

Antimicrobial action of ozone gas on surfaces and in the air

Ação antimicrobiana do gás ozônio em superfícies e na aeromicrobiota

Acción antimicrobiana del gas ozono en superficies y en la microbiota del aire

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Descriptores

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Abstract

Objective: Assess the antimicrobial action of ozone gas (O₃) on surfaces and artificially cooled ambient air.

Methods: Cross-sectional experimental/laboratory study carried out in ten rooms of a medical microbiology research lab, with class 2 biosafety risk. The demarcated surfaces on the floor, wall and counter were assessed in relation to the presence or absence of microorganisms, based on collections done with swabs dampened in sterile distilled water, before and after exposure to ozone gas produced by two different generators. After this procedure, each swab was inoculated on the surface of a Brain Heart Infusion Agar DIFCO® (BHI) culture, followed by incubation at 35°C for 24 hours. For the microbiological analysis of the air, a petri dish with BHI was openly exposed for one hour, before and after treatment with O₃ gas, and were incubated according to the same criteria.

Results: The antimicrobial activity of the O₃ gas produced by both generators was checked in all the areas investigated, with records indicating a decrease in the number of colony-forming units. The antimicrobial inhibition potential of the generators was close to the analysis criteria adopted, particularly for the floor and counter areas. Based on all the rooms and microbial inhibition percentages, in relation to the two generators, the results were: floor (100%), counter (90%), wall (50%) and air (70%).

Conclusion: The O₃ generators had antimicrobial potential as a procedure for controlling microorganisms present on surfaces and in artificially cooled ambient air, constituting a feasible sanitizer.

Resumo

Objetivo: Avaliar a ação antimicrobiana do gás ozônio (O₃) em superfícies e ar ambiente climatizado artificialmente.

Métodos: Estudo experimental/laboratorial e transversal realizado em dez salas de um laboratório de pesquisa em microbiologia médica, com risco de segurança biológica classe 2. As superfícies demarcadas do chão, parede e bancada foram avaliadas, quanto à presença ou ausência de micro-organismos, a partir de coletas feitas com *swab* umedecido em água destilada estéril, antes e após a exposição do gás O₃ gerado por dois equipamentos distintos. Após este procedimento, o *swab* foi inoculado na superfície do meio de cultura *Brain Heart Infusion Agar* DIFCO® (BHI), seguindo-se a incubação a 35°C por 24 horas. Para a análise microbiológica do ar, uma placa com BHI foi exposta aberta por uma hora, antes e após o tratamento do gás O₃, sendo incubadas segundo os mesmos critérios.

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Conflicts of interest: none.

Resultados: A atividade antimicrobiana do gás O_3 gerado por ambos os equipamentos foi constatada para todas as áreas investigadas, com registros de redução do número de Unidades Formadoras de Colônias. O potencial de inibição antimicrobiana dos aparelhos se manteve próximo para os critérios de análise adotados, com destaque para as áreas de chão e bancada. Considerando-se todas as salas e percentuais de inibição microbiana, frente aos dois equipamentos, os resultados foram: chão (100%), bancada (90%), parede (50%) e ar, 70%.

Conclusão: Os equipamentos geradores de gás O_3 apresentaram potencial antimicrobiano para medida de controle de microrganismos presentes em superfícies e ar ambiente climatizado artificialmente, sendo um sanitizante factível para utilização.

Resumen

Objetivo: Evaluar la acción antimicrobiana del gas ozono (O_3) en superficies y en el aire interior climatizado artificialmente.

Métodos: Estudio experimental/de laboratorio y transversal realizado en diez salas de un laboratorio de investigación en microbiología médica, con riesgo de seguridad biológica clase 2. Se evaluaron las superficies delimitadas en el piso, pared y mesa en cuanto a la presencia o ausencia de microorganismos, a partir de muestras recolectadas con hisopo humedecido en agua destilada estéril, antes y después de la exposición del gas O_3 generado por dos máquinas distintas. Luego de este procedimiento, el hisopo fue inoculado en la superficie del medio de cultivo *Brain Heart Infusion Agar* DIFCO® (BHI), y después incubado a 35 °C por 24 horas. Para el análisis microbiológico del aire, se expuso una placa con BHI abierta durante una hora, antes y después del tratamiento del gas O_3 , y luego se incubó con los mismos criterios.

