Original Article

Effectiveness of Gaseous Ozone as a Disinfectant for Nosocomial Pathogens in a Healthcare Emergency Room

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Abstract

Nosocomial infections are frequently caused by bacteria that are resistant to various antibiotics, resulting in the mortality or delayed recovery of hospitalized patients**.** Several studies have investigated the efficiency of ozone (O3) gas for the disinfection of surfaces to eliminate different nosocomial pathogens. In this study, the efficacy of O3 gas in a heavily contaminated healthcare facility was investigated using a low concentration of FDA-approved and human-safe O3. The total microbial loads on the air conditioning (AC) duct, wall, and tables after 1 month of O3 application were 0 CFU/100 cm^2, 1 CFU/ 100 cm^2, and 1 CFU/100 cm^2, respectively. Moreover, the total microbial loads on the AC duct, wall, and tables 2 months after O3 application were 0 CFU/m^2, 14 CFU/m^2, and 1 CFU/m^2, respectively. Finally, after the third month following O3 application, the microbial loads were 0 CFU/100 cm^2 on the AC duct, 7 CFU/100 cm^2 on the walls, and 54 CFU/100 cm^2on the tables. Overall results show that O3 gas controlled fungal growth, as it was decreased to minimal levels on some swabbed surfaces or even eliminated on most swabbed medical devices and work surfaces. Moreover, O3 is capable of eradicating nosocomial pathogens present in hidden areas even at low concentrations that match the levels approved by the FDA for human exposure. The study concluded that gaseous O3 can serve as an effective, safe, and cheap disinfectant. O3 could effectively work to eliminate both nosocomial bacteria and mould pathogens.

Keywords: Ozone, Nosocomial infections, Gram-positive, Gram-negative, Moulds

Introduction

Nosocomial infections are frequently caused by bacteria that are resistant to various antibiotics, and nosocomial infections are treated by selective resistant bacteria [1, 2]. Epidemiology of nosocomial infections have spread to ~5.7% of intensive care units in European hospitals, affecting more than three million patients. Accordingly, the mortality of patients or their delayed recovery from hospital treatment is an expected outcome [3-5]. Precisely, 1 out of 10 patients is infected by nosocomial infections by various pathogens during hospitalization, possibly resulting in significantly prolonged hospitalization and increased treatment costs; this scenario is further worsened for immunocompromised patients [3, 6]. Among the investigated healthcare facilities, neonatal hospitals reported the highest rates of nosocomial infections, followed by burn units. The most common nosocomial pathogens include *Staphylococcus aureus, Klebsiella, Escherichia coli, and Staphylococcus epidermidis* [7, 8]. *Enterobacteriaceae*, *S. aureus* (60% resistant to methicillin), *Pseudomonas aeruginosa*, coagulase-negative Staphylococci, and fungi [9].

Notably, medical devices have been considered the foci of nosocomial infections, such as stethoscopes, where several nosocomial pathogenic microbes have been isolated, including coagulase-negative Staphylococcus, Enterococci, E. coli, Klebsiella species, and *Acinetobacter* species. Nosocomial infections spread by medical devices include catheter-associated urinary tract infection (CAUTI), central line-associated bloodstream infection (CLABSI), and

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How to cite this article: Alshehri MA, Bahwerth FS, Althagafi ZA, Alsolami HA, Almalki AM, Saif A, et al. Effectiveness of Gaseous Ozone as a Disinfectant for Nosocomial Pathogens in a Healthcare Emergency Room. Arch Pharm Pract. 2021;12(4):17-24. https://doi.org/10.51847/UvHgz7UtJC

ventilator-associated pneumonia (VAP) [10-12]. Similarly, in Uganda, among swabbed equipment, about 19% of patient beds and infusion stands have been reported to exhibit the highest rates of bacterial contamination [13]. Some reusable and heat-sensitive medical devices are associated with decontamination failures, including endoscopes, as they cannot be autoclaved for sterilization; alternatively, they are subjected to deep decontamination using strong disinfectants, then the harmful chemicals are washed away with water. Nevertheless, such procedures can increase the possible re-contamination of devices by waterborne organisms, such as *P. aeruginosa* and mycobacterial species [14, 15].

Although ozone (O3) has been widely used in food and industrial sterilization protocols, it has only recently been implemented in healthcare disinfection protocols and studies. O3 is a highly reactive and colorless gas comprising three oxygen atoms, and owing to the mesomeric states of O3, it becomes dynamically unsteady [16]. It can be found in natural and manmade materials present in the Earth's stratosphere and troposphere. Depending on the atmospheric layer in which O3 is found, O3 affects life on Earth in a beneficial or deleterious manner [17, 18]. Several studies have been conducted to investigate the efficiency of O3 gas for disinfection against different nosocomial pathogens.

