

Original Article

Effect of ozone gas on cultures of *Candida albicans* and *Aspergillus fumigatus*: evaluation of two ozonation devices

Maicon Henrique Caetano¹, Adriano Menis Ferreira², Denise de Andrade³, Álvaro Francisco Lopes de Sousa^{3,4}, Liliane Moretti Carneiro⁵, Alessandra Lyrio Barbosa⁵, Herica Emilia Félix de Carvalho³, Marcelo Alessandro Rigotti², Margarete Teresa Gottardo de Almeida⁶

¹ Universidade Estadual Paulista (UNESP), Instituto de Biociências, Letras e Ciências Exatas (Ibilce), Campus São José do Rio Preto, São Paulo, Brazil

² Universidade Federal de Mato Grosso do Sul, Três Lagoas, Mato Grosso do Sul, Brazil

³ Human Exposome and Infectious Diseases Network (HEID); Universidade de São Paulo, Escola de Enfermagem de Ribeirão Preto, Ribeirão Preto, São Paulo, Brazil

⁴ Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Universidade de Lisboa, Lisboa, Portugal

⁵ Universidade Federal de Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil

⁶ Faculdade de Medicina de São José do Rio Preto, São José do Rio Preto, São Paulo, Brazil

Abstract

Introduction: *Candida albicans* and *Aspergillus fumigatus* are two important agents of Healthcare-associated infections. This study aimed to evaluate the antifungal activity of ozone (O₃) gas produced by two commercial devices against cultures of these two species.

Methodology: Sterile plastic plates were inoculated with *C. albicans* and *A. fumigatus* and placed on a countertop at three distances (30 cm, 1 m, and 2 m) and three positions in relation to the wall (near, middle, and away), considering the source of O₃. Plates were exposed to O₃ for one hour and incubated. After incubation, the counting of colony-forming units was performed. As a control, an inoculated plate was incubated, without being exposed to O₃. Tests were carried out with two different devices (namely, Mod.I and Mod.II), with the air conditioner on and off, in triplicate.

Results: Both devices showed antifungal activity. Mod. I presented better results, due to a higher flow rate. The best activity was on plates at 30 cm, middle position. Contrarily, on plates at 2m, near the wall, the inhibition activity was lower. The best results were obtained with the air conditioner off. *Candida albicans* was more sensitive to O₃ than *A. fumigatus*.

Conclusions: This method of decontamination by O₃ gas shows potential due to its fast and easy execution. The establishment of new protocols for hygiene and hospital disinfection using this approach should be considered, which may reduce environmental contamination by fungi and, consequently, the burden of fungal infections.

Key words: Ozone; ozonation; *Candida albicans*; *Aspergillus fumigatus*; disinfection.

J Infect Dev Ctries 2024; 18(3):473-479. doi:10.3855/jidc.15344

(Received 17 May 2021 – Accepted 25 August 2021)

Copyright © 2024 Caetano *et al.* This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Fungal infections are of enormous importance in the scenario of nosocomial infections at Health Care Institutions, with increasing morbidity and mortality rates. Microorganisms transmitted by air, water, and/or food can contaminate body surfaces and remain in the hospital environment, increasing the risk of infectious diseases. Several fungi may act as opportunistic or primary pathogens. In this context, *Candida* spp. and *Aspergillus* spp. are especially relevant [1-4].

Candida albicans is part of the normal microbiota. However, it can cause infections by rupturing the biological balance, and predisposing pathological, mechanical, physiological, or immunological

conditions [5-7]. *Aspergillus fumigatus* is a ubiquitous saprophytic fungus that releases millions of conidia into the environment. It is a common cause of opportunistic invasive infections when inhaled, along with being an allergen [8-9].

Due to its high oxidant power ozone (O₃) is used for antimicrobial activity during disinfection processes and/or sterilization [10]. Although the mechanisms of its action are not fully understood, O₃ is known to act on cell walls of microorganisms causing the oxidation of glycopeptides, glycoproteins, and amino acids, modifying permeability, and leading to cell lysis. Ozone is recombined with cytoplasmic elements when it reaches inside the cell, causing oxidation of amino

Table 1. Models of O₃ generators tested in this study: GEO 20000/AR-TD (Mod.I) and GEO 20000/AR (Mod.II).

