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In vitro evaluation of microbial contamination and disinfecting efficacy of 70% ethanol on archwires as received from different manufacturers

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Abstract
Introduction- The contamination of orthodontic appliances due to unhygienic manufacturing and packaging processes can lead to cross-contamination, posing significant risks in clinical settings. Despite literature highlighting the necessity for sterilization or disinfection of orthodontic appliances before oral use, this practice remains uncommon in routine clinical operations. Archwires are transferred directly from their original packaging to the patient's mouth without prior sterilization, as manufacturers do not indicate the need for such procedures.
Aim & Objectives-To evaluate the microbial contamination and disinfection efficacy of 70% ethanol on orthodontic archwires.
Materials & Method-A total of 65 archwires were obtained from three different manufacturers and divided into five groups: group 1 (3M Unitek; n = 15), group 2 (Ormco; n = 15), group 3 (Captain Ortho; n = 15), group 4 (negative control; n = 10), and group 5 (positive control; n = 10). The wires were placed in test tubes containing 10 ml of Brain Heart Infusion (BHI) broth to detect bacterial growth. Various biochemical and microbiological tests were conducted to analyze and identify the bacteria present. Archwires showing microbial contamination were then subjected to 70% ethanol to assess disinfecting efficacy.
Results-Microbial contamination was detected on archwires across all four groups. Identified bacteria included *S. aureus*, *S. epidermidis*, *E. coli*, *Klebsiella*, *Bacillus cereus*, and *Acinetobacter*. Microbial growth was observed in 20 of the 65 archwires, with 3M Unitek having the least contamination. Upon disinfection with 70% ethanol, *Staphylococcus species and E. coli* were effectively eliminated, while Bacillus remained resistant.
Conclusion-Orthodontic archwires from three manufacturers exhibited high bacterial contamination. While 70% ethanol effectively eliminated some microorganisms, it did not eliminate all. Hence, it is advisable to adopt suitable disinfection or sterilization methods in clinical practice before placing orthodontic archwires in the oral cavity.

Keywords: Disinfection, microbiology, orthodontic archwires

Introduction

Fighting infections in dental offices presents a persistent challenge due to microbial evasion of safety measures, increasing risks for both patients and professionals. Orthodontic treatments, aimed at enhancing dental aesthetics and function, involve appliances that hinder oral hygiene, promoting microbial adhesion and plaque formation. These conditions contribute to gingival inflammation and dental lesions. Unlike most dental instruments, orthodontic archwires, brackets, bands, and impression trays often arrive from manufacturers without sterilization, relying on assumed hygiene during production and transit. They are used as received from manufacturers, a practice that can lead to contamination during storage and handling in clinical settings, necessitating improved sterilization protocols to ensure patient safety. Additionally, the packaging labels for these materials typically do not indicate whether sterilization is required before clinical use. Although they are sealed in plastic containers, these containers do not always protect the contents from environmental contamination during transport and storage.

Orthodontic archwires are essential in orthodontic treatments, exerting mechanical forces through brackets to align teeth and maintain their positions. Vivek *et al* [1]. found high microbial contamination in 140 brackets from different manufacturers, including *Staphylococcus aureus*, *S. epidermidis*, and *Klebsiella*. However, there is limited research on bacterial evaluation of orthodontic archwires. This study aims to address this gap by assessing bacterial contamination on orthodontic archwires. Several studies [1, 10, 13] have investigated sterilization techniques for orthodontic archwires, including autoclaving, dry heat, and UV radiation. However, these methods are both time-consuming and costly, prompting consideration for alternatives like chemical sterilization or cold sterilization. 70% ethanol, widely available as a disinfectant, is considered effective for sterilizing orthodontic materials. It acts by denaturing proteins and lipids, disrupting cell membranes, and exhibiting bactericidal, fungicidal, and virucidal properties. However, it has limited effectiveness against bacterial spores, which is a notable drawback.

