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Comparative Evaluation of the Antimicrobial Efficacy of Probiotic, Herbal, Homeopathic and Conventional Toothpastes Against Streptococcus mutans

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Abstract

Aim: To find out the relationship between severe early childhood caries (s-ECC) and salivary counts of mutans streptococci (MS) and lactobacillus (LB) in preschool children of low socioeconomic status.

Materials and Methods: A cross-sectional study was conducted among 50 children aged 2–5 years selected from five Anganwadi centers in Bengaluru city. Twenty-five children diagnosed with severe early childhood caries (s-ECC) according to American Academy of Pediatric Dentistry criteria and 25 caries-free children were included. Whole unstimulated saliva samples were collected and cultured for mutans streptococci and lactobacilli. Statistical analysis was performed using Fisher’s exact test, Mann–Whitney test, and Spearman’s correlation coefficient.

Results: Twenty-one of 25 children with s-ECC were positive for mutans streptococci and 22 were positive for lactobacilli. Children with s-ECC showed significantly higher mean counts of mutans streptococci (12.2 vs. 4.16) and lactobacilli (8.4 vs. 3.8) compared with caries-free children ($P < 0.01$). A significant positive correlation was observed between caries experience and salivary levels of both microorganisms.

Conclusion: Severe early childhood caries is positively associated with increased salivary levels of mutans streptococci and lactobacilli in preschool children of low socioeconomic status. Early preventive and educational interventions are essential to reduce caries risk in this vulnerable population.

Keywords: Severe Early Childhood Caries (s-ECC), Mutans Streptococci, Lactobacilli, Salivary Micro flora, Preschool Children, Dental Caries, Low Socioeconomic Status, Caries Risk Assessment, Salivary Bacteria

Introduction

The oral cavity is a complex ecosystem characterized by warm and moist micro environments that provide favourable conditions for the growth and interaction of diverse microorganisms. Dental caries is one of the most prevalent oral diseases and remains an important global public health concern. [1] Dental caries results from a complex interaction between the host, diet, and microorganisms, with changes in the oral microbial community contributing to the initiation and progression of carious lesions. [1]

Among the microorganisms associated with dental caries, Streptococcus mutans and Streptococcus sobrinus have traditionally been recognized as important cariogenic bacteria. S. mutans possesses several virulence attributes, including adhesion to tooth surfaces, extracellular polysaccharide production, acid production, and acid tolerance, which facilitate biofilm formation and enamel demineralization. [8] Increased levels of S. mutans have also been associated with dental caries experience and severe early childhood caries. [8]

Dental caries is therefore closely associated with the development of cariogenic bio films and the production of acids that promote enamel demineralization. [1] The control of cariogenic microorganisms through effective oral-hygiene measures is an important component of caries prevention.

Toothpaste is one of the most commonly used vehicles for delivering antimicrobial and preventive agents to the oral cavity. Conventional fluoride dentifrices remain an important component of caries - preventive strategies, while herbal dentifrices have also demonstrated antibacterial and antiplaque effects. [2], [5] A randomized clinical trial by Shaalan and El-Rashidy demonstrated an antibacterial effect of Miswak herbal toothpaste compared with fluoride toothpaste in individuals at high risk of dental caries. [2]

In recent years, probiotics have attracted considerable interest in oral healthcare because of their potential to modify the oral microbial environment and contribute to the maintenance of oral health. Probiotic-containing toothpaste has been investigated for its antimicrobial potential, and an in-vitro study by Kataria and Singh evaluated the antifungal effectiveness of a novel probiotic toothpaste. [3] Clinical evidence has also examined probiotic fluoride dentifrices and their microbiological effects, suggesting a potential role for probiotic-containing dentifrices in oral healthcare. [6]

Furthermore, probiotic mouthwashes have been investigated for their ability to reduce dental plaque in both primary and permanent dentitions. [4]

Although different categories of dentifrices are commercially available, comparative information regarding their antimicrobial activity against S. mutans remains limited. Therefore, the present study aimed to compare the antimicrobial efficacy of probiotic, herbal, homeopathic, and conventional toothpastes against Streptococcus mutans using an in-vitro agar well diffusion

Aim

This study aims to compare the efficacy of Homeopathic, Herbal, Conventional and Ayurvedic pro biotic tooth paste on microbial action of Streptococcus mutans.

