DETERMINATION OF THE ANTIBACTERIAL ACTIVITY OF DIFFERENT TOOTHPASTES AGAINST PATHOGENIC BACTERIA ISOLATED FROM TOOTH GUMS

Sidra Farooq[1], Muatter Majid[2], Irshad Ullah[3], Arsalan Nadir[4], Amjad Khan[5], Rida Zainab Kayani[6], Faheem Ullah[7]

Correspondence

[1]Sidra Farooq, Assistant Professor, Abasyn University, Peshawar
: +92-343-9043858
: sidra.farooq@abasyn.edu.pk
[2]Student, Abasyn University
[3]Student, Abasyn University
[4]Student, Abasyn University
[5]Lecturer, Abasyn University
[6]Lecturer, Abasyn University
[7]Student, Abasyn University

How to cite this article

Farooq S, Majid M, Ullah I, Nadir A, Khan A, Kayani RZ, et al. Determination of the Antibacterial Activity of different Toothpastes against Pathogenic Bacteria Isolated from Tooth Gums. J Wazir Muhammad Inst Paramed Tech. 2023;3(1): 6-10

ABSTRACT

OBJECTIVES

This study aimed to determine the anti-bacterial activity of different toothpastes against the pathogenic bacteria isolated from gums.

METHODOLOGY

For this study, 100 gum samples were collected from dental clinics in Peshawar using sterile disposal swabs. The samples were transported to Peshawar's Abasyn University and streaked on Nutrient Agar plates. The obtained cultures were sub-cultured and processed for further identification by Gram staining and biochemical tests.

RESULTS

It was found that, out of a total of 100 samples, 60 were positive (40 males and 20 females), and 40 were negative. Among 60 samples, 9 species were identified, and both female and male samples showed a high prevalence of S. aureus. After the analysis of all 5 toothpastes' anti-bacterial activities, it was observed that all toothpastes showed activity against bacteria except toothpaste number 4. Those toothpastes that showed maximum activity were enlisted: TP-1 [Cellobiosococcus spp. (16.6 ± 0.57)], TP-2 [M. kristnae (16.6 ± 0.57)], TP-3 [M. mucilaginous (15.5 ± 0.86)], and TP-5 [K. pneumoniae (15.5 ± 0.86)].

CONCLUSION

The outcome of the studies concluded that the prevalence of bacteria isolated from male samples was higher and more pathogenic than in female samples.

KEYWORDS: *S. aureus, M. Kristnae, Cellobiosococcus Spp, K. Pneumoniae, Antibacterial Activity*

INTRODUCTION

Any natural habitat has microorganisms, as they coexist closely with both people and other living things. The microbial community contains bacteria, phages, viruses, fungus, and protozoans among its residents. Microscopic organisms have a wide range of functions throughout the ecosystem and are important to growing crops.[1] Bacteria may produce a vast variety of organic substances, have diversified genetics, and form symbiotic relationships with a variety of sea creatures. As a result, research on positive relationships among microorganisms and their hosts is gaining traction.[2] Numerous bacterial groups were identified and found to be effective in promoting crops. An important component of the typical micro-biome that colonies the epidermis and other mucous membranes of the individual's body is made up of anaerobes.[3] In addition to having enzymes that can render antimicrobial compounds inactive, genetic changes, metabolic bypasses, and peptides that can assist in shielding the targeted region, bacteria have evolved to become antimicrobial resistant in variety of areas.[4] About 250 strains of bacteria make up the typical human micro-biota. In general, such organisms have benefited humanity in the following ways: i) the micro-flora inhibits bacterial spores from colonizing human tissues and organs; (ii) The other way that the micro-flora can benefit people is by warding off infections by producing secondary.[5] Microbes that take advantage of opportunities might serve as a sign of good oral health. Many other non-commensal bacteria populate and thrive on advanced dental plaque, which is a type of glucan-based coating on the teeth. As a result, it is well recognized that dental plaque serves as a reservoir for diseases that prey on vulnerable individuals. Oral care can remove dental plaque.[6] Infancy is toothless, but as time passes, permanent teeth appear. This phase involves the formation of the permanent tooth structure, which also alters the microbiota in the mouth.[7] Neisseria, Strep, Actinomyces, Prevotella, and Veillonella are gram-positive and gram-negative obligatory and facultative anaerobic organisms found on the basal of the teeth.[8] The most noticeable residents of the mouth were bacteria. The enamel of the tooth is first colonized by Neisseria and Streptococcus species. Long-term brushing usage encourages infection by numerous bacteria, including Lactobacillus species, Streptococcus, and Staph. These microorganisms are thought to be the cause of certain illnesses that impair

