Effects of Different Times of Glutaraldehyde 2% on Bacillus subtilis Spores (In Vitro)

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1. Background
Healthcare providers (HCP) are in daily tangency with microorganisms in blood, saliva, laboratory specimens, and specimens which may possibly transfer infectious diseases. Persistent contact with microorganisms has extremely increased the morbidity rate of infectious diseases among HCP contrasted to that of society.¹ It is assessed that 14%–28% of dentists, 13% of assistants, and 17% of healthcare workers (HCWs) have been subjected to the hepatitis B virus (HBV), and more than 200 healthcare providers (HCPs) pass away annually in the United States from HBV infection caught from their work place.² Blood and saliva may port viruses, bacteria, and other pathogens that may reason diseases such as flu, HIV, pneumonia, tuberculosis (TB), HBV, and HIV. This peak the importance of infection rein at the workplace.³⁴ The use of manners to delete or reduction the number of contaminants on instruments, pecimens, and table tops is of higher importance. It is magistral to use bacterial solutions to delete or reduce the number of microorganisms on tools before autoclaving to exclude cross-infection.¹ Reusable tools that are hand washed before processing can be hazardous.⁵

The results showed that the use of the disinfectant substance glutaraldehyde 2% for disinfecting the equipment of a urology operating room with respect to conditions and factors affecting disinfection, such as concentration, reaction time, and the correct disinfection procedures, completely removed Staphylococcus aureus, Citrobacter, Pseudomonas aeruginosa, Escherichia coli, and Klebsiella. Glutaraldehyde 2% was effective on the studied pathogenic
Effects of Different Times of Glutaraldehyde 2% on Pseudomonas and Spores

1. Introduction

Tools should be decontaminated and then sterilized to remove microbes and spores. Omikkhoda et al and Camilla et al evaluated the effects of different disinfection methods on orthodontic pliers. They reported that glutaraldehyde was an acceptable disinfectant agent. Simoes et al studied the effect of glutaraldehyde on the control of mono- and dual-species biofilms of Bacillus cereus and pseudomonas in 2011. Another study done in 2008 by Retta and Saripanti showed the structure of a mathematic model for anticipating the activity of glutaraldehyde in destroying spores. In this model, the effect of glutaraldehyde was calculated exactly by changing factors such as the density of glutaraldehyde, the temperature, and the influence time of the substance.

2. Objective

Tools should be antisepted and then sterilized to eliminate microbes and spores. A literature search revealed no researches existing about disinfection of different instruments by glutaraldehyde 2%; thus, this study evaluated the effects of this solution on Bacillus subtilis spores to determine whether glutaraldehyde 2% can be effective in disinfecting dental settings. Experiments were conducted in 2016 at the OMFS and Microbiology Department of the Dental Branch of Islamic Azad University.

3. Methods

A glutaraldehyde 2% solution was combined in this experimental study. B. subtilis spores ATCC6633 KD were purchased from the laboratory research center. A spore suspension with normal opacity 1×10^8 CFU/ mL was prepared using frustrated physiological serum in accordance with McFarland 0.5 standard. Then a sterilized certain tube was used for preparing the solution (controls). In other tube 2 mL of newly opened flacon of glutaraldehyde 2% was combined to the test tube using a spay pipette and the tube was encased; then 1 mL of the spore solution with the same standard opacity was added to the test tube (test group) containing glutaraldehyde 2%. Because the producing factory claimed that glutaraldehyde 2% kills B. subtilis spores in 60 minutes, experiments were performed on nutrient agar at time intervals of 10, 15, 20, 25, 30, 40, and 60 minutes.

For sampling, a standard ring was Catch fire for 10 seconds and then the specimens were prepared after the loop chilled. A sample from the liquid was taken at each of the aforesaid time – points and the conveyed to nutrient agar (NA) immediately (without contact with glutaraldehyde for controls). Then the cultures were placed in an incubator for 24 hours at 37°C. Accompanying every set of samples into the incubator was a solid culture media. A lack of growth in this media confirmed sterilization.

The variables in this study included a plate of B. subtilis spores, glutaraldehyde 2%, the time of substance (proximity time), growth or lack of growth after contact with the disinfectant, and environment. The data about growth or lack of growth of B. subtilis was surveyed using the Kruskal-Wallis and Friedman tests.

