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Evaluation of Microbial Growth in Dental Operatory: An *in-Vitro* Study

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ABSTRACT

Introduction: There has been recent interest in using chemical fumigation in healthcare facilities due to concerns about the environment's role in causing healthcare-associated infections (HAIs). However, there have been incidents where fumigants have escaped, resulting in illness and even death among exposed workers and the general public. Therefore, it is crucial to thoroughly assess the benefits and risks before expanding the use of potentially hazardous technology in areas where vulnerable individuals are present. ***Aim:*** The objective of this study is to evaluate the microbial growth (CFU/m3) in the dental operatory at regular intervals following fumigation with 3-5% hydrogen peroxide. ***Methodology:*** The dental operatory was fumigated with hydrogen peroxide (3-5%) diluted with 1-1.5L of water for 1-2 hours. After 48 hours of fumigation, a nutrient agar plate was placed in the operatory. Similarly, nutrient agar plates were placed in the operatory after 72 hours, 5 days, and 7 days, with each plate being kept in the operatory for 24 hours. The agar plates were then examined under a microscope to calculate the microbial load at regular intervals, allowing the evaluation of microbial growth in the operatory after fumigation . ***Results:*** Prior to fumigation, the microbial count was 112 cfu/m3. After 24 hours of fumigation, the microbial count reduced to 24 cfu/m3, and after 72 hours of fumigation, it was 72 cfu/m3. ***Conclusion:*** Hydrogen peroxide proves to be an effective disinfectant as it significantly reduces the microbial count in the dental operatory by nearly fivefold. This study highlights the efficacy of fumigation in reducing the microbial count in the environment and lowering patient infection rates, considering the potential risks. Currently, there is a lack of consensus documents regarding safe fumigation exposure.

INTRODUCTION

In the 1960s, chemical fumigation was employed alongside standard environmental surface disinfection in hospital isolation rooms and other critical areas. The environment serves as a significant reservoir for MDRO (multidrug-resistant organisms) [1-3]. These organisms can remain viable on various lifeless surfaces for extended periods, ranging from days to months. Pathogens can be transmitted from the environment to patients directly through contact between patients and the contaminated surroundings, as well as indirectly through the hands of healthcare workers (HCWs). The persistent presence of pathogens in the environment is also believed to facilitate vertical transmission [4-6]. The communities of oral microorganisms, along with their interactions with the host, work to sustain a dynamic equilibrium within the oral microecosystem. Nevertheless, multiple factors can disrupt this balance, leading to dysbiosis of the oral microbiota, which, in turn, plays a role in the development of both oral and systemic diseases[7].

Initially, it was believed that surface disinfection alone was inadequate, and the introduction of a chemical fog would eliminate microorganisms present in hard-to-reach areas. However, over time, this approach fell out of favor due to concerns about its effectiveness [8,9,]. The Centers for Disease Control and Prevention (CDC), in its Guidelines for Environmental Infection Control in Health-Care Facilities, discourages the use of chemical fogging for general infection control in routine patient care areas[10-12].

Following the anthrax bioterrorism attack in 2001, there was renewed

interest in employing fumigants for microbial decontamination. In order to ensure complete eradication of anthrax from buildings, a fumigation technique was employed to eliminate bacteria and their spores [10-12]. Following the anthrax bioterrorism attack in 2001, there was renewed interest in employing fumigants for microbial decontamination. In order to ensure complete eradication of anthrax from buildings, a fumigation technique was employed to eliminate bacteria and their spores [13-15]. Based on the success achieved in eradicating anthrax through fumigation, healthcare officials are considering incorporating this technique in hospitals and similar institutional environments as a supplementary measure to routine cleaning methods [16,17].

Fumigation represents one of the infection control protocols that can effectively limit the transmission of such infections. Additionally, fumigation has been conducted using various other chemicals, including sodium hypochlorite, potassium permanganate, formaldehyde, ozone, superoxidized water (sterilox), glutaraldehyde, and chlorine dioxide, among others [18-20].