either oral or overall health.[9] A high incidence of mutans streptococci (MS) infections, where it often approaches 30% of the cultivable plaque-biofilm microbiota, characterizes ECC, a severe kind of tooth decay. Streptococcus mutans and, less commonly, Streptococcus sobrinus are the two main varieties of MS that are typically detected in people's wounds.[10] Toothpaste reduces stinky breath by removing stains brought on by beverage pigment. An antibiotic ingredient in toothpaste called triclosan serves to stop dental diseases. In managing oral disorders, its antibacterial and anti-inflammatory effects are advantageous.[11] The current study aimed to evaluate the antibacterial activity of different toothpastes against pathogenic bacteria isolated from the gums.

METHODOLOGY

One hundred samples of gum were taken from patients (male and female) at various Peshawar dental clinics using sterile disposal swabs. The labeled samples were immediately transported to Abasyn University Peshawar's microbiology lab and streaked on nutrient agar. The plates were then incubated for 24 hours at 37 °C. The first step after sub-culturing was the identification of bacteria. A microscopic examination is required to characterize a bacterium's phenotype. Depending on the structure of their cell membranes, bacteria are distinguished throughout the staining procedure. Gram-negative colonies tint red to pink and contain a thin peptidoglycan layer, while staphylococci shade blue to purple because of their thick peptidoglycan coating.[12] Biochemical Test: Depending on the differences in the biochemical characteristics displayed by several strains of bacteria, biochemical tests were used to determine the microorganisms. The following is a list of numerous biochemical experiments employed for Staphylococci and gram-negative bacterial detection.[13] Catalase Test: The presence of microbes that generate catalase was analysed using catalase testing. The hydrogen peroxide was neutralised by catalase, generated by facultative anaerobes and obligatory aerobes, and bubbling appeared. As a result, they signified a successful test.[14] Coagulase Test: This test was performed to determine whether bacteria could produce the coagulase enzyme. The enzymes will cause the blood fluid to clot.[15] Urease Test: The urease test identified bacteria that can produce the urease enzyme. The enzyme hydrolyses urea into NH3 and CO2.[13] Oxidase Test: An oxidase test was performed to identify bacteria with the capacity to synthesise the oxidase enzymes. The electron donor would be oxidised by oxidase, resulting in a deep purple colour.[16] Indole Test: Bacteria having the ability to produce tryptophanase were determined by the indole test. The enzymatic reaction produced indole gas, verified by Kovac's reagents.[17] Triple Sugar Iron Test: The TSI test was used to distinguish Enterbacteriacea members based on differences in carbohydrate fermentation patterns and hydrogen sulfide production.[18]

The antimicrobial effect of different toothpastes was assessed using Muller-Hinton agar. The extract was introduced into the wells made by a borer of 6 mm diameter, which already had bacteria. The plates were then incubated for 16 hours at 37°C. The results were analysed, and the zone of inhibition was measured in mm. Distilled water was used as negative controls, and Ciprofloxacin (5µg) was used as a positive control. All the tests were performed in triplicate.[19]

RESULTS

Out of 100 samples, 60 were positive (40 were male and 20 were female), while 40 were negative samples, as shown in figures 1 and 2. A total of nine species were isolated after the biochemical testing: Staphylococcus aureus, Staphylococcus xylosus, Staphylococcus warneri, Streptococcus salivarius, Streptococcus thermophiles, Micrococcus mucilaginous, Micrococcus kristnae, Klebsiella pneumoniae, and Cellobiosococcus spp., as mentioned in Table 1. The overall prevalence of these species, as well as from male and female samples were summarised in table 2.

