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Comparing the effectiveness of various sterilization methods on orthodontic instruments for bacteriological assessment

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Abstract

Introduction: Infection control is especially challenging in dentistry because of the high volume of patients and time-consuming procedures. Even though orthodontic patients are considered as low-risk patients for any cross-infection, every patient should be treated as a potential carrier.

Method: To provide information to Orthodontic office personnel which would serve as a reasonable guideline in selecting and using different sterilization methods, present in-vitro study was carried out to assess the quantitative differences in Gram Positive (Staphylococcus Aureus, Streptococcus Mutans) and Gram Negative (Escherichia coli) bacteria cultured from different Metallic (Weingart Plier), Non-metallic (Autoclavable Cheek Retractor), Cutting (Distal End Cutter) and Non-cutting (Explorer) instruments for comparing the effectiveness of various sterilization methods- Autoclave: Moist Heat Rapid Cycle, Hot Air Oven: Rapid Dry Heat, Chemical Immersion. in inhibiting bacteriological growth.

Result: Data was analyzed by Paired t-test and one-way ANOVA. Statistically highly significant ($P<0.001$) difference in bacterial adherence of E. Coli which was maximum in Distal end cutter followed by Cheek retractor, Weingart plier and minimum in Explorer,

however, which was also similar in Gram +ve S. Mutans and S. Aureus. On comparing the effectiveness of sterilization for Weingart plier, Distal end cutter, Explorer and Cheek retractor revealed a non-significant (P>0.05) difference.

Conclusion: Bacterial adherence found to be highly significant and maximum on Distal end cutter and minimum on Explorer. After sterilization, percentage reduction was almost equivalent in Autoclave and Hot Air Oven group, with a minor difference in Chemical immersion for all types of instruments.

Clinical Implications: Rapid cycle of Steam autoclave can be good, effective and less time-consuming method for sterilization of stainless steel metallic non-cutting instruments if proper measures are taken to prevent corrosion, Hot air oven being also quick for stainless steel metallic cutting instruments, while Chemical immersion for plastic instruments can be a good alternative option in non-allergic condition.

Keywords: Sterilization, Orthodontic Instruments, Autoclave, Hot Air Oven, Chemical disinfection, Gram positive and Gram negative bacteria.

Introduction

The concept of asepsis & sterilization and its role in the prevention of infection was put forward nearly two centuries ago. Effective sterilization and decontamination processes prevent cross infections and reduce the microbiological degree of contamination in the operative environment because of difficulty in identifying infected persons.

Approximately 280 bacterial species from the oral cavity have been isolated in culture and formally named [1]. Both patients and practitioners produce a substantial risk of transmitting infection. The most common micro-organisms pertaining to oral cavity are Staphylococcus Aureus, Streptococcus Mutans, Pseudomonas Aeruginosa, Clostridium Tetani, Candida Albicans, etc. [2].

Sterilization in orthodontics has been discussed and stressed through times in the dental literature. It has received much attention in the last few years mostly because of public awareness of communicable diseases. Thus, before beginning with any dental procedures, we should have clear basic goals of infection control. We must provide efficient and cost-effective treatment to patients by following various sterilization protocols.

Present in-vitro bacteriological study was designed because of clinical variations of microorganisms in oral cavity which differ from patient to patient, to identify best possible method for sterilization of metallic, non-metallic, cutting and non-cutting Orthodontic instruments which were contaminated by Gram +ve and Gram -ve bacterial pathogens.

Material & Methods

-Young subject was taken for sample collection of bacterial pathogens for in-vitro laboratory procedure,

Armamentarium

- Orthodontic instruments -Weingart Plier Distal End Cutter, Autoclavable Cheek Retractor and Explorer
- Autoclave, Hot Air Oven Laboratory Incubator
- Chemical Immersion by Chlorhexidine gluconate 0.3% v/v + Cetrimide IP 0.6% w/v (Cadlon).

30 numbers of each instruments were taken and sterilized by autoclave with conventional method- 121˚C for 15 minutes at 15 psi before they were exposed to Gram +ve and Gram -ve bacterial contamination through in-vitro laboratory procedure.

Fig. 1: Pure Bacterial Culture Plates

Isolation of Bacterial Pathogen:

- They were grown separately in Nutrient broth in 3 separate containers.
- Each instrument was dipped in different pure culture of bacteria separately for 24 hours. After that swab were taken in presence of flame to prevent air contamination and were cultured on nutrient plates.

