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The influence of gaseous ozone on the growth of fungi isolated from clinical and environmental samples

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Abstract

Background: Disinfection is performed in our laboratory using sodium hypochlorite, glutaraldehyde, alcohol, or hydrogen peroxide. All these products are known for their harmful side effects on human and ecological health. As a part of the search for new sterilization methods that respect the environment much more, we were interested in studying the antifungal activity of ozone gas on the most isolated fungi in our hospital "University Hospital Center Hassani Abdelkader of Sidi Bel Abbes".

Methods: This study focused on the antifungal activity of gaseous ozone on the most common fungal isolates in the hospital, with consideration of the duration of the exposure to ozone.

Results: The fungicidal effect of ozone gas on molds was more effective compared to its effect on yeasts, it makes it possible to inhibit the reproduction and fungal growth of molds, in particular the filamentous molds and the most commonly isolated dermatophytes. A longer exposure would seem to be more effective.

Conclusion: Ozone gas can be a new alternative for sterilization and disinfection techniques, especially filamentous fungi and dermatophytes.

Keywords: Disinfection, Sodium hypochlorite, Ozone, Antifungal, Environment

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Introduction

Disinfection is an operation that eliminates microorganisms carried by contaminated media. Different means of sterilization such as physical sterilization (dry or wet heat) and chemical sterilization are currently applicable in hospital environments. Each sterilization technique has certain limits, which can be, for example, the sensitivity of the product to be sterilized to the chosen technique, cost, toxicity, the need for the use of several instruments or several stages, etc. Chlorination of hospital wastewater can produce human carcinogenic by-products including trihalomethane compounds, some of which are cytotoxic, genotoxic, and mutagenic. These by-products can be harmful to flora and fauna and may have adverse effects on microorganisms and plankton present in these ecosystems (1,2). The entrance of high concentrations of chlorine into the wastewater can have an adverse impact on the proper functioning of the treatment system by treatment plants based on the use of bacterial strains for bioremediation. To protect human and environmental health, new alternative solutions must be developed. Recently, several studies have shown the decontaminating effect of ozone on different products and materials (3,4). As a part of the research and improvement of new sterilization methods, the most frequent isolates strains in our hospital were selected to test the effectiveness of ozone as an antifungal agent to fight against nosocomial infections.

Materials and Methods

Ten pathogenic species of fungal isolates (Figure 1) were provided by the laboratory of mycology and parasitology

from the university hospital center of Sidi Bel Abbes (Algeria). All isolates were identified using growth and colony characteristics, morphology, germ tube formation, and the sugar fermentation, according to the standard methods. They were maintained in culture on Sabouraud glucose (4%) agar medium. Fungal isolates and their origins are presented in Table 1.

The 10 fungal isolates that were the subject of this study are as follows: *Microsporum canis*, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Aspergillus terreus*, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria* sp, *Penicillium* sp, non-*albicans Candida, Rhodotorula* sp.

Fungal culture was suspended in 5 ml sterile 0.85% NaCl. Turbidity was adjusted to McFarland 0.5 scale, equivalent to 1.5×10^6 (CFU/mL).

Four sterile 96-well microplates were used for this study. In each plate, a 50% dilution series in Sabouraud broth medium was carried out for each fungal strain. This dilution series began at 1/20 in the first well and finished at a dilution of 1/2560 in the eighth well.

Figure 1. Fungal isolates tested from right to left: *Candia, Rhodotorula, T. rubrum, M. canis, T. mentagrophytes, A. terreus, Penicillium, A. niger, A. flavus, Alternaria*

Table 1. Origin of fungal cultures used in this study

Fungi culture	Sample origin	Diagnosed disease
M. canis	Hair and scalp scraping	Tinea capitis
T. rubrum	Nail scraping	Tinea unguium
T. mentagophytes	Skin scraping	Tinea corporis
A. terreus	Nail scraping	Tinea unguium
A. niger	Ear swab	Otomycosis
Candida sp	Mouth swab	Oral candidiasis
Alternaria sp	Hospital environment (air sedimentation)	None
Penicillium sp	Hospital environment (air sedimentation)	None
A. flavus	Hospital environment (surface)	None
Rhodotorula sp	Hospital environment (surface)	None

In the present study, ozone gas was freshly produced by an ozone generator. The concentration of ozone produced was 15 g/h.