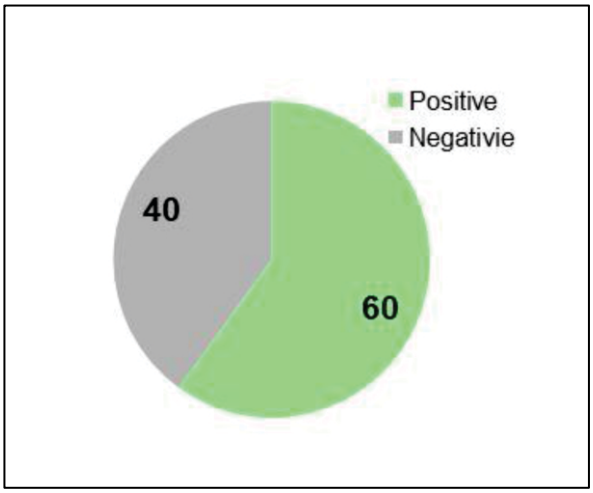

Figure 1: Overall Prevalence of Collected Samples, in Which 60 Were Positive and 40 Were Negative.

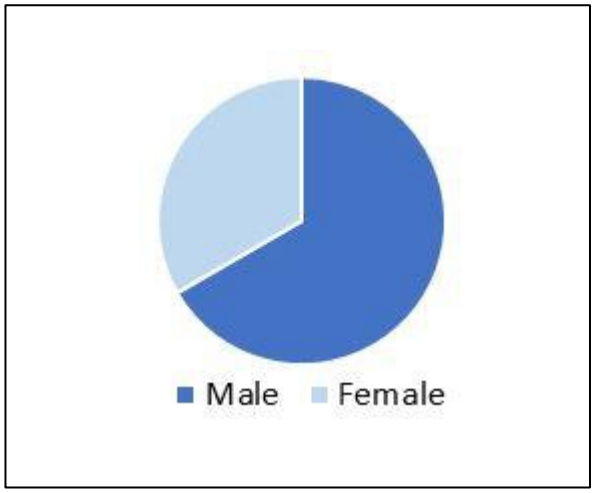

Figure 2: Prevalence of Positive samples out of 100 samples, 40 were Male, and 20 were Female.

Antibacterial Activity of Different Toothpastes:

Agar well diffusion was performed to determine the antibacterial activity of different brands of toothpastes against isolated species. Tooth paste1 showed good activity against Cellobiosococcus spp (16.6 ± 0.57), S. aureus (15.3 ± 0.57), S. warneri (15.1 ± 0.76), M. kristnae (14.8 ± 0.7), K. pneumoniae (14.83 ± 0.76), S. salivarius (11.3 ± 0.57), M. mucilaginous (11.3 ± 0.57), S. xylosus (10.8 ± 0.28), and S. thermophiles (10.7 ± 0.46). Tooth paste 2 showed good activity against M. kristnae (16.6 ± 0.57), Cellobiosococcus spp (15.5 ± 0.86), S. salivarius (15.3 ± 0.57), K.pneumoniae (15.1 ± 0.76), S. aureus (14.83 ± 0.76), S. xylosus (14.8 ± 0.7), S. warneri (11.3 ± 0.57), S. thermophiles (10.8 ± 0.28), and M. mucilaginous (10.7 ± 0.46). Tooth paste 3 showed good activity against M. mucilaginous (15.5 ± 0.86), S. xylosus (15.3 ± 0.57), K. pneumoniae (15.3 ± 0.57), S. aureus (15.1 ± 0.76), M.kristnae (14.83 ± 0.76), Cellobiosococcus spp (14.8 ± 0.7), S.thermophiles (12.6 ± 0.57), S.salivarius (10.8 ± 0.28) and S. warneri (10.7 ± 0.46). Tooth paste 4 showed no antibacterial activity. Tooth paste 5 showed good activity against K. pneumoniae (15.5 ± 0.86), S. thermophiles (15.1 ± 0.76), M. mucilaginous (14.8 ± 0.7), Cellobiosococcus spp (14.83 ± 0.76), S. xylosus (13.3 ± 0.57), M. kristnae (13.3 ± 0.57), S. salivarius (12.6 ± 0.57), S. warneri (10.8 ± 0.28), and S. aureus (10.7 ± 0.46). Ciprofloxacin antibiotic used as a positive control against isolates M.mucilaginosus (26mm) Cellobiosococcus spp (25mm), S.aureus (25mm), S.thermophilus (25mm), M.kristinae (25mm), S.salivarius (24mm), K.pneumoniae (24mm), S.warneri (22mm), and S.xylosus (20mm). Distilled water was used as a negative control that showed no zone of inhibition, summerized in table 3.

