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ORIGINAL ARTICLE

Comparative Study on the Effectiveness of the Use of Two Substances in the Disinfection of the Dental Environment by Thermonebulisation

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KEYWORDS

Quaternary ammonium;
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ABSTRACT: Stomatological practice demands a high biosafety risk due to the presence of pathogenic agents due to the production of aerosols during procedures, thus urging the implementation of a disinfection means such as Thermonebulisation to significantly eliminate microorganisms, The aim of this research was to compare the effectiveness of two disinfectant substances through thermonebulisation (quaternary ammonium and glutaraldehyde), 20 sterile swab samples from surfaces in public and private dental offices were used, the procedure was performed through thermonebulisation in a period of 5 min, and the sampling was done past the hour and a half of thermonebulisation, which were taken through Stuart transport for preservation, the samples were distributed in two groups of 10 for each disinfectant, where each 90 ml of disinfectant counted with 160 ml of distilled water, 160 ml of liquid glycerin,160 mml of industrial alcohol, It was found that in a thermonebulisation process there is greater effectiveness for quaternary ammonium with a confidence level of 100% due to the total elimination of colony-forming units of microorganisms, while glutaraldehyde only reduced the microbial load, It is concluded that quaternary ammonium proved to be more effective for the disinfection of the dental practice in contrast to glutaraldehyde.

INTRODUCTION

Stomatological practice involves taking measures to prevent and control cross-contamination, contracting an infectious disease at the dentist seems to be limited, but may be high if virulent microorganisms are present, if the environment is conducive to viral transmission or if the recipient is immune compromised, the knowledge acquired can be applied to prevent, minimise or eliminate health risks. [1,2].

Dental facilities take risks from open-mouth procedures, frequent exposure to saliva, blood and other body fluids, pathogens can be transmitted in dental environments by inhalation of microorganisms that remain suspended in the air for a long time, aerosols are the main cause of infection in dental facilities, in addition to causing the infected patient to cough and breathe, dental instruments, such as hand instruments, use high velocity air and water pressure to drive turbines causing dispersion of pathogenic microorganisms [3].

Coronavirus disease 2019 (COVID-19), a viral disease

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declared a pandemic by the World Health Organization (WHO) in January 2020, has caused dramatic changes in many sectors of society around the world. Its virulence and rapid spread have led many countries to adopt strict public health measures. Aerosols from dental procedures present a very high risk of infection, as the new coronavirus can survive in aerosol particles for up to 3 hours and on plastic or stainless steel surfaces for up to 72 hours.[4] Thermal fogging helps to meet the public health need for environmental disinfection, especially in frozen spaces during pandemics and other pathogens, by improving disinfection needs. [5]

The compound called glutaraldehyde (GA) has been cleared by the Food and Drug Administration's (FDA) Center for Devices and Radiological Health (CDRH) as a high-level disinfectant, used to sterilize heat-sensitive medical equipment in hospitals and medical centers.[6] Thus, its use has been widely experimented and practiced in disinfection processes.

A recent study in the dental field strongly suggests disinfecting alginate impressions with a 0.5% sodium hypochlorite solution using the spray disinfection process for 10 minutes. On the other hand, it strongly recommends disinfecting elastomeric impressions using a 0.5% sodium hypochlorite solution or 2% glutaraldehyde using the immersion method for 10 minutes. However, polyether prints should only be disinfected with 2% glutaraldehyde.[7]

Among the disinfection media, microwave irradiation and glutaraldehyde have shown acceptable antimicrobial efficiency against strains of *E. coli, P. aeruginosa, E. faecalis and S. aureus*, without causing detrimental damage to the physical properties of the impression material [8]. In the hospital context, glutaraldehyde is used as a biocidal agent. In Romania, recent public studies on the chemical composition of the biocides used have found in certain cases that glutaraldehyde, as the main component, is present in the biocides used. [9]

On the other hand, quaternary ammonium compounds (QACs) are widely used as disinfectants, antiseptics, preservatives and detergents, thanks to their antibacterial properties, and were the first biocides to be employed before phenolics or nitrogen products, their typical structure being composed of one or more quaternary ammonium ions, which are attached to four side substituents.[10] In laboratory studies, a variety of mechanisms have been identified by which bacteria can develop tolerance or resistance to quaternary ammonium QAC compounds and antibiotics. However, in real-world settings, the development of new tolerance or resistance is rare.[11]