Methodology

Commercially available toothpastes used in this study a. Probiotic Stim toothpaste b. Meswak toothpaste c. Hekla Lava Dental Cream d. Kidodent toothpaste.

2.2 Microorganisms used for antimicrobial assay Bacterial strain Streptococcus mutans (KBRC008) was used as test organisms for this study.

Bacterial strain was obtained from Kehloor Biosciences and Research Centre (KBRC), Ghumarwin, Bilaspur, Himachal Pradesh and the experiment was also conducted at KBRC under the supervision of Dr. Amit Kumar and Dr. Vikesh Bhatia. 2.3 Antimicrobial assay 2.3.1 Preparation of Inoculum Streptococcus mutans was freshly grown on nutrient agar slant. The bacterial inoculum was prepared by suspending 2- 3 loop full of isolate in 5ml of normal saline tube from freshly prepared slant. The tube was than vortexed for uniform suspension. The turbidity of the suspension was adjusted with 0.5 McFarland standard (1 x 108 cfu/ml) (Annexure- II). inoculum was further used for antibacterial assay.

Fig 1: Pure culture slant of Streptococcus mutans

2.3.2 Agar Well Diffusion Method The evaluation of antimicrobial activity of four toothpastes (A, B, C and D) was performed using agar well diffusion technique described by Sharma and co -workers (2012) with certain modifications. The Muller Hinton agar (MHA) plates were prepared and spread plated with the liquid inoculum of bacterial isolate using sterile cotton swabs (Annexure-III).

The plates were allowed to dry for 30 minutes after which equidistant wells of 6 mm in diameter were punched on MHA plates using a sterile cork borer. Wells of each plate were filled with testing materials (A, B, C and D) separately and allowed to stay for 15 minutes at room temperature for pre -diffusion to take place. The plates were then incubated for 24 hrs at 37°C. After incubation the zones of inhibition (in millimetre) was measured and recorded for analysis. This procedure was performed in replicate of three times for more accuracy and effectiveness.

Armamentarium

Equipment ’s Bacteriological Incubator, Laminar air flow, Auto clave, Weighing balance, Refrigerator Glassware ’s Petri plates, test tubes, conical flasks, measuring cylinders, beakers etc. were of borosilicate and were procured from the local market.

Chemicals and Media Ethanol, H2SO 4, BaCl 2, NaCl, Nutrient Agar, Muller Hinton Agar, Potato Dextrose Agar, Sabouraud dextrose agar (SDA) etc. were of HIMEDIA and NICE. Miscellaneous Inoculating loops (Hi - Media), Non- absorbent cotton and aluminium foils etc. were also procured from the local market.

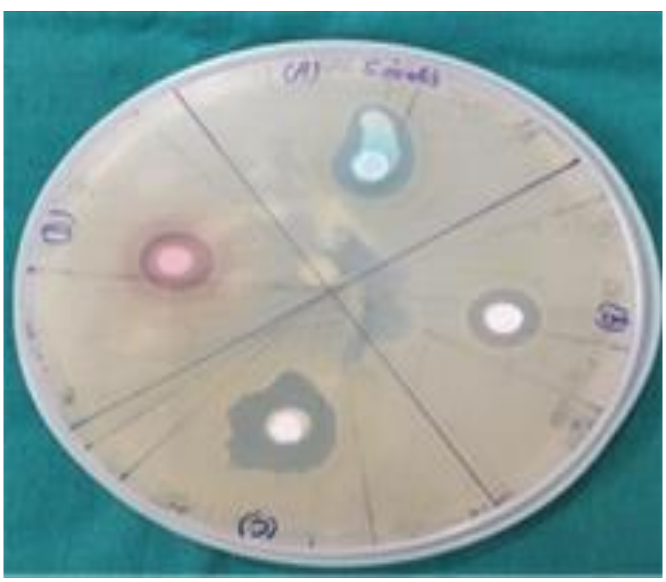
Results

3.1 Antimicrobial assay A total of four toothpastes (A, B, C and D) were evaluated for their antimicrobial potential against Streptococcus mutans. The zones of inhibition around the wells were recorded in millimetre (Fig. 2, 3, 4, 5; Table 2).

