

## **Antimicrobial Effect of Chlorinated Solutions in Tanks and Water Lines of Dental Office Units in Barranquilla, Colombia \***

**Efecto antimicrobiano de las soluciones cloradas en depósitos y líneas de agua de las unidades de consultorios odontológicos en Barranquilla, Colombia**

**Efeito antimicrobiano de soluções cloradas em tanques e linhas de água de consultórios odontológicos na Barranquilla, Colômbia**

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## ABSTRACT

**Background:** The characteristics of the water lines of dental units (LAUD) allow the creation of biofilm that can cause cross infections between patients and health personnel. The use of chlorinated antimicrobial solutions added to the water tank for other purposes has been recommended. **Purpose:** To evaluate the antimicrobial effect of chlorinated solutions in tanks and LAUD of Dental offices in Barranquilla (Colombia). **Methods:** Descriptive study, conducted in 13 Dental units where the presence of microorganisms and the effect of the chlorinated solution on the microorganisms found after 14 days of use of the solution were observed from a first sample. **Results:** In the first sample, mesophilic growth greater than 200 CFU was observed in 66.6 %, total coliforms greater than 2 CFU in 58 %, *E. Coli* in 16 %, *Pseudomonas* in 33 % and fungal growth in syringe spray in 66.6 %. In the second sample, 25 % mesophilic growth was observed in the triple syringe. In the sprayer syringe 8.3 % and in the high part 8.3 %, no growth of total coliforms or coliforms was observed. *E. coli*, growth of *pseudomonas* was observed in 16 % in triple syringe and of fungi in 8.3 %. **Conclusions:** The chlorinated solution had an antimicrobial effect in the water tanks of the dental units included in this study. The microorganisms isolated in the present study are of clinical importance because they are associated with cross infections and different types of infections in humans. **Keywords:** Barranquilla, Colombia; biosecurity; dental office; dentistry; hypochlorous acid; microbial contamination; water

## RESUMEN

**Antecedentes:** Las características de las líneas de agua de las unidades dentales (LAUD) permiten la creación de biopelícula que puede ser causa de infecciones cruzadas entre pacientes y el personal de salud. Se ha recomendado el uso de soluciones cloradas antimicrobianas adicionadas al depósito de agua con otros fines. **Objetivo:** Evaluar el efecto antimicrobiano de las soluciones cloradas en depósitos y LAUD de consultorios Odontológicos en Barranquilla (Colombia). **Métodos:** Estudio descriptivo, realizado a 13 unidades Odontológicas donde se observó a partir de una primera muestra la presencia de microorganismos y el efecto de la solución clorada sobre los microorganismos encontrados posterior a 14 días del uso de la solución. **Resultados:** En la primera muestra se observó crecimiento de mesófilos mayor a 200UFC en un 66.6%, coliformes totales mayor a 2 UFC en un 58 %, *E. coli*, en un 16 %, *Pseudomonas* en un 33 % y crecimiento de hongos en jeringa pulverizador en un 66.6 %. En la segunda muestra se observó crecimiento de mesófilos en jeringa triple en un 25 %. En jeringa pulverizador el 8.3 % y en pieza de alta 8.3 % no se observó crecimiento de coliformes totales ni de *E. coli*, se observó crecimiento de *pseudomonas* en un 16 % en jeringa triple y de Hongos en un 8.3 %. **Conclusiones.** La solución clorada tuvo efecto antimicrobiano en los depósitos de agua de las unidades odontológicas incluidas en este estudio. Los microorganismos aislados en el presente estudio revisten importancia clínica por estar asociados a infecciones cruzadas y diferentes tipos de infecciones en humanos. **Palabras Claves:** ácido hipocloroso; agua; Barranquilla, Colombia; bioseguridad; consultorio odontológico; contaminación microbiana; odontología

## RESUMO

**Antecedentes:** As características das linhas de água das unidades odontológicas (LAUD) permitem acriação de biofilme que pode causar infecções cruzadas entre pacientes e profissionais de saúde. Tem sido recomendado o uso de soluções antimicrobianas cloradas adicionadas à caixa d'água para outras finalidades. **Objetivo:** Avaliar o efeito antimicrobiano de soluções cloradas em tanques e LAUD de consultórios odontológicos em Barranquilla (Colômbia). **Métodos:** Estudo descritivo, realizado em 13 unidades odontológicas onde foi observada a partir de uma primeira amostra a presença de microrganismos e o efeito da solução clorada sobre os microrganismos encontrados após 14 dias de uso da solução. **Resultados:** Na primeira amostra foi observado crescimento mesofílico superior a 200 UFC em 66,6 %, coliformes totais superior a 2 UFC em 58 %, *E. Coli* em 16 %, *Pseudomonas* em 33 % e crescimento fúngico em spray de seringa em 66,6 %. Na segunda amostra foi observado crescimento mesofílico de 25 % na seringa tríplex. Na seringa pulverizadora 8,3 % e na parte alta 8,3 % não foi observado crescimento de coliformes totais ou coliformes. Em *E. coli*, foi observado crescimento de *pseudomonas* em 16 % em seringa tripla e de fungos em 8,3 %. **Conclusões:** A solução clorada apresentou efeito antimicrobiano nas caixas d'água das unidades odontológicas incluídas neste estudo. Os microrganismos isolados no presente estudo são de importância clínica porque estão associados a infecções cruzadas e diferentes tipos de infecções em humanos. **Palavras-chave:** ácido hipocloroso; água; Barranquilla, Colômbia; biossegurança; consultório odontológico; contaminação microbiana; odontologia

## INTRODUCTION

Water outlets from dental unit lines (LAUD) can be a potential source of infection for dental health personnel and patients (1). There are some determinants of microbial contamination in LAUDs, which are mainly: An exceedingly small lumen size (0.5-2 mm), shape of the pipes if they are smooth or twisted, quality of the materials from which the pipe is made and stagnation of water outside of working hours (2).