Numeric and qualitative variables were reported as mean (±SD) and count (%), respectively. Kruskal-Wallis and Friedman tests were used for statistical analyses, and P=0.05 was considered statistically significant. SPSS version 17 was also used for statistical analyses.

4. Results

The results indicated that, in all 8 repetitions, at the time intervals of 10, 15, 20, and 25 minutes, 10^2, 18.6 ± 3.4, 6.2 ± 1.4, and 2.1 ± 0.8 colonies grew, respectively; after 30 minutes, no growth was observed. Overall, colonies were numerous up to 10 minutes, and from 15–25 minutes the number of colonies was severely reduced. At 30 minutes, there was no more growth. The procedure for each time interval was repeated 8 times and continued as long as the culture result was positive. The glutaraldehyde used in all procedures came from a single bottle that was opened on the first day. For sampling, a standard ring was Catch fire for 10 seconds and then the specimens were prepared after the loop chilled. A sample from the liquid was taken at each of the aforesaid time – points and the conveyed to NA immediately (without contact with glutaraldehyde for controls) (Figure 1). Then the cultures were placed in an incubator for 24 hours at 37°C. Along with any series, a NA plate was placed inside the incubator and when no growth was looked, agar sterility was confirmed.

5. Discussion

Eliminating or reducing the number of microorganisms from reusable tools before autoclaving is significant in preventing cross-infection; in many offices reusable tools are still scraped before procedures. This may be perilous;
reusable tools should be fumigated and then sterilized via autoclave, gamma beam or ethylene oxide to remove the microbes and insistent spores. The spore is the most insistent from of the microbe. Bacterial spores are among the most insistent of all living cells to biocides. In a 2010 study performed to find a solution of the high level disinfectant aldeol to eliminate mycobacterial and bacterial spores, Miner et al investigated glutaraldehyde and orthophthalaldehyde solutions with different concentrations of alcohol, sodium, and potassium, Gailliet compounds and detergents with an alkaline pH and evaluated them on mycobacteria and bacterial spores in order to eliminate bacteria in an appropriate time and a temperature between 20-25°C. A concentration of less than 20% isopropanol, less than 8% potassium, and potassium acetate in composition with a lower concentration of glutaraldehyde 3.5% with an alkaline pH eliminated $6 \log_{10}$ mycobacteria in 10 minutes and at a temperature of 20°C. Similar solutions eliminated $6 \log_{10}$ B. subtilis in 30 minutes at a temperature of 25°C and in 60 minutes at 20°C. Ortho-phthalaldehyde spore paper properties did not increase in combination with isopropanol and potassium acetate. The researchers also stated that a high level disinfectant with the formulation of glutaraldehyde 5.3% in combination with isopropanol 20% and potassium acetate 8% eliminated mycobacteria in 10 minutes at a temperature of 20°C and $6 \log_{10}$ Bacillus subtilis spores in 60 minutes at 20°C. The results of this study were similar to those of the current study with one difference; in Miner et al, a temperature between 20-25°C was considered, but the current study was done at room temperature.

Da Silva et al evaluated the effects of 6 different disinfectants on the elimination of five species of bacteria and their effects on a resin acrylic surface texture. Performed in 2007, their study aimed to highlight the effects of disinfectant (sodium hypochlorite 1%, chlorhexidine digluconate 2%, chlorhexidine 2%, vinegar 100%, and a denture cleaning pill with a perborate sodium base and perborate sodium 3.8%) in disinfecting mycobacteria and of acetate and potassium acetate. The researchers also stated that a high level disinfectant with the formulation of glutaraldehyde 5.3% in combination with isopropanol 20% and potassium acetate 8% eliminated mycobacteria in 10 minutes at a temperature of 20°C and $6 \log_{10}$ Bacillus subtilis spores in 60 minutes at 20°C. The results of this study were similar to those of the current study with one difference; in Miner et al, a temperature between 20-25°C was considered, but the current study was done at room temperature.

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6. Conclusion

It seems that the density of 2% glutaraldehyde in 30 minutes time was enough to destroy B. subtilis spores. It is suggested that further research be done on higher level densities in order to destroy spores more strongly and quickly. It is also suggested that research be done on the shelf life of the solution and the reduction in its effect over time.

Authors’ Contributions

Study design: LD, MHKM; Data collecting: FN; Data analysis: RA, MR; Manuscript preparation: MHKM, FN, ZD.
Conflict of Interest Disclosures
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval
Not applicable.

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