Research Article

Comparative Evaluation of Antibacterial Activity of Chlorhexidine, Povidone Iodine and Glutaraldehyde for Disinfection of Orthodontic Appliances - An In-Vitro Study

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ABSTRACT:

Introduction: Orthodontists must take precautionary measures to protect patients from cross-infection as most materials supplied by manufacturers are not sterile.

Materials and methods : A sample of 108 as received brackets, molar tubes and archwires equally divided in four groups (3 experimental groups, Group 1- Chlorhexidine Gluconate, Group 2 – Povidone Iodine, Group 3 - Glutaraldehyde and one Control (group 4) were used in this study. Three samples of each (brackets, archwires, molar bands) were tested for CFUs of each micro-organism (*Staphylococcus aureus, Streptococcus mutans,* and *Lactobacillus acidophilus*) in each group. The samples were incubated and spread on blood agar and the colony forming units (CFUs/ml) enumeration were done before and after disinfection.

Results: Brackets and molar bands had least CFU/ml of *Staphylococcus aureus* after disinfection in Group 3 (p=0.000). Of all the materials contaminated by *Streptococcus mutans* (p= 0.000) and *Lactobacillus acidophilus*, the mean CFUs/ml were least in Group 2 (p<0.05).

Conclusion: 2% Glutaraldehyde was effective against *Staphylococcus aureus*. 5% Povidone iodine was effective against *Streptococcus mutans* and *Lactobacillus acidophilus*. The antimicrobial activity of 5% Povidone Iodine was most effective followed by glutaraldehyde when compared to chlorhexidine gluconate.

Keywords: Glutaraldehyde, Povidone Iodine, Chlorhexidine Gluconate, Disinfection, Orthodontic materials.

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INTRODUCTION

The oral microbial ecosystem is constantly exposed to exogenous foreign substances increasing the microbial load in the oral cavity.[1] Successional microbial colonization of bacteria, fungi, and viruses results in the development of polymicrobial biofilm in the oral cavity during orthodontic therapy.[2] The oral microbiome is crucial in maintaining oral as well as systemic health.

Previous studies revealed the presence of opportunistic pathogens namely, *Staphylococcus aureus, Lactobacillus spp* and *Escherchia. coli* in brackets and archwires received directly from the manufacturers. [3-5] Numerous healthcare centers are experiencing an increase in the number of nosocomial infections by multi-drug resistant gram-positive pathogens like *Staphylococcus aureus* and *Enterococcal spp*. [6] The hands of health care personnel, materials and equipment's are vectors for the nosocomial spread of germs. *Staphylococcus aureus* that is resistant to the antibiotic methicillin (MRSA) can spread through direct contact and dirty hands. It enters the bloodstream from organs and causes sepsis and pneumonia. [6,7] So, it is essential to decrease the microbial load by proper sterilization and disinfection before any orthodontic procedure especially bonding.

Vivek et al in his study on brackets showed that gram-positive and gram-negative bacteria could be effectively eliminated by 2% Chlorhexidine when compared to 0.2% Chlorhexidine.[4] No previous studies have compared the efficacy of different chemical disinfectants on brackets, archwires and molar bands. Hence this study was conducted to compare the antibacterial activity of 2% Chlorhexidine gluconate, 5% Povidone iodine and 2% Glutaraldehyde on orthodontic brackets, archwires and molar bands prior to bonding. The objective of the study was to compare the antibacterial activity of 2% Chlorhexidine gluconate, 2% Glutaraldehyde and 5% Povidone iodine against three different bacterial species *Staphylococcus aureus, Streptococcus mutans, and Lactobacillus acidophilus*.

MATERIALS AND METHODS

This study was conducted in Rajas Dental College with approval from the Ethical Committee (IRB approval number – RDCH/IRB/ EC/08/22). Using statistical power analysis G*Power software (version 3.1.9.2) and considering F tests - ANOVA: Fixed effects, omnibus, one-way; Power of the test (1-β error) as 94%, nu and large effect size (Cohen's f statistic) as 0.40; the total sample size (n) for the current study was estimated to be 108 (n). [8] For the assessment of colony forming units, a minimum of three samples (n=3) per material (brackets, archwires, molar bands) for each micro-organism (*Staphylococcus aureus* MTCC No 3160, *Streptococcus mutans* MTCC No 890 and *Lactobacillus acidophilus* MTCC No 10307 (Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh) were considered. A total sample size of 108 (n) was equally distributed in each of the four groups (3 experimental groups and 1 control group) for the evaluation of efficiency of disinfectants. The required sample size for the current study with 94% power was 27 samples per group.

