SSRG International Journal of Medical Science
ISSN: 2393–9117 / https://doi.org/10.14445/23939117/IJMS-V10I4P101

Volume 10 Issue 4, 1-7, Jul-Aug 2023


*Original Article*

# The Effectiveness of Glass Bead and Glutaraldehyde in Sterilizing the Endodontic Files Contaminated with Aerobic Microorganism: A Comparative Study

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Received: 29 May 2023 Revised: 03 July 2023 Accepted: 15 July 2023 Published: 26 July 2023

***Abstract*** *- In order to maintain asepsis and avoid cross-contamination from one person to another or tooth to another, sterilizing endodontic equipment is essential. In this work, the effectiveness of glass beads and glutaraldehyde in sterilizing endodontic files contaminated with infected pulp and dentinal tissue is assessed and compared. The diseased root canal contaminated samples of endodontic k-files. The samples were then aerobically delivered to the lab. It was determined that the files were microbiologically contaminated by incubating the files for 72 hours under aerobic conditions. The files were divided into three groups and sterilized as necessary while being carefully cleaned, dried, and sterilized using glass beads, glutaraldehyde, and an autoclave (as a control). After the instrument shaft was detached from the handle using a sterile autoclaved wire cutter, all sanitized endodontic K-files were put in separate test tubes containing 2.5 mL of Buffered Peptone Water to look for any microbial growth. Vortexing was followed by surface plating on Tryptic Soy Agar (TSA) plates, which were then incubated for 72 hours under aerobic conditions. Colony counting was used to assess the efficiency of the endodontic file sterilization methods. The findings showed that 6 of 31 (19.4%) files sterilized with glass beads and 7 of 31 (22.6%) samples sterilized with glutaraldehyde solution showed signs of microbial growth. The samples that went through autoclaving (the control) showed no signs of microbial development. Furthermore, there was no statistically significant difference in the sterilizing effectiveness of glass beads and glutaraldehyde. It was established that the endodontic files contaminated with infected pulp and dentinal tissue may be sterilized using glass beads and glutaraldehyde with comparable success.*

***Keywords*** *- Sterilization, Glass bead, Glutaraldehyde, Colony, Aerobic microorganism.*

## 1. Introduction

The root canal is cleaned and shaped with root canal files. Removing the infected pulp tissue, followed by removing soft dentine and eradicating germs from the root canal, is the goal of root canal cleaning and shaping.[1] According to earlier research, an infected root canal is always home to a large number of organisms.[2]Due to the close proximity of the tissues, microorganisms may travel from an infected tooth to a healthy pulp and enter the major and lateral canals, infecting a nearby tooth.[3] The oral cavity contains close to 700 different bacterial species, with any given person containing 100–200 of them.[4] Numerous bacteria are connected to intra-radicular, extra-radicular, and chronic infections.[5]

Stainless steel K-Type reamers and files, which come in two main designs—K-type instruments (K-files and K reamers) and Hedstrom files—are typically used to widen the root canal. These instruments could have various spiral patterns and cutting flute designs.[6] When compared to a file or reamer with a square blank, a triangular cross-sectioned file exhibits greater cutting and increased flexibility. The majority of times, the file is used, and the root canal is being filed or rasped with little to no rotation.[7] Although endodontic files are thin, tapered devices that are 21, 25, and 31 mm long, it is challenging to completely remove all of the biological material during resterilization operations because of the variances in their size and shape.[8] Microorganisms that are both aerobic and anaerobic can contaminate K- File.[9] Therefore, sterilization methods (such as autoclave, glass-bead, and 2% glutaraldehyde solution) have been employed in dentistry for a long time and have been assumed to eliminate bacteria from k-files. According to numerous research, 10 Sterilized files are thought to successfully clean, shape, prevent cross-infection, and get rid of bacteria.[2]

