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Ozone disinfection efficiency against airborne microorganisms in hospital environment: a case study

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Even though ozone has shown its potential for air disinfection in hospital environment, its more frequent use has earned attention only with the COVID-19 pandemic due to its proven antimicrobial effect and low cost of production. The aim of this study was to determine its antimicrobial efficiency against the most common bacterial species in a real-life setting, that is, in the air of one postoperative room of the General Hospital Dr Ivo Pedišić (Sisak, Croatia). Air was sampled for aiborne bacteria before and after treatment with the ozone concentration of 15.71 mg/m^3 for one hour. The most dominant Gram-positive bacteria of the genera *Micrococcus*, *Staphylococcus,* and *Bacillus* were reduced by 33 %, 58 %, and 61 %, respectively. The genus *Micrococcus* proved to be the most resistant. Considering our findings, we recommend longer air treatment with higher ozone concentrations in combination with mechanical cleaning and frequent ventilation.

KEY WORDS: aerosol; airborne bacteria; disinfection; hospital acquired infections; *Micrococcus spp.*

Hospital air contamination with microorganisms, multidrug-resistant in particular (1–5), has received more attention since the COVID-19 pandemic, as microorganisms can spread through the air on aerosol particles or liquid droplets (6, 7). These particles can be suspended in the air for over a week (7) and can settle on surfaces (8, 9). One of preventive measures against hospital acquired infections include ambient disinfection of hospital rooms (2, 5, 10–12), but they may not be efficient enough against antibiotic-resistant microorganisms, especially in a biofilm (2, 13–16). Furthermore, conventional disinfectants are often toxic and a burden in terms of hazardous waste.

In this respect, gaseous disinfectants stand out as the best choice for air disinfection (4, 10, 17), and ozone has already proven its antimicrobial effects through oxidation of nucleic acids, glycolipids, glycoproteins, sulphhydryl groups, enzyme amino acids, peptides, and proteins (18, 19). Although it is widely used in water disinfection or waste management, its use in hospital and similar environments is relatively rare and limited to disinfection of hospital linen and eradication of methicillin-resistant *Staphylococcus aureus* (20, 21). It was only since the COVID-19 pandemic that air disinfection in hospitals received more attention, as it was reported that ozone was successful against SARS-CoV-2 and other microorganisms on various surfaces (22, 23) and to have a potential use in "no-touch" automated room disinfection systems (24). In addition, ozone is very cheap to produce.

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However, save for a few studies (25), knowledge about its efficacy against airborne contamination in hospital settings is still scarce. The aim of this study was to gain more insight into its efficiency by determining air quality in a hospital room in terms of bacterial and mould load before and after treatment with ozone.

MATERIALS AND METHODS

Air sampling

Air was sampled in July in a recovery room for postoperative treatment located in the new wing of the Dr Ivo Pedišić General Hospital in Sisak, Croatia. The room (32.4 m^3; 4×3×2.7 m) was furnished with a stretcher, sink, desk, chair and a wardrobe. Ventilation in the room combines natural (windows) and centralised ventilation with a system using HEPA filters. Room temperature and relative humidity when the room is occupied by a patient are 23 °C and around 55 %, respectively. We made sure to have the same room temperature and relative humidity at sampling, so that the conditions are as close to real-life settings as possible.

Before ozone treatment, all ventilation holes in the room were sealed off and the central ventilation system was turned off. Air was sampled twice to get baseline (pre-ozone treatment) and post-ozone treatment measurements at three points (window sill, desk, and sink)

(Figure 1) using a mobile, 250 L air sampler (MAS-100, Merck, Berlin, Germany) which aspirates ambient air through a perforated lid. This air impacts the surface of a growth medium in standard size Petri dishes. Adhering microorganisms are then incubated and counted as instructed by the manufacturer.

