




## An in Vitro Effectiveness Evaluation of Chemical Agents for Toothbrushes Disinfection

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### Abstract

**Objective:** To evaluate in the vitro effectiveness of three chemical agents for toothbrush disinfection. **Material and Methods:** Sixteen new toothbrushes were evaluated, previously sterilized and classified in five experimental groups (n=3) and one item as control. Three chemical agents were assessed: 0.12% Chlorhexidine gluconate (CHX), essential oil mouth rinse (Listerine) and 3.5% Sodium hypochlorite (NaOCl). The five selected strains were inoculated on toothbrushes and incubated for a 24 hours period and 37°C temperature in aerobic conditions. The incubated toothbrushes were immersed for a 15 min period into selected chemical agents and after drying in a controlled air stream, again re-cultured into enriched broth. A comparison was made between the initial and final microorganisms density recovered after chemical disinfection based on Mc Farland scale. The data obtained was compared by descriptive analysis and ANOVA methodology. **Results:** 3.5% NaOCl was the most effective chemical agent for toothbrush disinfection followed by CHX; Listerine was not effective to eliminate the inoculated bacteria in toothbrushes. **Conclusion:** 3.5% NaOCl and 0.12% CHX are the most effective chemical agents for toothbrush disinfection and Listerine was only effective against *C. albicans*.

**Keywords:** Microbiology; Disinfection; Toothbrushing; Sodium Hypochlorite.

## Introduction

Toothbrushes are items conventionally used for oral health maintenance and for mechanical disorganization and elimination of dental biofilms. Several studies have demonstrated the sustained contamination rate of these items during its useful time; these investigations had established a neat relation between isolated microorganisms, water quality, fecal bioaerosol inside bathrooms and oral microorganisms [1-5].

Also, it has been well-described different protocols to control the microbial overgrowth and disinfection of toothbrushes by *in vitro* and *in vivo* studies, using different physical, chemical agents and methodologies with variable results on microbial elimination. Some of those methods include ultraviolet light (UVL), variable-time immersion in different disinfectants, antimicrobial sprays, microwave and automatic dishwasher [6-12].

Heavily contaminated toothbrushes could represent an important risk factor for the transmission of dangerous infectious pathogens among special hosts, such as intensive care patients, immunosuppressed patients, solid organ transplant candidates, oncologic patients and others [5].

The aim of this study was to evaluate *in vitro* the effectiveness of three chemical agents of three commonly used for toothbrush disinfection.

## Material and Methods

### Study Design and Sample

This was a descriptive, experimental, *in vitro* investigation. Sixteen new toothbrushes (Wisdom Toothbrush Company, Deerfield, IL, USA) were selected and previously sterilized following international standards (121°C, 1 ATM). Five groups were conformed by fifteen toothbrushes (n=3). Each group was inoculated with the selected microbial strains. As a control, 1 unused toothbrush was removed from its package, sterilized and directly inserted in microbiologic culture media without any microbial inoculation (negative control).

### Selection and Inoculation of Microbial Strains

The selected microbial strains for this investigation were: *Salmonella enterica* subsp. *enterica* serovar *typhimurium* ATCC 14028, *Proteus mirabilis* ATCC 4675, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739 and *Candida albicans* ATCC 10231. Nutritive broth (Oxoid, Thermo Fisher Scientific Inc., Hampshire, United Kingdom) was prepared and an aliquot of 10 mL was transferred to each of the sixteen capped test tubes. A  $2.0 \pm 0.2$  McFarland standard inoculum of the five selected strains was chosen to be inoculated in each of the test tubes and homogenized by 5 second vortex, then the sterile toothbrushes were immersed in the saturated broth and incubated at 37°C for 24 hrs. After that lapse, microbial grow was assessed by turbidity pattern and the new McFarland standard was obtained; after measuring the McFarland density, the toothbrushes were slightly air-dried in a 24°C stove for its inoculation in the selected chemical agents.

### Selection and Chemical Agents Immersion

For this study, three chemical agents were selected to assess its antimicrobial effectiveness: 0.12% Chlorhexidine gluconate (CHX: PeriDont, Oftalmi Laboratory, Caracas, Venezuela), essential oil mouth rinse (Listerine CoolMint, Jhonson & Jhonson S.A, Caracas, Venezuela) and 3.5% Sodium hypochlorite (NaOCl, Nevex, Clorox Corporation S.A, Caracas, Venezuela) provided by each fabricant in sealed plastic bottles.