Tests on disinfection with respirators and powered air purifiers measured the risk of surface damage from disinfectants based on hydrogen peroxide and quaternary ammonium chloride, where a minimal risk of damage was found for three of the disinfectants [12]. A sector of quaternary ammonium QAC derivatives and compounds are part of a recognized class of positively charged biocides with a broad range of antimicrobial activity, used as key components in surfactants, toiletries, cosmetics, fabric softeners, dyes, biological dyes, antiseptics, and disinfectants.(13) It is also known that a novel type of QAC was created by replacing the alkyl chain with zinc phthalocyanine (ZnPc), which is composed of a large aromatic ring and shares similar hydrophobic properties as the alkyl chain of QACs. as potent and environmentally friendly disinfectants. [14]

It is important to identify the dental procedures that cause droplets and aerosols resulting in contamination, and for each of them, to describe their pattern, dispersion and mode of sedimentation, in order to expose the importance of thorough disinfection. [15] In the practice of clinical dentistry, bioaerosols are routinely produced and distributed in the air, which is due to the operational instrumentation within an oral environment with the presence of salivary organisms. [16] making the environments a high-risk space for bacterial and viral infection. Thus, many studies show that there is a significant increase in the concentration of bacteria and viruses in the air. [17] Aerosols produced by high-speed dental instruments can promote the spread of pathogenic microorganisms. [18] similarly high-speed hand tools, air-water injectors and mechanical scalers are considered high-risk items in order to expose the importance of thorough disinfection. [19] Implementing appropriate protective measures against pathogens transmitted via droplets or aerosols from the oral cavity of patients is of great importance in dental practices. [20] The estimated dose of particles inhaled by dentists during surgery is significantly expressed, putting the clinical operator and staff at risk of direct exposure to environmental aerosols. [21]

Studies of aerosols in dentistry during the COVID-19 pandemic came into full force in 2020 and thanks to this event, the risk and exposure that exists in a dental practice, as well as the necessary means for ideal disinfection, became evident. [22]

The World Health Organization WHO states that the production of airborne particles that pose the greatest risk of infectious disease transmission is when people cough, sneeze, laugh or talk, producing large (diameter > 5 μm) and small (diameter ≤ 5 μm) droplets or aerosols. Larger droplets fall rapidly to the ground due to gravity; therefore, droplet transmission requires close physical proximity between infected and susceptible people [23]. currently studying the means and modes of infection, thermonebulisation presents itself as a major alternative to the means already identified. [24]

MATERIALS AND METHODS

The study has an experimental and observational approach with a transversal cut, taking samples of bacterial groups from dental surgeries in the city of Riobamba and then applying thermonebulisation with quaternary ammonium and glutaraldehyde, developing a statistical study of the effectiveness of disinfection of the products mentioned and the relationship of variables.

The study was carried out on 20 samples taken from the surfaces of both public and private dental surgeries, divided into two groups of 10 for quaternary ammonium and another 10 for glutaraldehyde, of which samples were taken specifically from the spittoon, the place where there is the greatest influx of bacterial microorganisms prior to the thermal fogging process.

The thermonebulisation of the surfaces of the clinics under study was carried out in a period of 5 min. and after 90 min. the swab was taken, giving a total of 40 samples, which were sent to the laboratory in a STUART transport medium for better preservation, according to Moffet et al . This medium is a semi-solid gel used for the transport and preservation of microorganisms, as it contains calcium chloride and sodium glycerophosphate which act as buffering agents, maintaining

osmotic equilibrium in the medium and sodium thioglycolate which prevents oxidative changes by maintaining a reduced atmosphere, while methylene blue is an indicator of oxidation- reduction state, and bacteriological agar is added as a solidifying agent to facilitate the subsequent culture of the isolated microorganisms [25], for a better recognition of the microorganisms it was decided to carry out the culture in blood agar, which is highly recommended for these studies, since it provides adequate growth of the great majority of both Gram- positive and Gram-negative bacteria; as well as fungi from a rich and supplemented base offering optimal conditions for development, facilitating the differentiation of the species of microorganisms, to be subsequently analysed and described in terms of their efficiency in disinfection and to develop a comparative statistic of the two substances studied.

