Ozone Therapy with Ozonized Water: An Alternative Process for the Sterilization of Manicure Pliers *In Vitro*

Ursulandrea Sanches Abelan[1], Morian Lauana Miguelão Canada[1], Dora Inés Kozusny-Andreani[2*], Renato Amaro Zângaro[3,4] and Rogério Rodrigo Ramos[2]

[1]*Centro Universitário de Rio Preto (UNIRP), São José do Rio Preto, Brazil*

[2]*Universidade Brasil, Fernandópolis, Brazil*

[3]*Biomedical Engineering Institute - Anhembi Morumbi University, São José dos Campos, Brazil*

[4]*Center for Innovation, Technology and Education-CITÉ, São José dos Campos, Brazil*

Abstract: The instruments' prophylactic measures in beauty salons are not effective and require an alternative method that is capable of eliminating the microorganisms adhered to the surface of the materials. The objective of the study was to evaluate the ozonized water effectiveness on pliers used by manicures. The study was performed out in a microbiology laboratory, 95 cuticle pliers were used in the sterilization with ozonized water. The treatments consisted of 5 groups: (A) no treatment, (E) pliers sterilized by commercial establishments, B05, B10, and B15 treated with ozonized water at different times (5, 10, 15 minutes). It was verified the presence of *C. Albicans* in 39,58% of non-sterilized pliers, 37,5% in those sterilized by manicures, in those treated with ozone for 5 minutes, 14,58%, and in 10 minutes, 8,33%. The *E. coli* and *S. aureus* were present in the non-sterilized pliers in 70% and 75% and, 30%, and 25% in the ones sterilized by manicures. The other microorganisms (*Micrococcus sp, Pseudomonas aeruginosa, Epidermophyton sp, and Proteus*) were present in nine occurrences, being 66.67% in the non-sterilized ones and 33.33% in the manicure ones. The B15 was efficient to eliminate all microbial agents. The sterilization of the instruments by ozonized water was able to inactivate microbial agents, showing that the time of 15 minutes was enough to present antimicrobial activity on the microorganisms. Further studies are suggested on the use of ozone in materials used in embellishment services on feet and hands, due to a few scientific studies in this area.

Keywords: Disinfection, Ozone Action, Prophylaxis, Esthetic Equipment, Beauty and Aesthetics Centers.

1. INTRODUCTION

Historical evolution shows that the search for beauty and aesthetics has undergone many changes, being currently the human goal and fulfillment [1]. As a result, the beauty and aesthetics market has been growing in recent decades, increasing the employer's labor force, among them, manicures and pedicures [2,3].

As a result of this growth, Brazilian Toiletry, Perfumery and Cosmetic Association's 2019 Yearbook [4] reports that in 2018 alone, created 5.4 million job opportunities, 1% more than in 2017 (5.3 million). The demand for personal care products, hygiene services, and beauty products, resulted in $ 30 billion in 2018, ranking Brazil in the fourth-largest global consumer market of personal care [4], even in a period of the financial crisis, is notoriously high the demand for beautification and use of services in beauty salons.

However, it is important to emphasize that the beautification procedures performed in commercial establishments are likely to transmit infectious diseases by pathogens, the most frequent being onychomycosis, onycholysis, onychodystrophy, hepatitis B and C, and acquired immunodeficiency syndrome [5,6]. These diseases can be transmitted during the process of the eponychium (cuticles) removal from the fingernails and toes, constituting a public health problem in Brazil and the world [7].

Being aware of this complexity, metallic instruments such as scissors, cuticle retractors, tweezers, and, mainly, nail pliers must be sterilized. This treatment is the only way to prevent the transmission of infectious diseases [8]. The sterilization efficiency depends on previous preparation, such as washing, drying, checking, packing, sterilization, and storage of the instruments [9,10].