The environmental conditions present inside the ducts of the dental unit can facilitate the proliferation of microorganisms and the subsequent formation of biofilm on the interior surface of the LAUD pipes. During the use of handpieces, particularly high-speed rotating instruments, aerosols or splashes containing biological material (saliva, blood, and dental plaque) and microorganisms are released (1). The water in these tubes creates the conditions for the growth of bacteria, which means that both professionals and patients are exposed to the risk of infection. Although bacteria are the most studied microorganisms in the water of dental units, other infectious agents such as prions, viruses, fungi and protozoa have been reported (3-4). In the handpieces and water lines of the dental unit, due to the retraction of oral fluids, prions and viruses such as HIV, hepatitis B and herpes simplex have been found (5-6).

Water is a major source of contamination for dental patients. This type of contamination has been divided into two: the first type comes from the main water supply and the second type adheres to the hose wall of the dental equipment to form a biofilm. The biofilm contaminates the equipment hoses and discharges microorganisms into the patient's mouth. The aerosols produced here can contaminate the environment, surfaces, instruments and healthcare personnel, putting public health at risk (7).

It is important to establish the microbiological quality of the water to reduce the risk of contamination, because the water will come into direct contact with patients and medical personnel, resulting in the provocation of diseases in special conditions or in people with a weakened immune system. .

In Colombia there is no specific law or surveillance entity that controls the quality of water in dental units. The studies that have been conducted on the microbiological quality of water are based on Resolution 2115 of 2007 and the Colombian technical standard NTC 813 for water for human consumption (8). The risk of cross-infections in dental settings can be addressed by implementing combined interventions to prevent contamination of LAUDs.

In our environment it is not common to add antimicrobial solutions to the water tanks of dental units. With the Covid-19 pandemic, due to the aerosols generated in Dental practice, the use of chlorinated solutions was recommended as a means to reduce the viral load possibly present in patients and reduce the risk for the dental professional and staff. Among the recommended chlorinated solutions is Hypochlorous Acid (HClO), which is considered among the most powerful natural disinfectants, non-toxic to humans, animals and plants, and is highly effective as an antimicrobial and fast-acting agent (9).

HClO is the active component of sodium hypochlorite without its adverse effects. In this way, it could be considered a powerful anti-plaque for use in the oral cavity, since it has demonstrated a high broad-spectrum antimicrobial effect in concentrations ranging from 0.1 to 2.8 mg/ml in an exposure period of 2 min (10). Adding chlorinated solution to the water tank of the dental units would allow irrigation of this solution through the water lines of the handpiece and triple syringe. Apart from the use to reduce the Covid-19 virus in dental aerosols, it would be interesting to know the effect that this HClO can have on the existing microbiota in the tanks and water lines of dental units. In Colombia there are few studies about this important topic, even the Dental Guild is unaware of it.

It would be interesting to know how much microbial contamination exists in the water tanks of the Dental units and take preventive measures to control it. The objective of this research was to identify the antimicrobial effect of chlorinated solutions in the water tanks of dental units.

## **MATERIALS AND METHODS**

The present study is a descriptive observational in vitro study. The universe was made up of Dental Units from the city of Barranquilla-Colombia. The sample was determined by random probabilistic sampling, 13 dental units participated. Once selected, permission was requested to obtain the samples, subsequently the owners of the participating dental units signed an informed consent. The inclusion criteria for this study were dental offices authorized by the health secretary of the city of Barranquilla, with dental units in good condition and without prior use of antimicrobial solution in the water tank. Offices with dental units without continuous use were excluded from this study.

With respect to ethical considerations, the provisions established in resolution 8430 of 1993 of the Colombian Ministry of Health, title IV related to the biosafety of research, chapter I of research with pathogenic microorganisms or biological material that may contain them, were followed.

### **Procedure**

The sample was taken at three different points in each dental unit:

1. High speed part water sample.
2. Triple syringe water sample.
3. Triple syringe spray mode water sample.

The samples were taken at two different times (Sample 1 and Sample 2): Sample 1 (M1) corresponds to the sample obtained on the first day of the visit, this sample was taken at the 3 points mentioned above (High speed piece, syringe triple and triple syringe sprayer). The water samples were preserved in a 500ml Schott brand glass bottle, which was sterilized following the parameters of the National Institute of Health (INS) (11).

After taking M1, the 500ppm Hypochlorous Acid solution was added, diluted 1/1 with purified water in the water tank of the dental unit. This solution was applied for 14 days every day in the morning in order to avoid the risk of contamination with the use of unpurified water. It should be noted that the auxiliary staff of each office was trained in the proper use and application of hypochlorous acid.

Sample 2 (M2) corresponds to the second sample that was conducted 14 days after M1, this was taken at the same water outlet points (High speed piece, triple syringe and triple syringe sprayer).