For ozone treatment we used a mobile ozone generator Mozone GPF 8008 (Mozone, Sisak, Croatia) releasing a mixture of air and ozone until ozone reached the concentration of 15.71 mg/m^3 in the air. Treatment with this concentration lasted for 1 h at room temperature of 23 °C and relative humidity of 60 %. The distance from the ozone generator and each sampling point was about 1.5 m. Ozone concentration was monitored continuously with a portable ozone detector (Keernuo GT901, Keernuo, Shanghai, China), also placed at 1.5 m from the generator. Room temperature and relative humidity were monitored with an Auriol 4-LD5531 radio-controlled weather station (Auriol, Berlin, Germany). After the ozone treatment was finished and ozone gas dissolved (in approximately 2 h), we took another air measurement for microorganisms. All experiments were done in triplicate on all sampling points.

Determination of total bacterial and mould count

Air was sampled directly on TSA agar (Biolife, Milano, Italy), chromogenic UTI agar (Brilliance^{TM}, Oxoid, Basingstoke, UK), and Saburaud dextrose agar (Biolife, Milano, Italy) for moulds. The chromogenic agar was also used for bacterial identification as described elsewhere (26, 27). Agar plates for bacterial identification were incubated in a BD400 incubator (Binder, Bohemia, NY, USA) at 36 °C for 48 h. Sabouraud dextrose agar plates were incubated at 30 °C for 10 days. After incubation, total bacteria and moulds were counted and expressed as CFU/250 L of air. All counts were done in triplicate.

Bacterial identification

Bacteria grown on the TSA and chromogenic UTI agar were identified by colony morphology and colour according to manufacturer's instructions. The final identification of the dominant species was done using the standard API20 Staph test (bioMérieux, Marcy-l´Etoile, France), Gram staining, catalase test, coagulase test, and oxidase test as described elsewhere (26, 27).

Data processing and statistical analyses

The total bacterial and mould counts are expressed as CFU/250 L of air, which is the capacity of the sampling device. They are estimates based on statistical corrections according to Feller's formula (28, 29), as follows:

$$Pr = N\left(\frac{1}{N} + \frac{1N}{N} - 1 + \frac{1}{N-2} \ldots \frac{1}{Nr} + 1\right) \quad (1)$$

where Pr is the probable (statistical) total number, r the number of colonies counted, and N the number of holes on the device head (N=400).

To evaluate the effect of ozone on the total bacterial and mould counts we relied on nonparametric Wilcoxon rank-sum test and set the significance to $p<0.05$.

RESULTS AND DISCUSSION

Ambient disinfection with ozone significantly reduced the total bacterial and mould count at all three sampling points (Table 1, Figure 2). Table 2 and Figure 3 show the identified bacterial cultures and their reduction after ozone treatment. The dominant bacterial strain before and after ozone treatment remained *Micrococcus* spp. at all three sampling points (Table 2). In contrast, several other authors reported the dominance of *Staphylococcus* spp. in hospital air (30–32).

Although we did not identify individual moulds, judging by the morphological properties of grown colonies, *Mucor* spp. seems to be one of the dominant species, which is in line with the findings of Ziaee et al. (33). Other authors reported the dominance of *Aspergillus* spp. and *Penicillium* spp. in the total fungal biomass (30, 34). Of course, our characterisation should be taken with reserve, as only further fungal identification would provide a more specific insight. In the meantime, one possible reason for the inconsistency between our bacterial and fungal findings and those of other studies

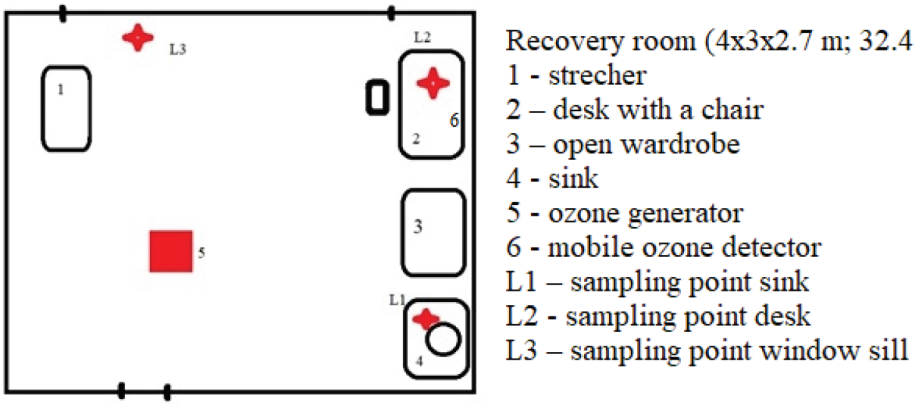

Figure 1 Recovery room layout

Table 1 Inhibition rates of total bacteria counts at the three sampling points

Sampling point	Before ozone treatment		After ozone treatment		Inhibition (%)
	r	*Pr*	*r*	*Pr*	
L1 – sink	413	2986	276	467	33
L2 – desk	367	992	151	189	58
L3 – window sill	309	591	118	140	61