Technical specifications	GEO 20000-AR/TD (Mod.I)	GEO 20000/AR (Mod.II)
Flow rate (m ³ /h)/ppm O ₃	200 / 2.0 ppm	100 / 2.1 ppm
Maximum relative humidity (%)	75	75
Working temperature (°C)	6 -35	5 – 40
Nominal power [MW]	135	127
Power voltage (V)	127	110
Weight (kg)	5.40	4.9
Maximum working area (m ²)		300
Dimensions (cm)	18 × 30 × 47.5	18 × 30 × 37

m³/h: Cubic meter per hour; %: Percentage; °C: Celsius Degree; W: Watt; V: Volt; Kg: Kilogram; m²: Square meter; cm: Centimetre; PPM: Parts per million.

acids and nucleic acids, resulting in cleavage and consequent cell death. Ozone can also collapse cellular enzymatic activity due to its action on the sulfhydryl compounds of enzymes and alter the purine and pyrimidine bases of nucleic acids [10-12].

This study aimed to compare the antifungal activity of O₃ gas produced by two commercial devices against cultures of *C. albicans* and *A. fumigatus*.

Methodology

Ozone generators – technical specifications

We used two devices from OZON® company (Cuiabá, Brazil), the GEO 20000/AR-TD (Mod.I) and GEO 20000/AR (Mod.II). Their main specifications are shown in Table 1.

Microbial inoculum and experiment site

The isolates tested were *C. albicans* (ATCC 90028) and *A. fumigatus* (environmental origin, characterized phenotypically by macromorphology and micromorphology and genotypically by PCR) [13], from the fungal culture collection of the Laboratory of Microbiology of the Department of Dermatological, Infectious, and Parasitic Diseases of the Faculty of Medicine of São José do Rio Preto (FAMERP), São Paulo, Brazil, where experiments were carried out.

The inoculums of *C. albicans* and *A. fumigatus* were prepared and adjusted, by spectrophotometry, in correspondence to the 0.5 McFarland scale of turbidity (1×10^6 – 5×10^6 cells/mL). For each species, ten sterile

plastic plates containing Brain Heart Infusion Agar (BHI) (Oxoid®, Basingstoke, Hants, UK) were inoculated with 100 µL of the inoculum, which was spread with a Drigalski spatula.

Ozonation procedure – generators Mod.I and Mod.II

The experiments were carried out on a 2.72 m long and 0.58 m wide granite countertop, in a room 9.0 m² in size. After inoculation, nine plates were placed on the surface of the countertop at three different distances from the O₃ gas generator: 30 cm, 1 m, and 2 m. At each distance, the flow targeting of O₃ was evaluated in three different positions, namely A (plates near the wall), B (in the middle), and C (plates away from the wall), as shown in Figure 1.

Plates were kept without a lid during O₃ gas exposure, which occurred in a closed environment for one hour. Subsequently, plates were closed and incubated at 35 °C. As a control, an inoculated plate was incubated according to the same criteria, without receiving O₃ treatment. *C. albicans* and *A. fumigatus*, were incubated for 24 hours and 48 hours, respectively. After incubation the number of colony-forming unit (CFU/0.1 mL) in each plate was observed and compared with the control group. The experiments were carried out in triplicate, including the control. Percentages of inhibition were calculated considering the mean CFU counting of the treatment plates in relation to the control.

Figure 1. Illustrative photographic image of the positions and distances of the plates in relation to the source of O₃.

