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Comparative evaluation of the antimicrobial efficacy among various mouthwashes against Streptococcus mutants - An in vitro study

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Abstract

Introduction: Prevention is always better than cure, a famous proverb and which works well in Paediatric dentistry Mouth wash have always been considered as an important anti -plaque chemical agent to reduce oral microbial count. The use of mouthwash has gained popularity in developing countries for the maintenance of oral hygiene

Aim: To comparatively evaluate antibacterial efficacy of various mouthwashes against Streptococcus mutans and their effect on tooth surface.

Material and methods: Agar diffusion method was used to evaluate the antimicrobial action of different mouthwashes. 15 Agar plate with 4 samples in each agar plate are used in this study: Group A: Kid do dent –R mouthwash, Group B: Chlohex Mouthwash, Group III: Listerine Mouthwash, and Group IV: Dionized Water.

The inhibition zones against S. Mutans were recorded and statistically assessed using one-way analysis of variance (ANOVA) test ($P < 0.05$).

Result: Significant antibacterial effect against S.Mutans was observed with Kiddodent –R Mouthwash followed by Chlohex mouthwash, Listerine Mouthwash and Dionized water.

Conclusion: kiddodent –R (CHX-ADS) found to be the best antimicrobial property against S. Mutans
as compared to Chlohex (CHX), Listerine mouthwash and Dionized water.

Introduction

Oral health is an essential part of general health and wellbeing. It is an important factor in an individual's quality of life. Dental caries is one of the most common oral health disease.According to WHO,Dental caries is a microbial multifactorial disease of calcified tissue of teeth, characterised by demineralization of the inorganic content anddissolution of the organic content. According to a study by Hery Mwakayoka (2017), Dental caries in infants and young children is prevalent worldwide, with prevalence ranging from 3.3%[1]. The factors that are currently associated with dental caries in children by inappropriate bottle feeding, frequent exposure to soft, sticky and sugar containing food,low saliva flow rate and high level of bacterial colonization etc.

The mouth contains a wide variety of oral bacteria,but only a few species of bacteria are believed to caused dental caries; streptococcus mutans and lactobacilli. After decades of research it has been established that plaque accumulation is a potential causative agent of dental caries as well as periodontal disease.Plaque has been consistently targeted with interventions with an exponential growth in the field of preventive dentistry. Oral inhabitants such as streptococcus mutans colonize in human dental plaque and play a key role in the initiation and progression of dental diseases such as dental caries[2].

Global antibiotic resistance by bacteria is becoming an amounting public health concern. To prevail over microbial drug resistance, researchers are looking forward for the development of alternatives and novel drugs in pursuit to discover new antimicrobial agent. Mouthwash has always been considered as an important anti -plaque chemical agent to reduce oral microbial count. The use of mouthwash has gained popularity in developing countries for the maintenance of oral hygiene[3].

Various types of mouthwashes such as Povidone Iodine, Alcohols, Benzylkonium chloride.Benzephonium Chloride,Cetylpyridinium Chloride and Chlorhexidine are commercially available. Amongall these Chlorhexidine is widely used nowadays.

Chlorhexidine was first developed in 1940 by Imperial chemical industries in England and marketed in 1954 as antiseptic for skin wounds and as presurgical disinfectant of mouth in dentistry .In 1969 Schroeder investigated plaque inhibition by CHX .Chlorhexidine gluconate is very potent chemoprophylactic agent,it has a broad spectrum action especially against streptococcus mutans .CHX is currently the most effective anti-microbial agent for reducing plaque and gingivitis .It act as an adjunct to oral hygiene and professional prophylaxis,post -oral surgery ,oral hygiene and gingival health benefits in mentally and physically handicapped patients ,denture stomatitis ,recurrent oral ulceration etc [4].

Mouthwashes like chlorhexidine have several side effects and its not indicated in childrendespite of good plaque control and antimicrobial effect such as taste alteration and Staining of teeth. Chlorhexidine ADS is an alcohol -free mouthwash. It consists of sodium

Hexametaphosphate and ascorbic acid which are active agents with synergistic effect increasing antiplaque activity and thus helps in preventing staining of the teeth and tongue.

Materials and Methods

The present agar well diffusion in vitro study was carried out in the department of Pediatrics and Preventive Dentistry, D.J college of Dental Sciences and Research Modinagar, Ghaziabad in collaboration with I.T.S Dental College, Murad Nagar.