There are different systems and disinfectants with different concentrations of hydrogen peroxide on the market for fumigation. In principle, hydrogen peroxide can be applied either as a vapor or as an aerosol. Vapors are generated from a 30% to 35% H_2O_2 solution, whereas for aerosols the concentration is often less, ranging from 5% to 12% H_2O_2 [21,22].

Compared to chlorine dioxide or formaldehyde, hydrogen peroxide presents a lower level of hazard. Hydrogen peroxide functions by generating destructive hydroxyl free radicals that can attack lipid membranes, DNA, and other essential components within cells. Catalase, produced by aerobic organisms and facultative anaerobes possessing cytochrome systems, can shield cells from metabolically produced hydrogen peroxide by breaking it down into water and oxygen. However, this defense mechanism becomes overwhelmed by the concentrations used for disinfection [23,24].

The inactivation of hydrogen peroxide demonstrates a bimodal pattern of killing, wherein low concentrations are presumed to cause damage to DNA, whereas high concentrations result in more severe damage to other cellular components. Also, Aerosolized hydrogen peroxide, is an economical, easy-to-use, and versatile fumigation method. The aim of the present study is to assess the level of atmospheric microbial growth (cfu/m^3) in a dental operatory at regular time intervals pre and post fumigation with 5-7% hydrogen peroxide [25,26].

MATERIALS AND METHODS

Pre-Requisites before Starting the Study

Ventilation should be completely restricted during the fumigation process. The nutrient agar plates must be prepared freshly before use. Only trained personnel should perform the fumigation procedure. The agar plate should not be handled with bare hands; proper precautions should be taken. The agar plate should be positioned one meter above the ground level and one meter away from the wall. Krishnan et al. proposed the utilization of table-top fans to ensure effective distribution of the hydrogen peroxide fumigant.

Types of Fumigators That Are Commercially Available

Thermal Fogging Machine, Mini Fogging Machines, Ulv Fogger (Used in Study) **(Figure: 2),** Medical Fogger Machine, Aerosol Disinfector. A ULV fogger is a cold fogging machine. It uses large volumes of air at low pressure to convert a liquid into droplets that can then be dispersed into the atmosphere. ULV stands for ultra-low volume because of the low volume of fluid that is required to create enough fog to cover very large areas.

METHOD

This experimental study was conducted in the dental operatory of the Department of Conservative Dentistry and Endodontics at SGT University. A nutrient agar plate was freshly prepared in the microbiology lab of SGT Medical College **(Figure 3).** To analyze the baseline atmospheric microbial contamination, a 90 mm diameter nutrient agar plate was exposed in the operatory before the start of the study to estimate the atmospheric microbial load in the dental operatory **(Figure 4).**

A diluted hydrogen peroxide solution with a concentration of 5-7% was prepared. The hydrogen peroxide was aerosolized at ambient room temperature **(Figure 6)** using an ULV fogger. The dental operatory was then fumigated for 60 minutes with hydrogen peroxide, carried out by trained personnel who followed appropriate precautionary measures. After fumigation, the operatory was left undisturbed for 12-15 hours to allow for maximum disinfection.

To evaluate the microbial load in the operatory post-fumigation, nutrient agar plates were placed at different time intervals. The first plate was placed 24 hours after fumigation **(Figure 5),** and the second plate was placed one week after fumigation **(Figure 6).** Each plate was left in the operatory for 12-15 hours. Subsequently, the plates were incubated for approximately 24 hours at 37 degrees Celsius, and the number of colonies on each plate was counted to determine the microbial count.