This study was designed to evaluate the sterility of new orthodontic archwires and detect any pathological microorganisms present. Given the easy accessibility of 70% ethanol, this research also assesses its efficacy as a disinfectant against both gram-positive and gram-negative

bacteria. Therefore, the study's purpose was to analyze microbial contamination on orthodontic archwires received directly from different manufacturers and evaluate the decontaminating efficacy of 70% ethanol using microbiological and biochemical tests.

Materials and methods

The in *in vitro* microbiological and biochemical investigations for this study were conducted at the Department of Orthodontics and Dentofacial Orthopedics at Pacific Dental College & Hospital, Debari, Udaipur. The study received approval from the institutional review board of Sai Tirupati University, Udaipur. The sample size was determined using G*Power software version 3.1, based on a study by Vivek *et al* [1], which indicated the need for 65 samples. Samples were collected from three commercially available brands.

The inclusion criteria were-1) Orthodontic archwires of same cross-section area (upper 0.017 x 0.025) 2) Type- NiTi archwires 3) Archwire packets straight from manufacturers (Ormco, 3M unitek, Captain ortho). Exclusion criteria were-1) Wires from open packets 2) Deformed wires.

The archwires were divided into 5 groups, which include

- Group 1-3 M Unitek (n=15)
- Group 2-Ormco (n=15)
- Group 3-Captain ortho (n=15)
- Group 4-Negative control group, Captain ortho archwires were sterilized in an autoclave, n=10
- Group 5- Positive control group,3 M Unitek archwires were contaminated with *S. aureus*, n=10)

All the samples were subjected to microbiological tests to determine the presence of bacterial growth and biochemical tests to identify the type of bacteria.

Microbiological tests

Packets from manufacturers were opened in the lab. Sterile tweezers were used to remove one wire from each packet, and a 1 cm distal end was cut using a sterilized cutter. These wire segments were immersed individually in test tubes containing 3 ml of sterilized brain heart infusion (BHI) broth and placed in an incubator for 48 h at 35 °C to evaluate the bacterial growth. The bacterial growth was assessed based on changes in the color/turbidity of the medium in each of the tubes. The tubes that were positive for bacterial growth were further subjected to biochemical analysis.

Biochemical tests

Biochemical analysis was performed for the tubes that exhibited bacterial growth. The organisms were grown on Blood and MacConkey agar medium using a streak plate technique. Later, the agar plates were incubated at 35 °C for 48 h. The colonies were counted in the colony counter from agar plates and the plates displaying growth of colonies were subjected to the gram staining protocol. The colonies were observed under a microscope to differentiate between gram-positive and gram-negative bacteria. Based on the morphological characteristics of the bacteria in each sample, the primary identification of bacteria was done and then they were subjected to biochemical tests for identification. Catalase test, coagulase test and indole test were performed to identify different gram-positive and gram-negative isolates involved in the contamination of the brackets.

Disinfection

In the second part, archwires were disinfected with 70% ethanol prepared by diluting 99.9% ethanol in a 7:3 ratio. Each wire was wiped with a sterile cotton pellet containing 10 drops of 70% ethanol, three times back and forth. The wires were then immersed in 3 ml BHI broth and incubated at 37°C for 48 hours, followed by repeating the above procedures.

Data recording and analysis

Each archwire was uniquely identified (1A to 1O for 3M Unitek, 2A to 2O for Ormco, and 3A to 3O for Captain Ortho) with 15 samples per company. Corresponding test tubes were labeled similarly to prevent errors. Colony counts were performed using a Labcare™ automated colony counter, and data was compiled into an Excel spreadsheet. The analysis compared microbial contamination among groups, with results presented in tables. A supplementary table detailed isolated organisms per group, visualized through pie charts. Additionally, a graph illustrated the effects of 70% ethanol on the organisms present on orthodontic archwires.