Fig. 2: Instruments Kept in Pure Culture Separately

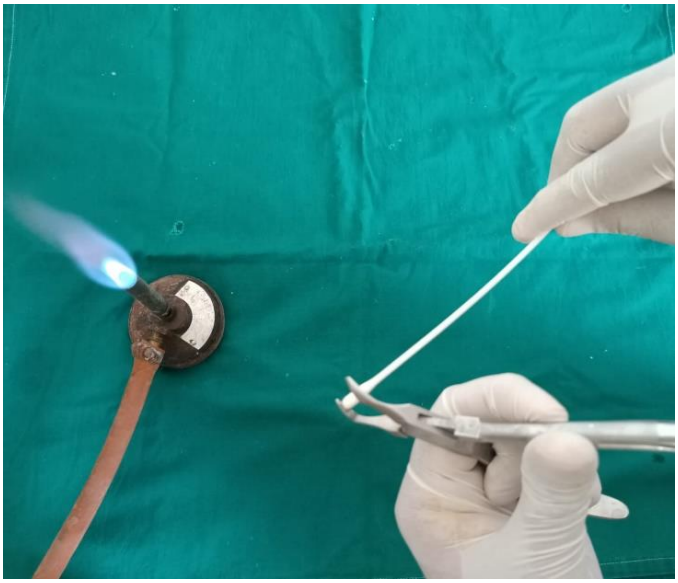


Fig. 3: Swab Taken from Different Orthodontic Instruments

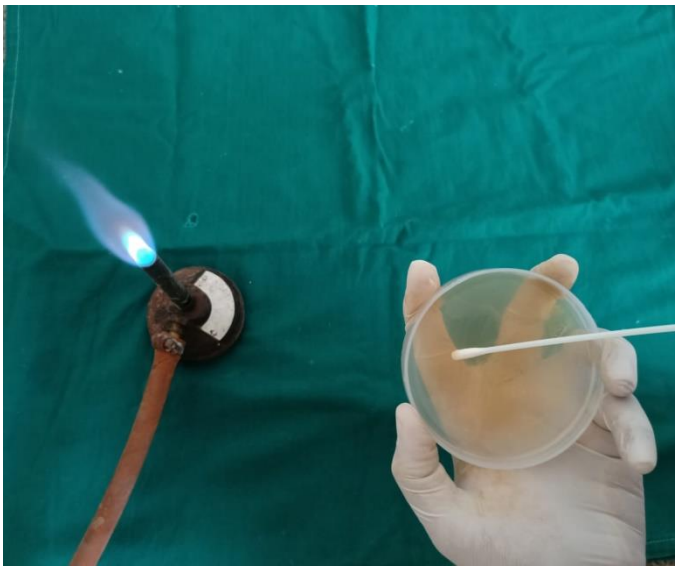


Fig. 4 Swab Cultured on Nutrient Plate

Culture Examination

- All plates were incubated aerobically for 24 hours at 37˚C for bacterial growth assessment in the incubator.
- E. Coli being a facultative anaerobe can be grown in aerobic condition as well. (Reference: Ali saadi et al., 2017) [3].

Fig. 5: Laboratory Incubator

Microscopic Examination: (Reference: Silvio D. Brugger et al., March 2012) [4]

- After the incubation period, individually all nutrient plates of 3 bacteria for different instruments were taken out sequentially to count Colony Forming Unit (CFU). For counting colonies, a manual method was used, i.e., with the help of a magnifying glass, all colonies were counted. Standardization was done in such a way that for over 200 colonies, plates were divided into equal 4 sectors and colonies were counted in 1 sector, making dot with marker on each colony. After one sector counting is completed, it was multiplied by 3 (remaining sectors) for total colonies.

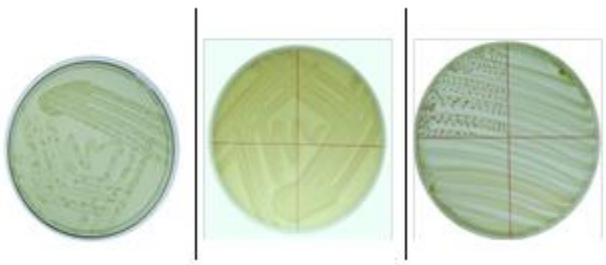


Fig. 6: Counting Colony Forming Unit

Sterilization Procedures

- Then each instrument was subjected to 3 different sterilization methods- Group A, B and C.

Group A	**Autoclave** (Moist Heat Rapid Cycle Sterilization) 134˚C, 15-20 psi pressure, for 6 minutes.
Group B	**Hot Air Oven** (Rapid Dry Heat Sterilization) 190˚C for 12 minutes
Group C	**Chemical Immersion** (Chlorhexidine gluconate 0.3% v/v + Cetrimide IP 0.6% w/v for 30 minutes)