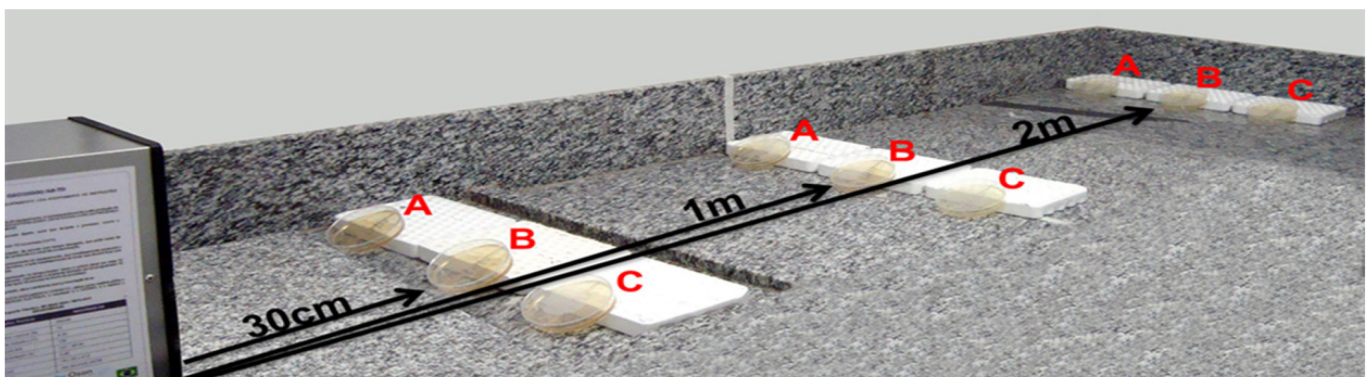


Figure 2. Arithmetic means and standard deviations of CFU counting of *C. albicans*, at 30 cm, 1 m, and 2 m; and positions A, B, and C. **D:** air conditioning on, Mod.I; **E:** air conditioning off, Mod.I; **F:** air conditioning on, Mod.II; **G:** air conditioning off, Mod.II. Ct. = Control.