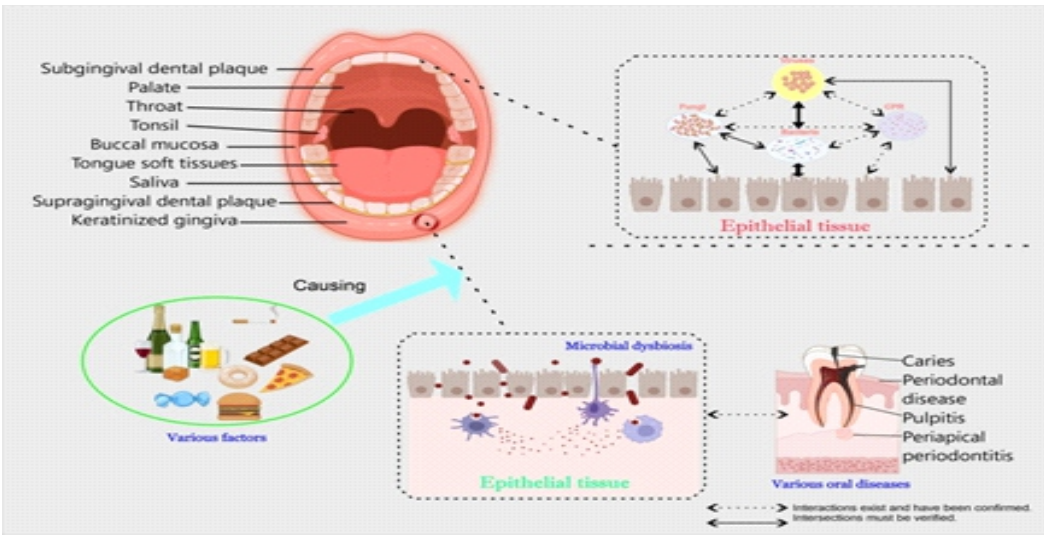

Figure 1: Structures of the well-regulated oral microbiome and its alterations in dysbiosis