The first plate was exposed to an ozone generator for 30 seconds, the second one for 60 seconds, and the third one for 120 seconds. The fourth plate not exposed to ozone was considered as a control (5).

Then, the 4 plates were incubated at a temperature of 28°C for 48-72 hours and a visual check of the fungal growth was carried out for each plate and each row, to observe the dilution for which no fungal growth was detected.

Results

The results of the present research indicate that ozone gas has an antifungal activity on all the strains used in this study. Its effectiveness depends on the sensitivity of the strains used and the duration of treatment.

Fungi treated with ozone gas can be classified into three classes: Very sensitive, moderately sensitive, and resistant.

The class of strains very sensitive to the fungicidal effect of ozone gas contains the filamentous species of molds (*A. niger*, *A. flavus*, *A. terreus*, *Penicillium sp*, and *Alternaria sp*); a total fungal elimination from well 2 was reported (Table 2).

The class of strains moderately sensitive to the fungicidal effect of ozone gas found in this study includes the species (*T. rubrum*, *T. mentagrophytes*, and *M. canis*) (Table 3). The effect of ozone is progressive and its effectiveness increases with the duration of exposure, however, it was found that the effect is more pronounced for *Trichophyton* genus (*T. rubrum* and *T. mentagrophytes*) compared to *Microsporum* genus (*M. canis*) and ozone gas completely eliminated *T. rubrum* after 120 seconds of exposure.

The class of resistant strains found in this study represents the yeast-like species (non-*albicans Candida* and *Rhodotorula* sp) and under such conditions (Table 4).

Discussion

Aspergillus niger, *A. flavus*, *A. terreus*, *Penicillium* sp, and *Alternaria* sp are considered as the filamentous fungi most sensitive to ozone gas. Other studies on the same strains used in the present study confirm our results. Vijayanandraj et al (6) report that germination of *A. niger* spores treated with ozone produce non-uniform and non-sporulating colonies, consisting of sterile mycelium.

The study conducted by Savi and Scussel (7) on *A. flavus* shows that prolonged exposure to ozone gas promotes an increase in the production of ROS, with an inhibition of the germination of conidia, deformations and ruptures of the structure of the hyphae, which leads to the death of hyphae. They suggest that the fungicidal effect may be the result of the oxidative activity of O_3, which leads to oxidative stress and apoptosis.

According to the study of Atony-Babu and Singleton

Table 2. Results of the growth of moulds isolates after treatment with ozone

Moulds	Ozone exposition	Dilution 1 :20	Dilution 1 :40	Dilution 1 :80	Dilution 1 :160	Dilution 1 :320	Dilution 1 :640	Dilution 1 :1280	Dilution 1 :2560
A. niger	No expo	+	+	-	-	-	-	-	-
	30 s	+	-	-	-	-	-	-	-
	60 s	+	-	-	-	-	-	-	-
	120 s	-	-	-	-	-	-	-	-
A. terreus	No expo	+	+	-	-	-	-	-	-
	30 s	+	-	-	-	-	-	-	-
	60 s	+	-	-	-	-	-	-	-
	120 s	+	-	-	-	-	-	-	-
A. flavus	No expo	+	-	-	-	-	-	-	-
	30 s	+	-	-	-	-	-	-	-
	60 s	-	-	-	-	-	-	-	-
	120 s	-	-	-	-	-	-	-	-
Penicillium	No expo	+	-	-	-	-	-	-	-
	30 s	+	-	-	-	-	-	-	-
	60 s	+	-	-	-	-	-	-	-
	120 s	+	-	-	-	-	-	-	-
Alternaria	No expo	+	+	-	-	-	-	-	-
	30 s	+	+	-	-	-	-	-	-
	60 s	+	-	-	-	-	-	-	-
	120 s	+	-	-	-	-	-	-	-

(+) growth, (–) no growth.