One of the older techniques is the glass-bead sterilizer, which is still used to sterilize hand instruments at the chair side, especially the working end of endodontic files.[11] In the dental clinic, it is also a typical quick method for sterilizing endodontic files while they are being used in the chair.[12] It frequently employs table salt, which contains sodium silicoaluminate, sodium carbonate, or magnesium carbonate in amounts of about 1% each. This causes it to pour more easily and not ignite when heated.[13] Salt can be substituted with glass beads; however, the beads must have a diameter of less than 1 mm because larger beads are ineffective at transferring heat to the endodontic instruments.[14]Heat transfer is prevented by the beads' wide air gaps between them.[11] At 437-465 $^0$F (260 $^0$C), the files can be disinfected in 5 to 15 seconds.[13] The bead sterilizer was used by several researchers between 1950 and 1970 to sanitize their samples in a matter of seconds completely.[13] Using a glass bead sterilizer for 45 seconds at 240 $^0$C and wiping the files with alcohol-soaked cotton contaminated with a readily accessible bacillus, some researchers discovered full sterility.[15]

Nevertheless, their effectiveness against polymicrobial endodontic infection is still unclear. Activation of chemical sterilization by 2% Sterilization can be achieved using a glutaraldehyde solution.[16] To achieve the full sporicidal effect, the immersion period must be lengthy (8–12 hours).[17] Glutaraldehyde baths must be covered and left in well-ventilated rooms due to their offensive smell and low toxicity.[10] Due to its strong smell, glutaraldehyde should be carefully washed off instruments with sterile distilled water before use.[18]Glutaraldehyde (more than or equal to 2%) likewise quickly eliminates the poliovirus, but it takes 40 to 60 minutes or longer to eradicate the tuberculosis germs.[19] They can also get rid of bacteria from the endodontic K file made of stainless steel.[20] Among glutaraldehyde's benefits are the fact that it will not corrode stainless steel and will not harm rubber or plastic surfaces.[10]

The high effectiveness of moist heat penetrating deeper than dry heat makes the autoclave the most effective way to sterilize endodontic files.[13] Typically, steam sterilization refers to the application of heat in an autoclave using saturated steam at a 15 PSI pressure to reach a minimum core temperature of 121 $^0$C. After the instruments being sterilized reach 121 $^0$C, the time is measured. To avoid infection, each instrument is carefully cleaned with antiseptic solutions before being placed into autoclavable pouches and vacuum sealed.[19] It is not yet known if they are capable of completely eliminating endodontic microbial growth from endodontic files.
Therefore, this study evaluated the effectiveness of glass beads and 2% glutaraldehyde solution in aerobic microbial elimination from endodontic files and compared their effectiveness with autoclave (as control) sterilization.

## 2. Materials and Methods

This quasi-experimental study was conducted jointly in the Department of Conservative Dentistry and Endodontics and at the Biological Hazard and Health Research Laboratory, Centre for Advanced Research in Sciences, University of Dhaka, Dhaka-1000, during the period of 12 months (September 2021 to August 2022).

### *2.1. Sample*

We used endodontic K-files with a 2% taper and a size 25 length. For the purpose of choosing endodontic files, the purposive sampling method was employed. The files that met the inclusion requirements were chosen for contamination from the diseased canal. The endodontic files were allocated into six groups by block randomization using a lottery approach after contamination. Participants with chronic periapical periodontitis of pulpal origin, infected root canals caused by pulp necrosis, and chronic periapical abscesses were chosen. Other inclusion criteria were large, straight root canals with sterile endodontic k files (size 25, 25 mm length, and 2% taper). Root canal therapy was performed on purpose because of a fixed prosthesis or overdenture, trauma to the critical pulp tissue. The study eliminated subjects with calcified root canals, bent canals, and broken endodontic files.

### *2.2. Study Labeling*

The following labeling was employed for simple clinical and laboratory tasks during plate spreading, dilution, incubation, and sterilization for aerobically transported materials (01A, 02A, up to 35A). Then, each group was split into three 35-file subgroups, one for glass-bead aerobics (1.2A, 2.2A up to 35.2A), one for aerobics using 2% glutaraldehyde solution (1.3A, 2.3A up to 35.3A), and one for aerobics using an autoclave (control) (1.1A, 2.1A up to 35.1A). Throughout the study's clinical and laboratory procedures, this labeling was applied.