Table 1: Identification of Bacteria Based on Biochemical Tests

Isolates	Gram Staining	Catalase	Coagulase	Oxidase	Citrate	Indole	Urease	TSI	H_2S Gas	Gas
S.aureus	+	+	+	-	+	-	+	A/A	-	-
S.xylosus	+	+	-	-	-	-	+	A/A	-	+
S.warneri	+	+	-	-	-	-	+	-	-	-
S.salivarus	+	-	+	-	-	-	+	A/A	-	-
S.thermophilus	+	-	+	-	-	-	+	-	-	-
M.mucilaginosus	+	-	+	+	-	-	+	A/A	-	-
M.kristinae	+	+	-	+	+	-	+	K/A	-	-
K.pneumoniae	-	+	-	-	+	-	+	A/A	-	+
Cellobiosococcus spp	+	+	-	+	-	-	+	K/A	-	-

Table 2: Overall Prevalence of Bacteria Isolated from the Male and Female Samples

Isolates	Overall prevalence of bacteria isolated from 60 samples	Prevalence of Bacteria Isolated from Male Samples	Prevalence of Bacteria Isolated from Female Samples
S. aureus	43%	40%	60%
M. mucilaginosus	20%	20%	20%
Cellobiosococcus spp	17%	20%	05%
S. xylosus	04%	05%	-
S. warneri	04%	05%	-
S. thermophilus	03%	-	05%
S. salivarus	03%	05%	-
M. kristinae	03%	-	10%
K. pneumonia	04%	05%	-

Table 3: Antibacterial Activity of Different Toothpastes

Isolates	Zone Diameter Interpretive Criteria (nearest whole mm)						
	Positive Control	Negative Control (Distilled Water)	TP-1	TP-2	TP-3	TP-4	TP-5
S. aureus	25mm	0	15.3 ± 0.57	14.83 ± 0.76	15.1 ± 0.76	0	10.7± 0.46
S. xylosus	20mm	0	10.8 ± 0.28	14.8 ± 0.7	15.3 ± 0.57	0	13.3± 0.57
S. warneri	22mm	0	15.1 ± 0.76	11.3 ± 0.57	10.7 ± 0.46	0	10.8± 0.28
S. salivarius	24mm	0	11.3 ± 0.57	15.3 ± 0.57	10.8 ± 0.28	0	12.6± 0.57
S. thermophiles	25mm	0	10.7 ± 0.46	10.8 ± 0.28	12.6 ± 0.57	0	15.1± 0.76
M. mucilaginous	26mm	0	11.3 ± 0.57	10.7 ± 0.46	15.5 ± 0.86	0	14.8 ± 0.7
M. kristnae	25mm	0	14.8 ± 0.7	16.6 ± 0.57	14.83 ± 0.76	0	13.3± 0.57
Cellobiosococcus spp	25mm	0	16.6 ± 0.57	15.5 ± 0.86	14.8 ± 0.7	0	14.83±0.76
K. pneumoniae	24mm	0	14.83 ± 0.76	15.1 ± 0.76	15.3 ± 0.57	0	15.5±0.86