It is extremely important to highlight the sterilizations by chemical products and equipment most used in beauty salons. The chlorhexidine digluconate is an antiseptic chemical used in the decontamination process of the instruments. ANVISA regulates a maximum concentration of 0.3% [11]. Chlorhexidine is a cationic agent, with a wide spectrum against Gram-positive and Gram-negative bacteria, and it acts against fungi and some types of viruses [12], given this, it is observed low viral toxicity. The enzymatic cleaner has the purpose of improving the quality of cleaning

*Address correspondence to this author at the Projected Road F1 s/n, Santa Rita Farm, Medical School, Brazil University, Box: 15613-899, Fernandópolis, Brazil; Tel: 055(17)3465-4200; E-mail: doraines@terra.com.br

E-ISSN: 2311-1755/20

and preserving the instruments. It acts on proteins and grease that are deposited and adhered to the material's surface, facilitating the material cleansing [13,14]. It is important to deny the use of the enzymatic agent for the instruments' sterilization, due to its function only for cleaning the metallic material.

As for equipment sterilizations, there are emphasized the autoclave and the sterilization oven. The autoclave uses pressurized saturated steam; the sterilization oven provides sterilization by dry heat. The dry heat sterilization requires a longer exposure time at high temperatures so that the penetration and distribution of heat in the instruments is uniformly eliminating the microorganisms, however, in case the exposure time and the temperature are lower than recommended, the result of this process suggests that the instruments were not sterilized. Another concerning factor is the establishment sizes, being small and inappropriate for washing, sterilizing, and storing the instruments [6,7,15,16]. It is also important to mention the factors linked to the difficulties in the sterilization process such as errors in sterilization due to lack of maintenance and poor use of the equipment, and it is good to remind the resistant organic material, in which the microorganism might resist the sterilization process [17].

As a consequence of the prophylactic measures not being totally effective and a constant preoccupation of the professionals, and even clients, in this area, it is important to emphasize the need of implementing an alternative treatment that is capable of eliminating the microorganisms adhered to the surfaces of the entire instruments [18]. Therefore, as proposed by the study, ozonized water was used for the sterilization of the instruments. Ozone (O_3) has been recommended for its high oxidation property in organic and inorganic compounds and, in this way, it can act as a sterilization agent, inactivating the microorganisms adhered to the most varied surfaces [19,20].

Ozone promotes the inactivation of bacteria, viruses, fungi, yeasts, and protozoa by disrupting the integrity of the bacteria's cell envelope through the oxidation of phospholipids and lipoproteins. In fungi, O_3 inhibits cellular growth in certain stages. Regarding the virus, O_3 damages the viral capsule and interferes with the reproductive cycle, interrupting virus-cell contact as a result of peroxidation. In this way, cells that are vulnerable to virus invasion become susceptible to oxidation and elimination by the body, which then replaces them with healthy cells [21,22].

In disinfection processes, many of the numerous compounds cause mutations and waste residue. The ozonization technique was performed through adequate practices, dosages, and exposure time, on the other hand, no residues were found, thus providing a very effective result, besides the proven germicidal properties of O_3. Other advantages would be low investment cost, easy application, and maintenance. The use of ozone can be in gas, aqueous, or oil form [17,23,24].

The ozone microbicidal properties in an aqueous solution allow it to be an option in the exchange of the chemical products in the equipment disinfection and sterilization. The effectiveness of ozone use in the process is linked to temperature, pH, salt concentration, amount of organic matter, microbial population, and exposure time [22,25-27]. One of the main ozone benefits is the use of washing and rinsing systems in a series of cycles of the external and internal surfaces of equipment [28].

Although the ozone inhibitory and lethal effects on pathogenic organisms have been observed since the last part of the 19th century, the processes for these effects have not yet been elucidated satisfactorily [22]. Ozone is a strong germicide that needs only a few micrograms for measurable antimicrobial activity. In low concentrations, ozone rapidly inactivates viruses, coliform bacteria, *Staphylococcus aureus*, *Aeromonas hydrophilia*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, and many other microorganisms [26,27,29].

It is in this context, the research presents the objective of evaluating the action effectiveness of the ozonized water on the instruments manipulated by manicures in the city of São José do Rio Preto, São Paulo.

2. MATERIALS AND METHODS

2.1. Study Design

In a randomized experimental study, 95 cuticle pliers were evaluated, supplied by 19 companies of São José do Rio Preto city, SP. The treatments consisted of 5 groups, the control group, the group with pliers sterilized by manicures, and the 3rd, 4th, and 5th group treated with ozonized water at different times (5, 10, and 15 minutes), carried out in a microbiology and chemistry laboratory of the Brasil University.