### **Sample collection protocol**

#### *High-speed piece*

Asepsis of the piece was conducted with sterile gauze soaked in Benzal®, the sterile bottle was uncovered, its head was introduced into the bottle, the pedal of the dental unit was pressed until 400ml of water was obtained. Subsequently, the lids of the jars were sealed with Kraft paper and labeled.

#### *Triple syringe with water expulsion*

Rigorous asepsis of the syringe was first conducted with sterile gauze soaked in Benzal®, then using sterile gloves, the water outlet button was pressed for 3 seconds, the bottle was uncovered, and the tip of the syringe was placed. instrument inside, the button of the triple syringe was pressed until 400 ml of water was obtained. He kept the mouth of the bottle covered with his other hand. From the beginning of asepsis until the end of the sample, a sterile field was maintained between the syringe and the bottle. Subsequently, the lids of the jars were sealed with Kraft paper and labeled.

### *Triple syringe with water/air sample (Sprayer)*

Sample collection following protocol for triple syringe sampling with water expulsion, with a variant the two buttons were pressed at the same time to obtain water/air at a time and until the required volume of water is obtained. Subsequently, the lids of the jars were sealed with Kraft paper and labeled. Finally, all samples were preserved and immediately transported to the microbiology laboratory of the Metropolitan University of Barranquilla.

### *Microbial isolation, quantification and identification*

The technique used was the membrane filtration method, which consists of filtering 100 ml of the samples under study for each of the parameters, namely: Total mesophilic count, determination of total coliforms, *Escherichia coli* (E. Coli), *Pseudomonas aeruginosa* (P aeruginosa) and fungi, incubation at the temperature indicated by the insert, reading and counting after 24-48 hours. m-Colibblue, TGE, and Cetrimide Agar for *Pseudomonas* media were used. The detection of *P. aeruginosa* was performed according to the international standard for water quality: detection and enumeration of *P. aeruginosa* method by membrane filtration (NF EN ISO 16266). Colonies were counted and examined under UV radiation.

Total coliform bacteria and *E. coli* were investigated according to the international standard for water. Quality: detection and enumeration of *Escherichia coli* and coliform bacteria—membrane filtration method (NF EN ISO 9308-1).

After applying the technique, the boxes were incubated in an inverted position and left for 24 hours at 36°C for mesophiles, coliforms and *P aeruginosa*, in the case of fungi, they were incubated at 30°C for 3 days. After the incubation time, the microorganisms were counted and identified.

## **Statistical analysis**

The data obtained was organized in an Excel matrix where the Colony Forming Units (CFU) present in the three sample points M1 and M2 were recorded. A descriptive analysis was conducted with absolute and relative frequency tables. Inferential statistics were applied, the variables were expressed qualitatively as absent or present and compared to define the relationship between variables, the SPSS statistical program was used and the McNemar test was applied with a p value <0.05 as statistical significance.

## **RESULTS**

A total of 13 dental units were included in this study, of which 1 was considered a negative control because it was using an antimicrobial solution and was office 8 (C8). Of the 12 units, the presence of microorganisms in the first sample (M1) and the effect of the chlorinated solution on the microorganisms in the second sample (M2) were identified. The microorganisms identified were Mesophiles, total coliforms, *E. Coli*, *P aeruginosa* and fungi. With respect to the mesophiles, the first sample collected M1 revealed growth of Mesophiles in a triple syringe greater than 200 CFU in 50 % (C3,C4,C6,C9,C12); In a spray syringe 66.6 % (C1,C2,C3,C4 ,C6,C9,C10,C12), finally; We showed growth of 58.33 % in the high part (C1,C2,C4,C6,C9,C10,C12). Table 1. In the second sample, mesophilic growth was observed in the triple syringe by 25 % (C7, C11, C12 ). In spray syringe 8.3 % (C12) and in discharge piece 8.3 % (C12). Table 1.

TABLE 1  
Mesophilic colony forming unit (CFU) counts in M1 and M2

Dental units	Triple syringe		Spray syringe		High part	
	M1	M2	M1	M2	M1	M2
C1	10	0	>200	0	>200	0
C2	45	0	>200	0	>200	0
C3	>200	0	>200	0	25	0
C4	>200	0	>200	0	>200	0
C5	1	0	0	0	5	0
C6	>200	0	>200	0	>200	0
C7	2	>200	0	0	75	0
C8	0	0	0	0	0	0
C9	>200	0	>200	0	>200	0
C10	0	0	>200	0	>200	0
C11	73	>200	62	0	48	0
C12	>200	>200	>200	>200	>200	>200
Percentage	50	25	66.6	8.3	58.33	8.3

Source: the authors.

Regarding total coliforms in 12 offices evaluated, it revealed growth of total coliforms in triple syringe greater than 2 CFU in 58 %; in syringe sprayer 33 %, finally, high part with 66 %. Regarding E. Coli, there was growth in the spray syringe with 16 % (C2) and in the discharge part with 16 % (C1, C2). In the second sample M2, no growth of E. Coli was observed at any of the collection points.

Regarding P aeruginosa, M1 showed growth of P aeruginosa in triple syringe by 25 % (C3, C11, C12); in spray syringe 33 % (C2, C3, C11, C12), finally, high part with 33 % (C3, C12, C13, C15). In the second sample (M2) growth of P aeruginosa was observed in triple syringe by 16 % (C11, C12). In spray syringe 8.3 % (C12) and in discharge piece 8.3 % (C12).