DISCUSSION

Every natural habitat contains microorganisms, and they interact closely with both people and other living things. Such species contain bacteria, archaea, viruses, fungi, and protists among their members.[1] Microbes, which represent one of the most common serious diseases worldwide, are the source of the communicable illnesses known as oral diseases. They result from bacterial damage to dental tissues. So if ignored, they may be extremely painful and disturbing. A wide variety of oral bacteria have been discovered in the mouth, including Lactobacilli and Streptococcus mutants, which are thought to be the cause of oral diseases. Other possible causes for tooth decay include not brushing their teeth on a routine basis, smoking, and poor oral hygiene. Diseases are mostly caused by "innate micro-flora," which is a microbial plaque composed of bacteria that is formed on the tooth surfaces.[20] Toothpaste reduces foul odors and eliminates stains brought on by beverage pigment.[11] This study was conducted at Abasyn University Peshawar to find out the antibacterial activity of different toothpastes against the pathogenic bacteria isolated from gums. The medical record data and 100 oral caries samples from patients who visited different dental doctors in the Peshawar region had been gathered by researchers using sterilized pincers.[20] In a recent study, 100 dental caries samples were collected from different dental clinics in Peshawar using sterile disposal swabs. The labeled samples were transferred to the Microbiology Laboratory of Abasyn University for further processing. Nine species were isolated and summarized in Table 1, where the prevalence of bacteria was also shown in the pie chart (Figures 1 and 2) and Table 2. Muller-Hinton agar media were used for the evaluation of the antibacterial activity of different toothpastes. The toothpaste extract was introduced into pre-existing bacteria-infested borer wells of 6 mm diameter. The plates were then incubated for 16 hours at 37°C. The results were analyzed, and the zone of inhibition was measured in mm. All the tests were performed in triplicate. It was discovered that TP-1 toothpaste had the strongest effect on Yersinia species. However, herbal toothpaste such as Dabur Red, Babool, and Himalaya is efficient and produces noticeable benefits.[20] In a recent study, isolated species were S. aureus (43%), S. xylosus (4%), S. warneri (4%), S. salivarius (3%), S. thermophiles (3%), M. mucilaginous (20%), M. kristnae (3%), Cellobiosococcus spp. (17%), and K. pneumonia (3%). It was observed that all toothpastes showed antibacterial activity against isolated bacteria, except TP-4. The maximum number of activities shown by toothpastes were enlisted: TP-1 against Cellobiosococcus spp., TP-2 against M. kristnae, TP-3 against mucilaginous, and TP-5 against K. pneumoniae. Ciprofloxacin was used as a positive control and showed the greatest activity against M. mucilaginous isolates (26 mm) and the least activity against S. xylosus (20 mm). Distilled water was used as a negative control that showed no zone of inhibition.

LIMITATIONS

In this study, further samples from other sites (nasal passage) were not collected. Other solvents can be used for negative controls. Other tooth pastes can be used to test antibacterial activity. As a result, other researchers are advised to use different toothpastes and different solvents with different concentrations and also categorize groups according to their ages.

CONCLUSIONS

According to the current study's findings, it was concluded that male' samples had the highest concentration of harmful bacteria, such as M. mucilaginosus and Cellobiosococcus spp 20% (due to poor oral hygiene), compared to female' samples, which has 60% more S. aureus. It was observed that all toothpastes show maximum activity against bacteria except Toothpaste number 4.