2.2. Treatment Methods

The treatments were composed of five groups, being 19 pliers for each group: group A - pliers without sterilization (control group); group E - pliers sterilized by the commercial establishments; group B05 - pliers submerged for 5 minutes in ozonized water; group B10 - pliers submerged for 10 minutes in ozonized water; group B15 - pliers submerged for 15 minutes in ozonized water.

2.3. Ozonation and Antimicrobial Effect of Ozonized Water

The synthesis of ozonized water was performed in a chemistry laboratory in a fume hood, according to the methodology described by Kozusny-Andreani *et al*. [26]. The ozone was produced through a generator (Ozon & Life). An oxygen cylinder supplied the pure oxygen and a silicone tube to the diffuser, generating 2ppm/min /L of ozonized water, conducted the ozone constantly produced by the equipment. To evaluate the ozone stylization efficiency, the pliers were submerged for 5, 10, and 15 minutes in ozonized water. The water ozonation and the pliers' treatment were conducted at a temperature of 25ºC. After treatment with ozonized water, the pliers were submitted to microbiological analysis in a microbiology laboratory, as described in subtitle 2.4, to evaluate the antimicrobial activity of ozonized water.

2.4. Microbiological Analysis of Specimens

After treatments, a sterile swab was swabbed over the cutting surfaces pliers, deposited in test tubes containing 10 mL of 0.5% sodium chloride (NaCl) solution. Then, 100 µL were inoculated in Petri dishes containing Sabouraud Dextrose Broth (Oxoid™) and Trypticase Soy Agar (Oxoid™), incubated at 35ºC for 24-48 hours for yeast and bacterial culture, while cultures of dermatophytes lasted up to 15 days. After these periods, the Colony-Forming Units (CFU) were counted. All tests were performed three times.

The microorganisms' identification was made by morphological methods, according to the methodology described by Trabulsi and Alterthum [30] for Gram staining and by Holanda *et al.* [31] for biochemical tests. Gram staining is a broad-spectrum method of staining on cell walls, where the Gram-positive bacteria were stained with the violet crystal and the Gram-negative with fuchsine. In the biochemical method, a colony was transferred in different agar mediums to obtain pure cultures. The Gram-positive bacteria were sub cultivated in Agar Baird-Parker (Oxoid™), for later identification by coagulase, catalase, NaCl 5%, oxidase, novobiocin, and DNA tests. Gram-negative bacteria were transferred to Agar Eosin Methylene Blue (Oxoid™) and identified by the API20E (Analytical Profile Index, BioMérieux™) system. For yeast identification, it was performed an evaluation of the germinating tube formation, urease tests, and carbohydrate fermentation.

2.5. Data Analysis

The data obtained were analyzed by chi-square tests; from Kruskal-Wallis with Dunn's post-hoc multiple comparison tests, using Minitab 15™ software. The statistical tests were evaluated with a significance level of 0.001 ($P<0.001$).

3. RESULTS

Microbiological analysis was performed on all pliers before treatment and several microbial agents "*Candida albicans, Escherichia coli, Staphylococcus aureus, Micrococcus sp, Pseudomonas sp, Epidermophyton sp, and Proteus sp*" were observed. The result of this analysis showed that all pliers were contaminated, mainly those sterilized by manicures.

Table **1** presents the descriptive statistics of the microorganisms count to the applied treatment. The results of this Table show the existence of a significant difference in the total number of the mesophilic count when pliers were compared to the applied sterilization treatment since the P-value found was lower than the significance level adopted for the test ($P=0.001$). The maximum number of CFU microorganisms detected in the non-sterilized and sterilized pliers were $2{,}00.10^9$, which was entirely eliminated in the 15-minute treatment of ozonized water.

Dunn's multiple comparison test demonstrated that non-sterilization treatments (A) and those sterilized by manicures (E) showed significant differences from water ozonized treatments for 5, 10, and 15 minutes. Restricting the analysis to the pliers which were submitted to treatment with ozonized water, it was possible to observe that the pliers treated with ozonized water for 5 minutes (B05) presented differences to the pliers ozonized for 15 minutes (B15), indicating that the treatment for 5-minute was not as effective as 15-minute, which eliminated all microorganisms (Table **1**).