Fungi were identified, in M1 it revealed fungal growth in triple syringe in 58.33 %, in spray syringe 66.6 %, finally; We show growth of 66.6 % in high-quality parts. In M2, fungal growth of 8.3 % was observed in triple syringe in (C14).

In the descriptive analysis of the results of the microorganisms found. We can observe that mesophiles, coliforms and fungi were those that occurred in a higher percentage (Figure 1).

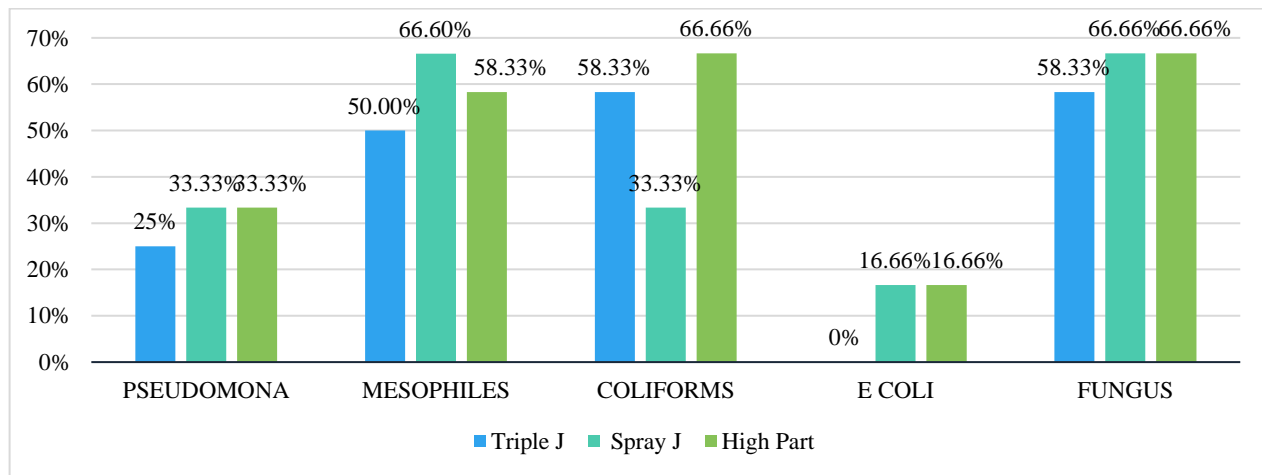


FIGURE 1  
Descriptive analysis of the microorganisms found  
Source: the authors.

After using the chlorinated solution, we observed the results of the microorganisms found in the descriptive analysis graph (Figure 2).

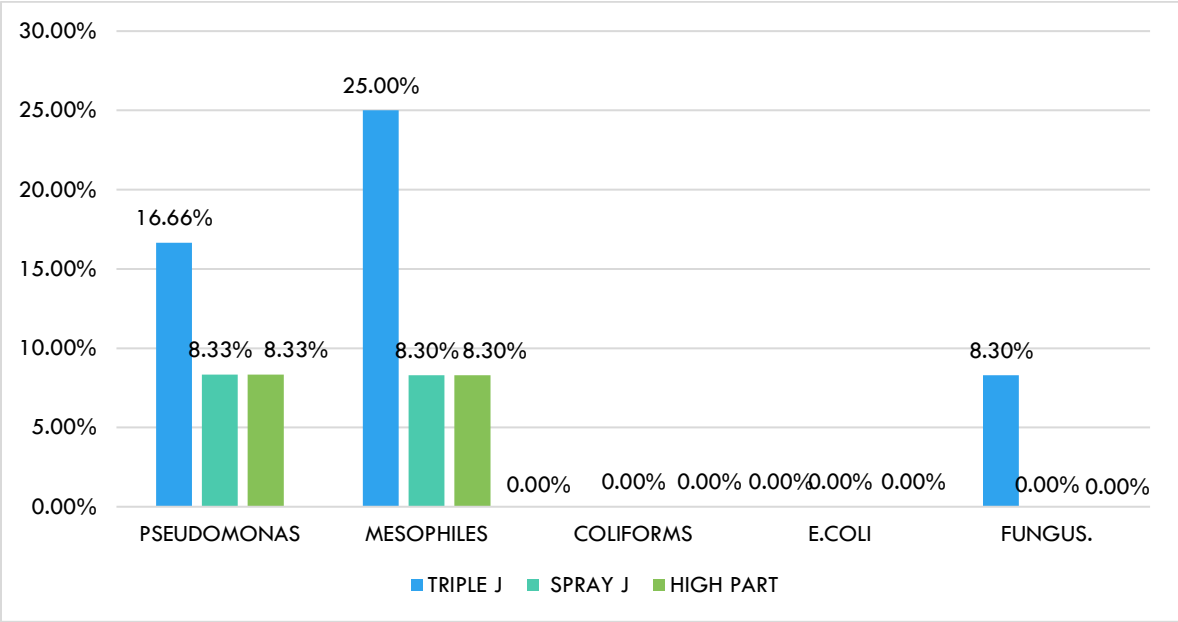


FIGURE 2  
Microorganisms found in M2 after the use of the chlorinated solution  
Source: the authors.