Figure 2: ULV fogger

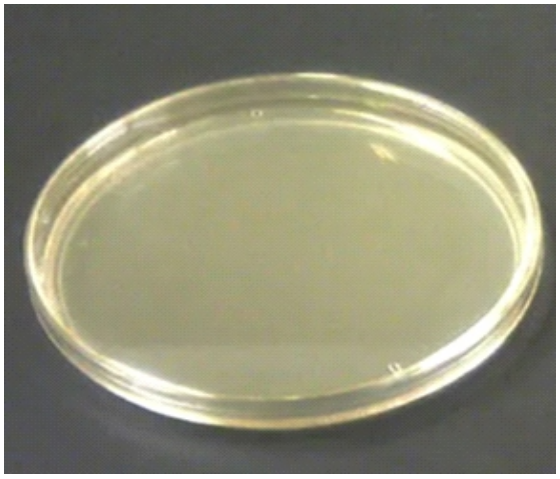

Figure 3: Freshly prepared nutrient agar plate

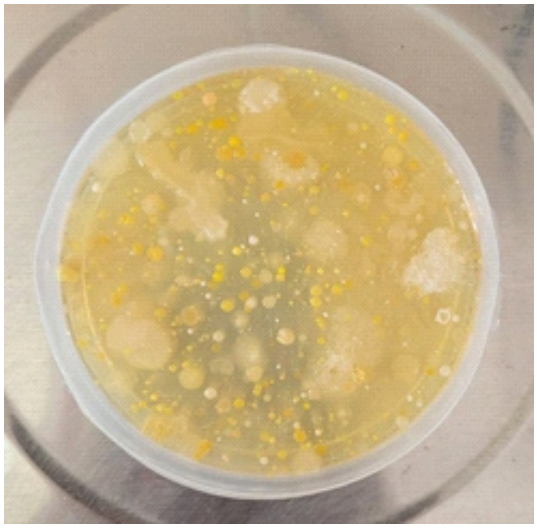

Figure 4: Nutrient agar plate without fumigation

Figure 5: After 24 hours of fumigation

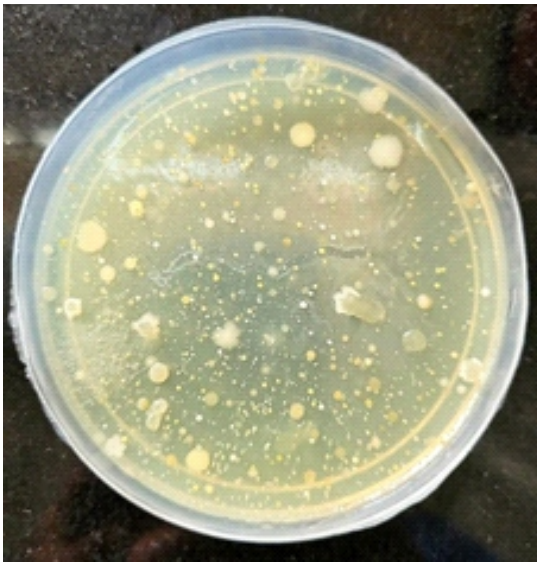

Figure 6: After 7 days of fumigation

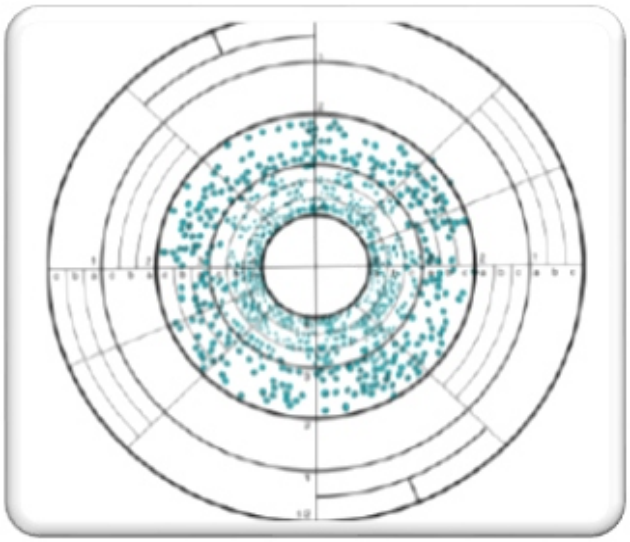

Figure 7: Dividing the plate for microbial counting.

Method of Microbial Counting

Manual microbial counting was performed by observing and counting colonies on the plates under transmitted light. To ensure optimal visibility of colonies, a LED-light source was used due to its high transparency. Care was taken to avoid any heat transfer to the sample during illumination using the light source.

Plates that contained over 200 colonies were typically counted by dividing them into equal sectors, as depicted in **figure 7.** The sectors ranged from 1/2 to 1/8 of the plate. To estimate the total colony-forming unit (CFU) count on the whole plate, one sector was counted, and the count was then multiplied by the total number of sectors.

During the process of colony counting, it is essential to consider factors such as magnification and illumination to ensure accuracy. Adequate magnification should be employed to clearly observe and distinguish individual colonies. Furthermore, proper illumination is crucial for optimal visualization of the colonies.

RESULTS

Data were calculated to obtain to determine values suitable for comparing the data to the scale of the Air Microbial Index (AMI) **(Figure 8).**

No. of cfu/m^3 determining the AMI:

50–25: LOW

26–50: MEDIUM

51–75: HIGH

>75: VERY HIGH

DISCUSSION

Chemical fumigation is currently being applied as a control measure for nosocomial infections in healthcare settings, given the challenges in thoroughly disinfecting rooms and equipment. There is a concern that conventional surface disinfection methods may not effectively reach all surfaces that come into contact with patients, whereas fumigation could reduce the risk of infection transmission [27-29].