Ten milliliters of each chemical agent was aliquoted and transferred to fifteen clean and sterile capped test tubes and the toothbrushes were vertically immersed inside for a 15 min lapse in a dry and controlled environment. After the immersion was completed, the toothbrushes were air-dried in a 24°C stove for 6 hours, simulating the overnight period. Brain Heart Infusion (3M Corporate, St. Paul, MN, USA) was prepared according the fabricant’s instructions and an aliquot of 10 mL was transferred to fifteen capped test tubes, all toothbrushes were again vertically immersed in this infusion and incubated at 37°C for 24 hrs. Microbial grow was assessed by measuring the turbidity pattern and a new McFarland standard was obtained.

### Ethical Aspects

This research was approved by the Bioethical Committee of the Dentistry School at the Universidad Central de Venezuela.

### Results

The microbial reduction of each toothbrush after immersion in the selected chemical agents is shown in Table 1. The 3.5% NaOCl was the most effective chemical agent against all the tested microorganisms based on McFarland standard before and after disinfection (Figures 1 to 4 and Table 2).

**Table 1. Inoculated microorganisms on toothbrushes and McFarland standard before and after chemical disinfection.**

Tube	Strain	McFarland Standard	McFarland (24hrs)	Chemical Agent	Final McFarland Standard
1	<i>S. enterica</i> subsp. <i>enterica</i> serovar <i>typhimurium</i>	2.0	4.3	0.12% CHX	1.4
2		2.0	4.2	Listerine	4.7
3		2.1	5.6	3.5% NaOCl	1.0
4	<i>Proteus mirabilis</i>	2.0	4.5	0.12% CHX	1.5
5		2.1	6.5	Listerine	6.5
6		2.1	3.4	3.5% NaOCl	1.2
7	<i>Pseudomonas aeruginosa</i>	2.0	4.4	0.12% CHX	1.4
8		2.0	3.3	Listerine	3.3
9		2.1	6.5	3.5% NaOCl	1.3
10	<i>Escherichia coli</i>	2.2	5.2	0.12% CHX	0.4
11		2.0	6.3	Listerine	6.9
12		2.2	6.6	3.5% NaOCl	1.0
13	<i>Candida albicans</i>	2.1	3.8	0.12% CHX	0.7
14		2.1	3.6	Listerine	1.1
15		2.0	3.6	3.5% NaOCl	0.8

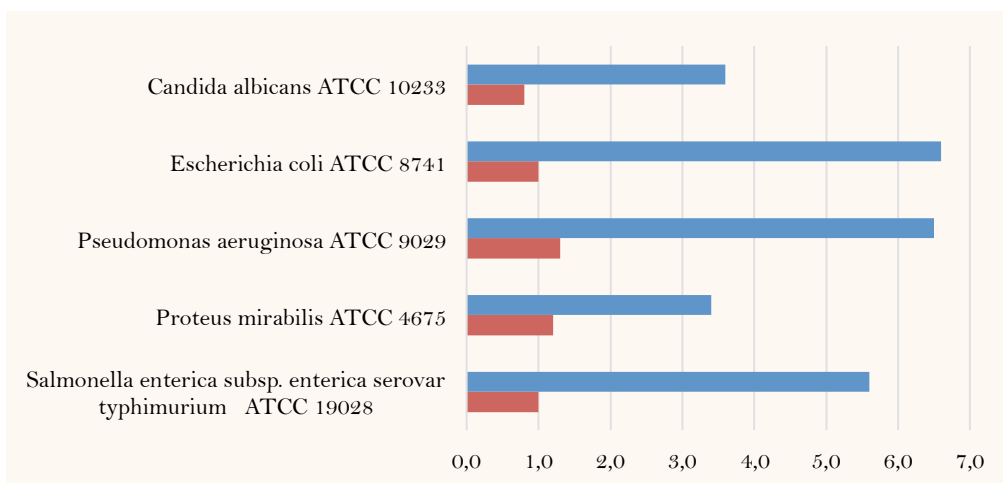


Figure 1. Average microbial growth based on McFarland standard before (blue bars) and after toothbrush disinfection with 3.5% NaOCl (red bars).