Automated identification of positive crops

21 strains were processed with the automated identification method PHOENIX ®Test PANEL NMIC/ID 406. The colonies were taken from the pure cultures, diluted to a McFarland® scale concentration between 0.5 and 0.6, the panels were inoculated and once inoculated, they were placed in the equipment. Diversity of bacterial species were identified such as *Pseudomonas aeruginosa*, *Bacillus cereus*, *Acinetobacter*, *Stenotrophomonas maltophilia*, *Pseudomonas putida*, and *E. coli*. Isolation and phenotypic identification of the fungi was conducted. Table 2.

TABLE 2	
Fungal species identified.	
C1. JT	Fusarium + Rhizomucor
C1 JS	Fusarium + Aspergillus fumigatus
C1 PA	Fusarium + Aspergillus fumigatus
C2 JT	Fusarium + Syncephalastrum
C2 JS	Fusarium + Rhizomucor + Penicillium SP
C2 PA	Fusarium + Aspergillus niger
C9 JT	Aspergillus fumigatus + Aspergillus niger
C9 JS	Aspergillus fumigatu
C10 JT	Aspergillus fumigatus
C10 JS	Candida SP Yeasts + Penicillium SP

Source: the authors.

Regarding the presence of microorganisms in the different points of the dental units, it was possible to identify that the most frequent microorganisms during M1 were mesophiles, 10 (83.3 %) in the triple

syringe and 11 (91.7 %) in the high-grade piece. speed, followed by total coliforms with 9 (75 %) in the high-speed piece and 8 (66.7 %) in the triple syringe. Fungi presented the same frequency in M1 at different points of the dental unit with 8 species corresponding to 66.7 % for triple syringe and spray syringe (Table 3).

TABLE 3  
Presence of microorganisms in M1 and M2 in the LAUDs

Microorganism	Sample	Triple Syringe	Spray S	High speed part
<i>Mesophiles</i>	M1	10 (83,3%)	9 (75%)	11(91,7%)
	M2	3 (25%)	1 (8,3%)	1 (8,3%)
<i>Coliforms</i>	M1	8 (66,7%)	6(50%)	9 (75%)
	M2	0 (%)	0 (%)	0 (%)
<i>E. coli</i>	M1	0 (%)	1 (8,3%)	2 (16,7%)
	M2	0 (%)	0 (%)	0 (%)
<i>Pseudomonas</i>	M1	3 (25%)	3 (33,3%)	4 (33,3%)
	M2	2 (16,7%)	1(8,3%)	1 (8,3%)
<i>Fungus</i>	M1	7 (58,3%)	8 (66,7%)	8 (66,7%)
	M2	1 (8,3%)	0 (%)	0 (%)

Source: the authors.

Regarding the different microorganisms identified, the mesophiles were the ones that presented a significant reduction when exposed to the chlorinated solution. Mesophiles were present in 7 triple syringes in M1 and after using the chlorinated solution there was a significant reduction in the microorganism, it was identified in 3 syringes in (M2) ( $p= 0.016$ ) (Table 4).

TABLE 4  
Presence and absence of microorganisms in M1 and M2 in the triple syringe of the dental units

		M2		<i>p-value</i>
		Absent	Present	
<i>Mesophiles</i>				
<b>M1</b>	<b>Absent</b>	2	0	0.016*
	<b>Present</b>	7	3	
<i>Coliforms</i>				
<b>M1</b>	<b>Absent</b>	4	0	
	<b>Present</b>	8	0	
<i>E. coli</i>				
<b>M1</b>	<b>Absent</b>	12	0	
	<b>Present</b>	0	0	
<i>Pseudomonas</i>				
<b>M1</b>	<b>Absent</b>	9	0	1,000
	<b>Present</b>	1	2	
<i>Fungus</i>				
<b>M1</b>	<b>Absent</b>	4	0	
	<b>Present</b>	8	0	

§: McNemar Test

\* Statistical significance

$p<0.05$

Source: the authors



In relation to the comparison of the presence of mesophiles before and after treatment with chlorinated solution in spray, it was present in 8 syringes in M1, which after the use of the chlorinated solution decreased significantly to only 1 unit (M2) ( $p= 0.008$ ) (Table 5).

TABLE 5  
Presence and absence of microorganisms in M1 and M2 in the spray syringe of the dental units

Microorganism		M2		p
		Absent	Present	
Mesophiles				
M1	Absent	3	0	0.008*
	Present	8	1	
Coliforms				
M1	Absent	6	0	
	Present	6	0	
E. coli				
M1	Absent	11	0	
	Present	1	0	
Pseudomonas				
M1	Absent	8	0	0,250
	Present	3	1	
Fungus				
M1	Absent	4	0	
	Present	8	0	

§: McNemar Test.

\* Statistical significance  $p<0.05$

Source: the author

In relation to the presence of microorganisms in the high-speed part, it was identified that mesophiles before and after treatment with chlorinated solution were present in 10 syringes in M1, which after the use of the chlorinated solution decreased significantly to only 1 unit (M2) ( $p= 0.002$ ). Table 6.

TABLE 6  
Presence /absence of microorganisms in M1 and M2 in the high-speed part of the dental units

Microorganism		M2		p
		Absent	Present	
Mesophiles				
M1	Absent	1	0	0.002*
	Present	10	1	
Coliforms				
M1	Absent	3	0	
	Present	9	0	
E. coli				
M1	Absent	10	0	
	Present	2	0	
Pseudomonas				
M1	Absent	8	0	0,250
	Present	3	1	
Fungus				
M1	Absent	4	0	
	Present	8	0	

§: McNemar Test.