Resultados: Se constató la actividad antimicrobiana del gas O_3 generado por ambas máquinas en todas las áreas investigadas, y se registró una reducción del número de unidades formadoras de colonias. El potencial de inhibición antimicrobiana de los dispositivos se mantuvo próximo a los criterios de análisis adoptados, con énfasis en el área del piso y mesa. Considerando todas las salas y porcentajes de inhibición microbiana, con las dos máquinas, los resultados fueron: piso (100 %), mesa (90 %), pared (50 %) y aire (70 %).

Conclusión: Las máquinas generadoras de gas O_3 presentaron potencial antimicrobiano como medida de control de microorganismos presentes en superficies y aire interior climatizado artificialmente, lo que lo convierte en un desinfectante factible para ser usado.

Introduction

The control of healthcare-associated infections (HAI) is a sensitive public health issue, since it results in morbidity, mortality and high costs, particularly in developing countries.⁽¹⁻³⁾

Environments participate in the transmission of microorganisms, with contamination of inanimate surfaces acting as potential reservoirs. Equipment and surfaces in hospital areas play a role in disseminating HAI, often as secondary reservoirs, and can promote cross-contamination.⁽⁴⁾ Likewise important in this context, the use of air conditioners as an artificial ambient cooling practice recycles air with microbial and aerosol particles, which impair air quality and are a risk factor for infectious disease.⁽⁵⁾

Cleaning and disinfection practices for environments, surfaces and equipment are part of infection control programs, in an effort to prepare environments and minimize disease risk.^(4,6,7) In this regard, the use of disinfectants is a standard practice for microbial control, including vaporization with formaldehyde, peracetic acid or chlorhexidine, sodium hypochlorite and formulations isolated or combined with hydrogen peroxide.⁽⁸⁾ There are disadvantages with these methods, such as high costs and preparation of labor, as well as the possibility that employees working close to the products will inhale toxic vapors.^(9,10)

In this context, ozone is presented in the triatomic form of oxygen (O_3) and has been used as a chemical element to control microorganisms in various segments of the health sector, particularly in hospital waste treatment,⁽¹¹⁾ pretreatment of dental cavities,⁽¹²⁾ disinfection of hemodialysis machines⁽¹³⁾ and disinfection of operating rooms,⁽¹⁴⁾ among others.

In the food sector, the sanitization process has been structured by ozone generators, resulting in adequate environments for cheese ripening processes.⁽¹⁵⁻¹⁷⁾

In terms of antimicrobial action, O_3 acts in the oxidation of glycopeptides, glycoproteins and amino acids of the cell wall, modifying permeability and causing cell lysis. When it penetrates the interior of the cell, O_3 recombines with cytoplasmic elements leading to the oxidation of amino acids and nucleic acids and, consequently, to cleavage and cell death. O_3 also promotes the collapse of cellular enzymatic activity, attacking the sulfhydryl enzyme groups, as well as modifying the purine and pyrimidine bases of nucleic acids.⁽¹⁸⁻²⁰⁾

Although it has been used in hospital environments for some time, little is known about the potential of this agent, particularly in the Brazilian care context, as seen by the scarcity of studies on the topic. Therefore, this study sought to assess the

antimicrobial action of ozone gas on surfaces and artificially cooled ambient air.

Methods

Type of study

This was a cross-sectional experimental/laboratory study carried out in ten internal rooms of a clinical microbiological research laboratory, which performs bacteriological and mycological tests as a part of its research projects. This environment adheres to biosafety criteria - Biosafety Level 2 (BN2), and has stable physical-chemical conditions (humidity, temperature and standard cleaning and disinfection). However, it is characterized as a critical area, with the possibility of microbial contamination. The rooms had common data in terms of physical space, lighting, temperature, humidity and circulation of people and were, therefore, subject to the same microbial risk conditions. The counters contained: ovens, lighting, water bathes, computers and wood cupboards.

Study protocol

Ten climate-controlled rooms, with an area of 9 m² underwent a microbiological analysis before and after exposure to O₃ gas, generated by two O₃ generators - GEO 20000/AR-TD (Mod. I) and GEO 20000/AR (Mod. II), manufactured by the company OZON® (Chart 1). The O₃ was produced through electrochemical discharge; the equipment was composed of two electrodes (high and low voltage) which are subjected to different action potentials, and the passage of air (O₂) between the two electrodes produces an electrostatic change, with generation of O₃. It should be noted that the environment in which O₃ was being generated was free of people. The experiments, conducted with the two generators I and II, occurred in six-month intervals.