Fig 2: Autoclaved Mullar Hinton Agar (MHA) media ready to pour in pet

Fig 3: Petri plates poured with Muller Hinton Agar media in laminar air flow chamber.

Fig 4: Zones of inhibition around the wells representing the antibacterial activity of A. Probiotic toothpaste B. Meswak toothpaste C. Hekla Lava Dental Cream and D. Kidodent paste against Streptococcus mutans.



Annexure - I

Following equipment's, chemicals and glassware were used in the study.

Equipment 's

Bacteriological Incubator, Laminar air flow, Autoclave, weighing balance, Refrigerator.

Glassware 's

Petri plates, test tubes, conical flasks, measuring cylinders, beakers etc. were of borosilicate and were procured from the local market.

Chemicals and Media Ethanol, H 2SO 4, BaCl 2, NaCl, Nutrient Agar, Muller Hinton Agar, Potato Dextrose Agar, Sabouraud dextrose agar (SDA) etc. were of HIMEDIA and NICE.

Miscellaneous Inoculating loops (Hi - Media), Non-absorbent cotton and aluminium foils etc. were also procured from the local market.

Annexure - II

Details of standards used in the study.

Normal saline

Normal saline was prepared by adding 0.85g of NaCl in 100 ml of water.

0.5 McFarland standard

Ingredients	Quantity Barium chloride
Dihydrate	0.05 ml of 1.175%
Sulphuric Acid	9.95ml of 1% McFarland

standard was made by mixing specified amounts of barium chloride and sulphuric acid together. A 0.5 McFarland standard was prepared by mixing 0.05ml of 1.175% barium chloride Dihydrate, with 9.95ml of 1% of sulphuric acid.

Annexure - III

Composition of media used in this study

Nutrient agar (NA)

Ingredients	Quantity
Peptone	5.0g Beef extract

3.0g Sodium chloride 5.0g Agar

15.0g Distilled water 1000 ml

Ph 7.4 - 7.6

Ingredients were heated to dissolve and sterilized in autoclave at 121oC for 15 minutes at 15 lb Pressure. About 15- 20 ml of media was poured into sterile Petri -dishes.

Muller Hinton Agar (MHA)

Ingredients	Quantity
Casein acid hydrolysate	17.5gBeef heart infusion
2.0g Soluble starch	1.50g
Agar	17.0g Distilled water1000 ml
pH	7.3 +-0.2

Ingredients were heated to dissolve and sterilize in autoclave at 121degree C for 15 minutes at 15 lbs. pressure. About 15- 20 ml of media was poured into sterile petri -dishes.

Potato Dextrose Agar

Ingredients	Quantity
Infusion from potatoes	200.0 g
Dextrose (Glucose)	20.0 g
Agar	15.0g
Distilled water	1000 ml
pH after sterilization	5.6±0.2

Ingredients were heated to dissolve and sterilize in autoclave at 121degree C for 15 minutes at 15 lbs. pressure. About 15- 20 ml of media was poured into sterile petri –dish.

Discussion

The present in-vitro study compared the antimicrobial activity of probiotic, herbal, homeopathic, and conventional toothpastes against Streptococcus mutans. The findings demonstrated variation in the antimicrobial activity of the tested dentifrices, with Hekla Lava Dental Cream demonstrating the greatest zone of inhibition, followed by probiotic toothpaste, whereas Meswak and Kidodent demonstrated comparatively smaller zones of inhibition.