\* Statistical significance  $p<0.05$

Source: the authors.

## DISCUSSION

At the national level there are few articles that discuss and conduct a detailed study of the problems of tanks and hoses in dental units.

The proliferation of biofilm in these water conduits provides an ideal environment for the colonization of microorganisms, these being the main sources of reproduction of opportunistic pathogens that secrete polymeric matrices that protect them from the outside and that usually have an impact on the final result of a treatment. dental (7).

The objective of this work was to determine the antimicrobial effect of chlorinated solutions in water tanks in dental units. Samples were taken in fifteen units initially to observe the microbiological state of the water lines and final samples after adding the chlorinated solution for fourteen days in the water tanks. The samples were from the handpiece and the triple syringe in jet and spray mode. Two offices were removed from the study due to problems when using the chlorinated solution in the water tank. One office was used as a negative control.

The results of the initial samples report a high microbial contamination of 66 % of mesophiles, total coliforms and fungi. *Pseudomonas* presented a significant value of 33.33 %, while *E. Coli* values were low at 16.66 %.

These results indicate that the water quality of these units does not comply with Resolution 2115 and NTC 813 (12); by finding these microorganisms above normal counts. The presence of *Pseudomonas* is indicative of contamination even though it is not contemplated in the standard. (13). The main source of bacterial factors that allow biofilm growth in dental unit water systems is the local or municipal water supply, with saprophytic bacteria content in drinking water. However, there are other sources of contamination in dental equipment. The equipment includes surgical aspiration systems, turbines, micromotors and modules with syringes operated by water, air and spray (7).

Biofilm is a community of bacteria and other microorganisms that secrete a matrix of exopolysaccharides to protect them from external influences, forming a layer that helps them overcome adverse conditions. In LAUDs, the main factors that support this colonization are the small diameter of the hoses (1/8 to 1/16 inches), the quality of the plastic with which they are made, the time that the water can remain stagnant inside and the Absence of anti-reflux valves in the handpieces, together with the low water pressure and the small flow rate used, all of this favors the accumulation of bacteria in the units' drinking water distribution system.

The proven presence of a wide variety of microorganisms of clinical importance constitutes an obvious health problem, which threatens not only the health professional, but also the patients who come into contact with the water in dental offices.

Checking the presence of these microorganisms in water units constitutes a health problem both for patients due to the risk of cross infections and for the health professional, numerous international studies (14-15) and two studies at international level national (8,16) have previously verified this contamination and for this reason they make recommendations to place antimicrobial solutions in water tanks.

A study carried out in the city of Cali (Colombia) during 2006 reports that, in the majority of samples taken from a University Dental clinic, the number of CFU of *Staphylococcus* and *Candida albicans* were above the permitted level, in contrast with the most probable number (MPN) of coliform bacteria, which in all dilutions turned out to be negative, they did not obtain *E. coli* counts, however, they affirm that there is contamination by total coliforms and report the presence of *Klebsiella* spp (16).

The study conducted in the rural dental clinic of the Autonomous University of Zacatecas (Mexico) reports fecal coliform counts and the identification of *E. coli* (15).

Another study conducted in the city of Bogotá (Colombia) in 2013 determined that the water intended for use in the dental units of a University Dental clinic does not comply, with regard to microbiological

characteristics, with the provisions of Resolution 2115 of 2007. and the Colombian Technical Standard 813 (NTC 813) because it exceeds the acceptable limits for total coliforms and Enterococcus, and also presents a significant count of Pseudomonas, which can cause disease in patients with a depressed immunological state (8).

In this study, the effectiveness of the chlorinated solution added to the tanks was proven by presenting an effectiveness of 100 % in the second sample against total Coliforms and E coli, 91.7 % on Fungi (C14), 83.34 % on Pseudomonas (C14 , C15) and 75 % on mesophiles C10, C14, C15) It should be noted that offices C14 and C15 were the ones that presented positive results for mesophiles, fungi and Pseudomonas in the second sample, it is possible that they did not follow the placement instructions of the chlorinated solution in the water tanks. Even so, the effectiveness of the chlorinated solution as an antimicrobial presented a significant value with a  $p < 0.05$ .

Chlorinated solutions have been used in Dentistry in mouthwashes (10), irrigating solution in endodontics compared to sodium hypochlorite (17) and similar solutions are recommended to be used as antimicrobials in the water tanks of dental units (18). Patel M. *et al*, in 2016 demonstrated that at 5 ppm chlorine dioxide (ClO<sub>2</sub>) can kill 99.95 % of the mixed culture biofilm grown in vitro in sections of LAUD hoses and is drinkable at this concentration , therefore has the potential to be used as a disinfectant in these lines and to be used in treatment water, and conclude that further research is required to evaluate the disinfectant effectiveness of ClO<sub>2</sub> in a normal clinical environment and to determine whether it could be in adverse corrosion of equipment (19).

Water quality is of utmost importance because patients can ingest and aspirate it during procedures. Additionally, patients and dental staff are exposed to water via mucocutaneous and contact via projection of aerosols generated containing microorganisms. Dental aerosols can disperse more than one meter around the patient and remain in the air for 30 minutes (20).

The microorganisms isolated in the present study are of clinical importance because they are associated with different types of infections in humans.

