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Hydrogen Peroxide-Silver 6%: an Effective Eco-Friendly Disinfectant for Contaminated Instruments

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Abstract- *Background and aim:* Healthcare workers are often in contact with micro-organisms present in blood, saliva, laboratory samples and specimens which may potentially transfer infectious diseases. Use of methods to remove and or decrease the number of contaminants on instruments, samples and tabletops are of paramount importance in infection control. Important properties of a good disinfectant aside from the ability to kill microbes include being safe , noncorrosive or damaging to instruments, odorless, colorless, economical and eco-friendly. This study was done to assess effectiveness and longevity of 6% Hydrogen Peroxide-silver for disinfection prior to sterilization.

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Hydrogen Peroxide-Silver 6%: an Effective Eco-Friendly Disinfectant for Contaminated Instruments

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Abstract- Background and aim: Healthcare workers are often in contact with micro-organisms present in blood, saliva, laboratory samples and specimens which may potentially transfer infectious diseases. Use of methods to remove and or decrease the number of contaminants on instruments, samples and tabletops are of paramount importance in infection control. Important properties of a good disinfectant aside from the ability to kill microbes include being safe , noncorrosive or damaging to instruments, odorless, colorless, economical and eco-friendly. This study was done to assess effectiveness and longevity of 6% Hydrogen Peroxide-silver for disinfection prior to sterilization.

Materials and Methods: Using sterilized physiological serum a spore suspension of Bacillus subtilis of 1×10 cfu/ml was prepared on McFarland culture medium. A sterilized capped tube was used for preparation of the suspension (control group); in another tube 2 ml of a newly opened bottle of 6% hydrogen peroxide silver was poured in the test tube using a sterilized pipette and the tube was recapped; then , 1 ml of the spore suspension was added to the test tube (test group). On the first day nine samples were taken (at 30, 60,90, 120, 180, 240,300, 360, and 420 minutes) and repeated three times. Also the same number of cultures were taken from the control groups at all the aforementioned time points . All54 samples (study and controls) were transferred to the culture medium after the required time points elapsed. All cultures were placed in an incubator for 24 hours at 37°C and assessed for growth. This procedure was repeated daily using the same batch of 6% hydrogen peroxide silver for 10 days.

Results: The batch of 6% Hydrogen peroxide silver re-opened once daily was effective on Bacillus subtilis up to 8 days consecutive days use after contact for 30 minutes. There was no effect on day 8 even after 420 min.All controlsamples taken at the same time points showed viability and growth of Bacillus subtilis.

Conclusion: This study indicated that although 6% Hydrogen peroxide silver has a short 30 minutes disinfection time,it has a short-lived shelf life of only 7 days (provided it is opened just once per day). It seems prudent that a fresh bottle be used daily and instruments be soaked longer than 30 for greater guarantee of disinfection prior to sterilization.

Keywords: hydrogen peroxide silver 6%; bacillus subtilis; disinfection.

I. Introduction

Healthcare providers (HCP) are in daily contact with microorganism in blood, saliva, laboratory samples and specimens which may potentially transfer infectious diseases. Continuous contact with micro-organisms has considerably increased the incidence of infectious diseases among HCP as compared to that of the society (1); 14 to 28 percent of dentists, 13 percent of assistants and 17 percent of percent of HCW have been exposed to HBV (2) and annually more than 200 HCP in the USA die from infection from HBV from their work environment(3) . Blood and saliva may harbor viruses, bacteria and other pathogens that may cause diseases such as flu, HSV, pneumonia, TB, HBV and HIV. ThIshighlights the importance of infection control at the workplace (1,4).

Use of methods to remove and or decrease the number of contaminants on instruments, samples and tabletops are of paramount importance in this regard.

Use of bactericidal solutions removing or reducing the count of microorganisms on instruments before autoclaving is imperative in preventing cross-infection. Reusable instruments that are hand-washed before processing can be hazardous (1). Thus, instruments should be disinfected and then sterilized to eliminatemicrobes and spores (5-7). We found no study available regarding disinfection time or shelf life of 6% hydrogen peroxide silver (HPS). So we decided to study the effect of this solution on spores of Bacillus subtilis to see if 6% hydrogen peroxide silver can be effective in disinfection of dental settings. The null hypothesis was that 6% HPS is not an effective disinfectant for the dental setting.

II. Materials and Methods

Nano-Silver disinfectant an H_2O_2- Based solution (Nano-trade Company, Czech Republic) was assessed in this study. Bacillus subtilis (BS) spores ATTCC6638 KD were purchased as spores with culture medium

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(Brown co, UK, certification by FDA and biologic test standards number 11138). A spore suspension with normal opacity 1×10CFU/ML was prepared using sterilized physiological serum according to McFarland 0.5 standard. Then a sterilized sealed tube was used for preparing the suspension (controls); in another tube 2 ml of a newly opened bottle of 6% hydrogen peroxide silver was poured in the test tube using a sterilized pipette and the tube was recapped; then, 1 ml of the spore suspension with the same standard opacity was added to the test tube (test group) containing 6% HPS.

On the first day samples were taken from the tube after 30, 60, 90, 120,180, 240, 300, 360, and 420minutes and repeated three times. Also the same number of cultures were taken from the control groups at all the aforementioned time points. All 54 samples (study and controls) were transferred to the culture medium after the required time points elapsed. All cultures were placed in an incubator for 24 hours at 37°C and assessed for growth. This procedure was repeated daily using the same batch of 6% hydrogen peroxide silver for 10 days.