The 108 samples were divided into 4 groups as follows:

Group 1 – Chlorhexidine Gluconate (2% Asep-Rc Anabond Stedman Pvt Ltd Chennai, TamilNadu): 27 samples total - 3 samples per material contaminated by three microorganisms.

Group 2 – Povidone Iodine (5% NicodinTM, Nanz Med Science Pvt Ltd, Sirmour Himachal Pradesh): 27 samples total - 3 samples per material contaminated by three microorganisms.

Group 3 – Glutaraldehyde (2.45% (ASP Cidex TM, ALLIANCE formulations Solan, Himachal Pradesh): 27 samples total - 3 samples per material contaminated by three microorganisms.

Group 4 – Control (Untreated): 27 samples total - 3 samples per material contaminated by three microorganisms. The contaminated brackets, archwires and molar bands were collected in 5ml of nutrient broth (Figure-1) and were incubated for 24 hours at 37° C and the turbidimetric measurement of bacterial growth was measured by UV spectrophotometer (Systronics PC based UV-VIS spectrophotometer 119 Ahmedabad). The optical density (OD) values were 0.524, 0.391 and 0.596 which shows the enhanced turbidity of bacterial growth in nutrient broth. After incubation, the contaminated broth was diluted with distilled water in the ratio of 1:100 dilution and 20μl was spread on blood agar (Figure-2), since the primary isolation of *Staphylococcus aureus* (zone of clear beta hemolysis), *Streptococcus mutans* (alpha or gamma hemolysis) and *Lactobacillus acidophillus* (exhibit alpha hemolysis) are best done in blood agar medium.[9-11] The colony forming units (CFUs/ml) were enumerated before disinfection. Based on the previous studies[4,12] the contaminated brackets, archwires and molar bands in the nutrient broth were washed with sterile distilled water and then disinfected with 2% Chlorohexidine gluconate (Group 1), 5% Povidone Iodine (Group-2) and 2% Glutaraldehyde (Group 3) (Figure-3) for 10 minutes and then incubated for 24 hours at 37°C. After incubation, the broth was diluted with distilled water in the ratio of 1:100 and 20μl was spread plated on blood agar medium and incubated at 37°C for 24 hours and the colony forming units (CFU/ml) were enumerated after disinfection.

CFUs (colony-forming units) are calculated by the formula.

$$\text{CFU/ml} = \frac{\text{(Number of bacterial colonies counted on plate x Dilution Factor)}}{\text{Volume of culture plate}}$$

Colony-forming units (CFU) enumeration were done by marking of the colonies on the reverse of the plate using colony counter.[13]

Figure 1 - Infected samples in nutrient broth

Figure 2 - Sample spread into agar plate

Figure 3 - Disinfectants

STATISTICAL ANALYSIS

Data regarding the colony forming units in experimental and control groups were entered into Microsoft Excel and analyzed using IBM SPSS Statistics for Windows, Version 20 (IBM Corp., Armonk, N.Y., USA). Data was investigated for normality using the Shapiro-Wilk test and it showed a normal distribution. Descriptive statistics were derived as mean, standard deviation and 95% confidence interval. The colony forming units (per ml) between the experimental and control groups were analyzed using One-way analysis of variance (ANOVA) followed by multiple pairwise comparisons with Tukey's Honest significant difference test. The level of statistical significance was determined at $p < 0.05$.

RESULTS

On the brackets and molar bands, the mean CFU/ml of *Staphylococcus Aureus* was least in the Glutaraldehyde group (5.13, 1.95). The least CFU/ml was found on archwires disinfected with Povidone iodine (0.12) (Table-1) (Graph-1). In the post hoc analysis, the mean differences in the CFU/ml of *Staphylococcus aureus* on brackets, between Chlorhexidine gluconate and Povidone Iodine; Chlorhexidine gluconate and Glutaraldehyde groups were statistically significant ($p = 0.000$) (Table-1)(Graph-1). On the archwires, a statistically significant mean difference in CFU/ml were observed between Chlorhexidine gluconate and Glutaraldehyde ($p = 0.000$); Povidone Iodine and Glutaraldehyde ($p = 0.000$)(Table-1)(Graph-1).