Fig 1: Orthodontic archwires from 3 different manufacturers – 3M Unitek, Ormco, Captain

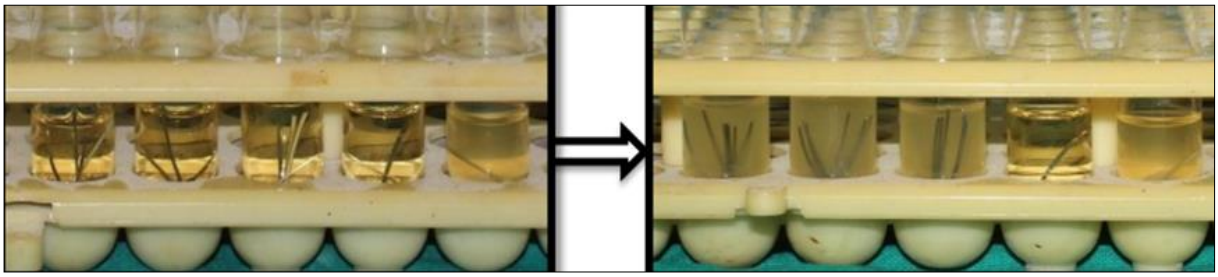


Fig 2: Bacterial growth was assessed based on changes in the Turbidity of the medium in each tube

Fig 3: Plates showing colonies

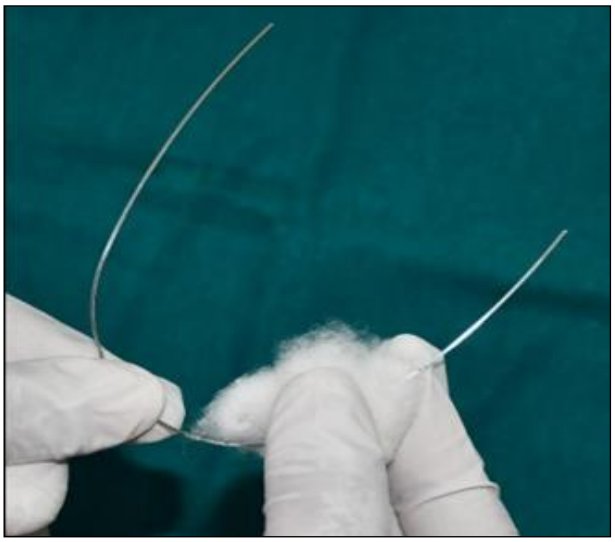


Fig 4: Orthodontic archwire being disinfected using a sterile cotton swab saturated with 70% ethanol

Results

The microbiological tests revealed that none of the samples in group 4 showed any darkening or turbidity of the BHI medium, indicating no bacterial growth. Conversely, all specimens in group 5 exhibited a darkened medium, confirming bacterial presence. Group 3 had the highest number of contaminated archwires, while group 1 had the least contamination (Table 1).

The microorganisms isolated in group 3 were higher compared to those in groups 1 and 2 The most predominant microorganisms isolated from groups were *Staphylococcus epidermidis*, *Staphylococcus aureus* followed by *Klebsiella pneumoniae* and *Bacillus cereus*. (Table 2).

The table 3 demonstrates the efficacy of 70% ethanol on microorganisms found on orthodontic archwires from all the 3 groups. *Staphylococcus aureus* and *S. epidermidis* were completely terminated across all groups. *Klebsiella* showed reduced counts to 40% in groups 2 and 3, indicating partial resistance. *Bacillus cereus* showed no reduction in groups 2 and 3, indicating resistance. *E. coli* was completely terminated in group 2. *Acinetobacter* exhibited an 80% reduction in group 3. Overall, ethanol was highly effective against *Staphylococcus* species and *E. coli* but less effective against *Klebsiella*, *Bacillus cereus*, and *Acinetobacter*.