Table **2** shows the microbial agents and the percentage variation found in the evaluated treatments,

Table 1: Descriptive Statistics of the Total Count of Mesophilic Microorganisms about the Treatment Applied

Treatment	$\bar{x} \pm s$	Md	Min	Max	*P-value
A	$4{,}21.10^8 \pm 8{,}22.10^8$	$6{,}60.10^{4\ a}$	$1{,}50.10^3$	$2{,}00.10^9$	<0,001
E	$2{,}10.10^8 \pm 6{,}19.10^8$	$3{,}60.10^{4\ a}$	$1{,}00.10^3$	$2{,}00.10^9$	
B05	$1{,}05.10^8 \pm 4{,}50.10^8$	$0{,}00^{\ b}$	0,00	$2{,}00.10^9$	
B10	$3{,}67.10^3 \pm 1{,}29.10^4$	$0{,}00^{\ bc}$	0,00	$7{,}80.10^4$	
B15	0,00±0,00	$0{,}00^{c}$	0,00	0,00	

A: non-sterilized pliers, E: pliers sterilized by manicures, pliers treated with ozonized water for 5 (B05), 10 (B10), and 15 (B15) minutes. *P-Value referring to the Kruskal-Wallis test at 5% significance.

Table 2: Percentage Variation (%) of Microorganisms in Comparison to the Evaluated Treatments

Microorganisms	Treatment	CFU (percentage)	*P-value
Candida albicans	A	19 (39,58%)	=0,001
	E	18 (37,50%)	
	B05	7 (14,58%)	
	B10	4 (8,33%)	
	B15	0 (0,00%)	
Escherichia coli	A	7 (70,00%)	=0,001
	E	3 (30,00%)	
	B05	0 (0,00%)	
	B10	0 (0,00%)	
	B15	0 (0,00%)	
Staphylococcus aureus	A	6 (75,00%)	=0,001
	E	2 (25,00%)	
	B05	0 (0,00%)	
	B10	0 (0,00%)	
	B15	0 (0,00%)	
Others *Micrococcus sp, Pseudomonas sp, Epidermophyton sp* e *Proteus*	A	6 (66,67%)	=0,001
	E	3 (33,33%)	
	B05	0 (0,00%)	
	B10	0 (0,00%)	
	B15	0 (0,00%)	

A: non-sterilized pliers, E: pliers sterilized by manicures, B5: B10, B15: pliers treated with ozonized water for 5, 10, and 15 minutes, CFU: Colony-Forming Units.

with the presence of a significant association between the utilized treatments and the occurrence of microorganisms (P=0.001). The results of this Table show a significant presence of *Candida albicans* in the majority of the analyzed pliers, with 48 occurrences. Of these occurrences, 39.58% (n=19) were identified in the non-sterilized pliers (A), 37.50% (n=18) in those sterilized by manicures (E), in pliers treated with ozonized water for 5 minutes (B05), 14.58% (n=7) and in 10 minutes (B10) 8.33% (n=4). It was possible to observe the decrease in the occurrence of this microorganism with the ozonized water treatment; however, in the periods evaluated, the pliers still presented contamination by *Candida albicans*. For the pliers, which were submitted to the 15-minute ozonized water treatment, there was no microbial growth.

In the non-sterilized pliers (A) and sterilized (E) by manicures (Table **2**), there was the presence of *E. coli*, 70% of them were found in the non-sterilized pliers and the other 30% in those sterilized by manicures; *Staphylococcus aureus*, 8 occurrences (A, 75% and E,

25%) and the other microorganisms found, called others (*Micrococcus sp, Pseudomonas sp, Epidermophyton sp, and Proteus sp*) in 9 occurrences, being the pliers without sterilization with 66.67% and sterilized by manicures with 33.33%. For the pliers that were submitted to treatment with ozonized water for 5, 10, and 15 minutes, there was no microbial growth (*E. coli, S. aureus, M. sp, P. sp, E. sp, and P. sp*).