Pr – probable (statistical) total bacterial count; *r* – number of counted colonies

could be that our measurements took place in a recovery room of a relatively new hospital wing that had been operational for three months only and had received no more than 20 patients by the time of our study.

The efficiency of ozone treatment was not even across the three sampling points but was the most efficient at the window sill (Table 1). This points to an unequal distribution of ozone gas across the room, which is required for an even effect, as reported by Blanco et al. (35) and Ito Kazuhide (36). However, considering that the ozone generator was placed at equal distance from all three sampling points, we believe that this difference may be owed to the fact that its exhaust was directed towards the window.

Furthermore, ozone treatment did not affect all identified bacterial strains equally. *Bacillus* spp. turned out to be the most sensitive to ozone at all three sampling points, which is a very interesting finding, considering that *Bacillus* spp. sporulates when exposed to unfavourable environmental conditions, disinfection included (37). It seems that only the vegetative / cultivable forms of *Bacillus* spp. were present in the room, as ozone is very effective against vegetative bacteria and inhibits sporulation. However this has been reported at very high ozone concentrations which are not adapted to healthcare settings (37–39). *Staphylococcus* spp. turned out to be very resistant, which is also surprising, considering that Russell et al. (16) found Gram-positive cocci to be more sensitive to disinfectants (16). However, our results are in line with reports claiming that Gram-positive bacteria are less sensitive to gaseous ozone than the Gram-negative ones (40).

Overall, our findings confirm high ozone efficacy against airborne bacteria in hospital settings reported earlier (25). However, its application as ambient disinfectant has certain limitations, as ozone gas it toxic to humans and can affect the respiratory system (41). The Croatian standards limit its concentration to 0.39 mg/m^3 over no more than 8 h a day (42). Furthermore, it has a specific and strong odour and can be corrosive if used very frequently (24). Some of these issues can be resolved with personal protective equipment when applying ozone and by neutralising (quenching) it with agents like magnesium thiosulphate to remove it from air (43).

CONCLUSION

To sum up, ozone gas at the applied concentration and exposure time reduced bacterial and mould contamination of the recovery room air but did not remove their presence entirely. To achieve effective air hygiene in the hospital environment it is necessary to combine mechanical cleaning of surfaces, conventional disinfectants, regular ventilation, and ozone for final disinfection. Considering the lack of national standards for microbiological indoor air quality, studies like this one provide some insight into the issue and alternative solutions. Further investigation should involve longer exposure time and higher ozone concentrations to get to know better its effects against airborne microorganisms in a hospital environment.

Acknowledgements

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We wish to express our gratitude to the Dr Ivo Pedišić General Hospital for providing facilities and to the Mozon d.o.o. company from Sisak for providing the ozone generator and expert guidance.

