the same concentration, the maximum inhibition zone was observed to *Escherichia coli* compared to other microorganisms, while at different concentration *Streptococcus mutans* showed the significant antimicrobial activity.[30]

Limitations

We were limited in determining the level of sensitivity of each toothpaste due to the absence of guidelines similar to those for antibiotics. We were unable to determine why some toothpastes are sensitive while others are not. The study was conducted at one hospital; thus, we cannot generalise the findings nationwide.

CONCLUSION

The study evaluated the antimicrobial properties of toothpastes and mouthwash on microorganisms isolated from patients with dental decay at Ruhengeri Referral Hospital in Rwanda. *Streptococcus mutans* and *Lactobacilli spp* were the most common bacteria. White dent and SONATEC were effective in inhibiting growth of microorganisms, but Colgate and ABC dent were less effective. The study highlights the importance of choosing toothpastes with proven antimicrobial activity to effectively combat oral pathogens and maintain oral health. Further research is needed to understand the mechanisms behind these effects.

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Peer Reviewed

Competing Interests: None declared.

Funding: The study did not receive any funding.

Received: 04 April 2024; **Accepted:** 17 March 2024

Cite this article as Joseph M, Guillain C, Ndabikunze F, Yadufashije C. Toothpastes Inhibitory Effects on Microorganisms Isolated from Dental Decay of Patients Attending Ruhengeri Referral Hospital, Musanze, Rwanda. *East Afr Science J.* 2024: 6(1): 117-125. https://doi.org/10.24248/easci.v6i1.103