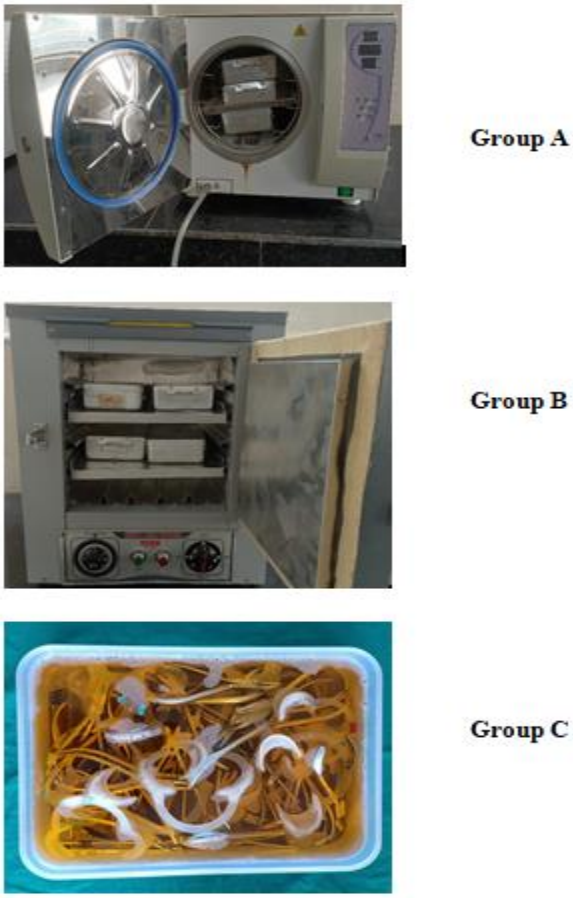


Fig. 7: Instruments Kept for Sterilization

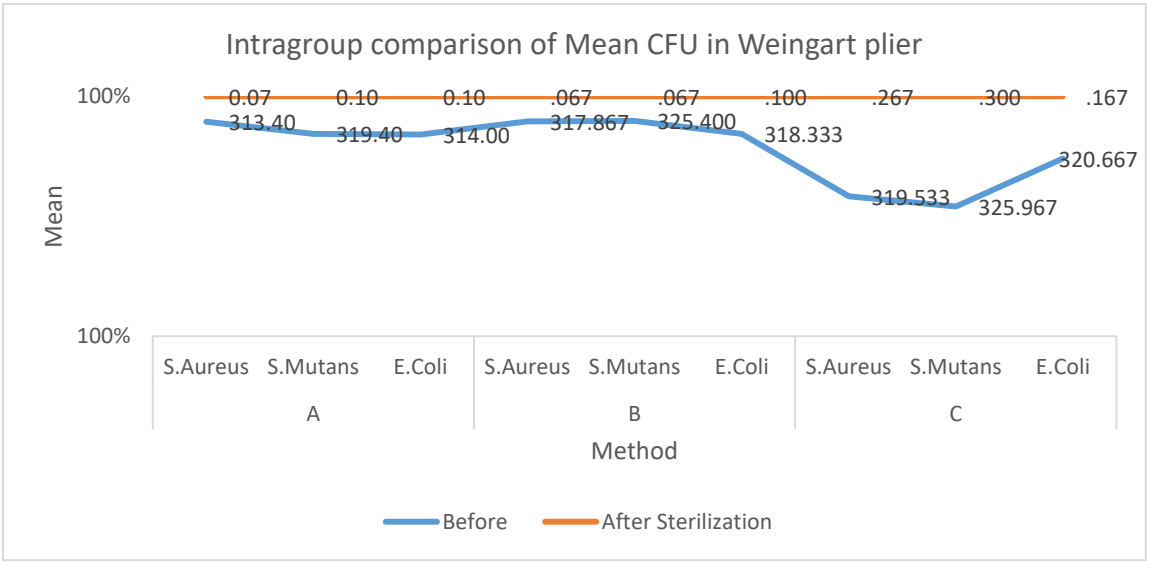
After this swab was taken again and cultured on the nutrient plate in a sterile environment that was incubated for 24 hours at 37˚C in an incubator to check the reduction in colony forming unit for different bacteria.