For the microbiological investigation of the wall, floor and counter surfaces, the collection was done using a swab pre-moistened in sterile distilled water, which was placed in contact with a specific area of 30X30 cm² quadrant. The swab was then immediately inoculated on the surface of a culture

Chart 1. Models 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)
Ozone flow (m ³ /h)/ppm	200/2 ppm	100/2.1 ppm
Maximum relative humidity (%)	75	75
Working temperature (°C)	6 - 35	5 - 40
Output power (W)	135	127
Supply voltage (V)	127	110
Weight (kg)	5.40	4.9
Maximum work area (m ²)	300	300
Dimensions (cm)	18x30x47.5	18x30x37

M³/H - Cubic meter per hour; % - Percentage; °C - Degree Celsius; W - Watt; V - Volt; kg - Kilogram; M² - Square meter; CM - Centimeter; PPM - Parts per million

medium containing BHI and incubated at 35°C for 24 hours. In the next stage of the experiment, the O₃ generators located on the floor in the center of the rooms was turned on for an hour. The investigation site was kept shut and sealed throughout the sanitization procedure, without any interference. At the end of this period, a new collection was performed, using another swab, placed this time in contact with the surface diametrically opposite the quadrant, following the same analysis procedures as in the first stage. Figure 1 illustrates the investigated sites, as well as the arrangement of the ozone generator.

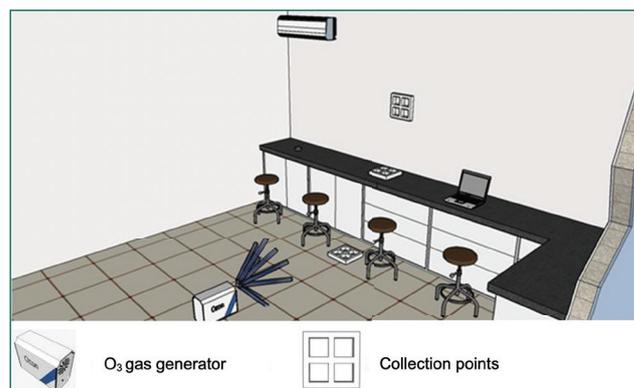


Figure 1. Illustration of the arrangement of the ozone generator and collection points

The ambient air samples were collected through a simple sedimentation technique, before and after exposure to O₃ gas, and constituted an investigation parameter, with exposure of Petri dishes, containing BHI, kept open for one hour and incubated at 35°C for 24 hours (Figure 2). The temperature and humidity of the rooms were monitored.

Antimicrobial action was determined by the number of colony-forming units (CFU) on the sur-

face of the Petri dish, from inocula from the floor, counter and wall surfaces and ambient air, before and after treatment with O₃.

The classic and consensual recommendation of safe methods for disinfecting surfaces entails prior cleaning of the site, followed by disinfection with a microbicide agent.⁽²¹⁾ In the present study, the surfaces were analyzed without any prior cleaning process, since the objective was to determine microbial reduction.

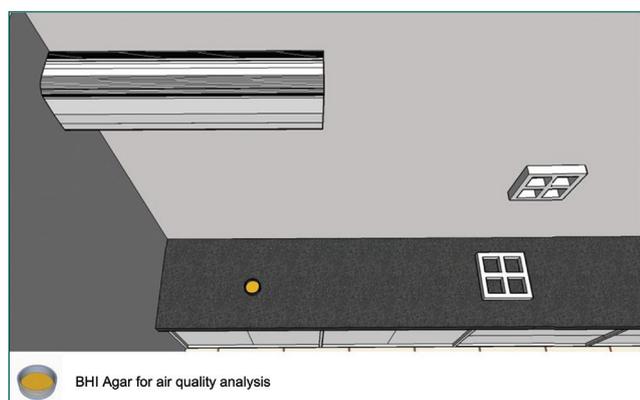


Figure 2. Illustration of the arrangement of the Petri dish for the air analysis.

Data analysis

The collected data was consolidated in SPSS (20.0) software and underwent a statistical analysis, using the paired student's t-test, to compare two samples (before and after) and determine whether there was a significant difference in a variable between the two groups of interest. The Wilcoxon test, a non-parametric method for comparing two samples,

was used to verify the overall efficiency of the ozone generators.

Results

The antimicrobial action of the ozone gas was effective for all the areas studied, there was a reduction in the CFU count, in relation to the two generators (Tables 1 and 2).

In terms of the ozone activity from generator I, on the floor of all the rooms, there was a reduction in microbial load (CFU) and a negative count in room 1. The findings were the same for the counters, except for room 4, which maintained the same colony count before and after exposure to ozone. As for the walls, there was a 50% reduction in microbial contamination. In the ambient air analysis, contamination was reduced in seven rooms; in the other rooms no microorganisms were detected either before (B) or after (A) exposure to O₃.