- **Bacillus cereus** identified in 31.81 %. It is a catalase positive bacillus, used in antibiotic analysis. They are the etiological agent of two well-differentiated food poisoning syndromes: 1) the diarrheal type characterized by abdominal pain with diarrhea from 8 to 16 hours after eating contaminated foods (meat, vegetables, pasta, desserts, cakes, sauces and milk) and 2) the emetic type characterized by nausea and vomiting occurring 1 to 5 hours after eating the offending food, predominantly oriental dishes with rice (although sometimes involved in pasteurized cream, milk pudding, pasta, and reconstituted powdered products).

They also cause serious infections of various types, usually, but not always, in immunocompromised patients (21).

- **Pseudomonas aeruginosa** Identified in 27.27 % of the crops. Widely distributed in soil, water, sewage, intestine of mammals and plants. It can cause diseases in humans, as well as in certain animals, insects and plants. Associated with 5 % of all community-acquired infections, infections are usually limited to hospitalized patients with predisposing factors (22).

A comparative analysis with research that preceded the present study has also reported finding P aeruginosa in dental units. This study not only reported its presence but also its association as the cause of oral infections in 2 medically compromised dental patients (23).

- **Acinetobacter** identified in 13.63 % of positive cultures. It is generally considered non-pathogenic in healthy individuals but can cause infection in immunocompromised patients. Multiresistant strains may appear mainly in the Acinetobacter Baumannii/calcoaceticus complex and some strains of Acinetobacter haemolyticus. (24).

- **Stenotrophomonas maltophilia** identified in 9.09 % of positive cultures. Isolated from water, soil, animal sources, plant material and all types of human clinical samples. Occasionally opportunistic, in humans, associated with urinary and respiratory tract infections, post-operative and post-traumatic wound infections, endocarditis, septicemia, meningitis, mastoiditis, conjunctivitis and ecthyma

gangrenosum. High incidence of infections in patients with solid malignant tumors, leukemia and lymphoma (25).

- **Pseudomonas putida** identified in 9.09 % of positive cultures. Isolated from soil, water, plant and animal sources, hospital environments and human clinical samples. Rarely opportunistic, associated with human infections of the extremities, bacteriuria, septic arthritis, and sepsis after blood transfusion. Recently recognized as a pathogen in cancer patients (26).

- **Pantoea agglomerans** identified in 4.54 % of positive cultures, it is found in water, soil, plants, food, animals and humans. It is isolated in humans as an opportunistic pathogen in urine, sputum, wounds and burns (27).

- **E. coli** identified in 9.09 % of positive cultures, it is a type of bacteria that lives in the intestine. Most E. coli do not cause problems, but some types can make you sick and cause diarrhea. One of them causes traveler's diarrhea. The worst type of E. coli causes hemorrhagic diarrhea and can sometimes cause kidney failure and even death. This generally occurs in children and adults with weakened immune systems (28).

Its presence is not frequent in the water tanks of dental units, however a study conducted in the rural dental clinic of the Autonomous University of Zacatecas (Mexico), reports counts of fecal coliforms and the identification of E. coli (15).

Microbiological studies on dental units are performed either for the prevention or reduction of the density of bacterial contamination in LAUDs, however, the existence of fungi in these systems requires more attention. During dental treatment, direct contact with water contaminated with fungi such as Candida, Aspergillus or inhalation of aerosols from a high-velocity handpiece can cause various respiratory infections, such as asthma, allergies and wounds to the mucous membranes, especially in patients immunocompromised and dental personnel (29).

In the study by Göksay D *et al*, in 2013 (14) they investigated the number and colonization of fungi in LAUDs in the city of Istanbul (Turkey). Water samples were collected from air-water syringes, high-speed parts, and inlet water from 41 dental units. The count of aerobic mesophilic fungi in high-speed parts was higher than in inlet water and in air-water syringes. Non-sporulating fungi were found in 7 dental units. The isolated fungi were identified as *Penicillium waksmanii*, *Cladosporium* spp., *Penicillium* spp., *Candida fameta*, *Cryptococcus laurentii*, *Candida guilliermondii*, *Penicillium verrucosum*, *Aspergillus pseudoglaucus*, *Penicillium decumbens* and *Acremonium* sp. Some of these fungal genera are known as opportunistic pathogens that lead to respiratory diseases such as allergic rhinitis. This study showed the importance of regular control of mycological contamination in the water of dental units.

In the present study, the presence of fungi was found in 66.66 %, that is, in 8 of 12 dental units studied from samples taken from the discharge piece and from a spray syringe, and 58.33 % in 7 of twelve units in a triple syringe, regardless of the number of colonies present.

The positive cultures were reseeded in specific media and the following fungi were phenotypically identified:

- **Fusarium** Identified in 33.33 % of positive cultures. It is a saprophytic fungus, a natural inhabitant of soil and decomposing organic material. It is known for its opportunistic phytopathogenic capacity capable of generating disease in humans, especially in immunocompetent patients, causing superficial or localized infections and in immunocompromised patients. It is also associated with high rates of morbidity and mortality. It causes superficial (keratitis, onychomycosis), localized (endophthalmitis, sinusitis) and disseminated infections. It is genetically toxic. Sometimes they cause infections in the normal patient (Keratitis, onychomycosis, etc.). However, increasingly serious infections are being described in immunosuppressed patients, which is why its importance has grown exponentially. Infections by the *Fusarium* genus are included within hyalohyphomycosis, that is, those caused by opportunistic fungi that present septate hyaline hyphae (30).