One study demonstrated that O3 can efficiently destroy bacteria such as *Bacillus subtilis*, *P. aeruginosa*, *E. coli*, *S. aureus*, *methicillin-resistant S. aureus* (MRSA), and *Candida albicans*, and can be used for disinfecting moulds [19]. Moreover, the efficacy of O3 application as a healthcare furniture sterilizer has been proven due to the dramatic decline in the MRSA growth curve by using high concentrations of O3. The above studies have been conducted under standard and quintessential scientific conditions. In this study, the efficacy of O3 gas in a heavily contaminated healthcare facility was investigated using a low concentration of FDA-approved and human-safe O3. As it is the most highly crowded ward in a hospital, an emergency room was selected for the study.

Materials and Methods

Study Area and Period

The study was approved and funded by Najran University, which is located in Saudi Arabia (NU/MID/18/028) to be conducted at two private hospitals in an area affected by COVID-19 cases during the pandemic lockdown. Both hospitals are crowded and treat a diversity of different nationalities. The research was conducted over 2 weeks, from 1-14 May 2020. The study was conducted in an emergency department, as it admits most of a hospital's patients with various medical conditions, including upper respiratory tract infections.

Sample Size and Sampling Techniques

A total of 297 swab samples were taken before synthetic gaseous O3 application: 1 week after O3 application and 2 weeks after O3 application (99 samples each time). Swabs were taken from medical equipment and work surfaces (walls, drawers, floors, etc.). After receiving informed consent from each participant, they were provided with national identification cards.

Specimen Collection and Identification of Pathogens

The method used for examination of surfaces was swabbing of a 100 cm^2 area by using a sterile swab moistened in 10 ml of neutralizing diluent, which enabled enumeration of the micro-organisms per m^2. For the enumeration test, a swab was used in buffered peptone water (BPW) as a diluent. The sample comprised a swab in a tube of 10 ml neutralizing buffer, which is considered to be a 10^0 dilution (neat sample). The neutralizing buffer and swab tip was transferred to a sterile bag with wire closures and 1 in 10 dilutions were performed by adding 90 ml of buffered peptone water (BPW). The sample was homogenized for 2 min in a stomacher. Twenty ml were transferred to a universal container, which was equivalent to a 10^{-1} dilution and provided a lower limit of detection of 100 CFU per swab by plating 1 ml. Swab specimens were collected from several surfaces, including medical equipment surfaces and work surfaces, subcultured on (sheep blood agar, plate colony agar (PCA) Saudi Industrial company, KSA). The plates were incubated aerobically, at 35–37°C for 24 hr and released for bacterial growth. Then aerobic Gram-positive bacilli were initially identified based on colony characterization, hemolysis pattern, Gram staining of the colonies, and API CHB Medium. Further identification was made with a catalase test, mannitol fermentation, and coagulase test. For identification of Gram-negative bacteria, the following tests were done: catalase, oxidase, urease, indole, citrate utilization, lysine decarboxylation, glucose and lactose fermentation, gas and H2S production, and motility tests. All biochemical test reagents were purchased from Oxoid Ltd. Company, UK. Colony count ≥20 CFU/diaphragm was considered significant contamination [15].

The data were entered and analyzed using SPSS version 25.0 computer software. Comparisons were made using the Chi-square test. A *P*-value of <.05 was considered indicative of a statistically significant difference.

Ethical clearance was secured from the Research Ethics Committee of Najran University (442-42-37841-DS). Permission was also obtained from the medical directors of the two selected hospitals.

Results and Discussion

Preliminary Study of O3 Gas as a Disinfectant

To identify the effectiveness of O3 in healthcare facilities for microbial disinfection, an O3 gas application experiment was carried out in the air conditioning (AC) ventilation ducts of

the laboratory and medical waste rooms. As reported in **Table 1**, the total microbial loads in AC duct, wall, and tables before O3 application were 4715 CFU/100 cm^2, 5664 CFU/100 cm^2, and 3505 CFU/100 cm^2, respectively. While the total microbial loads on the AC duct, wall, and tables after 1 month of O3 application were 0 CFU/ 100 cm^2, 1 CFU/ 100 cm^2, and 1 CFU/ 100 cm^2, respectively. Moreover, the total microbial loads on the AC duct, wall, and tables after 2 months of O3 application were 0 CFU/100 cm^2, 14 CFU/100 cm^2, and 1 CFU/100 cm^2, respectively. Finally, after the third month of O3 application, the microbial loads were 0 CFU/cm^2 from the AC duct, 7 CFU/100 cm^2 from the walls, and 54 CFU/100 cm^2 from the tables. This significant reduction in microbial growth after O3 application means that O3 has a notable effect on disinfection processes at healthcare facilities.