4. DISCUSSION

The importance of this study is due to Brazilian beauty and aesthetics companies offer the population numerous services and in many of them using instruments that, if not sterilized properly, can transmit contagious diseases. According to the Municipal Health Secretary of São José do Rio Preto town, the process of materials sterilization used by manicures represents a possible solution to minimize the transmission of diseases such as AIDS, Hepatitis, Onychomycosis, among other infections [32]. Nevertheless, it is important to emphasize that the sterilization of the instruments performed in Rio Preto establishments were not efficient to inactivate certain microorganisms as "*Candida albicans, Escherichia coli, Staphylococcus aureus, Micrococcus sp, Pseudomonas sp, Epidermophyton sp, and Proteus sp*". It is suggested a failure in the prophylaxis and the need to reinforce the constant equipment monitoring or even the renovation of the instruments sterilization method to avoid the pathogenicity of the infectious agent in the beauty salons.

Through *in vitro* experiments, it was verified that the groups not treated with ozonized water had an occurrence of *C. Albicans, E. coli, S. aureus, Micrococcus sp, Pseudomonas sp, Epidermophyton sp, and Proteus* (Table **2**). The groups treated with ozonized water were able to reduce and eliminate the presence of microorganisms in pliers (Table **1**). This result suggests that ozonized water can be considered and recommended as an alternative method for the cuticle pliers' sterilization. Thus, the use of ozone in an aqueous solution can act as a sterilizing agent due to high antimicrobial efficiency. For Ekren and Ozkomur [33], O_3 is considered as an antimicrobial agent, having a direct effect on the plasmatic membrane and enzymatic systems, with high efficiency and speed, due to its highly oxidizing activity, another advantage is that it makes bacterial resistance impossible.

Table **2** illustrates the percentages of the microorganisms cases found concerning the evaluated treatments, with the presence of a significant relation between the treatments used for the pliers' sterilization, and the microorganisms' occurrence (P=0.001). The relation is in the fact that there was a drastic reduction in the number of occurrences when the pliers that were not sterilized (A) and sterilized by manicures (E) are compared with the pliers that were submitted to ozonized water (B05, B10, and B15). This result shows the effectiveness of the treatment with ozonized water to sterilize the pliers used by manicures, being that the treatment B15 eliminated the total count of mesophiles, guaranteeing the sterilization of these instruments.

In treatments with ozonized water for 5 and 10 minutes, 14.58% and 8.33% of pliers (Table **2**), only *C. Albicans* were found. The occurrence of this microorganism in non-sterilized pliers (A, 39.58%) and those sterilized by manicures (E, 37.50%) was numerically very close and well above the value found in ozone-treated pliers. These results are relevant because they are yeasts that live in nails and are part of the normal microbiota, however, in adverse situations and the Brazilian tropical climate, they can invade the tissues and become pathogenic [34].

The results show the presence of *E. coli*. Even being less incident in treatment A and E (Table **2**), it is important to emphasize that this microorganism is a pathogenic bacteria involved in outbreaks of food-borne diseases and presents virulence factors that make them capable of causing intestinal diseases, as well as infections in the genitourinary tract, meningitis, and septicemia [35]. It should be noted that *E. coli* is a producer of Shiga or Vero toxigenic toxin, this microbial agent should be highlighted due to its link to a broad spectrum of human diseases, ranging from mild to severe bloody diarrhea (hemorrhagic colitis) that can evolve to complications such as Hemolytic Uremic Syndrome, renal failure and Thrombocytopenic Purpura [36,37].

When ozonized water is used, its effectiveness against *Escherichia* coli and *Candida albicans* present in human radicular channels and the disinfection of blood dialyzers was observed. The ozonized aqueous solution showed its efficacy in eliminating *E. coli* and significantly reduced the presence of *C. Albicans* [10,38]. Similar results were described by Dosti *et al.* [39], against *Enterobacter cloacae* and *Enterobacter aerogenes* (industrialized food) cultures, when ozonized for 10 minutes, were almost eliminated and, in some cases, completely eliminated. It should be noted that the effects are similar to the *in vitro* study;

however, the ozonized water on the pliers with a 15-minute treatment was able to eliminate all microorganisms, aiming that this treatment can be considered to inactivate the microorganisms.