### *2.3. Transport Media and Petri Plate Preparation*

There were two different kinds of media prepared: one was for transportation, and the other was for culture. HiMedia (Model Number M614-500G) created a transport medium from commercially available Buffered Peptone Water. The following manufacturer recommendations, 20 grams of powder were thoroughly mixed with 1000 mL of distilled water. To preserve the sample aerobically and transfer it to the lab, 2.5 mL of autoclaved screw cap tubes were filled to a capacity of 5.

All of the screw cap tubes were then sterilized by being autoclaved at 15 PSI pressure (121 °C) for 15 minutes in a big beaker, and they were then incubated at 37 °C overnight in an incubator. They were then inspected for contamination, parafilm sealed, placed inside a ziplock bag, and stored in a refrigerator between 2 and 4 °C until needed. Liofilchem created Tryptic Soy Agar using commercially available Tryptic Soy Agar. Following manufacturer recommendations, 20 grams of powder were thoroughly mixed with 500 mL of purified/distilled water. The bottles were then autoclaved for 15 minutes at 121 °C and 15 PSI of pressure. They were autoclaved and then kept fresh in a 45 $^o$C drier before being put into Petri dishes.

Spreading BPW was done on commercially available plastic Petri plates, which were also examined for microbial development both before and after sterilization. The plastic package was sprayed with 70% ethanol after being removed from the 500-piece cartoon. They were then moved into Biosafety Cabinet II while being ventilated. After that, the plastic bag was exposed to UV rays for 30 minutes to sanitize the surface. Plates were then unsealed, placed into Biosafety Cabinet II, and each dish received 20 mL of TSA at a temperature of 45 ºC. The TSA plates were given enough time to dry. After labeling, all plates were stored overnight at 37 ºC in an incubator. All incubated plates were examined the following day in Biosafety Cabinet II under suitable lighting for signs of contamination. A plate was discarded if any contamination was discovered on it. After that, the leftover TSA plates were stored for use at 2-4 °C in a refrigerator.

### *2.4. Sample Contamination*

In order to contaminate the endodontic files, patients who were receiving root canal therapy for infected teeth were sought out. Each participant's permission was sought after providing the specifics. For standardization for each diseased root canal, all of the endodontic files (K-files of sizes 10, 15, 20, and 25mm long utilized in this study) were pre-sterilized in an endodontic pouch by autoclaving for 30 minutes at 121 °C at 15 PSI. With a rubber dam, isolation was first accomplished. Each tooth's access cavity was constructed in accordance with the anatomy of the root canals after adequate isolation.

To make it easier to insert a number 25 K-file approximately up to the apical third of the root canal, as was presumed from the pre-operative radiograph, the canals were negotiated successively with numbers 10, 15, and 20 K-files after the access cavity had been prepared. Then, a 25-K file (a total of 3 pieces for aerobic transportation) was put into the root canal using a watch winding motion. When the file was pulled out, the canal walls of each tooth were rasped, and then these files were sequentially transferred to screw-cap tubes. The samples (K-files) were then immediately put into a test tube with a screw lid that held 5 mL and almost 2.5 mL of autoclaved buffered peptone water for aerobic transit. After that, the tubes were parafilm-sealed, tagged, and stored in a cocksheet tube holder. Afterwards, the holder was placed inside a zip-lock plastic bag, which helped transfer the k-file samples aerobically and within two hours to the lab.

### *2.5. Sample Processing*

In the lab, plastic surfaces were cleaned with 70% ethanol and tissue paper was placed atop a zip-lock plastic bag. The plastic bag was then transported into the biosafety cabinet-II, and all of the screw cap test tubes had their plastic bags and parafilm removed. For the uniform mixing of microorganisms into BPW, all tubes were vortexed for 20 seconds. Then, for all samples, a 10-fold dilution was performed using micropipettes (1000 L/1 mL) into a subsequent 5 mL screw cap tube containing 2.25 mL of BPW; for samples that had been transported aerobically, an additional 0.25 mL was added with the aid of a micropipette. Here, sterile tips from autoclaved tubes were utilized to transfer BPW, and each tube's tip was replaced after each transfer.