Table 1: Comparison of Microbial contamination between the different study groups

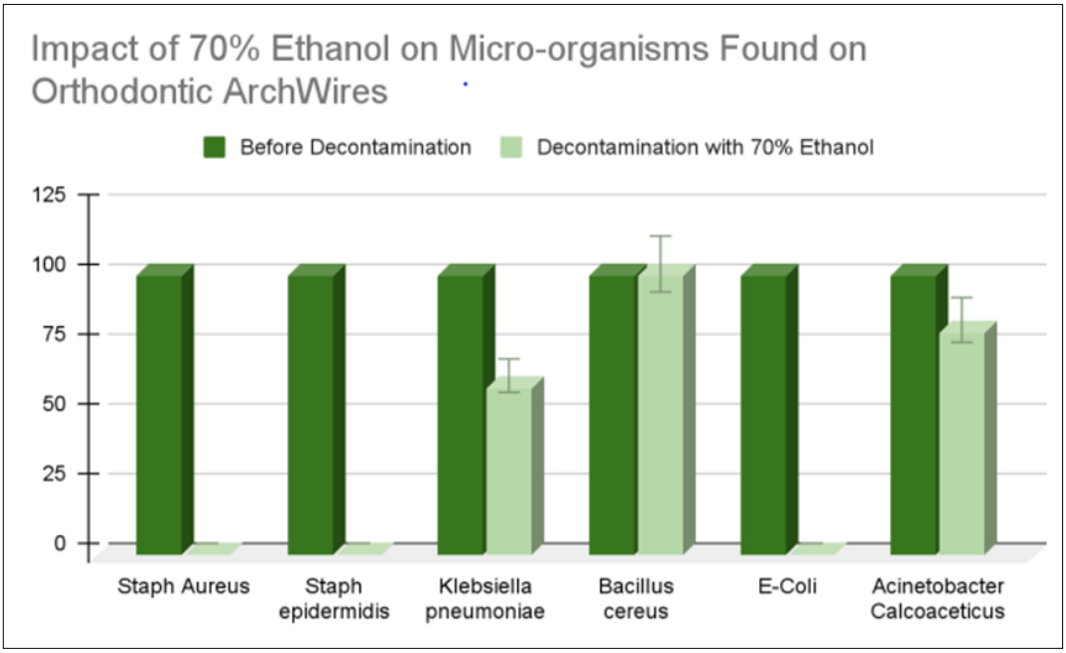
Groups	Microbial Contamination	
	Absent, n (%)	Present, n (%)
Group I (15) 3M Unitek	13 (86.7%)	2 (13.3%)
Group 2 (15) ormco	8 (53.3%)	7 (46.6%)
Group 3 (15) Captain Ortho	4 (26.6%)	11 (73.3%)
Group 4 (10) Negative Control Group	10 (100%)	
Group 5 (10) Positive Control Group		10 (100%)

Table 2: Micro-Organisms isolated from the different groups

Organisms	Group 1, n (%) 3M Unitek	Group 2, n (%) Ormco	Group 3, n (%) Captain Ortho
Staphylococcus Aureus	1 (6.6%)	3 (20%)	4 (26.6%)
Staphylococcus epiderm idis	1 (6.6%)	1 (6.6%)	3 (20%)
Klebsiella pneumoniae		2 (13.3%)	2 (13.3%)
Bacillus cereus		2 (13.3%)	2 (13.3%)
E-Coli		2 (13.3%)	
Acinetobacter Calcoaceticus			3 (20%)

Table 3: Impact of 70% Ethanol on Microorganisms found on Orthodontic Archwires among different groups

	Group 1 (3M Unitek)		Group 2 (Ormco)		Group 3 (Captain Ortho)	
	Mean Bacterial Count (cfu/ml) before and after decontamination with 70% Ethanol					
	Before	After	Before		Before	After
Staph. Aureus	3.1×10^3	o	1.04×10^3		4.2×10^3	
Staph. Epidermidis	2.4×10^3	o	3.2×10^2	o	4.8×10^2	
Klebsiella pneumomae			2.5×10^3	1.5×10^3	4.4×10^3	2.6×10^3
Bacillus Cereus			1.77×10^3	1.77×10^3	5.8×10^2	5.8×10^2
E.C01i			3.15×10^1			
Acinetobacter calcoaceticus					9.3×10^2	7.5×10^2