Micrococcus sp, *Epidermophyton sp*, and *Proteus* and *Pseudomonas aeruginosa* microorganisms were found, in smaller numbers, in non-sterilized treatments (A) and in those sterilized by manicures (E), being absent in ozonized water treatments at different times. As in studies performed by Curtiellas *et al*. [40] and Prabakaran *et al*. [20], the ozone action was registered in the decrease or inactivation of *E. coli*, the same was reported by researchers Estrela *et al*. [41], against the culture of *S. aureus*, which was also confirmed in this study.

5. CONCLUSION

The ozonized water was efficient in sterilizing cuticle pliers when applied for 15 minutes. The pliers' sterilization methods used by the companies were not efficient to eliminate all microorganisms, since the results showed the presence of several microbial agents, presenting a contamination risk for clients. The literature regarding the use of ozone in materials used in beauty and aesthetic services on feet and hands procedures is practically null, so there is a need for more studies about ozonized water to sterilize cuticle pliers.

ACKNOWLEDGEMENT

To the Centro Universitário de Rio Preto for the support and to the Professors and collaborators of the Laboratory of Microbiology and Chemistry of the University Brazil for the contribution of the master's research.

DECLARATION OF POTENTIAL CONFLICT OF INTEREST

The authors declare no conflict of interest.

FINANCING

There was no funding for this study.

REFERENCES

[1] Gallas JC, Cancellier ÉLPL, Vargas SML, Rossetto CR. Comportamento estratégico no setor de beleza e estética baseado na tipologia de Miles e Snow. Rev Organiz Contexto 2015; 11: 119-41. https://doi.org/10.15603/1982-8756/roc.v11n22p119-141

[2] Garbaccio JL, Oliveira AC. O risco oculto no segmento de estética e beleza: uma avaliação do conhecimento dos profissionais e das práticas de biossegurança nos salões de beleza. Texto contexto – enferm 2013;22:989-98. https://doi.org/10.1590/S0104-07072013000400015.

[3] Garbaccio JL, Oliveira AC. O risco oculto no segmento de estética e beleza: uma avaliação do conhecimento dos profissionais e das práticas de biossegurança nos salões de beleza. Texto Contexto - Enferm 2013; 22: 989-98. https://doi.org/10.1590/S0104-07072013000400015

[4] Associação Brasileira da Indústria de Higiene Pessoal, Perfumaria e Cosméticos. Anuário ABIHPEC 2019. August 2019. Available from: https://abihpec.org.br/publicacao/anuario-abihpec-2019/

[5] Covisa. Coordenação de Vigilância em Saúde. Beleza com segurança: Guia técnico para profissionais. São Paulo: Covisa, 2009.

[6] Brasil. Ministério da Saúde. Departamento de doenças de condições crônicas e infecções sexualmente transmissíveis. Available from: http://www.aids.gov.br/pt-br

[7] Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Hepatites virais: o Brasil está atento / Ministério da Saúde, Secretaria de Vigilância em Saúde, Departamento de Vigilância Epidemiológica. 3rd ed. Brasília: Ministério da Saúde, 2008.

[8] Oliveira-Filho AB, Pimenta AS, Rojas MF, *et al*. Likely transmission of hepatitis C virus through sharing of cutting and perforating instruments in blood donors in the State of Pará, Northern Brazil. Cad Saúde Pública. 2010; 26: 837-44. https://doi.org/10.1590/S0102-311X2010000400025

[9] Anders PS, Tipple AFV, Candé TA, Barros CA, Miranda PV, Pimenta FC. Tubos de látex: esterilidade pós-reprocessamento em vapor saturado sob pressão. Rev Eletrônica Enferm 2009; 11: 280-5. https://doi.org/10.5216/ree.v11.46958

[10] Huth KC, Quirling M, Maier S, *et al*. Effectiveness of ozone against endodontopathogenic microorganisms in a root canal biofilm model. Int Endod J 2009; 42: 3-13. https://doi.org/10.1111/j.1365-2591.2008.01460.x

[11] Anvisa. Agência Nacional de Vigilância Sanitária. A Anvisa esclarece sobreas soluções tópicas com clorexidina: Medicamento ou cosmético? Available from: http://antigo.anvisa.gov.br/documents/33868/2658967/Clorexidina+-+Cosm%C3%A9tico+ou+Medicamento.pdf/5255cb84-0b7b-44fb-8404-3e0e046c9fe5

[12] Bednarek RS, Nassereddin A, Ramsey ML. Skin Antiseptics. StatPearls [Internet]. 2020. [Updated 2020 Nov 25]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK507853/

[13] Vieira FP, Freitas LK, Siqueira HCH, Silva JRS, Moura NO. Avaliando a eficiência da esterilização dos equipamentos utilizados nos serviços de manicure e pedicure: possíveis ações do enfermeiro. VITTALLE 2011; 23: 33-42.