Table 3. Results of the growth of dermatophytes isolates after treatment with ozone

Dermatophytes	Ozone exposition	Dilution 1 :20	Dilution 1 :40	Dilution 1 :80	Dilution 1 :160	Dilution 1 :320	Dilution 1 :640	Dilution 1 :1280	Dilution 1 :2560
T. rubrum	No expo	+	+	+	+	+	+	-	-
	30 s	+	+	+	+	-	-	-	-
	60 s	+	+	+	-	-	-	-	-
	120 s	-	-	-	-	-	-	-	-
T. canis	No expo	+	+	+	-	-	-	-	-
	30 s	+	+	+	-	-	-	-	-
	60 s	+	+	+	-	-	-	-	-
	120 s	+	-	-	-	-	-	-	-
T. menta	No expo	+	+	+	-	-	-	-	-
	30 s	+	+	-	-	-	-	-	-
	60 s	+	-	-	-	-	-	-	-
	120 s	+	-	-	-	-	-	-	-

(+) growth, (–) no growth.

(8), ozone is more effective in reducing the growth of spore inoculum than mycelium in *Aspergillus nidulans*, thus, spore production was nil in this species after continuous low-level exposure of O_3.

Pathogenic fungi synthesize melanin to be more virulent, these melanins are destroyed after ozonation of pathogenic *Aspergillus* species as reported by Zotti et al (9).

As for *Penicillium*, ozone gas eliminates its sporulation and reduces its incidence rate to 98% (10,11).

Trichophyton and *Microsporum* genus are considered as fungi with medium sensitivity to the fungicidal effect of ozone gas. Similar results showed that ozone gas is effective in eliminating more than 99% of dermatophyte fungi in particular *T. rubrum* and *T. mentagrophytes*, which are considered to be the most commonly isolated pathogens in patients with onychomycosis and tinea

Table 4. Results of the growth of yeast isolates after treatment with ozone

Yeasts	Ozone exposition	Dilution 1 :20	Dilution 1 :40	Dilution 1 :80	Dilution 1 :160	Dilution 1 :320	Dilution 1 :640	Dilution 1 :1280	Dilution 1 :2560
Candida	No expo	+	+	+	+	+	+	+	-
	30 s	+	+	+	+	+	+	-	-
	60 s	+	+	+	+	+	+	-	-
	120 s	+	+	+	+	+	-	-	-
Rhodotorula	No expo	+	+	+	+	+	+	+	-
	30 s	+	+	+	+	+	+	-	-
	60 s	+	+	+	+	+	+	-	-
	120 s	+	+	+	+	+	-	-	-

(+) growth, (–) no growth.

pedis (12).

The results of this study show that yeasts are the most resistant strains to treatment with ozone gas. The results suggest that these species are more tolerant to the fungicidal effect of ozone gas. A slight inhibitory effect compared to the control is observed despite prolonged treatment. Another study carried out on 50 strains of *C. albicans* confirms the results of the present study, indicating that ozone was very effective in eliminating *C. albicans* but at 210 seconds of exposure. The study also shows that ozone resistance was much higher among vaginal and nail isolates, even after 60 minutes (13). Another study also shows that the clinical strains of *Candida* were more resistant to ozone than the non-clinical isolates (14).

Conclusion

The fungicidal effect of ozone gas on molds is more interesting compared to yeasts, which makes it possible to inhibit the reproduction and the fungal growth in particular the filamentous molds and the most commonly isolated dermatophytes. A longer exposure would seem to be more effective. The results of the present study could be exploited in the establishment of an alternative antifungal based on ozone therapy rather than the chemistry of drugs and the disinfection and sterilization of equipment. This technique eliminates all human and ecological risks resulting from the massive use of chemicals for sterilization.

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Authors' contribution

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Competing interests

The authors declare that they have no conflict of interests.

Ethical issues

The authors do not confirm that this work is original, it was done in the central laboratory of the university hospital center, and has not been published elsewhere, nor it is currently under consideration for publication elsewhere.

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