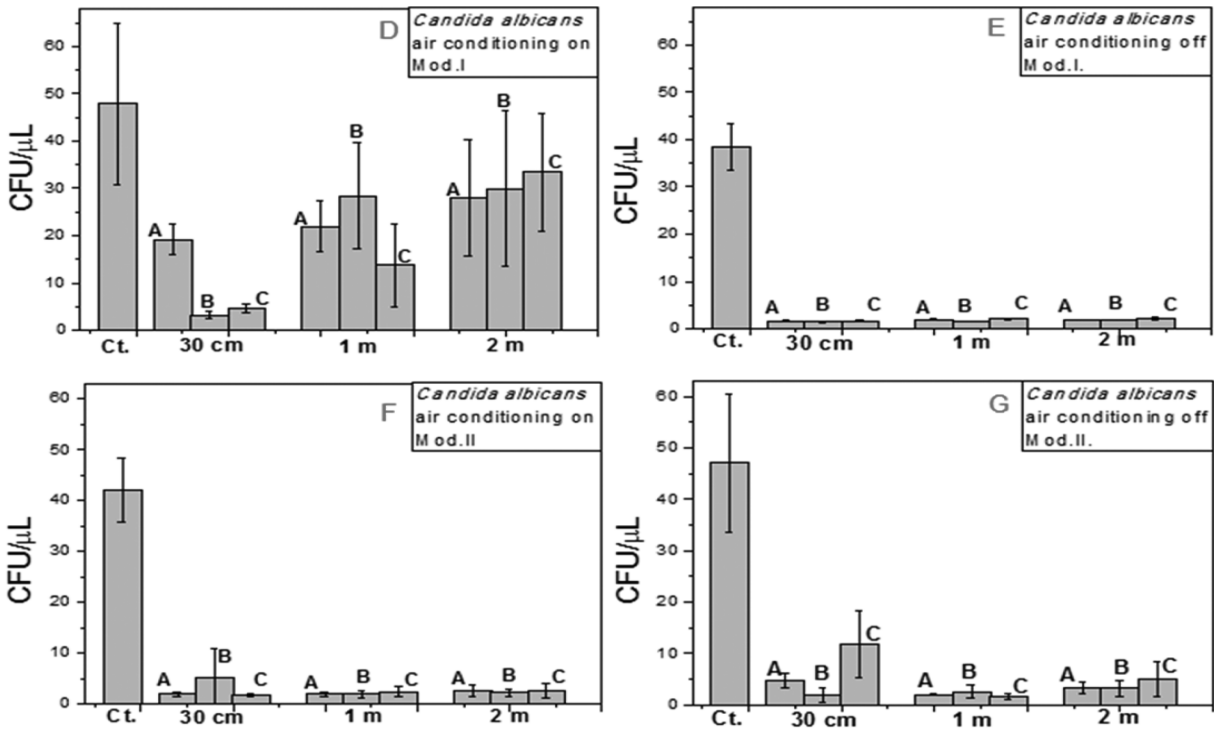
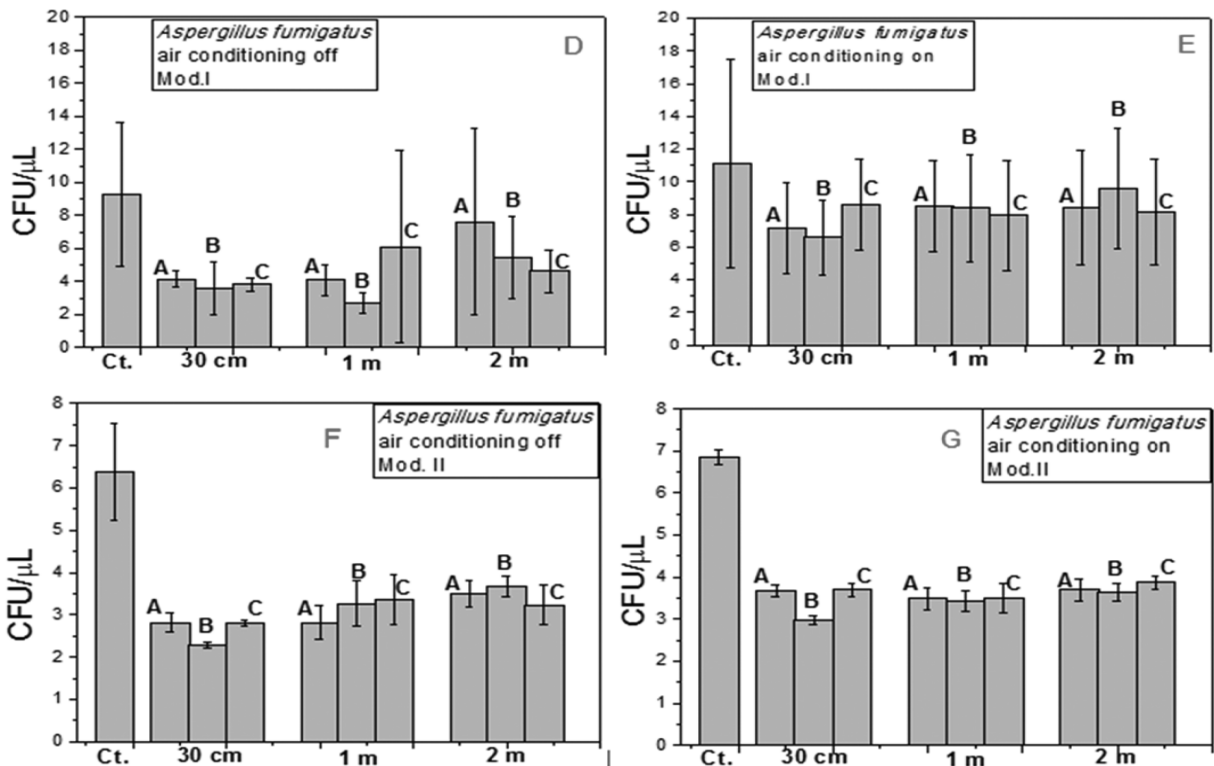


Figure 3. Arithmetic means and standard deviations of CFU counting of *A. fumigatus*, at 30 cm, 1 m, and 2 m; and positions A, B, and C. **D:** air conditioning on, Mod.I; **E:** air conditioning off, Mod.I; **F:** air conditioning on, Mod.II; **G:** air conditioning off, Mod.II. Ct. = Control.



The procedure described above was carried out at two distinct times: with air conditioning turned on and off. When turned on, air conditioning was set to 20 °C and maximum fan power. Temperature and humidity were monitored.

The data were subjected to statistical analysis, according to the arithmetic mean of fungal growth of triplicate tests. Then, the chi-squared test (χ^2) was applied to verify the associability and dependence between the variables: positions (A, B, and C) and distances (30 cm, 1 m, and 2 m), with the air conditioning turned on and off.