On days two, three, four, five, six, seven, eight, nine and ten, each day 54 samples were taken from the tubes containing Nano-silver (from the bottle re-opened once daily since day one) added to BS and incubated for 24h.

For sampling, a standard loop was flamed for 10 seconds and then the samples were prepared after the loop cooled. Each culture sample was repeated three times. A sample from the suspension was taken at each of the aforementioned time-points and then transferred to the nutrient agar (NA) immediately (without contact with Nano-silver for controls). Culture was done using 1 cc of the spore-contaminated solution added to nutrient agar using the linear technique. Then the cultures were placed in an incubator for 24 hours at 37 degree centigrade. Along with each series, a NA plate was placed inside the incubator and when no growth was observed, agar sterility was assured. On day two, the procedure was repeated in the same fashion except that samples were taken from the tube starting at the time it took HPS to kill the spores on day one. This procedure was repeated for 10 days.

III. Results

On the first day, in the 27 samples taken from the tubes containing HPS and BS, no growth was seen on agar in the samples which had 30 min of contact or more; the 27 controls were all positive.

On days two, three, four, five, six and seven, in the 54 samples (27 study and 27 controls) were taken each day; from the tubes containing HPS (from the bottle re-opened once daily since day one) and added to BS, no growth was seen on agar in the samples which had 30 min of contact or more; the 27 controls were all positive on all days.

On day eight, in the 27 study samples (study group) taken from the tubes containing HPS added to BS growth was seen at all time points (30 min and up to 420 minutes), on agar plates 24-48 h later. Growth was observed in all control samples at all stages every day indicating the viability of BS (at all stages). The growth of the control spores showed that the spores were viable; the case spores showed no-growth up until the eighth day. In other words, samples taken on the eighth day showed growth in all samples (24-48 h later on agar plates)even after 420 minutes of contact with the HPS solution. The bactericidal effect of HPS was apparently lost.

IV. Discussion

Destroying or decreasing the number of microorganisms on reusable instruments before autoclaving is important in preventing cross-infection; in many offices reusable instruments are still hand-washed before processing (1). This may be dangerous; reusable instruments should be disinfected and then sterilized via autoclave , Gamma ray or ethylene oxide to kill the microbes and resistant spores. The spore is the most resistant form of the microbe. Bacterial spores are among the most resistant of all living cells to biocides (8).

Many studies have assessed similar solutions (9-16), but we only found one study regarding the shelf-life of Nano-silver disinfectant, an H_2O_2- Based solution (Nano-trade Company, Czech Republic) , with concentration of 2% (17).We sought to study the effect of 6% Nano-silver solution on spores of Bacillus subtilis to see if 6% hydrogen peroxide silver can be an effective disinfectant in the workplace and if so for how long. This solution is for disinfection and not for sterilization. To assess how long it can be used after opening it once a day. Because this solution is natural with no color or odor it does not ruin instruments. It is noncorrosive and is odorless contrary to sodium hypochlorite. It does not stain either. However it is very volatile. There are many brands of disinfectants with different properties on the market; consumers have no idea about their real effectiveness or properties. Nano-silver disinfectant an H_2O_2- based solution with silver ions. Important properties of a good disinfectant aside from the ability to kill microbes includes being safe, noncorrosive, should not cause discoloration or damage to instruments and be economical and eco-friendly. This study was done to assess both effectiveness and longevity of 6% HPS for disinfection. Nano-silver is a newly marketed product and the producer claims that it has high level of disinfection without mentioning its shelf-life. As Nano-silver is an H_2O_2- based solution its effectiveness is based on releasing oxygen and thus, is short-lived.

Silver has strong antibacterial effects and does allow bacteria thrive and reacts with SH groups to create oxidative enzymes. Silver is known to exhibit a strong toxicity to a wide range of micro-organisms. Bactericidal effect of silver ions on micro-organisms is well known, but the mechanism is only partially understood (18). A proposed theory describes that ionic silver strongly interacts with thiol groups of vital enzymes and inactivates them and has extensive use in many bactericidal applications (18).

A recent study was done to assess the shelf-life of 2% hydrogen peroxide-silver, It was indicated that the effect time was 180 minutes and efficacy of the solution was up to 4 days (17), However 6% HPS in our study was more potent and disinfected in a shorter time (30 min) and was effective for a longer time after opening proving that the 6% solution is preferable.

The cultures were taken at 30 to 420 on day one because the exact time of effectiveness on the BS spore was unknown. Lack of growth of BS spores after contact with Nano-silver on day one was 30 min. This time period of half an hour is reasonable for disinfection of instruments comparing to the three hours waiting time of the 2% hydrogen peroxide-silver solution; Additionally , as the disinfection is ineffective after 7 seven days , a new bottle must be opened in less than 7 days to assure safety.

V. Conclusion

Instruments should be soaked in 6% hydrogen peroxide-silver for at least half an hour before being sterilized in an autoclave; 6% hydrogen peroxide-silver will not disinfect after this. Thus this refutes the null hypothesis provided a fresh bottle is used daily for greater guarantee of disinfection in a shorter time period.

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