Graph 1: Impact of 70% Ethanol on Micro-Organisms Found on Orthodontic archwires

Discussion

Infection by microorganisms is a significant concern for dental practitioners, highlighting the need to sterilize all materials before use in the oral cavity. However, orthodontic wires and brackets are often used directly from the manufacturer's packaging. Assessing the potential contamination of these materials is crucial for establishing sterilization protocols to protect patient and practitioner health.

This study aimed to evaluate the bacterial load on orthodontic archwires from three different manufacturers and determine the efficacy of 70% ethanol in eliminating microbial contamination, Ensuring the safety and efficacy of orthodontic treatments while minimizing infection risks associated with orthodontic appliances is essential.

In this study, bacterial contamination of orthodontic archwires was assessed through aerobic culture methods and key biochemical reactions. In contrast, Gerzson *et al* [2]. used DNA extraction and PCR for bracket sterility, offering more sensitive and accurate detection. Similarly, Purmal *et al* [3]. employed DNA extraction, PCR, and sequencing to analyze microbial contamination of orthodontic buccal tubes. While PCR offers high sensitivity and specificity, it is technique-sensitive, expensive, and requires expert knowledge, posing practical and financial challenges for routine clinical implementation.

The samples in this study consisted of orthodontic archwires (nitinol) from 3M Unitek, Ormco, and Captain Ortho. Results showed bacterial contamination on archwires as received from manufacturers, confirming findings from previous studies on different orthodontic appliances. Purmal *et al* [3]**.** Reported biological contamination of orthodontic buccal tubes, and Vivek *et al* [1]. Found microbial contamination on brackets from four manufacturers. This contamination likely results from unhygienic practices during manufacturing, packaging, and transportation. None of the three manufacturers provided labelling indicating sterility or instructions for sterilization before clinical use, contributing to the potential for contamination.

In this study, we observed significant differences in microbial contamination among archwires from three manufacturers. Specifically, 3M Unitek exhibited low levels of contamination (13.3%), compared to Ormco (46.6%) and Captain Ortho (73.3%). This discrepancy could be attributed to packaging methods: 3M Unitek's archwires were individually packaged, reducing cross-contamination risk, whereas Ormco and Captain Ortho packaged their archwires in groups of ten, increasing contamination likelihood. Similarly, Hassan Suha Saad *et al* [4]. found varying contamination levels in archwires from different companies, suggesting that packaging methods play a crucial role in microbial contamination. Hence, manufacturers should improve packaging quality to minimize contamination risks.

In this study, microbial contamination was detected on orthodontic archwires. The predominant micro-organisms isolated were *Staphylococcus epidermidis* and *Staphylococcus aureus*, likely from skin contact during manufacturing or packaging. These findings are similar with

studies by Azeredo Fabiane *et al* [5]. who found various *Staphylococcus species* on orthodontic pliers and Dos Santos Gerzson *et al* [2]. who identified similar bacteria on orthodontic brackets. Barker *et al* [6]. Also found significant contamination on orthodontic materials including archwires and brackets mainly due to skin contact during handling.

Staphylococcus epidermidis, a common skin flora, can cause endocarditis, particularly in individuals with prostheses or immunosuppressed conditions, according to Levinson and Jawetz [7]. *Staphylococcus aureus*, found in all archwire groups, is pathogenic due to its extracellular factors and toxins, leading to serious conditions like endocarditis and osteomyelitis. Oliveira *et al* [8]. Highlighted that these pathogens in biofilms can cause nosocomial pneumonia.