Table 1: Intergroup comparison of colony forming units/ml of Staphylococcus Aureus species on the brackets, archwires and molar bands

Staphylococcus Aureus		n	Mean + SD (x 104)	Post hoc analysis†		
				Comparisons	Mean difference	p-value
Brackets	CHX Gluconate	3	31.73 + 2.36	CHX vs PI	23.6	0.000*
	Povidone Iodine	3	8.06 + 0.28	CHX to Glu	26.6	0.000*
	Glutaraldehyde	3	5.13 + 0.86	PI vs Con	-25.6	0.000*
	Control	3	33.66 + 4.16	Glu vs Con	-28.5	0.000*
	Total	12	19.65 + 13.84			
Archwires	CHX Gluconate	3	1.71 + 0.37	CHX vs Glu	-17.2	0.000*
	Povidone Iodine	3	.12 + 0.02	CHX vs Con	-22.6	0.000*
	Glutaraldehyde	3	18.93 + 3.78	PI vs Glu	-18.8	0.000*
	Control	3	24.33 + 0.57	PI vs Con	-24.2	0.000*

	Total	12	11.27 + 11.13	Glu vs Con	-5.4	0.036*
Molar Bands	CHX Gluconate	3	4.90 + 0.56	CHX vs Glu	2.9	0.007*
	Povidone Iodine	3	5.84 + 0.78	CHX vs Con	-6.1	0.000*
	Glutaraldehyde	3	1.95 + 0.67	PI vs Glu	3.9	0.001*
	Control	3	11.00 + 1.00	PI vs Con	-5.2	0.000*
	Total	12	5.92 + 3.47	Glu vs Con	-9.1	0.000*

*Statistically Significant ($p<0.05$), F value – ANOVA test, †Tukey's Honest Significant Difference test

Graph 1: Colony forming units/ml of Staphylococcus Aureus species on the brackets, archwires and molar bands.
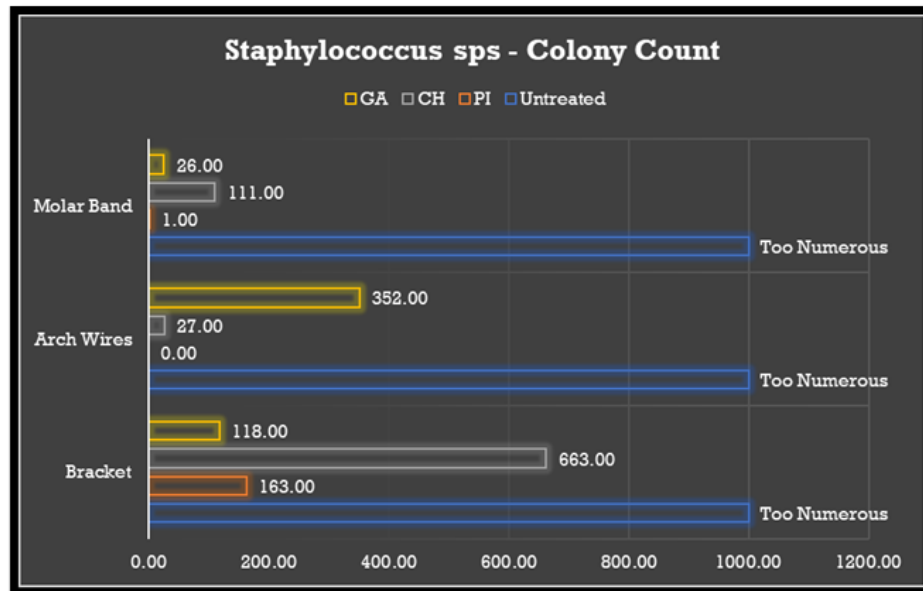

The mean CFU/ml of *Streptococcus Mutans* on the brackets, archwires and molar bands was found to be the least in the Povidone iodine group (6.37, 0.29 and 0.37) respectively (Table-2)(Graph-2). In the post hoc analysis, the CFU/ml of *Streptococcus Mutans* on brackets, showed mean differences between Povidone Iodine and Chlorhexidine gluconate; Povidone Iodine and Glutaraldehyde groups were statistically significant ($p=0.000$, 0.002) (Table-2)(Graph-2). On the archwires and on molar bands, a statistically significant mean difference in CFU/ml was found between Povidone Iodine and Glutaraldehyde ($p=0.000$) and ($p=0.013$)(Table-2)(Graph-2).