Before surface plating into TSA plates, the screw cap tubes were vortexed for 20 seconds to ensure that all microorganisms in the samples and BPW were mixed uniformly. Thirty minutes later, all the tubes underwent another vortexing. 0.1 mL of BPW was then applied to the Tryptic Soy Agar plates using a micro-pipette (100 L/0.1 mL) and sterile autoclaved tips. Spreading was done with the sterile glass spreader into the bio-safety cabinet under airflow up until homogenous mixing of 0.1mL solution over solid TSA plates. The plastic Petri plates (90mm 5mm) with the inoculations were then incubated for 72 hours at 37 °C in an incubator to promote the growth of aerobic microorganisms. After 72 hours, colony-by-colony counting was used to validate the presence of the bacteria. To reduce spreading errors, all samples underwent double plating.

### *2.6. Pre-Sterilization Disinfection of Samples*

The contaminated sample files were shaken, whipped, and scrubbed with a brush to remove debris while being submerged in detergent-mixed water separately for each participant and separately for samples that were sent by air. They were then each put into a different beaker that contained autoclaved distilled water individually. Here, they received a thorough rinsing. They were then mechanically dried using sterile gauge pieces. They were then cleaned with a 70% isopropyl alcohol pad (available commercially). Then, using a block randomization and lottery approach, the aerobically transported files were split into three groups: glass bead, glutaraldehyde, and autoclave (used as a control). Three contaminated files were taken from each diseased channel and divided into three groups. In this manner, three aerobic subgroups of 35 files each were created out of a total of 35 blocks (from 35 diseased canals). The files were then given unique group labels.

### *2.7. Sample Sterilization*

Finally, the three groups of files (aerobically transported) were sterilized by three different methods (glass bead sterilizer, 2% glutaraldehyde, and autoclave as control):

Glass-bead sterilization: From a cold start, the glass-bead sterilizer was operational in 60 seconds. Less than 1mm diameter beads were used for the lesser sizes. Life Steriware Glass Bead Sterilizer, the glass bead sterilizer employed in this instance, operated at a temperature of 250 °C. The glass bead sterilizer was turned on once the chosen endodontic files were positioned around the edge of the sterilizer. The files were then sterilized for 45 seconds at 250 °C. The remainder of the procedure was then carried out with the glass bead sterilizer in the bio-safety cabinet.
Utilizing Johnson & Johnson's Cidex Dialdehyde Solution, which contains 2% activated glutaraldehyde, for sterilization: According to the manufacturer's

recommendations, sodium bicarbonate was first added to the solution to activate it. A 10 mL activated solution was added to a 15 mL screw cap autoclaved tube. Each file was then inserted into a different tube, and the tube was labeled. After that, the file containing the tubes was placed in a test-tube stand and maintained in a secure location in the lab for 12 hours. After twelve hours, the files were completely cleansed with autoclaved distilled water and then moved into the biosafety cabinet to complete the process.

All files had the label "autoclave aerobic" on them. After that, the files were separated into endodontic bags and stored in a big beaker. They were then placed into the autoclave (ALP Co, Ltd, Japan) after that. Temperature, pressure, and timing for the autoclaving process were predetermined. After 15 minutes of being static at 121 $^0$C with a pressure of 15 PSI, the temperature began to fall. The beaker was eventually taken out of the autoclave and moved to the bio-safety cabinet to complete the treatment once the temperature dropped below 79 $^o$C.