The observed antimicrobial activity of the herbal dentifrice is consistent with previous evidence indicating that herbal toothpastes can possess antibacterial and antiplaque properties.[2,5] Shaalan and El-Rashidy reported an antibacterial effect of Miswak herbal toothpaste in a randomized clinical trial involving individuals at high risk of dental caries.[2] Similarly, Al-Kholani demonstrated differences in the clinical efficacy of herbal and conventional dentifrices in the management of established gingivitis.[5]

These findings support the potential role of plant-derived ingredients in commercially available dentifrices as adjunctive antimicrobial agents.

Probiotic-containing dentifrices represent a relatively recent approach to oral healthcare. [3,6] Kataria and Singh investigated the antimicrobial potential of a novel probiotic toothpaste in an in-vitro study, while Kaur et al. evaluated a probiotic fluoride dentifrice using clinical and microbiological parameters.[3,6] The antimicrobial activity observed with the probiotic toothpaste in the present study is therefore consistent with the growing interest in probiotic-based oral-hygiene products. However, the antimicrobial mechanisms and clinical relevance of probiotic dentifrices may vary according to the probiotic strain, formulation, viability of microorganisms, and duration of exposure.

The importance of targeting S. mutans is supported by previous pediatric dental literature. Ramamurthy et al. demonstrated an association between severe early childhood caries and increased salivary levels of mutans streptococci and lactobacilli in preschool children. [8] Therefore, evaluating the antimicrobial activity of dentifrices against S. mutans may provide useful preliminary information regarding their potential to influence cariogenic microbial populations.

The present findings should, however, be interpreted within the limitations of an in-vitro agar well diffusion model. The diameter of the inhibition zone can be influenced by the diffusion characteristics of individual toothpaste ingredients within the agar medium and does not necessarily reproduce the complex conditions of the oral cavity.

Furthermore, antimicrobial activity demonstrated in vitro cannot be directly extrapolated to clinical caries-preventive efficacy. Clinical studies are therefore required to determine whether the differences observed between the tested dentifrices translate into meaningful reductions in cariogenic bacterial levels or caries risk.

Overall, the findings suggest that the tested dentifrices exhibited differing degrees of antimicrobial activity against S. mutans, with Hekla Lava Dental Cream showing the greatest inhibition under the conditions of the present assay. Further in-vivo investigations are required to evaluate the clinical effectiveness, safety, and long-term oral-health benefits of these formulations.

Conclusion

Probiotic toothpastes contain viable microorganisms that confer various health benefits when administered in adequate quantities. Probiotic toothpaste is a relatively new concept and may be useful in the prevention of periodontal diseases. The present study aimed to evaluate the antimicrobial activity of herbal, conventional, Ayurvedic and homeopathic toothpaste, and we got our results with Hekla lava being the most potent toothpaste against streptococcus mutans. Its promising agent kreosotum has phenolic com pounds which is antiseptic and anti-microbial. it also contains tar acids and creosote oils which is helpful in soothing inflamed tissues and healing ulcerated areas. Kreosotum in liquid form can be given for children with delayed teething. Further investigations are required to gain a clearer understanding of its efficacy within the oral environment and to determine whether it can serve as a substitute for conventional toothpastes in oral healthcare. Based on the findings of this study, it can be concluded that both probiotics and fluoride toothpastes are effective in promoting oral health.

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Legend Tables

Table 1: Combinations of testing materials used for antimicrobial assay.

Sn.	Combination of testing materials	Test Code
1.	Probiotic toothpaste with xylitol by STIM	A
2.	Meswak toothpaste by Dabur	B
3.	Hekla Lava Dental Cream by wheezal homoeo pharma	C
4.	Kidodent with Xylitol by Indoco Remedies Ltd.	D

Table 2

Toothpaste	Mean Zone of Inhibition mm)
Hekla Lava Dental Cream	27.33 ± 0.58
Probiotic Toothpaste	22.00 ± 0.00
Meswak Toothpaste	18.33 ± 0.58
Kidodent Paste	18.33 ± 0.58