[14] Anvisa. Agência Nacional de Vigilância Sanitária. Resolução da Diretoria Colegiada - RDC Nº 15, de 15 de março de 2012. Available from: https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2012/rdc0015_15_03_2012.html

[15] Melo FC, Isolani AP. Hepatite B e C: do risco de contaminação por materiais de manicure/ à prevenção. SaBios: Rev Saúde Biol 2011; 6: 72-8.

[16] Yoshida CH, Oliveira RA, Coelho PG, Fonseca FLA, Filipini R. Process of instrument sterilization in shops with manicure and pedicure services. Acta Paul Enferm 2014; 27: 18-22. https://doi.org/10.1590/1982-0194201400005

[17] Fitria S, Buntat Z, Nawawi Z, Sidik MAB, Jambak MI, Yuniarti D. Antibacterial potency of ozonated water against *Escherichia coli*. J Pure Appl Microbiol 2019; 13: 637-41 https://doi.org/10.22207/JPAM.13.1.73

[18] Malik SN, Ghosh PC, Vaidya AN, Mudliar SN. Hybrid ozonation process for industrial wastewater treatment:

Principles and applications: A review. J Water Process Engineering 2020; 35: 101193. https://doi.org/10.1016/j.jwpe.2020.101193

[19] Murphy L. Ozone-the latest advance in sterilization on of medical devices. Can Oper Room Nurs J 2006; 24: 28-38. Available from: https://pubmed.ncbi.nlm.nih.gov/16869464/

[20] Prabakaran M, Tamil SS, Merinal S, Panneerselvam A. Effect of ozonation on pathogenic bactéria. Adv Appl Sci Res 2012; 3: 299-302.

[21] Hashemi M, Jalili P, Mennati S, *et al*. The effects of prolotherapy with hypertonic dextrose versus prolozone (intraarticular ozone) in patients with knee osteoarthritis. Anesth Pain Med 2015; 5: e27585. https://doi.org/10.5812/aapm.27585

[22] Martínez-Sánchez G. Ozonized water, background, general use in medicine and preclinic support. Ozone Therapy Global J 2019; 9: 33-60. Available from: http://revistaespañoladeozonoterapia.es/index.php/reo/article/view/146

[23] Song Y, Liu D, Lu Q, *et al*. An Atmospheric-pressure large-area diffuse used for disinfection application. IEEE T Plasma Sci 2015; 43: 821-27. https://doi.org/10.1109/TPS.2015.2393952

[24] Huang Y, Kou Y, Zheng C, Xu Y, Liu Z, Yan K. *Escherichia coli* inactivation in water using pulsed discharged. IEEE T Plasma Sci 2016; 44: 1-6. https://doi.org/10.1109/TPS.2016.2559802

[25] Galdeano MC, Wilhelm AE, Goulart IB, *et al*. Effect of water temperature and pH on the concentration and time of ozone saturation. Braz J Food Technol 2018; 21: e2017156. https://doi.org/10.1590/1981-6723.15617

[26] Kozusny-Andreani DI, Andreani G, Prado LFA, *et al*. *In vitro* inactivation of pathogenic bacteria by the use of ozone in different exposure times. Rev Cubana Med Trop 2018; 70: 34-44.