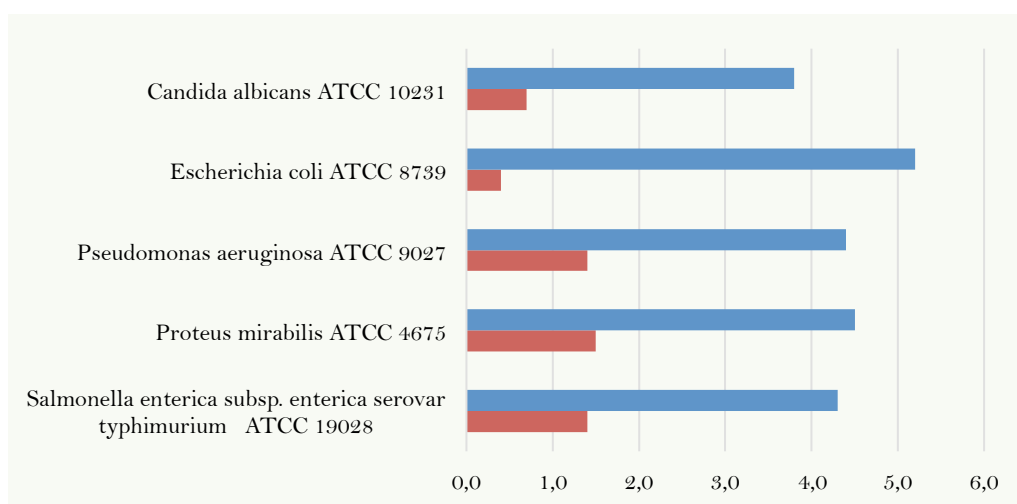


Figure 2. Average reduction on microbial growth based on McFarland standard before (blue bars) and after toothbrush disinfection with 0.12% CHX (red bars).

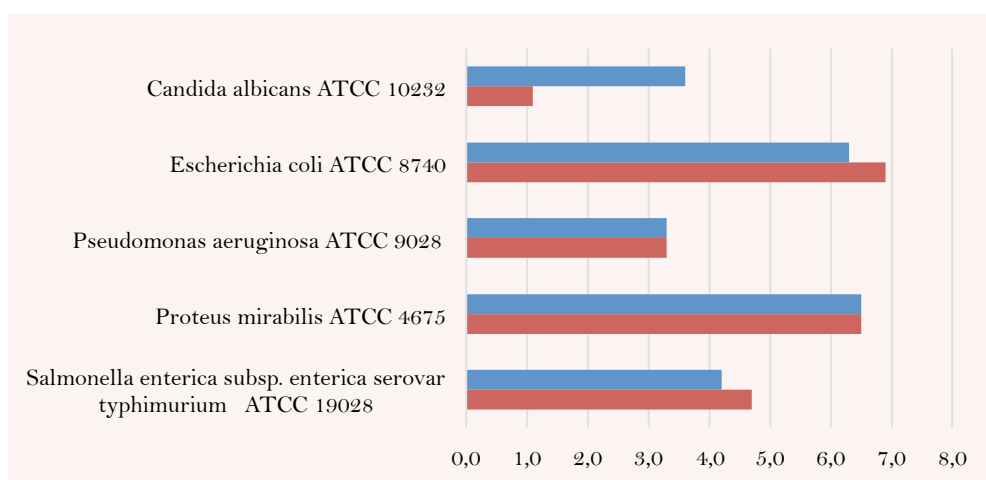


Figure 3. Average microbial growth based on McFarland standard before (blue bars) and after toothbrush disinfection with Listerine (rede bars).

In preliminary results there is a superior average microbial reduction using 3.5% NaOCl but slightly superior than 0.12% CHX. Microbial reduction with Listerine was very low as shown in the Figure 4 and Table 2.

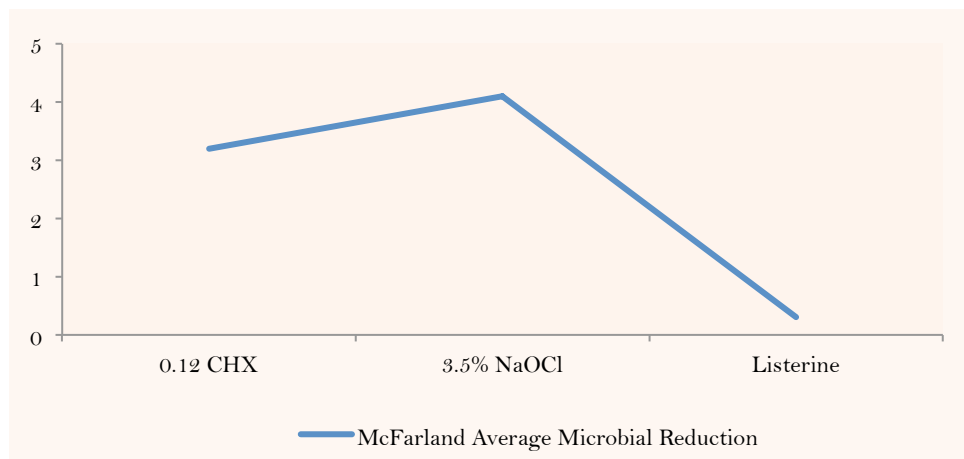


Figure 4. Average microbial reduction.