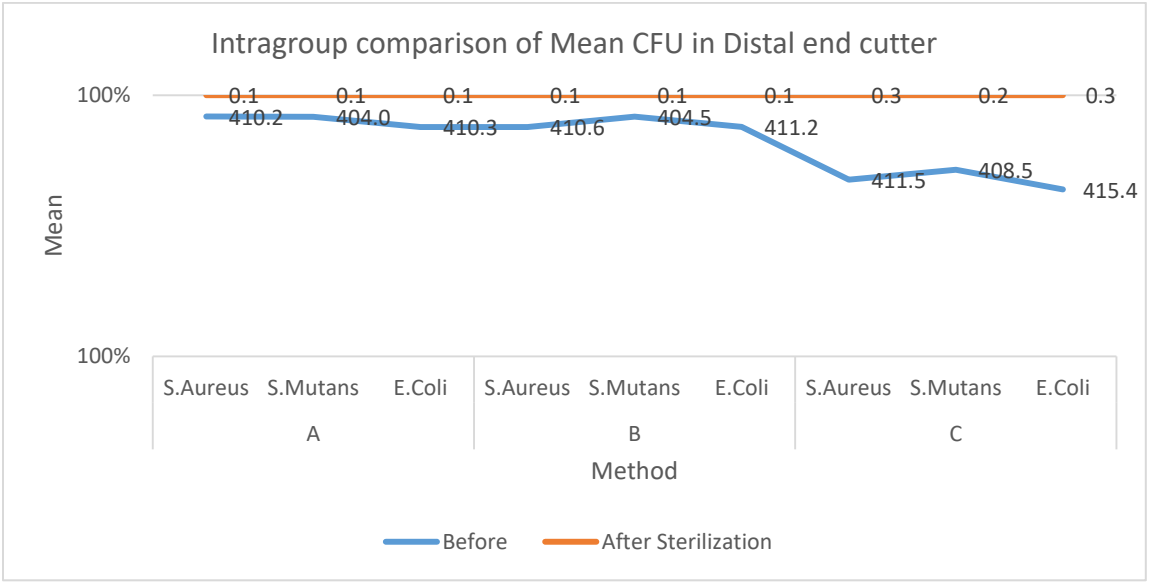
Counting for reduction in colonies was done with the same method as described above.

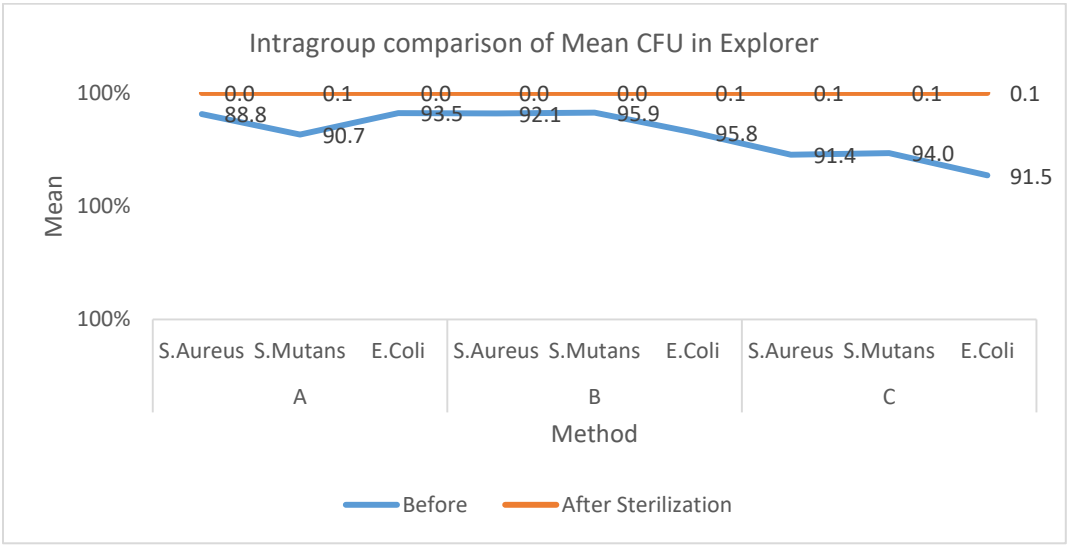
Fig. 8: Colony Forming Unit Counted with Help of Magnifying Glass