CONFLICT OF INTEREST: None

FUNDING SOURCES: None

REFERENCES

1. Findley K, Grice EA. The skin microbiome: a focus on pathogens and their association with skin disease. Plos pathogens. 2014 Nov 13;10(11):e1004436.
2. Ritchie KB, Schwarz M, Mueller J, Lapacek VA, Merselis D, Walsh CJ, Luer CA. Survey of antibiotic-producing bacteria associated with the epidermal mucus layers of rays and skates. Frontiers in microbiology. 2017 Jul 5;8:1050.
3. Shenoy PA, Vishwanath S, Gawda A, Shetty S, Anegundi R, Varma M, Mukhopadhyay C, Chawla K. Anaerobic bacteria in clinical specimens–frequent, but a neglected lot: a five year experience at a tertiary care hospital. Journal of clinical and diagnostic research: JCDR. 2017 Jul;11(7):DC44.
4. Temple A, Riley H, Ramachandran A, Hoff W. Pan Genomes of Pathogenic Bacteria. Research Reports from Life Science Freshmen Research Scholars. 2017 Oct 4;3(1).
5. Orji FA, Ugbogu OC, Ugbogu EA, Barbabosa-Pliego A, Monroy JC, Elghandour MM, Salem AZ. Pathogenic flora composition and overview of the trends used for bacterial pathogenicity identifications. Microbial pathogenesis. 2018 Aug 1;121:139-46.
6. Shahcheraghi SH, Ayatollahi J, Lotfi M, Bafghi AF, Khaleghinejad SH. Application of nano drugs in treatment of leishmaniasis. Glob J Infect Dis Clin Res 2 (1): 018-020.
7. Li F, Tao D, Feng X, Wong MC, Lu H. Establishment and development of oral microflora in 12–24 month-old toddlers monitored by high-throughput sequencing. Frontiers in cellular and infection microbiology. 2018 Dec 4;8:422.
8. Chawla R, Shetty K, Prakash A, Rathore A, Saroch SJ. Orthodontics and oral microflora: synergism or parasitism. J Interdiscipl Med Dent Sci. 2018;6(234):2-5.
9. Okafor AC, Igwesi SN, David EE, Okolo VK, Agu K. Presence of bacteria with pathogenic potential among already-used toothbrushes from university students. American Journal of Life Science Researches. 2016 Jul 24;4(3).
10. Hajishengallis E, Parsaei Y, Klein MI, Koo H. Advances in the microbial etiology and pathogenesis of early childhood caries. Molecular oral microbiology. 2017 Feb;32(1):24-34.
11. Bhattacharjee S, Nath S, Bhattacharjee P, Chouhan M, Deb B. Efficacy of toothpastes on bacteria isolated from oral cavity. International Journal of Medicine and Public Health. 2018;8(2).
12. Froböse NJ, Bjedov S, Schuler F, Kahl BC, Kampmeier S, Schaumburg F. Gram staining: a comparison of two automated systems and manual staining. Journal of Clinical Microbiology. 2020 Nov 18;58(12):e01914-20.
13. Shoaib M, Muzammil I, Hammad M, Bhutta ZA, Yaseen I. A mini-review on commonly used biochemical tests for identification of bacteria. A Mini-Review on Commonly used Biochemical Tests for Identification of Bacteria. 2020 Jun 14;54(1):8-.
14. Christensen JJ, Ruoff KL. Aerococcus, Abiotrophia, and other aerobic catalase-negative, Gram-positive cocci. Manual of clinical microbiology. 2015 May 15:422-36.
15. Rajan BM, Kannabiran K. Extraction and identification of antibacterial secondary metabolites from marine Streptomyces sp. VITBRK2. International journal of molecular and cellular medicine. 2014;3(3):130.
16. Kavitha K, Ragunathan L, Elantheriyan P, Gopalakrishnan K, Gopala K, Balamurugan I, Navya R, Marcella S, Venkatachalam G. The burden of mycobacteria species among children from postvaccination abscess in Southern India. International Journal of Mycobacteriology. 2021 Oct 1;10(4):358-.
17. Albadri AT. Characterization and Molecular study to detect Multidrug Resistance Bacteria Isolated from Patients with Diabetic Foot Ulcers in Wasit Province (Doctoral dissertation, Ministry of Higher Education) (Doctoral dissertation, M. sc Thesis, Wasit University, Wasit, Iraq).
18. Kafilzadeh F, Dehdari F. Amylase activity of aquatic actinomycetes isolated from the sediments of mangrove forests in south of Iran. The Egyptian Journal of Aquatic Research. 2015 Jan 1;41(2):197-201.
19. Bouyahya A, Abrini J, El-Baabou A, Bakri Y, Dakka N. Determination of phenol content and antibacterial activity of five medicinal plants ethanolic extracts from North-West of Morocco. J Plant Pathol Microbiol. 2016;7(342):2.
20. Bibi K, Rehman A, Fatima A, Ali G, Ishaq MS, Samad A, Asim M, Ullah S, Siddiqui MF. Assessment of antibiotics and fluoride toothpastes against pathogenic bacteria associated with dental caries. Abasyn Journal of Life Sciences. 2018;1(1):1-0.

CONTRIBUTORS

1. ***Sidra Farooq*** **-** *Concept & Design; Supervision; Final Approval*
2. ***Muatter Majid*** **-** *Data Acquisition; Data Analysis/Interpretation; Drafting Manuscript*
3. ***Irshad Ullah*** **-** *Data Acquisition; Drafting Manuscript*
4. ***Arsalan Nadir*** **-** *Drafting Manuscript*
5. ***Amjad Khan*** **-** *Critical Revision; Final Approval*
6. ***Rida-E-Zainab Kayani*** **-** *Final Approval;*
7. ***Faheem Ullah*** **-** *Critical Revision*

LICENSE: JGMDS publishes its articles under a Creative Commons Attribution Non-Commercial Share-Alike license (**CC-BY-NC-SA 4.0**).
COPYRIGHTS: Authors retain the rights without any restrictions to freely download, print, share and disseminate the article for any lawful purpose.
It includes scholarlynetworks such as Research Gate, Google Scholar, LinkedIn, Academia.edu, Twitter, and other academic or professional networking sites.