Table 2. Reduction of microbial grown based on McFarland standard.

Chemical Agent	N	Mean	SD	Minimum	Maximum
0.12% CHX	5	3.6	0.80	2.90	4.80
3.5% NaOCl	5	4.08	1.50	2.20	5.60
Listerine	5	0.28	1.27	-0.60	2.50
Total	15	2.57	2.04	-0.60	5.60

## Discussion

Toothbrushes are items prone to contamination, thus can be considered as reservoir not only for oral microorganisms, but other microorganisms from environment and bioaerosols of the place where are kept during its useful time; in consequence many protocols have been established for its disinfection [5,6,10].

Recently, Enterobacteria are increasingly associated to oral cavity, as transient microbes or even primary pathogens in immunocompromised hosts. This fact has become a keystone to develop chemical and physical strategies to diminish the microbial burden in toothbrushes bristles and the risk these bacteria may represent for special hosts [3,5].

Based in our results, we observed a solid microbial reduction rate with 3.5% NaOCl whit superior efficacy against *C. albicans* and *E. coli*. A previous study evaluated the disinfection effectiveness of different chemical agents on toothbrushes and found that 2% NaOCl was the second most efficient against several bacteria [7]. Similarly, NaOCl was tested as disinfectant against diverse microorganisms and it was more effective on *E. coli* [11]. NaOCl antimicrobial capacity was evaluated at 0.08% concentration for 15 minutes toothbrush immersion and showed that this procedure satisfactory eliminated *E. coli*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Streptococcus*

*pyogenes* from the toothbrush surface [13]. Our results are similar to those described in the literature [11,13] and the superior effectiveness of NaOCl above other chemical agents was also evident in this research.

Our results demonstrate a similar microbial reduction capacity for NaOCl and CHX, being NaOCl slightly superior to CHX; nonetheless Listerine media reduction was lower in comparison to the first two agents. Low standard deviation was observed related to equality of population variances.

Likewise, it is important to consider the innocuously interaction among toothbrushes bristles and the selected chemical agent for disinfection [14]. To our knowledge, there are no studies about the deleterious impact of NaOCl on toothbrushes bristles; however another physical and chemical methods such as electrical dishwashers and microwave described in some literature, may represent a disturbance to lifelong use of toothbrushes and compromise its ability to biofilm elimination [6-9,11].

In this investigation, 0.12% CHX antimicrobial effect against selected microbial strains was successfully demonstrated; specifically best results were observed on *E. coli* and *C. albicans* microbial burden reduction. CHX is a cationic agent with wide antimicrobial spectrum and it is considered as gold standard in comparison with other chemical agents. It has been established by previous authors, the effectiveness of CHX in the reduction of *S. mutans* on toothbrush surface [15,16]. In 2000 not only CHX but also NaOCl were proposed by several authors as ideal chemical agents for toothbrush disinfection [2,15-17].

Essential oil derivatives (Listerine) are chemical agents traditionally considered as trustable antiseptics and disinfectants with no secondary effects on host's oral cavity [1,6,8,9]. Listerine is a mouth rinse composed by menthol, ethanol, thymol, eucalyptol (essential oils) and methyl salicylate as active agents; previous investigations reported that this mouth rinse has wide range of disinfectant properties together with CHX [18]. Our results differ from those reported in consulted literature; in our case the average microbial reduction of Listerine was the lowest in comparison to CHX and NaOCl. Interestingly, we observed that *E. coli* and *S. enterica* growing rate was superior after the toothbrush immersion in Listerine. The only exception was observed against *C. albicans*, which had a lower growing rate after the immersion; this last result is similar to the observations reported in 2016 in a literature review that referred to Listerine as a superior chemical agent along with CHX in *Candida* biofilm elimination [19].

## Conclusion

Diverse chemical agents are available to be used as toothbrush disinfectants; 3.5% NaOCl and 0.12% CHX are the most effective for toothbrush disinfection against the microbial strains used in this study and Listerine was only effective against *C. albicans*. We suggest toothbrush immersion in non-diluted solutions of NaOCl or CHX for appropriate disinfection of these items.

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**Conflict of Interest:** The authors declare no conflicts of interest.

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