### *2.8. Final Sample Processing, Spreading Plates, Incubation, and Colony Counting*

The sterile endodontic files (samples) were transported into a 2.5 mL BPW containing screw cap test tube separately for each group, labeled with the date, and vortexed for 20 seconds before surface plating of aerobically transported samples. The shaft of the sterilized endodontic files (samples) was removed from the handle using a sterile autoclaved wire cutter and removed from the handle using sterile tweezers. Each sample was put in its own screw cap tube. Then 0.1mL of BPW was spread out on TSA plates (90mm 5mm) in the presence of airflow, and the plates were incubated for 72 hours at 37 °C in the incubator. The Petri plates were examined for microbial colonies after the incubation period, and a colony count was performed to evaluate the effectiveness of the sterilization techniques. Sterilization was determined to be the most effective method for eliminating the aerobic endodontic bacteria if there was no microbial development. It is possible to conclude that the sample (endodontic file) was still contaminated if there was microbial growth on Petri plates.

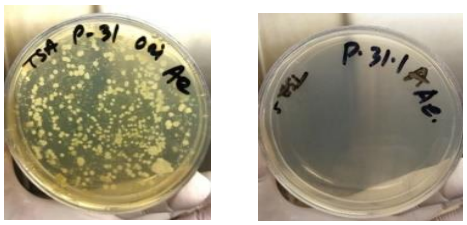
This method allowed for the contamination and sterilization of 105 endodontic files (K-files 25, 25mm long) from 35 diseased root canals for the study. Twelve of them (from four infected canals) had to be discarded because of procedural mistakes (accidentally falling to the floor during washing, slippage from the wire cutter, cutting the shaft of the file without burning the active portion of the wire cutter, completely contaminating petri plates after incubation, etc.). The remaining 93 files were divided into three groups, Glass Bead (B-1), Glutaraldehyde (C-1) and Autoclave (A-1), to interpret the results from the remaining 93 files.

### *2.9. Statistical Analysis*

The proper method and system were used for the computer-based statistical analysis. To gather all the relevant details on selecting diseased teeth, file incubation, and the results were recorded as CFU/mL in the master data sheet. The statistical program SPSS (Statistical Package for the Social Sciences), version 26.0 (IBM, Armonk, New York), was used to analyze the data. Once finished, tables with the data were displayed. The Chi-square test was used to compare the percentages of files that were sterilized using three distinct procedures (two experimental and one control), and a P-value of less than 0.05 was deemed statistically significant.

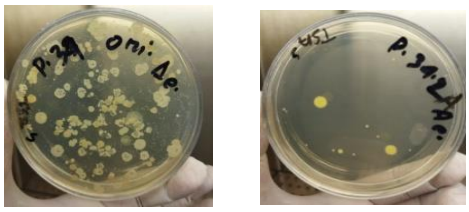
## 3. Results

Endodontic files that were transported aerobically showed no microbiological growth after autoclaving (control), whereas microbial growth was observed in 6 of 31 (19.4%) files sterilized by glass beads and 7 of 31 (22.6%) by glutaraldehyde solution. There was no statistically significant difference in the sterilizing ability between the glass-bead and glutaraldehyde groups for files that were moved aerobically. However, for aerobically carried file sterilization, the differences in sterilizing effectiveness between the autoclave versus glass bead and autoclave against glutaraldehyde groups were statistically significant (Table 1). Figures 1-3 show the microbial growth before and after sterilization. It was found that the sample was totally free of microorganism autoclaving, but remnants of microorganisms were seen even after glass bead and glutaraldehyde sterilization.



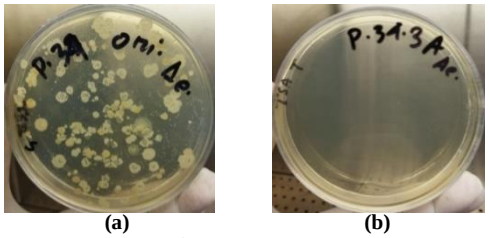
**(a)** **(b)**

**Fig. 1 Microbial growth of aerobically transported sample before (a) and after autoclave sterilization (b)**



**(a)** **(b)**

**Fig. 2 Microbial growth of aerobically transportedsample before (a) and after glass-bead sterilization (b)**



**(a)** **(b)**

**Fig. 3 Microbial growth of aerobically transported sample before (a) and after (b) glutaraldehyde sterilization**

**Table 1. Comparison between the effectiveness of individual sterilization groups in aerobically transported sample (endodontic K-files) sterilization (n=93)**