The thermonebulisation equipment used was the American Xtreme 1500 W in which the liquid consisting of 160 ml of distilled water, 160 ml of liquid glycerine, 160 ml of industrial alcohol and 90 ml of either quaternary ammonium or glutaraldehyde was placed and the disinfection process was carried out for 5 minutes.

For the collection of samples after Thermonebulisation, sterile swabs of the CITOSWAB® brand recommended by the clinical laboratory Chávez & Robles, Sterile swabs were used.

The analysis of the samples was carried out in the clinical laboratory Chávez & Robles, with the assistance of BQF. Luis Chávez and MsC. Jimena Robles, who used the CFU colony forming unit counting technique.

RESULTS

Table 1 shows that a count of the type and quantity of microorganisms in the dental surgeries prior to thermonebulisation revealed the presence of several bacterial groups: *Aspergillus candidus, Aspergillus fumigatus, Enterococcus faecalis with a higher incidence and Staphylococcus epidermidis, Escherichia coli and Staphylococcus aureus*. The percentage of concentration of the bacterial groups in the sampling areas was recorded, where *Enterococcus faecalis*, recorded the highest accumulation in all clinics and the lowest in *Aspergillus fumigatus.*

Table 2 shows that in the statistical analysis there is no significant difference ($p<0.05$) between the accumulation of microorganisms and the dental procedures performed, rejecting the hypothesis with a confidence level of 95.

Table 1. Micro-organisms present in the dental office before the thermonebulisation process.

	Frequency		Percentage	Percentage valid	Cumulative percentage
Micro-organisms	*Aspergillus candidus*	3	11.1	11.1	11.1
	Aspergillus fumigatus	2	7.4	7.4	18.5
	Enterococcus faecalis	8	29. 6	29.6	48.1
	Escherichia coli	4	14.8	14.8	63.0
	Staphylococcus aureus	3	11.1	11.1	74.1
	Staphylococcus epidermidis	7	25.9	25.9	100.0
	Total	27	100.0	100.0	

`Source: Data processed in SPSS

Table 2. Cross-table Micro-organism by dental procedures.

		Endodontics	Extractions	Operations	Tartrectomies	Total
Micro-organisms	*Aspergillus candidus*	1	4	0	0	5
	Aspergillus fumigatus	2	0	2	0	4
	Enterococcus faecalis	3	0	4	4	11
	Escherichia coli	1	0	2	3	6
	Staphylococcus aureus	2	1	0	2	5
	Staphylococcus epidermidis	1	2	6	0	9
Total		10	7	14	9	40

Source: Data processed in SPSS

Table 3 represents the resistance of the bacterial group Enterococcus Faecalis with 23.08%, while Staphylococcus Epidermidis with 15.08% and Escherichia Coli, Staphylococcus Aureus, Aspergillus Candidus, and Aspergillus Fumigatus with a similarity of reduction of 15.38%.

Table 3. Micro-organisms present in the dental office after the glutaraldehyde thermal fogging process

	Frequency		Percentage	Percentage valid	Cumulativepercentage
Micro-organisms	*Aspergillus candidus*	2	15.4	15.4	15.4
	Aspergillus fumigatus	2	15.4	15.4	30.8
	Enterococcus faecalis	3	23.1	23.1	53.8
	Escherichia coli	2	15.4	15.4	69.2
	Staphylococcus aureus	2	15.4	15.4	84.6
	Staphylococcus epidermidis	2	15.4	15.4	100.0
	Total	13	100.0	100.0	

Source: Data processed in SPSS

Table 4 shows a comparison of the effectiveness of the reduction of microorganisms in dental surgeries by means of the thermonebulisation process between quaternary ammonium and glutaraldehyde, proving the

100% efficiency of quaternary ammonium in the presence of microorganisms with respect to glutaraldehyde.

Table 4. Comparative data on the effectiveness of quaternary ammonium thermal fogging with respect to glutaraldehyde.

Type		**Statistician**	**Standard error**
Glutaraldehyde	Media	583.3333	207.61174
	Medium	.0000	
	Variance	2586158.192	
	Minimum	.00	
	Maximum	10000.00	
	Asymmetry	4.157	.309
	Kurtosis	20.581	.608

Source: Data processed in SPSS

Comparison of the Effectiveness of Quaternary Ammonium and Glutaraldehyde in Thermal Fogging for the Elimination of Common Microorganisms in the Dental Office The primary objective of this study was to compare the effectiveness of two disinfectant agents—quaternary ammonium and glutaraldehyde—when applied through thermal fogging to eliminate the most frequently identified microorganisms in dental offices. These microorganisms are considered the main contributors to infections and failures in oro-stomatological treatments.