Klebsiella pneumoniae and *Bacillus cereus* were frequently isolated from groups 2 and 3, while *Escherichia coli* was found in group 2, and *Acinetobacter calcoaceticus* in group 3. Rastogi *et al* [9]. found similar results, noting higher contamination in benchtop-exposed materials compared to those received directly from manufacturers, with bacteria like *Klebsiella* and *E. coli. Klebsiella pneumoniae*, a respiratory pathogen, often transmits through contaminated hands in healthcare settings. *E. coli*, a common gastrointestinal colonizer, causes various infections, including UTIs and pneumonia. *Bacillus cereus* is associated with foodborne illnesses and nosocomial outbreaks in immunosuppressed patients.

Anil Ardeshna *et al* [10]. found bacterial contamination including *Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae, Bacillus licheniformis*, and *Bacillus cereus* on orthodontic appliances. Both 70% ethanol and 2% glutaraldehyde effectively eliminated all micro-organisms However, discrepancies emerge with our study as *Bacillus cereus* persisted with ethanol, and *Klebsiella pneumoniae* showed reduced susceptibility in our study.

Madhu Sudhan and Saqib Hassan *et al* [11]. compared glass bead sterilization and 70% ethanol on molar bands. Glass bead sterilization achieved 100% microbial growth absence, whereas 70% ethanol didn't completely eliminate microorganisms, similar to our findings with Bacillus cereus spores persisting despite ethanol treatment.

The ineffectiveness of 70% alcohol against *Bacillus cereus* spores may be due to their protective spore coat, which hinders alcohol penetration. In contrast, glass bead sterilization uses high temperatures to disrupt bacterial proteins, while 2% glutaraldehyde penetrates cell walls and cross-links proteins, effectively killing bacteria. The observed differences and similarities in the effectiveness of disinfectants may be attributed to their different mechanisms of action or variations in contact time with the disinfectant.

Vivek *et al* [1]. found that lower concentrations of chlorhexidine effectively eliminated gram-positive but were less effective against gram-negative strains like *Klebsiella pneumoniae*, indicating concentration-dependent efficacy compared to 70% ethanol.

Studies by JA Staggers *et al* [12]., C. Pernier *et al* [13]., and Sridhar Kannan *et al* [14]. Confirmed that autoclaving is consistently effective for completely decontaminating various orthodontic archwires.

In this study, we found that while 70% ethanol eliminated some bacteria, it was not universally effective. In contrast, 2% glutaraldehyde, glass bead sterilization, and autoclaving were more reliable methods. Chlorhexidine's efficacy varied with concentration, with higher concentrations proving more effective than 70% ethanol. Despite its limitations, ethanol was chosen for its efficiency, cost-effectiveness, and ease of use in daily routines for disinfection purposes.

Our findings underscore the need for rigorous sterilization of orthodontic materials straight from manufacturers to prevent cross-contamination among patients. Proper sterilization protocols are essential to ensure patient safety before clinical use.

However, this study has limitations. The small sample size may limit generalizability, and the focus on aerobic cultures excludes anaerobic microorganisms. While 70% ethanol was effective as a disinfectant for orthodontic archwires, exploring alternative sterilization methods is needed. Future research should use larger samples and advanced techniques like PCR for comprehensive bacterial identification.

Conclusion

1. Microbial contamination was found on orthodontic archwires from all three manufacturers: Group 1 had the least contamination, followed by Group 2 and Group 3.
2. Microbiological and biochemical tests confirmed the presence of bacteria including *Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae, Bacillus cereus, E. coli and Acinetobacter calcoaceticus* on the orthodontic archwires.
3. The study demonstrated that 70% ethanol effectively eliminated *Staph. Species* and *E. coli* from the orthodontic archwires, indicating its potential as a disinfectant. However, *Klebsiella pneumoniae, Acinetobacter calcoaceticus*, and *Bacillus cereus* showed persistence despite treatment with ethanol.

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