Results

The O₃ generators (Mod.I and Mod.II) showed antifungal activity in the two species studied. Plates showed a reduction in the number of CFU when compared with the control.

The Mod.I generator showed greater efficiency in the reduction of microbial load, considering distance and targeting parameters ($p < 0.05$). The results of the Mod.II devices also showed significant reductions ($p < 0.05$), except for *C. albicans* at 2 m ($p = 0.3581$) and *A. fumigatus* at 1 m ($p = 0.5985$) and 2 m ($p = 0.4874$), proving the associability between the variables.

Considering only the parameters of distances and positions, plates at 2 m showed the worst antifungal result for *A. fumigatus*, and plates B, at 30 cm, showed the best antifungal result for *C. albicans*, as shown in Figures 2 and 3.

Table 2 (Mod.I) and Table 3 (Mod.II) show the percentage values of inhibition for all variables. The

highest inhibition values occurred for *C. albicans* for both devices, with significant statistical differences ($p < 0.05$). The inhibition values of *A. fumigatus* cultures were less expressive, around 50%.

Antifungal activity of O₃ was better for the tests performed in the room with air conditioning turned off, for both fungal species. The percentages of inhibition were 83% and 78% for Mod.I and Mod.II, respectively. When the air conditioning was on, the values were lower, 28% and 67%, respectively.

Considering the environmental factors during the experiments, the average temperatures recorded were 21 °C and 25 °C, and the average humidity was 58% and 53%, with the air conditioning turned on and off, respectively.

Discussion

Considering the relevance of *C. albicans* and *A. fumigatus* as agents of nosocomial infections, our study evaluated the antifungal activity of O₃ gas on the surface of culture media contaminated by these two species. Our experiments considered different distances and positions of the fungi related to the source of O₃ and tested two devices with different specifications. In addition, we observed the interference of air conditioner flow on the antifungal activity of the O₃.

Our data showed significantly lower numbers of CFU on the plates exposed to O₃, as compared to the control, for both devices (Mod.I and Mod.II), and all variables (species, distance, position, air conditioning on and off).

Table 2. Percentage of inhibition obtained by the arithmetic mean of the CFU, at 30 cm, 1 m, and 2 m of distances; and positions A, B and C; with the air conditioner on and off; in comparison with the control (Mod.I).

		Air conditioner off			Air conditioner on		
		30 cm	1 m	2 m	30 cm	1 m	2 m
<i>C. albicans</i>	A	90	96	93	60	55	42
	B	97	95	94	94	41	38
	C	76	97	90	91	72	31
<i>A. fumigatus</i>	A	56	56	19	36	24	25
	B	62	72	42	41	25	14
	C	59	35	51	23	29	27

A: plates near the wall; B: plates at the middle; C: plates away from the wall.

Table 3. Percentage of inhibition obtained by the arithmetic mean of the CFU, at 30 cm, 1 m, and 2 m of distances; and positions A, B and C; with the air conditioner on and off; in comparison with the control (Mod.II).

		Air conditioner off			Air conditioner on		
		30 cm	1 m	2 m	30 cm	1 m	2 m
<i>C. albicans</i>	A	96	95	96	96	96	94
	B	97	96	96	97	96	95
	C	96	95	95	96	95	94
<i>A. fumigatus</i>	A	57	57	46	47	50	47
	B	65	49	43	57	50	48
	C	57	48	50	46	48	44

A: plates near the wall; B: plates at the middle; C: plates away from the wall.

Statistically, Mod.I device showed greater efficiency in microbial load reduction, regardless of the fungal species tested, which could be explained by the different flow rates of the devices. Although O₃ concentrations in part per million (ppm) are virtually the same, Mod.I O₃ generator has twice the flow when compared with Mod.II. Consequently, more ozone comes in contact with the samples being tested.