Fumigation is being considered due to the ability of gases and vapors to penetrate hard-to-reach areas. However, this characteristic also necessitates the sealing of ventilation ducts, plumbing fixtures, doors, windows, and any other openings with a material that can resist penetration. The feasibility of fumigating leaky rooms with aerosolized hydrogen peroxide has been established [30]. Hydrogen peroxide, despite its limitations, offers clear advantages over other fumigants. It is environmentally friendly and enhances personnel safety since it does not produce toxic end products [31,32]. The short cycle times associated with hydrogen peroxide fumigation result in quick turnaround times, thereby increasing the availability of fumigation zones for users and their intended purposes [33]. However, it is crucial to consider the limitations of hydrogen peroxide as a fumigant, as they will impact the technical installations required in a facility. It is important to note that aerosol generation during fumigation can pose a significant health hazard to dentists and dental assistants. It can potentially cause infectious diseases such as influenza, tuberculosis, meningitis, or severe acute respiratory syndromes [34].

CONCLUSION

The present study provides evidence of atmospheric microbial contamination during dental treatment procedures. It is recommended

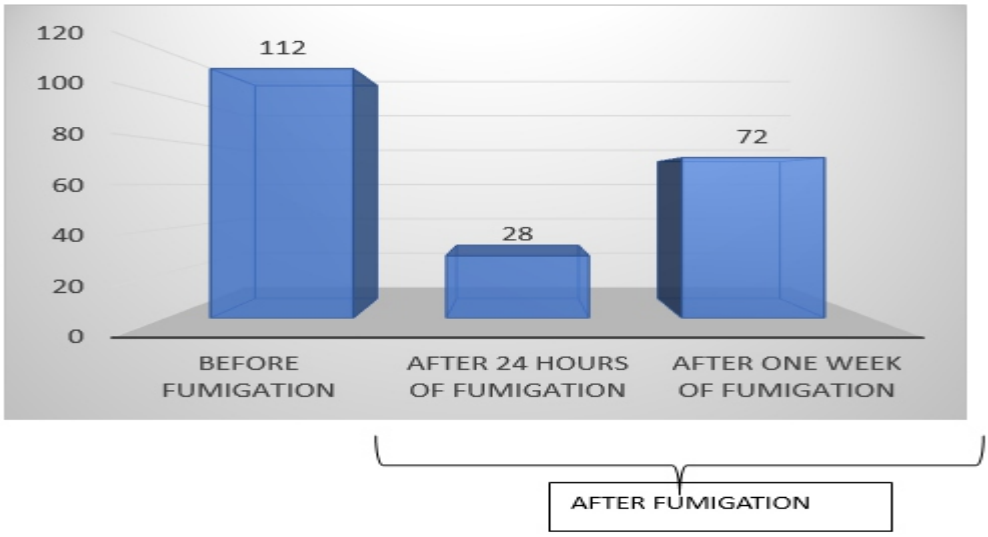

Figure 8: Representing the Microbial Load After and Before Fumigation

Table 1: Advantages and disadvantages of hydrogen peroxide

	DISADVANTAGES	ADVANTAGES
HYDROGEN PEROXIDE	• No activation required • May enhance removal of organic matter and organisms • No disposal issues • No odor or irritation issues • Does not coagulate blood or fix tissues to surfaces • Inactivates *Cryptosporidium*	• Material compatibility concerns (brass, zinc, copper, and nickel/silver plating) both cosmetic and functional • Serious eye damage with contact

to conduct frequent fumigation of the dental operatory. Hydrogen peroxide has proven to be an effective disinfectant, significantly reducing the microbial count in the operatory by nearly fivefold. While hydrogen peroxide has been utilized as a fumigant for several years, recent data support its broad spectrum of activity and diverse applications. These advancements highlight hydrogen peroxide as a low-cost, versatile, and robust fumigant, potentially leading to expanded usage in various settings. Further studies are warranted to isolate specific types of microorganisms and determine the most suitable materials for fumigation.

Limitations of the Study

Catalase is an enzyme that plays a crucial role in protecting cells from hydrogen peroxide by metabolically breaking it down into water and oxygen. However, the concentrations of hydrogen peroxide used for disinfection purposes can overwhelm this natural defense mechanism. Therefore, it is important to have trained personnel operating the fumigating machine to ensure proper handling and effective disinfection.

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