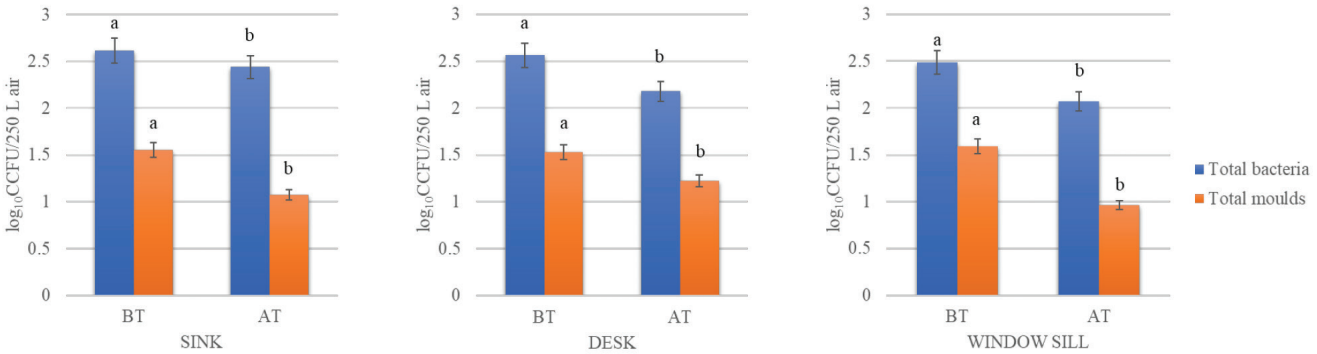

Figure 2 Total bacterial and mould counts before (BT) and after (AT) 1-hour treatment with ozone at the concentration of 15.71 mg/m^3. Different letters denote significant differences between groups ($p<0.05$; nonparametric Wilcoxon rank-sum test)

Table 2 Inhibition rates by identified bacteria at the three sampling points.

Bacteria by identified genera	Sink					Desk					Window sill				
	Before ozone treatment		After ozone treatment		Inhibition (%)	Before ozone treatment		After ozone treatment		Inhibition (%)	Before ozone treatment		After ozone treatment		Inhibition (%)
	r	*Pr*	*r*	*Pr*		*r*	*Pr*	*r*	*Pr*		*r*	*Pr*	*r*	*Pr*	
Micrococus spp.	380	1189	260	419	31	345	791	141	174	59	275	464	105	122	61
Staphylococus spp.	15	15	10	10	33	2	2	2	2	0	4	4	4	4	0
Bacillus spp.	13	13	4	4	69	10	10	3	3	70	20	20	0	0	100
Acinetobacter spp.	5	5	2	2	60	10	10	5	5	50	10	10	9	9	10

Pr – probable (statistical) total bacterial count; *r* – number of counted colonies

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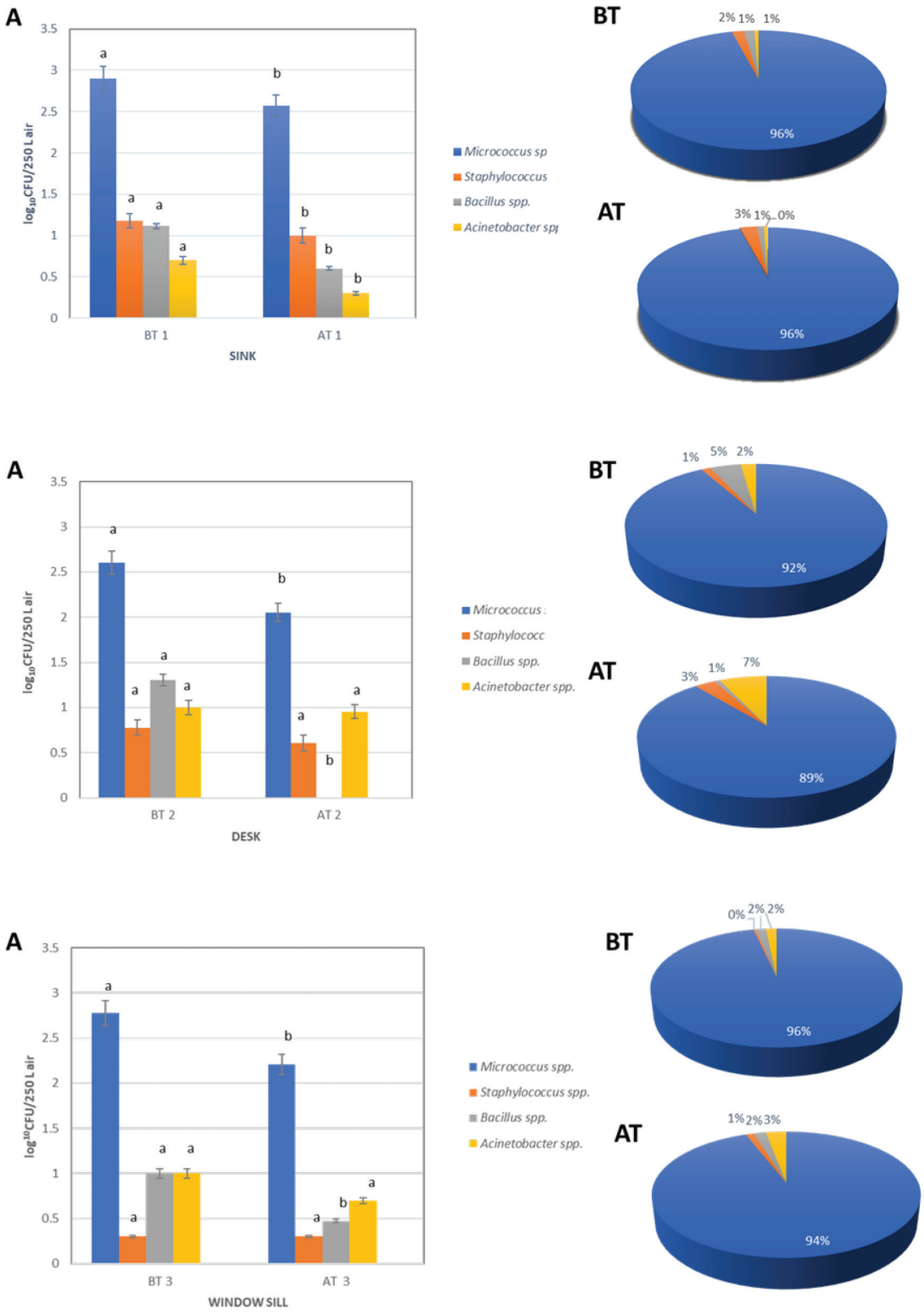