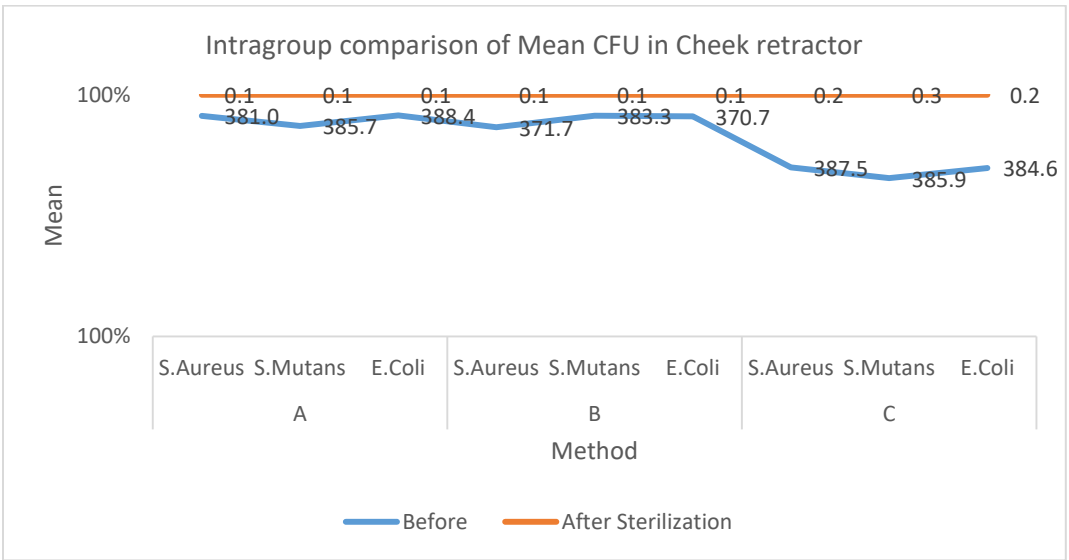
Results

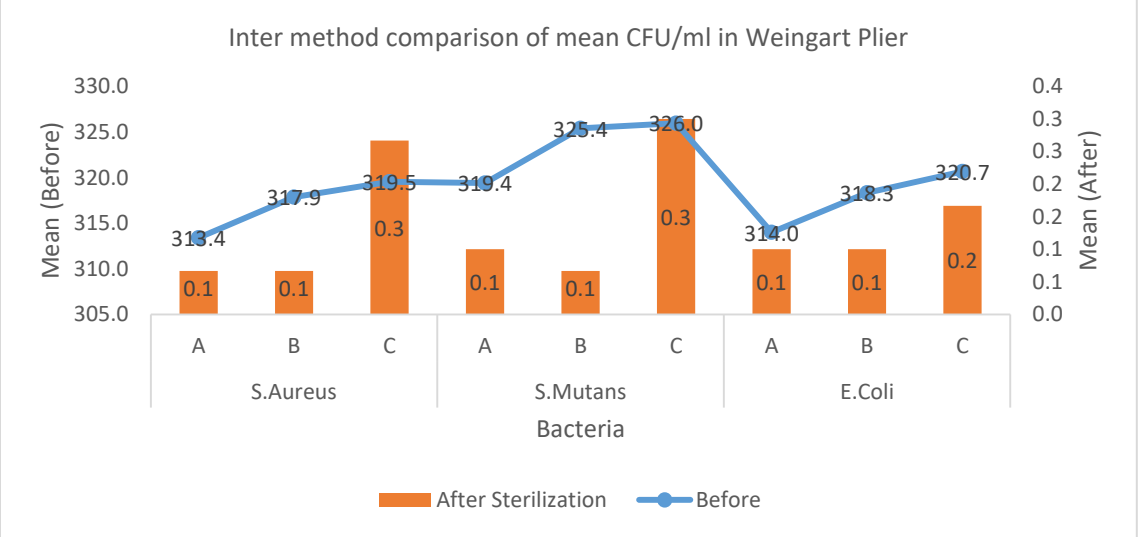
Data was analyzed using SPSS version 23. Descriptives, paired t test and one way ANOVA was done.