[27] Carvalho MML, Kozusny-Andreani DI, Batigália F, *et al*. Comparative analysis of *in vitro* antibacterial effectiveness of ozonized and non-ozonized vegetable oils on *Neisseria gonorrhoeae*. J Mod Med Chem 2020; 8: 41-48 https://doi.org/10.12970/2308-8044.2020.08.05

[28] Ribeiro PH, Faroni LRD'A, Finger FL, Cecon PR, Heleno FF, Santos RR. Ozônio como agente fitossanitário na conservação pós-colheita da bata-baroa. Braz J Food Technol 2017; 20: e2016137. https://doi.org/10.1590/1981-6723.13716

[29] Wolf C, von Gunten U, Kohn T. Kinetics of Inactivation of waterborne enteric viruses by ozone. Environ Sci Technol 2018; 52: 2170-177. https://doi.org/10.1021/acs.est.7b05111

[30] Trabulsi LR, AlterthumF. Microbiologia. São Paulo: Atheneu, 2008.

[31] Holanda CMCX, Arimateia DS, Neto RM. Manual de bacteriologia e de enteroparasitos. Natal: EDUFRN, 2017.

[32] Secretaria Municipal de Saúde. Subsecretaria de Vigilância, Fiscalização Sanitária e Controle de Zoonoses. Superintendência de Vigilância e Fiscalização Sanitária em Saúde. Guia de Orientação Sanitária para Institutos de Embelezamento e Esteticismo. Rio de Janeiro: SMS, 2013. Available from: http://www.rio.rj.gov.br/dlstatic/10112/5796274/4151158/ManualSalao_Livreto.pdf

[33] Ekren O, Ozkomur A. Influence of ozone and paracetic acid disinfection on adhesion of resilient liners to acrylic resin. J Adv Prosthodont 2016: 8: 290-5. https://doi.org/10.4047/jap.2016.8.4.290

[34] Sousa CS, Torres LM, Azevedo MPF, *et al*. Ozônio na esterilização de produtos para assistência à saúde: revisão integrativa da literatura. Rev Esc Enferm 2011; 45: 1243-9. https://doi.org/10.1590/S0080-62342011000500030

[35] Sousa CP. The versatile strategies of *Escherichia coli* pathotypes: a mini review. J Venom Anim Toxins incl Trop Dis 2006; 12: 363-73. https://doi.org/10.1590/S1678-91992006000300002

[36] Caldorin M, Almeida IAZC, Peresi JTM, Alves EC. Ocorrência de *Escherichia coli* produtora de toxina Shiga (STEC) no Brasil e sua importância em saúde pública. BEPA, Bol Epidemiol Paul (Online) [periodical on the Internet]. 2013; 10: 4-20 [citado 2020 Feb 15]: Available from: http://periodicos.ses.sp.bvs.br/scielo.php?script=sci_arttext&pid=S1806-42722013000200001&lng=pt

[37] Mora A, Blanco JE, Blanco M, *et al*. Antimicrobial resistance of Shiga toxin (verotoxin) producing *Escherichia coli* O157:H7 and non-O157 strains isolated from humans, cattle, sheep and food in Spain. Res Microbiol 2005; 156: 793-806. https://doi.org/10.1016/j.resmic.2005.03.006

[38] Canada MLM, Zangaro, RA, Kozusny-Andreani DI. A eficiência da água ozonizada no reprocessamento de dialisadores sanguíneos. In: Encontro de Pós-Graduação e Iniciação Científica, 2013, Fernandópolis. Anais... Fernandópolis: Unicastelo, 2013; 1: 279.

[39] Dosti B, Guzel-Seyndim Z, Greene A. Effectiveness of ozone, heat and chlorine for destroying common food spoilage bacteria in synthetic media and biofilms. Int J Dairy Technol 2005; 58: 19-24. https://doi.org/10.1111/j.1471-0307.2005.00176.x

[40] Curtiellas V, Gutiérrez M, Sánchez E, *et al*. Characterization of *E. coli* cell lysis by ozone. In: IOA 17th World Ozone Congress, 2005, Strasbourg. Anais... Strasbourg: IOA , 2005: 3; 3-8.

[41] Estrela C, Estrela CRA, Decurcio DA, Silval JA, Bammann LL. Antimicrobial potential of ozone in an ultrasonic cleaning system against *Staphylococcus aureus*. Braz Dent J 2006; 17: 134-38. https://doi.org/10.1590/S0103-64402006000200010

Received on 31-10-2020 Accepted on 05-12-2020 Published on 11-12-2020

DOI: https://doi.org/10.12970/2311-1755.2020.08.05