The results obtained from the antimicrobial activity of the ozone gas with generator II are presented in Table 2. Once again, antimicrobial activity was evident, as shown by the reduction in CFU for all the variables examined, such as the air and surfaces, before and after exposure to O₃ gas. The amount of CFU only remained constant in two rooms (4 and 9) for the two investigation periods.

Regardless of the generators, microbial control was higher for the floor and counter areas. In addition, considering all the areas investigated, Mod. I

Table 1. Numerical presentation of CFU and percentages obtained before (B) and after (A) exposure to ozone (Generator I) in ten rooms

Location	Room 1	Room 2	Room 3	Room 4	Room 5	Room 6	Room 7	Room 8	Room 9	Room 10
Floor										
B (CFU)	1	7	2	19	9	17	110	10	11	30
A (CFU)	0	5	1	4	6	10	105	7	5	25
%	100	29	50	79	33	41	5	30	55	17
Counter										
B (CFU)	10	7	10	2	32	30	18	3	12	44
A (CFU)	7	5	5	2	31	21	2	2	6	10
%	30	29	50	0	3	30	89	33	50	77
Wall										
B (CFU)	1	0	1	1	1	14	0	3	2	1
A (CFU)	0	0	1	1	1	8	0	0	0	0
%	100	0	0	0	0	43	0	100	100	100
Air										
B (CFU)	0	1	1	3	1	11	0	2	0	6
A (CFU)	0	0	0	1	0	6	0	0	0	3
%	0	100	100	67	100	45	0	100	0	50

B - Before; A - After; % - Percentage of microbial reduction

Table 2. Numerical presentation of CFU and percentages obtained before (B) and after (A) exposure to ozone (Generator II) in ten rooms

Location	Room 1	Room 2	Room 3	Room 4	Room 5	Room 6	Room 7	Room 8	Room 9	Room 10
Floor										
A (CFU)	89	479	309	94	193	182	782	186	55	114
D (CFU)	22	39	68	74	162	69	167	131	53	74
%	75	92	78	22	16	62	79	30	4	35
Counter										
A (CFU)	23	36	0	281	5	15	9	73	26	54
D (CFU)	16	19	0	55	4	2	5	4	7	41
%	30	47	0	80	20	87	45	95	73	24
Wall										
A (CFU)	3	0	0	1	0	4	1	3	1	1
D (CFU)	0	0	0	1	0	3	0	1	1	0
%	100	0	0	0	0	25	100	67	0	100
Air										
A (CFU)	0	1	1	0	1	4	0	0	2	1
D (CFU)	0	0	0	0	0	0	0	0	0	0
%	0	100	100	0	100	100	0	0	100	100

B - Before; A - After; % - Percentage of microbial reduction

and Mod. II had statistically significant differences ($p < 0.05$). When assessed separately, in relation to each one of the surfaces and ambient air, the results were not significant except for the wall ($p = 0.0639$) and counter ($p = 0.1267$), respectively.

It should be noted that generators I and II had distinct ozone gas flows and concentrations, i.e., 200 and 100 m³/h - 2.0 and 2.1 ppm of ozone gas, respectively. The temperature and humidity recordings of the rooms were 21°C and 58% for the two investigation periods.

Discussion

This study has limitations associated with the study design (experimental-laboratory and cross-sectional). The environmental condition of the laboratory rooms was considered as a parameter of analysis and inference for the hospital environment. However, this does not detract from the importance of the research as a prototype for the evaluation of microbiological action, in two periods of investigation - before and after exposure to ozone. In this sense, the researchers sought to mimic the reality of environments, in terms of the occurrence of microorganisms present on surfaces and in the air, introduce the sanitization process and then develop environmental hygiene protocols.

The practical applicability of O₃ gas in hospital environments could improve microbiological conditions, and thereby prevent or help reduce HAI rates. Furthermore, the portable nature of the

equipment makes the sanitization process mobile and feasible for monitoring specific hospital areas.

It is known that O₃ is part of the disinfection and sanitization routine of other environments,⁽²²⁾ with controls for bacteria and fungus.^(15,17) However, this study expands what is already known about the gas, in that halting microbial growth or reducing the CFU count on Petri dishes after the application of O₃ gas to any of the surfaces analyzed, compared to the control group, proves the effectiveness of the chemical compound in microbial control processes. Similar findings were reported in a study on the decontamination of operating rooms in a veterinary hospital.⁽¹⁴⁾ The two studies clearly demonstrated the potential of O₃ gas in environmental disinfection processes, since the decrease in the count of mesophilic aerobes, molds and yeasts occurred frequently.