In terms of experimental conditions suitable for microbial load reduction, the most promising results observed for *C. albicans* was from samples at 30 cm, position B (central), while in case of *A. fumigatus*, samples at 2 m and position A (plates near the wall), represented the least favorable condition. This may be related to the way the gas flows. In laminar flow, particles move orderly, always maintaining the same relative position. When there is a turbulent flow, these particles move randomly and irregularly [14]. Therefore, the wall may have caused a disorder of the O₃ molecules, which could have interfered with the flow, decreasing the antifungal activity when compared with the dishes near the wall.

Regarding the use of an air conditioner, the best antifungal activity for both devices was observed when the air conditioner was turned off, for both fungal species. Here, the airflow may have interfered again. The prolonged exposure to the gas resulted in more affected surfaces. However, when the air conditioning was turned on, it captured room air and filtered it before throwing it again into the environment [15], resulting in a variation in the flow of O₃ gas upon reaching the surface, thereby decreasing its antifungal activity.

According to the literature, factors such as ozone concentration, microbial species, and time of exposure, may lead to different antimicrobial effects of O₃ [16,17]. In our study, these factors were kept constant in all experimental conditions, minimizing possible biases. According to the observed values, cultures of *C. albicans* were more sensitive than *A. fumigatus*, which is in concordance with existing literature [16,18]. *A. fumigatus* can grow and survive in humid environments and extreme temperatures, by profuse dispersion of conidia [9]. According to Santana *et al.* [7], *C. albicans* is less adapted to diverse environmental conditions outside the human body than *A. fumigatus*.

Several other studies have shown the antimicrobial activity of O₃ gas against fungi, with different times of exposure and concentrations. Variables were analyzed to inhibit yeasts and filamentous fungi, germination tube formation, biofilm, and fungus spores, proving that O₃ gas in well-established protocols shows excellent antimicrobial activity [5,12,19-23]. Zotti *et al.* [24]

observed phenotypic changes in the colony of *A. flavus* and *A. niger* after exposure to O₃ gas, with a decrease in growth rates for both fungi and a change in their natural pigmentation. Consequently, O₃ gas can be expected to inhibit pigments and protein synthesis, with possible impairment of virulence factors of pathogenic fungal species.

It is worth mentioning that, although the two devices tested here generate low concentrations of O₃, people should not remain in the environment while the devices are on. Ozone can be toxic when inhaled at high concentrations or longer periods, thus impairing respiratory health [25].

Surface cleaning is crucial for the control of healthcare-associated infections (HAI) [26-29]. Unfortunately, health services proper surface cleaning and disinfection is often overlooked. These practices should be discussed and implemented by the Hospital Infection Commissions, together with nursing and cleaning services, developing activities related to environmental hygiene protocols, supervision, and training [4,30-32]. Finally, it is important the search for new products, methods, and practices for surface disinfection, for which O₃ gas appears as a promising alternative.

Conclusions

Our study proved the antifungal potential of O₃ gas produced by two different devices, according to the described criteria. This is a satisfactory procedure for decontamination purposes due to its fast and easy execution. This procedure provides a new perspective for the disinfection of surfaces aiming at better control of microbial dispersion. The incorporation of this procedure into hospital hygiene and disinfection protocols may decrease contamination rates and, consequently, fungal infections in health facilities. The action of O₃ on fungal control is important and further studies should be conducted to optimize the best protocols against this group of microorganisms.

Acknowledgements

We would like to thank Dr. Fernando Ferrari for carrying out the statistical analyses. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001, Universidade Federal de Mato Grosso do Sul-UFMS/MEC, Brazil, and National Council for Scientific and Technological Development – CNPq Brazil.