Division of samples

A total of 60 wells in 15 plates of brain heart infusion culture media were prepared for the study, which were divided as follows into four groups having 15 wells in each group:

GROUP 1: Kiddodent -R Mouthwash (n= 15)

GROUP 2: Dr. Reddy's Chlohex Mouthwash (n= 15)

GROUP 3: Listerine Mouthwash (n= 15)

GROUP 4: Dionized water (n=15)

Various types of mouthwashes used are

1) Chlorhex Mouthwash (Dr.Reddy's) was obtained from medical store

2) Listerine Mouthwash (Johnson and Johnson's) was obtained from medical store

3) Kiddodent R Mouthwash with ADS System (Powdered sodium hexametaphosphate)was obtained from Avarice quality laboratory, New Delhi.) will be prepared in Unimarck Laboratory,Haridwar

Methodology

Preparation of New Mouthwash (Kiddodent –R)

a) Glycerinand bromopol were added in a tank and stirred well. After that PEG400, sodium sachrine and RH 40 were added in the tank.

b) After 30 minutes 0.12% chlorhexidine gluconate was added and mixed properly,

c) Sorbitol was diluted with distilled water and added to the main container. In other containers, Sodium benzoate and Sodium saccharine were dissolved, filtered and added to the main container. Sodium hexametaphosphate was diluted with water, filtered and added to the main container.

d) Color, which comes in powder form, was boiled in 20ml distilled water in a beaker and cooled down. Then, sodium hexametaphosphate was added to it followed by TWEEN 80. Constant Stirring was done constantly to avoid the turbidity (Figure 1).

e) Flavor was added in the solution and after adding it boiling was done at 100^0 Con a hot plate for 10 minFinally, flavouring agent and colour were dissolved separately, filtered and added to the main container. The total volume was adjusted using distilled water. The final product was analyzed and filled in suitable bottles. (Figure 2)

Figure1: Sodium Hexametaphosphate

Figure 2: Kiddodent –R

Agar-diffusion test

The bacterial stock culture S. Mutans was obtained and culture was grown overnight in brain heart infusion (BHI) broth and inoculated in Mueller- Hinton agar plates. Inoculation was performed by using sterile swab brushed across the media. One round well, 4mm of depth X 6mm of diameter was punched in each agar plate and the mouthwashes were added to the wells. Agar plates were incubated at 370 C for 24 hours in an incubator. The diameter of bacterial inhibition zones around each well was recorded to the nearest size in mm. The results were tabulated and statistically analyzed using analysis of variance (ANOVA) 7,8\

Results

On statistical analysis group A Kiddo dent –R showed the best result with the mean value 23.14followed by Group B Chlohex Mouthwash, with mean value 19.10 followed by Listerine Mouthwash with mean value 8.80 and group D Deionized water with mean value 0.00 (Table 1)

Graph -1 shows the mean distribution of study samples with the standard deviation. Out of total samples of 60,

Group A Kiddodent –R (n=15) shows a mean of 23.14 Group B Chlohex (n=15) showed a mean of 19.10 Group C Listerinemouthwash (n=15) had a mean of 8.80 and Group D Deionized water (n=15) 0.00.

On applying one way ANOVA, we found that inhibition zone in all the groups was statistically significant (p=0.001) (Table 2)

When intercomparison of various groups were done using Tukey's test (Post Hoc tests) Groups A versusGroup B, Group C and GroupD, Group B versus Group A, GroupC, GroupD, Group C versus Group A, Group B Group D, Group D versus Group A, Group B and Group C and was statistically significant (p=0.001) (Table 3, Graph 1).

Groups	N(Sample Size)	Mean	Standard deviation	Std. Error Mean
Group A	15	23.14	1.552	0.214
Group B	15	19.10	0.961	0.131
Group C	15	8..80	1.373	0.182
Group D	15	.00	.000	0.000

Table 1: Mean values of inhibition zone in various groups

ANOVA					
	Sum of Squares	df	Mean Square	F	p value
Between Groups	3964.050	3	1321.350	3.6753	0.000 *
Within Groups	20.133	56	0.360		
Total	3984.183	59			
* p value < 0.05 Significant					

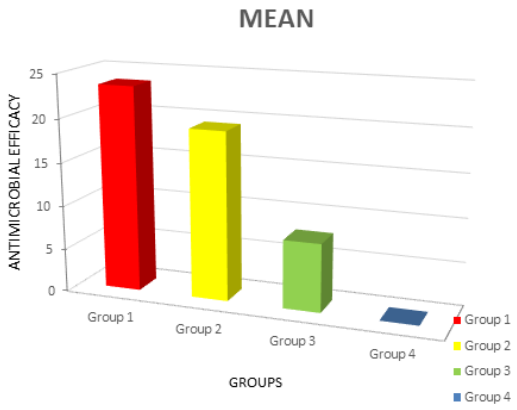
Table 2: Comparison of Means of inhibition zone in various groups.