In terms of the microbial elimination process by O₃ gas, it is known that cell destruction occurs through oxidation of structural elements, without specifying targets present in bacterial or fungal cells.⁽¹⁸⁾ In this sense, multi-drug resistant organisms can be eliminated, with significant advantages, when compared with mechanical disinfection methods that use liquid disinfectants for environmental surfaces in healthcare facilities, including hospital environments, where it is common to use other chemical compounds in liquid form.

Biological samples which indicate that environments or surfaces are probable reservoirs for HAI transmission are essential in epidemiological studies.^(23,24) In hospitals, terminal cleaning is done in

areas and surfaces close to or in contact with patients, after their departure, whether through their death, transfer or end of isolation,⁽²⁴⁻²⁷⁾ and, despite efforts, disinfection and cleaning results are not always satisfactory.^(28,29) The use of O₃ gas in the study provided scientific proof regarding its microbial control potential, making it a compound that can be used as a sanitization procedure for healthcare environments.

In this study, differences were noted between the two O₃ generators in terms of antimicrobial potential under the same conditions in the sanitization of environments, particularly in relation to flow and output power of the devices. The results indicated significant differences for certain parameters; however, in general terms, the sanitization potential of the two models was nearly the same. Innovative protocols could be created to improve the antimicrobial pattern detected in the study.

CFU values varied from one room to another. This shows that an environment does not always have the same microbiological conditions, which is to be expected considering hospital environments. Due to the demarcation of the areas investigated, before and after treatment with O₃ gas, only a limited surface area was sampled, which does not ensure that the results would be the same throughout the extension of the surfaces. However, sanitization practices using ozone, generated by portable equipment, certainly enables implementation of new microbial control measures in hospital environments.

The floor and counter surfaces had higher CFU counts and, at the same time, corresponded to the areas of greater microbial inhibition by ozone. Microbial particles are dense in relation to air, which normally contributes to the permanence of potential pathogens on the floors of nosocomial environments.⁽³⁰⁾ In this sense, portable equipment can be moved close to areas of high environmental contamination and reduce the microbial load.

According to the literature, the antimicrobial effect of O₃ depends on certain factors, such as exposure time, concentration, temperature and humidity.⁽³¹⁾ However, in the current study, these criteria were assessed and maintained under equal experimental conditions, thereby minimizing possible

biases. In this sense, other studies are needed that consider other parameters of temperature, relative humidity, concentration and exposure time.

When inhaled in high concentration, O₃ can be toxic and has respiratory health risks.⁽³²⁾ The two pieces of equipment tested generate a small amount of ozone (2.0 - 2.1 ppm) and, during their use, no one should remain in the environment.

Little importance was given to the training of the group from the Surface Cleaning and Disinfection Service in Health Service, which plays an essential role in reducing HAI. Therefore, cleaning and disinfection practices for environments, equipment and surfaces must be part of Hospital Infection Control Committees, along with nursing and cleaning services, carrying out activities related to environmental hygiene protocols, supervision and training of teams.^(33,34) Another important point in the utilization of technologies that do not use mechanical action for disinfecting environments and surfaces is that they do not ensure that adjacent areas will be disinfected. Nor do they replace mechanical cleaning and disinfection activities.⁽³⁵⁾

Finally, the search for new products or methods and hospital practices for disinfecting surfaces and reducing air microbiota through artificial cooling has been increasing over time, and ozone gas is a promising compound. There are still not many studies in the literature that address this object of research and those that do have highly varied experimental conditions, which suggests the need to create well-designed protocols for microbial control.

Conclusion

The findings of the present study demonstrate the antimicrobial potential of O₃ gas, produced by two generators and according to the criteria set forth, ensure antimicrobial action. Applying ozone to environments is a practical procedure for sanitizing surfaces and artificially cooled air. It appears that this technology is feasible for use in various segments which seek to reduce microbial density. This resource could be used in protocols for sanitizing hospital environments and surfaces, due to its quick

and easy execution and ability to control microbial development, an essential condition for maintaining microbiologically safe environmental quality.

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Collaborations

Caetano MH, Siqueira JPZ, Andrade D, Sousa AFL, Rigotti MA, Diniz MO, Almeida WA, Ferreira AM, Almeida MTG contributed to the study design, data analysis and interpretation, writing of the article and critical review of the intellectual content. All the authors approved the final version for publication.

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