Table 2: Intergroup comparison of colony forming units/ml of Streptococcus Mutans species on the brackets, archwires and molar bands

Streptococcus Mutans		n	Mean + SD (x 104)	Post hoc analysis†		
				Comparisons	Mean difference	p-value
Brackets	CHX Gluconate	3	45.86 + 9.15	CHX vs PI	39.5	0.000*
	Povidone Iodine	3	6.37 + 0.39	CHX vs Glu	16.9	0.014*
	Glutaraldehyde	3	28.96 + 2.80	PI vs Glu	-22.6	0.002*
	Control	3	55.46 + 2.83	PI vs Con	-49.1	0.000*
	Total	12	34.16 + 19.93	Glu vs Con	-26.5	0.001*
Archwires	CHX Gluconate	3	24.09 + 6.13	CHX vs PI	23.8	0.001*
	Povidone Iodine	3	.29 + 0.13	CHX vs Con	-32.1	0.000*
	Glutaraldehyde	3	35.50 + 6.00	PI vs Glu	-35.2	0.000*
	Control	3	56.10 + 2.33	PI vs Con	-55.8	0.000*
	Total	12	28.99 + 21.39	Glu vs Con	-20.6	0.002*
Molar Bands	CHX Gluconate	3	2.59 + 0.89	CHX vs Con	-9.9	0.000*
	Povidone Iodine	3	.37 + 0.12	PI vs Glu	-4.6	0.013*
	Glutaraldehyde	3	4.95 + 0.36	PI vs Con	-12.1	0.000*
	Control	3	12.53 + 2.50	Glu vs Con	-7.6	0.001*
	Total	12	5.11 + 4.91			

*Statistically Significant (p<0.05), F value – ANOVA test, †Tukey's Honest Significant Difference test