References

- Santos-Junior AG, Ferreira AM, Frota OP, Rigotti MA, Barcelos L da S, Lopes de Sousa AF, de Andrade D, Guerra OG, Furlan MC (2018) Effectiveness of surface cleaning and disinfection in a Brazilian healthcare facility. *Open Nurs J* 12: 36-44. doi: 10.2174/1874434601812010036.
- Nakamura HM, Caldeira SM, Avila MAG de (2013) Incidence of fungic infections in surgery patients: a retrospective approach. *Rev SOBECC* 18: 49-58. [Article in Portuguese]
- Paramythiotou E, Frantzeskaki F, Flevari A, Armaganidis A, Dimopoulos G (2014) Invasive fungal infections in the ICU: how to approach, how to treat. *Molecules* 19: 1085-1119. doi: 10.3390/molecules19011085.
- Sales V, Oliveira E, Célia R, Gonçalves F, Melo C (2014) Microbiological analysis of inanimate surfaces in an Intensive Care Unit and patient safety. *Rev Enferm Ref IV Série*: 45-53. doi: 10.12707/RIII1293.
- Mathé L, Van Dijck P (2013) Recent insights into *Candida albicans* biofilm resistance mechanisms. *Curr Genet* 59: 251-264. doi: 10.1007/s00294-013-0400-3.
- Mayer FL, Wilson D, Hube B (2013) *Candida albicans* pathogenicity mechanisms. *Virulence* 4: 119-128. doi: 10.4161/viru.22913.
- Santana DP, Ribeiro EL, Menezes ACS, Naves PLF (2013) New approaches on virulence factors of *Candida albicans*. *Rev Ciênc Méd Biol* 12: 229-233. doi: 10.9771/cmbio.v12i2.6953. [Article in Portuguese]
- Kwon-Chung KJ, Sugui JA (2013) *Aspergillus fumigatus*-what makes the species a ubiquitous human fungal pathogen? *PLoS Pathog* 9: e1003743. doi: 10.1371/journal.ppat.1003743.
- Van De Veerdonk FL, Gresnigt MS, Romani L, Netea MG, Latgé JP (2017) *Aspergillus fumigatus* morphology and dynamic host interactions. *Nat Rev Microbiol* 15: 661-674. doi: 10.1038/nrmicro.2017.90.
- Caetano MH, Siqueira JPZ, Andrade D, Sousa FL, Rigotti MA, Diniz MO, Almeida WA, Ferreira AM, de Almeida MTG (2021) Antimicrobial action of ozone gas on surfaces and in the air. *Acta Paul Enferm*. 34: eAPE02712. doi: 10.37689/actape/2021AO02712.
- Hunt NK, Mariñas BJ (1999) Inactivation of *Escherichia coli* with ozone: chemical and inactivation kinetics. *Water Res* 33: 2633-2641. doi: 10.1016/S0043-1354(99)00115-3.
- Tormin SC, Navarrini A, Almeida JOCF, Travassos LHR, Negri MVG, Silva RA (2016) Analysis of bactericidal effect of ozone on multi-resistant bacteria. *Arq Médicos dos Hosp e da Fac Ciências Médicas da St Casa São Paulo* 61: 138-141.
- de Hoog GS, Guarro J, Gené J, Figueras MJ (2011) Atlas of clinical fungi. CD-ROM version 3.1. CBS-KNAW Fungal Biodiversity Centre.
- Vasques EJ, Menegasso P, De Souza M (2016) Exploring the connection between fluid mechanics and the kinetic theory. *Rev Bras Ensino Fis* 38: 1307. doi: 10.1590/S1806-11173812096. [Article in Portuguese]
- Silva DP, Nazare DL, Muniz JWC, Camara CNS (2014) Hospital-acquired infections associated with poor air quality in air-conditioned environments. *Rev Epidemiol e Control Infecção* 3: 153-157. [Article in Portuguese]
- Kim JG, Yousef AE, Dave S (1999) Application of ozone for enhancing the microbiological safety and quality of foods: a review. *J Food Prot* 62: 1071-1087. doi: 10.4315/0362-028X-62.9.1071.
- Lüddecke F, Heß S, Gallert C, Winter J, Güde H, Löffler H (2015) Removal of total and antibiotic resistant bacteria in advanced wastewater treatment by ozonation in combination with different filtering techniques. *Water Res* 69: 243-251. doi: 10.1016/j.watres.2014.11.018.