The oral cavity provides a favorable environment for the proliferation of multiple microorganisms that, under normal physiological conditions, maintain a balanced ecosystem and contribute to the protection of anatomical structures. Approximately 12 different microbial families have been detected coexisting in the oral cavity. However, even slight alterations in pH can lead to pathogenic transformations, resulting in opportunistic infections such as candidiasis and respiratory diseases, which affect a significant proportion of dental patients.[26] Thermal fogging is a disinfection technique in which a chemical substance is dispersed in a confined space using a thermal fogger, ensuring complete coverage of the area. This method has been widely employed to disinfect various environments, both within and outside healthcare settings, utilizing agents such as hypochlorous acid, quaternary ammonium, glutaraldehyde, and hydrogen peroxide. Previous studies have demonstrated that quaternary ammonium exhibits high efficacy in the disinfection process [27, 28], which aligns with the findings of the present study.

Piovesan et al. reported that glutaraldehyde is highly effective due to its broad-spectrum activity against bacteria, mycobacteria, fungi, viruses, and spores. It is commonly used for disinfecting medical instruments. Meanwhile, quaternary ammonium is one of the most widely used disinfectants due to its broad-spectrum antimicrobial properties, particularly against bacteria, fungi, and viruses, including coronaviruses. Their study found that quaternary ammonium was the most effective disinfectant, capable of completely eliminating microorganisms commonly present in dental offices, such as Enterococcus faecalis, Escherichia coli, Staphylococcus epidermidis, Staphylococcus aureus, Aspergillus candidus, and Aspergillus fumigatus.[29] These findings are consistent with those of Zambrano and Luna, who also identified these microorganisms in dental settings, primarily originating from patients' gastrointestinal and respiratory tracts.[30] The present study supports these results, demonstrating that quaternary ammonium was more effective than glutaraldehyde at similar concentrations. It successfully eliminated *E. faecalis, E. coli, S. epidermidis, S. aureus, A. candidus*, and *A. fumigatus* from dental office surfaces, further reinforcing its superior disinfectant properties.

CONCLUSIONS

The comparative study on the effectiveness of two disinfectant substances used in dental environment disinfection through thermal fogging demonstrates that this procedure is effective and represents a viable alternative to traditional disinfection methods commonly employed in dental offices. Furthermore, the study highlights the superior efficacy of quaternary ammonium for this purpose, suggesting it as a primary option to be considered in clinical disinfection protocols.

The application of thermal fogging after dental procedures reduces or eliminates microorganisms in the dental office, thereby preventing both patients and dental practitioners from becoming infected and contracting diseases that could be detrimental to their health.

Following dental procedures such as scaling, surgeries, extractions, and endodontic treatments, the proliferation of microorganisms within the dental environment is inevitable due to the use of rotary instruments. The most frequently identified microorganisms in this study included Enterococcus faecalis, Escherichia coli, Staphylococcus epidermidis, while Staphylococcus aureus, Aspergillus candidus, and Aspergillus fumigatus were detected in smaller quantities.

The disinfection procedure using thermal fogging has proven to be highly effective in combating microorganisms in the dental office, as it is capable of completely eliminating bacteria that pose health risks, thereby preventing potential infections.

Quaternary ammonium used in thermal fogging has demonstrated superior effectiveness by completely eradicating microorganisms present on surfaces and within the dental environment, whereas glutaraldehyde only significantly reduces the number of colony-forming units (CFUs).

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ETHICAL CONSIDERATION

Written informed consent was obtained from all study participants.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Abbreviations

According to the World Health Organization (WHO), dental aerosols may contain microorganisms that are potentially pathogenic.

Glutaraldehyde (GA) was used as a high-level disinfectant in the procedures.

Quaternary ammonium compounds (QACs) are commonly used in dental clinics as surface disinfectants.

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) conducted the evaluation.

The photosensitizer used was zinc phthalocyanine (ZnPc), which has been extensively studied for its antimicrobial properties.

Availability of data and materials

The datasets used and analysed during the present study are available via the author upon reasonable request.

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