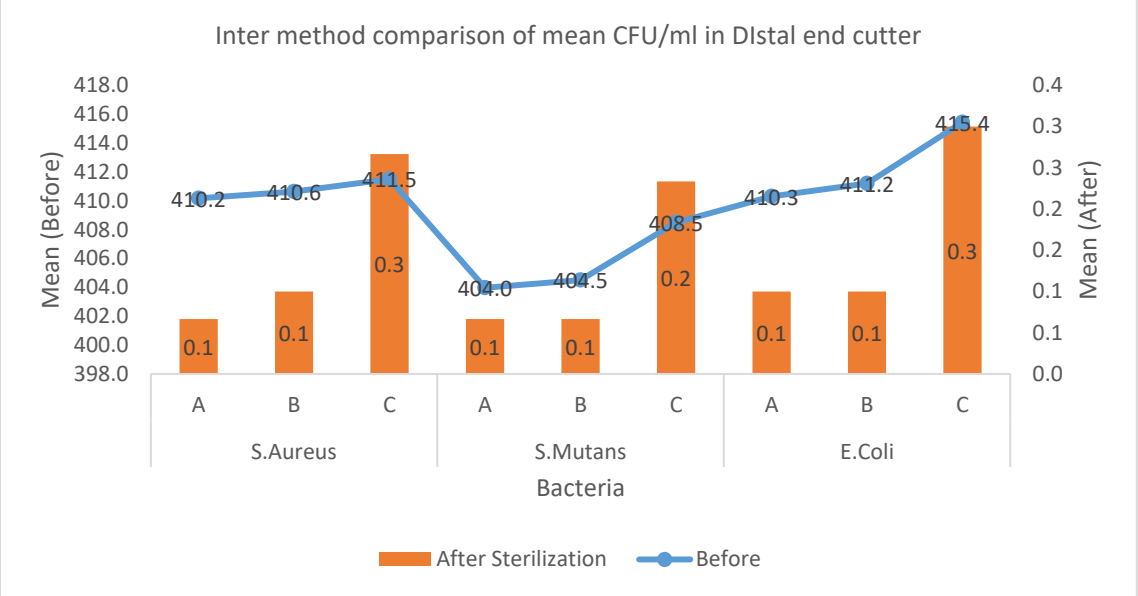


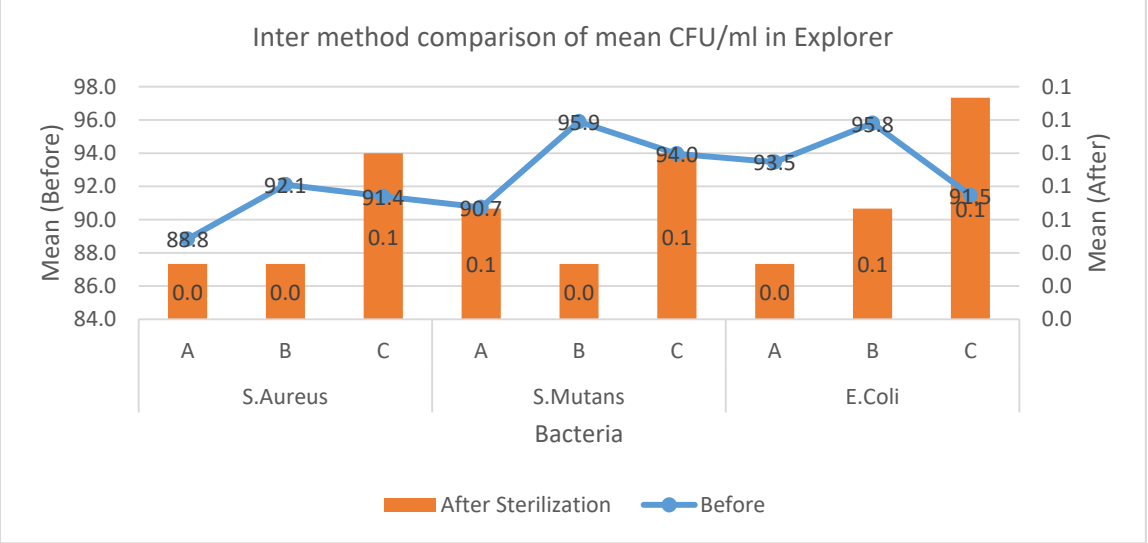


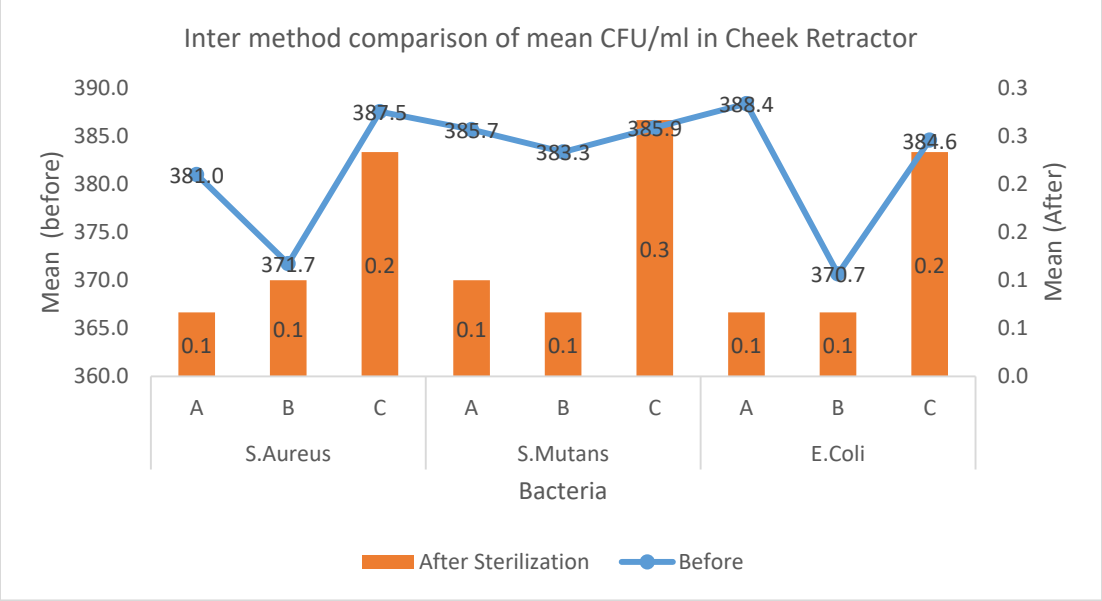












Discussions

Comparison of different instruments, which were exposed to in-vitro laboratory cultured Gram +ve and Gram -ve bacteria and that were sterilized with various methods (Group A, B, C) by mean CFU/ml. Before sterilization, bacterial adhesion for Weingart plier depicts more mean value for S. Mutans and least for S. aureus and intermediate for E. coli and also standard deviation, whereas after sterilization mean and standard deviation was equal for S. aureus and S. Mutans in Group B, equal for E. Coli in Group A and B, which was statistically highly significant (P<0.001) by mean CFU/ml with 100% reduction for Group A and B and 99.9% for Group C. On assessing bacterial adhesion before sterilization for Distal end cutter showed more mean and standard deviation for E. Coli and less for S. Mutans. After sterilization, 100% reduction for Group A and B for all three bacteria which was statistically highly significant (P<0.001). But Group C showed a 99.9% reduction, which was almost similar for all three bacteria. Explorer reveal maximum bacterial adhesion for E. Coli and less for S. Aureus before sterilization. But after sterilization there was 100% reduction for S. Aureus in Group A and B which was statistically highly significant (P<0.001) and 99.9% reduction for S. Mutans in Group A, E. Coli in Group B and for all bacteria in Group C by mean CFU/ml. Bacterial adherence of E. Coli in Group A, S. Mutans for Group B and S. Aureus for Group C was more by mean CFU/ml for Cheek retractor before sterilization. Reduction was similar in Group A and B after sterilization for Gram +ve and Gram -ve bacteria, which was statistically highly significant (P<0.001) but 99.9% reduction in Group C for all bacteria.

In the present study, bacterial adherence of E. Coli was maximum in Distal end cutter followed by Cheek retractor, Weingart plier and minimum in Explorer, however, which was also similar in Gram +ve S. Mutans and S. Aureus bacteria for which surface roughness of cutting edge in Distal end cutter and less surface area of Explorer may be contributing factor for variations in bacterial adherence.

Mirjam Kozmos et al. (2021) [5] found that bacterial adhesion for S. mutans has a significant influence on surface roughness, wettability and charge of dental material.

Intermethod comparison of various types of sterilization for different instruments showed statistically non-

significant difference (P>0.05) in mean CFU/ml for Gram +ve (S. Mutans and S. Aureus) and Gram -ve (E. Coli) for Weingart plier. Like way Distal end cutter, Cheek retractor and Explorer also showed statistically non-significant difference.