- Restaino L, Frampton EW, Hemphill JB, Palnikar P (1995) Efficacy of ozonated water against various food-related microorganisms. *Appl Environ Microbiol* 61: 3471-3475. doi: 10.1128/aem.61.9.3471-3475.1995.
- Zargarán M, Fatahinia M, Zarei Mahmoudabadi A (2017) The efficacy of gaseous ozone against different forms of *Candida albicans*. *Curr Med Mycol* 3: 26-32. doi: 10.29252/cmm.3.2.26.
- Wollheim C, Da Silva Gonçalves E, Cristina Lopes K, Bega A (2020) Microbicide effect of gaseous ozone on *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. *Rev Ibero-Americana Podol* 2: 121. doi: 10.36271/iajp.v2i1.22. [Article in Portuguese]
- Carolina Ferro A, Paula Ferreira A, Utiana A, De Araujo J, Neves M, Bega A (2019) The inhibitory activity of ozone gas in solid half and half net on *Candida albicans*, in vitro. *Rev Ibero-Americana Podol* 1: 30-35. doi: 10.36271/iajp.v1i1.4. [Article in Portuguese]
- Hayran Y, Deniz ST, Aydın A (2020) Antimicrobial activity of ozone against pathogenic oral microorganisms on different denture base resins. *Ozone Sci Eng* 42: 43-53. doi: 10.1080/01919512.2019.1626215.
- Hudson JB, Sharma M (2009) The practical application of ozone gas as an anti-fungal (anti-mold) agent. *Ozone Sci Eng* 31: 326-332. doi: 10.1080/01919510903043996.
- Zotti M, Porro R, Vizzini A, Mariotti MG (2008) Inactivation of *Aspergillus spp.* by ozone treatment. *Ozone Sci Eng* 30: 423-430. doi: 10.1080/01919510802487997.
- Nuvolone D, Petri D, Voller F (2018) The effects of ozone on human health. *Environ Sci Pollut Res* 25: 8074-8088. doi: 10.1007/s11356-017-9239-3.
- Frota OP, Ferreira AM, Guerra OG, Rigotti MA, Andrade D de, Borges NMA, Almeida MTG de (2017) Efficiency of cleaning and disinfection of surfaces: correlation between assessment methods. *Rev Bras Enferm* 70: 1176-1183. doi: 10.1590/0034-7167-2016-0608.
- Souza ME, Ferreira H, Zilly A, De Mattos ALA, Pereira LSG, Silva RMM (2019) Disinfection conditions of inanimate surfaces in intensive therapy units. *Rev Pesqui Cuid é Fundam Online* 11: 956. doi: 10.9789/2175-5361.2019.v11i4.951-956.
- Boyce JM (2016) Modern technologies for improving cleaning and disinfection of environmental surfaces in hospitals. *Antimicrob Resist Infect Control* 5: 10. doi: 10.1186/s13756-016-0111-x.
- Santos Oliveira BA, Rigonato EM, Sousa AFL, Ferreira (2021) Correlation between surface cleaning and disinfection methods in an emergency room. *Open Nurs J* 15: 103-108. doi: 10.2174/1874434602115010103.
- Furlan MCR, Ferreira AM, Da Silva Barcelos L, Rigotti MA, De Sousa AFL, Dos Santos Junior AG, De Andrade D, De Almeida MTG, Da Silva Barreto M (2019) Evaluation of disinfection of surfaces at an outpatient unit before and after an intervention program. *BMC Infect Dis* 19: 355. doi: 10.1186/s12879-019-3977-4.
- Giroti ALB, Ferreira AM, Rigotti MA, de Sousa AFL, Frota OP, de Andrade D (2018) Hospital infection control programs: assessment of process and structure indicators. *Rev da Esc Enferm* 52: e03364. doi: 10.1590/s1980-220x2017039903364.
- Furlan MCR, Ferreira AM, Rigotti MA, Guerra OG, Frota OP, De Sousa AFL, De Andrade D (2019) Correlation among

monitoring methods of surface cleaning and disinfection in outpatient facilities. *Acta Paul Enferm* 32: 282-289. doi: 10.1590/1982-0194201900039.

Corresponding author

Prof. Margarete Teresa Gottardo de Almeida, PhD.
Head of the Microbiology Lab, Faculty of Medicine of São José do Rio Preto,
5416 Brigadeiro Faria Lima Av., 15090-000,
São José do Rio Preto, São Paulo, Brazil.
Tel: +55 17 3201 5843
Email: margarete@famerp.br

Conflict of interests: No conflict of interests is declared.