Graph 2: Colony forming units/ml of Streptococcus Mutans species on the brackets, archwires and molar bands.
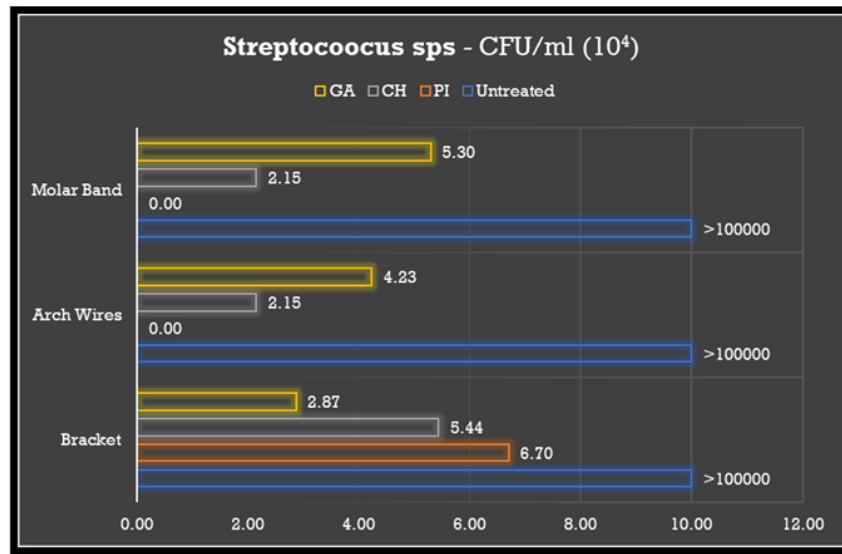
The mean CFU/ml of *Lactobacillus Acidophilus* on brackets, archwires and molar bands was found to be the least after disinfection with povidone iodine (0.47, 0.36 and 0.49). The intergroup comparisons among the disinfecting agents, showed that the differences between the mean CFU/ml values after disinfection were statistically significant for brackets, archwires and molar bands ($p \le 0.05$). In the post hoc analysis, the *Lactobacillus Acidophilus* CFU/ml counts for brackets, archwires and molar bands showed that there was a statistically significant difference between all the groups ($p < 0.05$)(Table-3)(Graph-3).
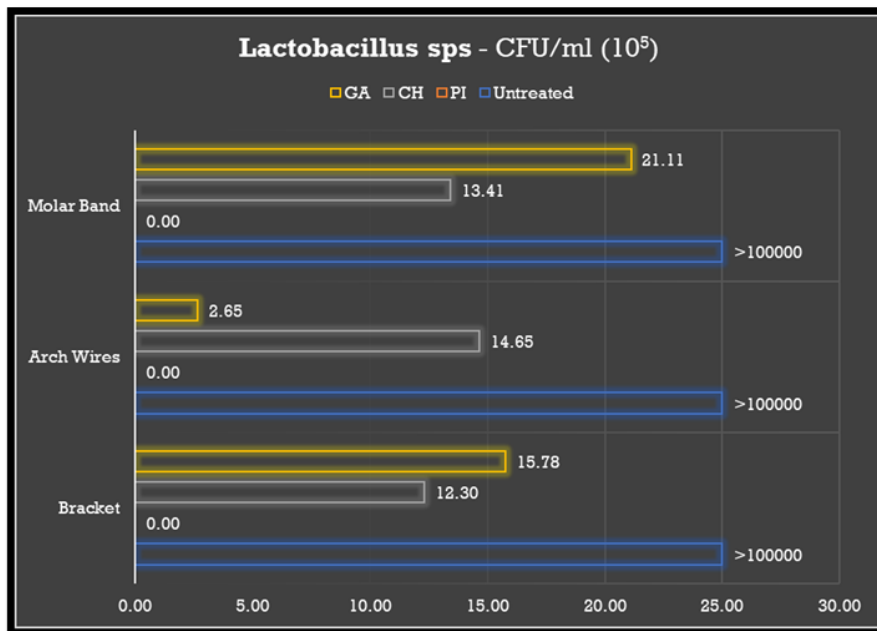
Table 3: Intergroup comparison of colony forming units/ml of Lactobacillus Acidophilus species on the brackets, archwires and molar bands.

Lactobacillus Acidophilus		n	Mean + SD (x 10⁴)	Post hoc analysis†	
				Comparisons	p-value
Brackets	CHX Gluconate	3	112.00 + 10.14	All comparisons	<0.05
	Povidone Iodine	3	.47 + 0.07		
	Glutaraldehyde	3	144.66 + 12.02		
	Control	3	180.08 + 16.13		
	Total	12	109.30 + 70.93		
Archwires	CHX Gluconate	3	132.33 + 12.27	All comparisons	<0.05
	Povidone Iodine	3	.36 + 0.08		
	Glutaraldehyde	3	36.23 + 10.01		
	Control	3	175.29 + 7.81		
	Total	12	86.05 + 74.10		

Molar Bands	CHX Gluconate	3	122.86 + 12.13	All comparisons	<0.05
	Povidone Iodine	3	.49 + 0.03		
	Glutaraldehyde	3	200.96 + 10.57		
	Control	3	271.58 + 18.22		
	Total	12	148.97 + 105.56		

*Statistically Significant (p<0.05), F value – ANOVA test, †Tukey's Honest Significant Difference test

Graph 3: Colony forming units/ml of Lactobacillus Acidophillus species on the brackets, archwires and molar bands.

DISCUSSION

The need for disinfection of as received brackets and archwires have been reported in various studies and most of the literature revealed the presence of *Staphylococcus aureus, Streptococcus mutans, Lactobacillus, Klebsiella pneumoniae, Bacillus licheniformis*, and *Escherichia coli* as bacterial contaminants.[3,4,5] Chemical disinfection reduces the microbial load and the disinfectants used most frequently for cold sterilization are Glutaraldehyde, Hydrogen peroxide, Alcohol, Povidone iodine and Chlorhexidine.[14] So, in the present study the efficacy of three disinfectants, Chlorhexidine, Povidone iodine and Glutaraldehyde were evaluated against the most commonly isolated organisms (*Staphylococcus aureus*, *Streptococcus mutans*, and *Lactobacillus acidophilus*) from as received brackets, archwires and molar bands.