Andrea Wichelhaus et al. (2006) [6] found efficacy of different disinfection methods after clinical use of S. Aureus and E. Coli contaminated Orthodontic pliers (Weingart pliers and Distal end cutters) and observed that chemical methods were less effective. Maria Reggiani Azevedo Carvalho et al. (2015) [7] observed that there were statistically non-significant differences (P>0.05) regarding antimicrobial efficacy of 70% isopropyl alcohol, 2% glutaraldehyde and 0.25% peracetic acid for S. Mutans, S. Aureus and Candida Albicans on Distal end cutter.

But Camilla Machado Feitosa de Almeida et al. (2012) [8] found that 2% glutaraldehyde was more efficient for Orthodontic pliers that were contaminated by S. Aureus and S. Salivarius. Also, Shifa Jabar et al. (2020) [9] found that 2% glutaraldehyde solution, quaternary ammonium compound-based wipes and foam spray to be equally effective (P<0.05) for disinfection of Distal end cutter contaminated with strains of Pseudomonas Aeruginosa, Staphylococcus Aureus and Streptococcus Salivarius. And Nagaraj Venkatachalam et al. (2020) [10] found no bacterial growth on Orthodontic instruments (Weingart plier, Distal end cutter) after disinfecting in all four disinfectants (Dettol, Savlon, Bacillol and Durr Dental solutions) as all were equally effective on 15 minutes immersion.

Rajeev Lall et al. (2018) [11] in their comparative study using biological indicators and conventional swab test method on Orthodontic instruments by different sterilization methods (Steam Autoclave, Hot air oven, Cold Sterilization- Bioclenz-G i.e., 2% glutaraldehyde and Ethylene dioxide), biological indicators for steam autoclave or chemical vapor sterilization contain spores of Bacillus stearothermophilus and spores of Bacillus subtilis for dry heat or ethylene oxide sterilization and stated that biological indicator was a more reliable and accurate method for monitoring sterilization.

Maryam Omidkhoda et al. (2016) [12] observed statistically non-significant results (P=0.026) in a comparative study of different sterilization methods on Orthodontic markers that was contaminated with S. Aureus, E. Coli and Candida Albicans, but Autoclave and Glutaraldehyde were the best methods for disinfecting Orthodontic markers.

In the present study, rapid cycle of Autoclave and Hot air oven was found to be more effective than Chemical immersion with Cetrimide and Chlorhexidine combination in reduction of Gram +ve and Gram -ve bacteria for Weingart plier, Distal end cutter, Explorer and Cheek retractor. So, chemical immersion can also be preferred for plastic instruments like cheek retractor because of all chances of deformation due to heat sensitivity.