Table 3: Inter comparison of various groups were done using Bonferroni

(I)GROUP	(J)GROUP	Mean Difference (I-J)	Std. Error	P value	95% Confidence Interval	
					Lower Bound	Upper Bound
GROUP A	B	2.000*	.219	0.000*	1.40	2.60
	C	4.667*	.219	0.000*	4.07	5.27
	D	20.600*	.219	0.000*	20.00	21.20
GROUP B	A	-2.000*	.219	0.000*	-2.60	-1.40
	C	2.667*	.219	0.000*	2.07	3.27

	D	18.600*	.219	0.000*	18.00	19.20
GROUP C	A	-4.667*	.219	0.000*	-5.27	-4.07
	B	-2.667*	.219	0.000*	-3.27	-2.07
	D	15.933*	.219	0.000*	15.33	16.53
GROUP D	A	-20.600*	.219	0.000*	-21.20	-20.00
	B	-18.600*	.219	0.000*	-19.20	-18.00
	C	-15.933*	.219	0.000*	-16.53	-15.33

Graph 1: Bar diagram showing mean antimicrobial efficacy of all the groups.



Discussion

The mouth is considered as the mirror of the body and the health of the oral cavity has been closely associated with systemic health. Periodontal disease is the most frequent oral disease in the world. It consists of a bacterial inflammatory process in the periodontal tissue that results from the accumulation of dental plaque on the external surface of the tooth. It is widely accepted in dentistry that plaque containing a combination disease. Although mechanical plaque control can effectively prevent gingivitis if conscientiously applied, the wide distribution of gingivitis existing in the general population suggests that additional measures may prove beneficial [5.] Chemotherapeutic agents have been shown to be useful adjuncts to daily oral home care in the control of plaque and gingivitis1 Beginning in the 1960's the preventive and therapeutic studies of oral antimicrobials began to shift from caries, which was beginning to respond dramatically to fluorides, to gingivitis and periodontitis - where plaque and calculus were considered the dominant etiologic on of pathogenic micro-organisms is a principal etiological factor associated with periodontal factor in periodontal diseases [6].

Dental caries is one of the most prevalent childhood diseases[51]; people especially children are susceptible to the disease, because of the frequent intake of sticky food, lack of proper oral hygiene habits and inappropriate methods of feeding. Chlorhexidine mouthwashes were first introduced in 1976 but its active ingredient chlorhexidine had been used since 1957, as an antiseptic agent and is still considered one of the most effective antiplaque agents in dentistry.[7] Chlorhexidine is the most widely known mouthwash, available in the concentration 0.12% and it significantly reduce plaque and gingivitis. The antibacterial properties of Chlorhexidine have been demonstrated in-vitro as well as in-vivo studies,[8]providing enough evidence on the superiority of Chlorhexidine, thus making it the gold standard.[9] However, Chlorhexidine is known to have various side effects ranging from more common effects such as alteration in patient taste sensation and staining of teeth to certain less common effects such as mucosal erosion or parotid swelling.[10] So there arises a need for better mouthwash with fewer side-effects.

Comparison between Kiddodent -R, Dr. Reddy s Chlohex andListerine mouthwash hasn't been explored yet. Thus in the present study, Kiddodent –R mouthwash, Dr. Reddy's Chlohex mouthwash and Listerine mouthwash were taken as experimental groups

whereas dionized water was taken as control and analysed antimicrobial efficacy against S. mutans by agar diffusion method