Figure 3 Changes in bacterial counts and prevalence (%) by identified genera before (BT) and after (AT) ozone treatment (15.71 mg/m^3) at the three sampling points. Different letters denote significant differences between groups ($p<0.05$; nonparametric Wilcoxon rank-sum test).

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Utjecaj plinovitog ozona na kakvoću zraka u bolničkom okružju

Bolničke infekcije mogu se prenijeti zrakom pa je higijena zraka u bolničkim sobama važan preventivni čimbenik. Standardne metode dezinfekcije nisu dovoljne za potpuno uništavanje bakterija u zraku te je potrebno pronaći odgovarajuću metodu dezinfekcije zraka. Primjena plinovitih dezinficijensa pokazala se učinkovitom, a ozon je potencijalni kandidat za dezinfekciju zraka u bolničkom okružju zbog dokazanog antimikrobnog učinka i niske cijene proizvodnje. Svrha ovog istraživanja bila je utvrditi antimikrobni učinak plinovitog ozona na kakvoću zraka bolničke sobe i identificirati standardne bakterije, raspršene u zraku. Istraživanje je provedeno u bolničkoj sobi Opće bolnice "Dr. Ivo Pedišić" u Sisku. Zrak je bio uzorkovan na različitim mjestima u prostoriji prije i nakon tretmana ozonom u trajanju od 1 sata / 15, 71 mg/m^3. Nadalje, identifikacija i kvantifikacija vrsta bakterija provedena je u uzorkovanom zraku na različitim lokacijama. Dominirale su gram-pozitivne bakterije iz rodova *Micrococcus*, *Staphylococcus* i *Bacillus*. Nakon tretmana, broj bakterija u zraku smanjen je 33 %, 58 % i 61 %, ovisno o lokaciji uzorkovanja, a najotpornijima su se pokazale bakterije iz roda *Micrococcus*. Plinoviti ozon prouzročuje značajno smanjenje mikroorganizama koji su raspršeni u zraku u česticama prašine i kapljicama u bolničkoj sobi. Preporučuje se produljeno djelovanje s većim koncentracijama ozona u kombinaciji s mehaničkim čišćenjem i čestim prozračivanjem.

KLJUČNE RIJEČI: aerosol; bakterije u zraku; bolničke infekcije; dezinfekcija; *Micrococcus spp.*