Claire Thompson (2002) [13] suggested in his review that dental instruments should be autoclaved wherever possible but not feasible for smaller departments and recommended that dental mirrors and cheek retractors be cleaned using a high-level cold disinfectant (alcohol or chlorine-based).

Shilpa Kalra et al. (2015) [14] in a review article about infection control in Orthodontic office described special consideration for Orthodontic pliers as high-quality stainless-steel pliers can be sterilized by steam, dry heat, chemical vapour and ethylene oxide gas. Steam autoclave is not preferred for low-quality pliers, as it may damage the material.

Downside of previous literature about Autoclave which causes tarnish, corrosion, decrease in cutting efficiency because of repeated cycles of instruments, preheating and post cooling time for metallic instruments. Chemical immersion also having drawbacks like allergy, toxicity, surface discoloration, clogged hinge joint of instruments.

Limitation of Study

Other more commonly found Gram +ve and Gram -ve organisms in the oral cavity were not taken into consideration.

Conclusion

Distal end cutter had maximum bacterial adhesion of E. Coli and S. Aureus bacteria but Weingart plier had more of S. Mutans adhesion, and Explorer had almost similar number of S. Mutans and E. Coli, whereas almost equivalent of S. Aureus, S. Mutans and E. Coli bacteria in Cheek retractor. Bacterial adherence varies with type of instrument.

Number of colonies does not have any influence on the effectiveness of sterilization methods.

Autoclave and Hot-air oven were found to be more efficient than chemical immersion but non-significant.

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Legend Tables

Bacteria		Method	Before			After Sterilization		
			Mean	SD	P Value	Mean	SD	P Value
Gram +ve	S. Aureus	A	313.40	25.25	0.652 NS	0.07	0.25	0.145 NS
		B	317.867	26.4180		.067	.2537	
		C	319.533	27.6988		.267	.6915	
	S. Mutans	A	319.40	28.24	0.601 NS	0.10	0.31	0.168 NS
		B	325.400	27.4849		.067	.2537	
		C	325.967	27.8202		.300	.7944	
Gram - ve	E. Coli	A	314.00	26.36	0.634 NS	0.10	0.31	0.717 NS
		B	318.333	27.7555		.100	.3051	
		C	320.667	27.9227		.167	.4611	

Table 1: Intermethod comparison of CFU/ml in Weingart Plier

Bacteria		Method	Before			After Sterilization		
			Mean	SD	P Value	Mean	SD	P Value
Gram +ve	S. Aureus	A	410.2	22.8	0.975 NS	0.1	0.3	0.203 NS
		B	410.6	22.6		0.1	0.3	
		C	411.5	25.1		0.3	0.7	
	S. Mutans	A	404.0	20.7	0.684 NS	0.1	0.3	0.164 NS
		B	404.5	21.0		0.1	0.3	
		C	408.5	24.1		0.2	0.6	
Gram -ve	E. Coli	A	410.3	26.2	0.749 NS	0.1	0.3	0.207 NS
		B	411.2	27.7		0.1	0.3	
		C	415.4	29.6		0.3	0.7	

Table 2: Intermethod comparison of CFU/ml in Distal End Cutter

Bacteria		Method	Before			After Sterilization		
			Mean	SD	P Value	Mean	SD	P Value
Gram +ve	S. Aureus	A	88.8	16.0	0.706 NS	0.0	0.2	0.438 NS
		B	92.1	15.1		0.0	0.2	
		C	91.4	16.7		0.1	0.3	
	S. Mutans	A	90.7	17.3	0.500 NS	0.1	0.3	0.594 NS
		B	95.9	15.1		0.0	0.2	
		C	94.0	18.7		0.1	0.3	
Gram - ve	E. Coli	A	93.5	17.3	0.669 NS	0.0	0.2	0.446 NS
		B	95.8	17.7		0.1	0.3	
		C	91.5	20.9		0.1	0.4	

Table 3: Intermethod comparison of CFU/ml in Explorer

Bacteria		Method	Before			After Sterilization		
			Mean	SD	P Value	Mean	SD	P Value
Gram +ve	S. Aureus	A	381.0	19.1	0.092 NS	0.1	0.3	0.239 NS
		B	371.7	36.4		0.1	0.3	
		C	387.5	25.1		0.2	0.6	
	S. Mutans	A	385.7	20.8	0.919 NS	0.1	0.3	0.203 NS
		B	383.3	34.3		0.1	0.3	
		C	385.9	22.4		0.3	0.7	
Gram - ve	E. Coli	A	388.4	21.3	0.079 NS	0.1	0.3	0.164 NS
		B	370.7	29.3		0.1	0.3	
		C	384.6	25.0		0.2	0.6	

Table 4: Intermethod comparison of CFU/ml in Cheek Retractor