A sample size of 60 wells in 15 plates of brain heart infusion culture media were selected after confirming statistical validity for the study. Similar studies were done by Parkar SM (2013)[11] where they had used a similar sample size and Teh JY et al (2015)[12]had taken a sample size of 50 wells in 10 plates of brain heart infusion agar,to evaluate antimicrobial efficacy of various mouthwashes. S.mutans was choosen as the microbial indicator for this study as it has already been established that S. mutans is the leading cause of dental caries and is considered to be the most cariogenic of all of the oral streptococci. S. mutans plays a major role in the tooth decay by metabolizing sucrose to lactic acid and has ability to metabolize dietary sucrose and synthesize glucan by cell surface and extracellular glucosyl transferase which in turn helps the establishment of S. mutans in the dental plaque.[13].Similarly,Pathan MM et al (2017) 14, Ronanki S et al (2016), [15] Pires JR et al (2007)[16], Prabhakar J et al (2014)[17], Moeintaghavi A et al (2012) and Nuuja T et al (1993) [18]choose S.mutans as the microbial indicator to evaluate antimicrobial efficacy of various mouthwashes in an in-vitro set up. In each Brain heart infusion agar plate 4 wells (4mm of depth X 6mm of diameter) were made using a disposable dropper cut end. A sufficient depth was required for placing the 50 µL of the mouthwash in to the wells as a substantial amount of material was required to diffuse in the agar plate and illicit an evident clear inhibition zone lacking bacterial growth. Thomas A et aL (2015)[19]has conducted a study on comparison of the antimicrobial efficacy of various mouth rinses on cariogenic microbes, where they punched out wells with 4mm of depth and 5mm of diameter on brain heart infusion agar and placed 50 µL of the mouthwash in to the wells. The similar depth of agar wells was used to evaluate the antibacterial efficacy of different mouthwashes by Müller HD (2017)[20], Pires JR et al (2016)[16] and Shah et al (2018)[21] Chlorhexidine (CHX) is a bisbiguanide antiseptic that is a symmetrical molecule consisting of four chlorophenyl rings and two biguanide groups connected by a central hexamethylene bridge. The compound is a strong base and is Di cationic at pH levels >3.5, with the two positive charges on either side of the hexamethylene bridge. Indeed, it is the Di cationic nature of CHX, making it extremely interactive with anions, that is relevant to its efficacy, safety, local side effects, and difficulties with formulation in products

Out of all the contents present in Listerine mouthwash benzoicacid, exhibits definite antimicrobial efficacy against S.mutans, whereas in Kiddo dent -R contains sodium hexametaphosphate mouthwashwhich has a definite antimicrobial action and ant staining efficacy and Dr. Reddys Chlohex mouthwash consists of 0.12% chlorhexidinefor antimicrobial action. This might be reason why Listerine mouthwash has significantly lower antimicrobial efficacy than Kiddodent –R and chlorhexidine mouthwash. The studies related to inter-group comparison of chlorhexidine and Listerine have been carried out by Bhat N et al (2017)[22], Dabholkar CS (2014), S ingh A et al (2013)[23], Biswas G et al (2014)[24] Mishra R et al (2014)[25] and Pathan MM (2017)[26]which showed that Listerine mouthwash has lower antimicrobial efficacy compared to chlorhexidine mouthwash. While contradictory results have been discussed in the studies done by PrasadKARV et al **(**which showed that there is no significant difference between the antimicrobial efficacy of chlorhexidine and Listerine mouthwashes. In the present study, the highest zone of inhibition was seen with kiddodent –R

mouthwash against s. mutans which was higher but comparable to the other two experimental mouthwashes (Chlohex Mouthwash and Listerine mouthwash). Kiddodent–R mouthwash contains sodium hexametaphosphate which might be responsible for its better antimicrobial efficacy.Murphy et al. Da Camara et al. [26]who proved that Sodium Hexameta

phosphate is characterized by potent, and various biological properties including efficient anti-microbial and anti-biofilm actions. Sodium Hexametaphosphate probably have multiple mechanisms of antibacterial action.

Chlohex mouthwash showed antibacterial efficacy which was lower but comparable to that of kiddodent – R. Antibacterial activity of Chlohex mouthwash is primarily due to presence of component like 0.12% chlorhexidinegluconate.

Listerine mouthwash showed antibacterial efficacy which was lower than that of both other group used in study (kiddodent –R) and (Chlohex) mouthwash. because of the presence of benzoic acid and alcohol.Inclusion of benzoic acid and alcohol in Listerine mouthwash is still controversial. Although a drug or a component within a mouth rinse may not cause adverse reactions in the short term, it may produce pathological changes with chronic usage. Winn DM, Blot WJ, McLaughlin JK, et al (2019)has been suggested that alcohol may function as a carcinogenic agent or may alter intact metabolism, thus rendering cells more susceptible to carcinogens.

Group four Distilled water showed no antimicrobial efficacy against S. mutans, thereby authenticating the validity of the present study.

Conclusion

Within the limitation of this in vitro study the following conclusions were drawn: -

Antimicrobial efficacy was seen in all the mouthwash namely Kiddodent –R,Chlohex, andListerine mouthwash. Maximum antimicrobial efficacy was seen in kiddodent R followed by Chlohex then Listerine mouthwash and was least in Deionised water. No zone of inhibition was seen with dionized water thus authenticate the study. Therefore, it can be concluded that Kiddodent -R mouthwash can be an alternative to Chlorhexidine mouthwash and Listerine mouthwash.

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