| Microbial growth pattern | Group | | | Glass bead vs. glutaraldehyde | Autoclave vs. Glass bead | Autoclave vs. glutaraldehyde |
|---|---|---|---|---|---|---|
| | Glass bead (n=31) No. (%) | Glutaraldehyde (n=31) No. (%) | Autoclave (Control) (n=31) No. (%) | p-value | p-value | p-value |
| No growth | 25(80.6%) | 24(77.4%) | 31(100.0%) | $0.755^{ns}$ | 0.009* | 0.004* |
| Growth | 6(19.4%) | 7(22.6%) | 0(0.0%) | | | |
| Total | 31(100.0%) | 31(100.0%) | 31(100.0%) | | | |

*Data were expressed as frequency and percentage*
*p-value obtained by Chi-square test, *significant, ns= not significant*

## 4. Discussion

The effectiveness of sterilizing endodontic files using a glass bead sterilizer, 2% glutaraldehyde solution, and autoclaving (control) was confirmed in the current investigation. Instead of employing commercially available microorganisms (single microorganisms), all the endodontic files were contaminated with diseased pulp and dentinal tissue by polymicrobial endodontic infection from infected root canals.[2] Aerobic cultures were used to test for contamination and sterilizing effectiveness, and colony counts using colony counters provided further assurance.[21] The study's findings supported the difference in efficacy between glass bead, chemical (using 2% glutaraldehyde), and autoclave sterilization of endodontic files. The sterilization rate, however, varied amongst the groups as follows: 31 of 31 (100%) files in the autoclave, 25 of 31 (80.6%) files in glass beads, and 24 of 31 (77.4%) files in glutaraldehyde. Additionally, there was no statistically significant difference between the sterilization efficiency of glass beads and glutaraldehyde. However, there were statistically significant differences between the autoclave, glass bead, and glutaraldehyde. As a result, it can be said that autoclave sterilization was more effective than glass bead and glutaraldehyde sterilization at sterilizing endodontic files.

Rajkumar and Lakshminarayanan,[15] who discovered that autoclaving the files by either putting them in an endodontic instrument box or a synthetic sponge at 121 °C and 15 PSI of pressure and achieved total sterilization, provide support for the conclusion. Hurtt and Rossman,[22,] examined the efficacy of autoclave, glutaraldehyde, and salt sterilization for eradicating Bacillus stearothermophillus contamination from hand files. They came to the conclusion that while autoclaving created germ-free tools, similar to the findings of the present investigation, glutaraldehyde solutions and glass beads could not totally sterilize the endodontic hand files.

The findings were comparable to those of the studies conducted by Hurtt and Rossman[22] (1996) and Sheth et al.[24] but did not coincide with those of Hubbard et al.[23] and Rajkumar and Lakshminarayanan.[15] Furthermore, despite using smaller (less than 1mm) beads that improved heat conduction, the glass bead sterilizer (Group B) failed to sterilize the samples for the aerobic microbes completely.[14] Furthermore, extreme dry heat harms bacteria in their vegetative and spore stages.[25] The findings of the current investigation were comparable to those of the study done by Kumar et al.[20] They claimed that because moist heat penetrates bacteria more effectively than glass beads, the effectiveness of glass bead sterilization was insufficient to sterilize the object completely. Low penetrating heat was also caused by dead air spaces between the beads,[14] which could have led to insufficient sterilization of the glass beads in our investigation. In spite of this, the outcome did not match that of the Rajkumar and Lakshminarayanan investigations.[15] After wiping the files with cotton dipped in alcohol, it was discovered that the glass bead sterilizer (45 seconds at 240 °C) had achieved 100% sterilization.