The results of this study showed that brackets and molar bands when disinfected with 2% Glutaraldehyde showed less bacterial growth (mean CFU/ml - 5.13 and 1.95)(Table-1)(Graph-1). Least CFUs/ml was found on archwires disinfected with Povidone iodine (0.12). The design of brackets and molar bands have more retentive areas when compared to archwires which has less retentive surfaces for microbial growth. In the present study, Glutaraldehyde was effective against *Staphylococus aureus* for disinfecting brackets and molar bands when compared to Povidone iodine which was effective in disinfecting archwires. Sowmithra et al reported that 2%

Glutaraldehyde was found to be effective in eliminating the entire microflora in orthodontic brackets before using them on patients.[12]

Andreshna et al studied the efficacy of UV, dry heat, steam autoclave, ethyl alcohol, and 2% Glutaraldehyde in eliminating bacterial contamination.[15] In this study, Glutaraldehyde was also effective in eliminating bacterial contamination. In this study, the disinfecting efficacy of Chlorhexidine gluconate was low as the mean CFUs/ml were high in all the three groups (Tables 1-3). In the present study, 2% Chlorhexidine gluconate was not effective against the three species which is contradictory to Vivek et al who reported that use of 2% Chlorhexidine gluconate was effective in disinfecting brackets[4] and Evans et al reported that Chlorhexidine gluconate mouthrinse was effective against *Streptococcus mutans* and *Lactobacillus spp*.

Povidone iodine is a broad-spectrum antimicrobial solution effective against a variety of pathogens including *Staphylococcus aureus* and also effective against fungi, viruses, and protozoa. In the present study, Povidone iodine was effective against *Staphylococcus aureus* for disinfecting archwires (Table-1). Povidone iodine was effective against *Streptococcus mutans* and *Lactobacillus acidophilus* in disinfecting all the brackets, archwires and molar bands (Tables 2 and 3). Previous studies have reported that all the sealed and unsealed brackets, archwires and molar bands, received directly from the manufacturers were contaminated with various organisms.[16,17] According to Lucas et al, both aerobic and anaerobic bacteria can produce bacteremia as a result of orthodontic treatment.[18]

In vivo studies comparing the effects of Povidone iodine gargle and Chlorhexidine gluconate mouthwash on six different bacterial strains showed that Povidone iodine reduced the viable cell count. This indicated a quick bactericidal action of Povidone iodine gargle against strains was superior to the outcomes for Chlorhexidine gluconate mouthwash.[19] Evans et al reported that 10% Povidone iodine inhibits the growth of *Streptococcus mutans* intraorally.[20] In accordance with the previous studies, the results of the present invitro study also shows that the antibacterial activity of 5% Povidone iodine for 10 minutes was effective when compared to 2% Chlorhexidine gluconate for disinfecting the orthodontic brackets, archwires and molar bands. The limitation of this study was that the microorganisms were not isolated from the received brackets, archwires and molar bands but in this study, these materials were contaminated with three types of cariogenic bacteria.

CONCLUSION

2% Glutaraldehyde was effective against *Staphylococcus aureus* and 5% Povidone iodine was effective against *Streptococcus mutans* and *Lactobacillus acidophilus* for disinfecting brackets, archwires and molar bands. The antimicrobial activity of 5% Povidone Iodine was most effective followed by Glutaraldehyde when compared to Chlorhexidine Gluconate.

SOURCE OF FUNDING

CONFLICT OF INTEREST

The authors have no conflict of interests to declare.

REFERENCES

1. Sedghi L, DiMassa V, Harrington A, Lynch SV, Kapila YL. The oral microbiome: Role of key organisms and complex networks in oral health and disease. Periodontol 2000. 2021 Oct; 87 (1):107-13.

2. Saad HS, Nidhal HG, Hassan AG. Evaluation of microbial contamination of different orthodontic as received arch wires from manufacturers. Int J Med Sci Public Health 2017;6 (12):13-8.

3. Dos Santos Gerzson DR, Simon D, Dos Anjos AL, Freitas MP. In vitro evaluation of microbial contamination of orthodontic brackets as received from the manufacturer using microbiological and molecular tests. Angle Orthod. 2015 Nov; 85(6): 992-6.