• **Aspergillus fumigatus** Identified in 22.22 % of positive cultures. In immunocompromised hosts, *A. fumigatus* represents the main cause of morbidity and mortality. This patient population is expanding due to the increasing use of transplants, it is the most common invasive mold in these patients and in those being treated with immunosuppressants and myeloablative therapies for autoimmune and neoplastic diseases and human immunodeficiency virus/AIDS. Mortality rates exceed 50 % in high-risk groups, such as leukemic and hematopoietic patients receiving stem cell transplants. *A. fumigatus* antigens are associated with asthma, the prevalence of which is increasing in the developed world (31).

• **Rhizomucor** Identified in 11.11 % of positive cultures. Zygomycoses are deep, subcutaneous infections caused by fungi belonging to the class Zygomycetes. There is a subdivision of these into two orders: mucorales and entomophthorales. Mucorales because infections called Mucormycosis, caused entirely by molds. Human beings have an effective and rapid response against zygomycetes, therefore, for infection to occur, the spores must exceed the phagocytosis capacity of the monocyte-macrophage and polymorphonuclear cells, which will lead to the development of hyphae. which then causes vascular invasion (32).

Vascular invasion causing necrosis of infected tissue and perineural invasion are the most frustrating features of these infections. Zygomycosis is often fatal.

There are few reports of human infections due to *Rhizomucor* spp. So far, cases of cutaneous, pulmonary, rhinofacial and disseminated zygomycosis due to *Rhizomucor pusillus* have been reported in neutropenic patients with hematological malignancies (33). It should be noted that primary skin infections due to *Rhizomucor variabilis* have been reported in healthy individuals; and has recently been associated as an opportunistic infection in patients who have suffered from the SAR COVID-19 virus) resulting in extensive damage with involvement of the upper jaw (34).

• **Penicillium SP** identified in 11.11 % of positive cultures. It is a hyaline, saprophytic filamentous fungus belonging to the phylum Ascomycota. Opportunistic pathogen that causes respiratory infections and local or superficial infections such as: pneumonia, keratitis, endophthalmitis, otomycosis, endocarditis, esophagitis and skin and surgical wound infections. *Penicillium* is one of the main causative agents of mold-related allergies in buildings (35).

• **Aspergillus niger** identified in 11.11 % of positive cultures. *A. niger* is the third most common *Aspergillus* species that is associated with human diseases. It has been linked to the formation of the “fungal ball in the lung.” This ball is created by the growth of *A. niger* in the lungs. However, they do not invade lung tissue. Symptoms may present as lung disease and wheezing; some people are asymptomatic, while others may experience weight loss, shortness of breath, fever, tiredness, chest discomfort or maybe coughing up blood. If *A. niger* invades the maxillary sinuses, also forming “fungal balls”, symptoms may include nasal congestion, inflammation, headache, facial pain and drainage (36).

• **Syncephalastrum** Identified in 5.55 % of positive cultures. They are basal fungi that belong to the order mucorales. They are found in different types of organic substrates, exclusively in soil and manure in tropical and subtropical areas. It has pathogenic potential because it can grow at 37°C; Although, few cases of medical mycoses have been reported, mainly relating to immunocompromised patients, prolonged use of corticosteroids or poorly controlled diabetics. Infections of nails, skin and soft tissues, and respiratory rhinosinus infections have been described (37).

• **Candida SP yeasts** Identified in 5.55 % of positive cultures. It is a fungus that lives almost everywhere, even inside your body. The immune system usually keeps fungi under control. If you are sick or take antibiotics, they can multiply and cause an infection. *Candida* infections affect different parts of the body in different ways: Thrush or oral candidiasis is a fungal infection that causes white patches in the mouth.

It usually does not cause any harm under certain conditions; however, *Candida* infects mucous membranes and moist areas of the skin, they are opportunistic and in immunocompromised patients can cause more serious infections, *Candida* in the blood can be life-threatening. danger (38).

Considering that infectious diseases could occur when humans and microbes come into contact, all health entities have the responsibility to reduce this possible contact particularly when it could take place between patients and microorganisms in a health institution, hence the importance of the following preventive measures:

Use and implement the use of antimicrobial solutions in water tanks that are in the Colombian market, in routine use in our dental practice to reduce the risk of cross infections between patient, dentist and health personnel.

- Change hoses eventually, at least every two years.
- Drainage and daily cleaning of the water storage tank of the dental units.
- Daily disinfection between patients of the discharge piece and triple syringe of the dental units.
- Implement the use of high quality, smooth and non-kinked hoses.
- At the beginning and end of the workday, let water and air flow through the unit hoses for 3 minutes.
- Between patients, let water and air pass through the hoses for 30 seconds.
- Preferably use filtered, boiled or sterilized water in the storage tanks of the units.
- Raise awareness among all staff to adopt asepsis and antisepsis measures.
- When choosing the antimicrobial solution, verify which type of microorganisms it is most effective on.
- Cleaning and disinfection of the dental area must be conducted before and after each day.

## CONCLUSIONS

The chlorinated solution was effective as an antimicrobial placed in the water tanks. Water quality is of utmost importance because patients can ingest and aspirate it during procedures. The microorganisms isolated in the present study are of clinical importance because they are associated with different types of infections in humans and the risk of cross infections. According to the studies conducted and the results obtained in this research, it is important that the Dental Guild becomes aware of the importance of taking preventive measures since the health of patients and office staff is exposed.

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