It was previously established that the mechanism of action for the 2% activated alkaline glutaraldehyde solution's chemical sterilization involved denaturing proteins and alkylating bacterial nucleic acids. The other mechanism of action involves the cross-linking of proteins at the exterior and interior of a bacterial cell, which prevents the movement of materials, the activity of enzymes, and the creation of RNA, DNA, and proteins.[26] The results of the current investigation, which are essentially identical to those of Hurtt and Rossman's earlier work, showed that 24 of 31 (77.4%) files were sterilized by submerging in 2% activated glutaraldehyde solution for 12 hours after following the required pre-sterilization disinfection protocol.[22] Additionally, 77.4% of the files were entirely sterile, which is almost identical to research done by Venkatasubramanian et al.[19], Yenni et al.[12] and Raju et al.[27]. Due to some unidentified bacterial element that may have made the remaining 22.6% of the file resistant to glutaraldehyde sterilization. However, the outcome differed from the Kumar et al.[20] investigations, which claimed that 2% glutaraldehyde demonstrated total sterilization.

The mechanism of autoclaving sterilization was explained by Boyd et al.[28.] They said that whereas

autoclave often killed bacteria by coagulating proteins, it did so only under extreme circumstances. Because less alterations in nucleic acids, enzyme inactivation, and cytoplasmic membrane modification were seen, the microbes likely perished before the coagulation took place.[19]

The current investigation also found that aerobic culture was necessary after sterilization to completely protect the 31 (100%) endodontic files in an endodontic pouch from polymicrobial endodontic microbiota. This study's findings concurred with those of Yenni et al.[12], Raju et al.[27], Rajkumar and Lakshminarayanan [15,] Hurtt and Rossman [22], and Velez et al.[29], who discovered complete sterility by autoclaving after performing aerobic incubation after contaminating with commercially available microorganisms.

The results between glass beads and glutaraldehyde in the event of aerobic contamination were not statistically significant because both demonstrated only partial sterility. Microbial growth was discovered in 6 of 31 (19.4%) glass bead-sterilized files and 7 of 31 (22.6%) glutaraldehyde-sterilized files transferred aerobically. However, there was no microbial development in the autoclave-treated files. It was statistically significant that the autoclave group differed from the glass bead and glutaraldehyde groups.

Several researchers did not agree with the findings of the current study. For instance, research by Al-Jamell et al.[13] revealed that glass beads were 96.74% sterile, while autoclaved sets were 99.66% sterile. According to Kumar et al.[20], other procedures, including using glass beads, were unreliable for the resterilization of endodontic files, while autoclaving and glutaraldehyde (2%) demonstrated full sterilization. The endodontic k-files were contaminated from infected root canals during routine endodontic work during the removal of the infected pulp and dentinal tissue, which may account for the variations between the present study and earlier ones. In the current investigation, files were contaminated by various aerobic, facultative, and obligate anaerobic bacteria due to the polymicrobial nature of the endodontic infection.[30] However, the majority of earlier research was conducted on just one or two particular spores or bacteria. Because some of the germs may be resistant to glass beads (dry heat) or 2% glutaraldehyde (chemical), neither of these two methods could completely eradicate all of these different types of microbes.

Numerous approaches can be used to analyze contamination and sterilization, including turbidity comparison, colony counting in culture procedures, and PCR techniques for molecular detection.[21] However, the efficiency of the frequently used procedures of glutaraldehyde, glass beads, and autoclave sterilization was assessed in the current investigation using aerobic culture followed by colony counting techniques.[31] If only a live organism is present in the sample following sterilizing in culture procedures, microbial growth happens. However, turbidity can develop even in the absence of a living thing, which could explain discrepancies in earlier research findings.[31] Colony counting was done on the Petri plates both before and after sterilization after 72 hours of incubation in aerobic conditions at a temperature of 37 ºC. The incubation duration in other investigations ranges from 1 to 21 days.[27] On the basis of the current and prior investigations, it can be concluded that autoclave effectiveness is not comparable to that of glass beads or glutaraldehyde contaminated with infected pulp and dentinal tissue.

## 5. Conclusion

The study's findings showed that when it comes to sterilizing endodontic files contaminated with infected pulp and dentinal tissue, glass beads and glutaraldehyde are equally effective.

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