4. Vivek Aithal PR, Akshai Shetty KR, Dinesh MR, Amarnath BC, Prashanth CS, Roopak MD. In vitro evaluation of microbial contamination and the disinfecting efficacy of chlorhexidine on orthodontic brackets. Prog Orthod. 2019 May 1; 20(1):17.

5. Brusca MI, Chara O, Sterin-Borda L, Rosa AC. Influence of different orthodontic brackets on adherence of microorganisms in vitro. Angle Orthod. 2007; 77:331–336.

6. Khan HA, Baig FK, Mehboob R. Nosocomial infections: Epidemiology, prevention, control and surveillance. Asian Pac J Trop Biomed. 2017 May 1;7(5):478-82.

7. Umar D, Basheer B, Husain A, Baroudi K, Ahamed F, Kumar A. Evaluation of bacterial contamination in a clinical environment. J Int Oral Health. 2015 Jan;7(1):53-5.

8. Faul, F., Erdfelder, E., Lang, A.-G. & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior Research Methods, 39, 175-191.

9. Zhang H, Zheng Y, Gao H, Xu P, Wang M, Li A, Miao M, Xie X, Deng Y, Zhou H, Du H. Identification and Characterization of Staphylococcus aureus Strains with an Incomplete Hemolytic Phenotype. Front Cell Infect Microbiol. 2016 Nov 18;6:146.

10. Wolff L, Liljemark WF. Observation of beta-hemolysis among three strains of Streptococcus mutans. Infect Immun. 1978 Feb;19(2):745-8.

11. Goldstein EJ, Tyrrell KL, Citron DM. Lactobacillus species: taxonomic complexity and controversial susceptibilities. Clin Infect Dis. 2015 May 15;60 Suppl 2: S98-107.

12. Sowmithra Devi S, Ashwin Mathew George, Rajesh kumar, Need for Disinfecting New Orthodontic Brackets-A Reality Check, J Res Med Dent Sci, 2022, 10(5): 61-65.

13. Da Silva, N., H. Taniwaki, M., Junqueira, V.C.A., Silveira, N., Okazaki, M.M., & Romeiro Gomes, R.A. (2018). Microbiological Examination Methods of Food and Water: A Laboratory Manual, 2nd Edition (2nd ed.). CRC Press.

14. Tweij-Thu-Alfeqar Razzaq AJ, Shnan D, Ali AB. Sterilization of Surgical Tools: Removing Bacterial Endospores with a Combination of Povidone-iodine, Chlorhexidine Gluconate, Ethanol, and Methanol. J Pure Appl Microbiol. 2019;13(4):2499-506.

15. Ardeshna A, Chavan K, Prakasam A, Ardeshna D, Shah D, Velliyagounder K. Effectiveness of Different Sterilization Methods on Clinical Orthodontic Materials. J Indian Orthod Soc. 2022; 0(0).

16. Musaddique SS, Ajit K, Ashwith H, Nasim M, Mohamed F. Evaluating the sterility of orthodontic materials as received from the manufacturer and that exposed to clinic environment-in vitro study. South Eur. J. Orthod. Dentofac. Res. 2018 Oct 17;5(2):30-7.

17. Purmal K, Chin S, Pinto J, Yin WF, Chan KG. Microbial contamination of orthodontic buccal tubes from manufacturers. Int J Mol Sci. 2010 Sep 16;11(9):3349-56.

18. Lucas VS, Omar J, Vieira A, Roberts GJ. The relationship between odontogenic bacteraemia and orthodontic treatment procedures. Eur J Orthod 2002 Jun 1; 24(3):293-301.

19. Rahn R, Schneider S, Diehl O, Schäfer V, Shah PM. Preventing post-treatment bacteremia: comparing topical povidone-iodine and chlorhexidine. J Am Dent Assoc. 1995 Aug;126(8):1145-9.

20. Evans A, Leishman SJ, Walsh LJ, Seow WK. Inhibitory effects of antiseptic mouthrinses on Streptococcus mutans, Streptococcus sanguinis and Lactobacillus acidophilus. Aust Dent J. 2015 Jun; 60 (2):247-54.

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