A total of 297 swabs samples were taken from medical devices or work surfaces in an emergency room at the most crowded hospital in Makkah City in the Kingdom of Saudi Arabia (KSA). About 9٩ samples, were taken at three-time intervals, before O3 application and after 1 week and 2 weeks. To categorize and identify the distribution of isolates on medical items, the isolates from the first 99 swabs (before O3 application) are shown in **Table 1**. In total, 213 isolates were isolated from swabbed medical items, in which 173 isolates were of Gram-positive bacteria, 27 isolates were of moulds, while 15 isolates were of Gram-negative bacteria.

Table 1. The total growth of microbial loads on laboratory surfaces before (at 0 times) and after O3 application

Surfaces	0	1st	2nd	3rd
	Time (CFU/100 cm^2)	Month (CFU/100 $cm^{2)}$	month CFU/100 cm^2	Month CFU/100 cm^2
AC duct	4715	0	0	0
Wall	5664	1	14	7
Tables	3505	1	1	54

The effectiveness of the disinfection of O3 gas on nosocomial infection pathogens and normal floral microorganisms is shown in **Table 2**. The total CFU/100 cm^2 of each isolate, on average, is presented to simplify data visualization **(Figure 1)**. The total average growth of isolated microorganisms was significantly affected by O3 gas application 2 weeks after application. The total average isolate growth before O3 application was ~696.4X10^3 CFU/100 cm^2; while the total average isolates growth 1 week after O3 gas application was ~76.4X10^3 CFU/100 cm^2. However, the total average isolate growth declined significantly 2 weeks after O3 gas application to ~7.8X10^3 CFU/100 cm^2.

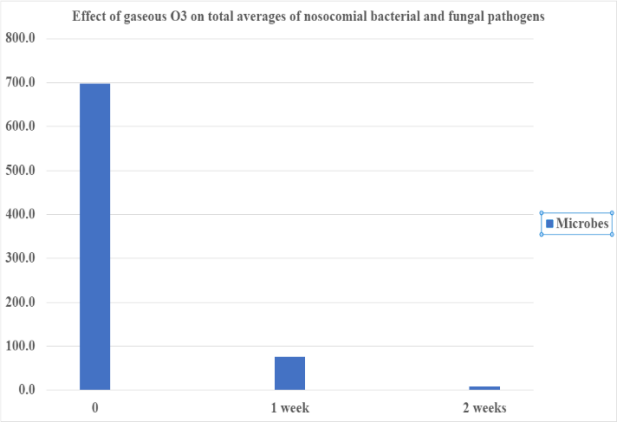

Figure 1. Effect of gaseous O3 on total averages of nosocomial bacterial and fungal pathogens.

O3 Gas Differently Controlled Bacterial Nosocomial Growth on Treated Medical Device Surfaces

The extent to which O3 gas can control both Gram-positive and Gram-negative bacterial nosocomial pathogens and normal floral growth is shown in **Table 2**. Isolate growth loads of the bacteria on medical devices and work surfaces before O3 gas application and after 1 week and 2 weeks of O3 gas, the application is reported in **Table 3**.

The growth loads were measured by CFU/100 cm^2 before O3 gas application and at 1 week and 2 weeks after O3 gas application and shown in **Figure 2**. For door knobs, commonly known to be highly contaminated, bacterial loads before O3 gas application were ~80.0X10^3 CFU/100 cm^2, while 2 weeks after O3 gas application, the growth was significantly reduced to ~1.0X10^3 CFU/100 cm^2. The sink was reported to be contaminated with ~80.0X10^3 CFU/100 cm^2 before O3 gas application, while 2 weeks after application the bacterial growth loads were at ~1.5X10^3 CFU/100 cm^2. The drawers were also swabbed and were found to be the most contaminated surface with ~69.5X10^3 CFU/100 cm^2 before O3 gas application, and the loads then decreased to ~12.6X10^3 CFU/cm^2 at 1 week then ~1.8X10^3 CFU/100 cm^2 at 2 weeks after O3 gas application. Oxygen pipes were the second most highly contaminated surface with ~58.6X10^3 CFU/m^2, and this significantly decreased to ~1.1X10^3 CFU/100 cm^2 after 2 weeks of O3 gas application. Interestingly, patients' beds were heavily contaminated with ~52.0X10^3 CFU/100 cm^2 before O3 gas application; the growth of the bacteria was then significantly reduced to ~1.8X10^3 CFU/100 cm^2 after 2 weeks of O3 gas application. Blood pressure devices and their attachments were contaminated with ~51.4X10^3 CFU/100 cm^2 before O3 gas application, and then this load declined to ~0.9X10^3 CFU/100 cm^2 after 2 weeks of O3 gas application. Electrical plugs were contaminated with ~65.4X10^3 CFU/100 cm^2 before O3 gas application, and then these numbers decreased to ~0.9X10^3 CFU/100 cm^2 after O3 gas application. These results suggest that O3 gas proved to be a good disinfectant, even for the farthest and smallest surfaces, and those most difficult to

clean and sterilize. The aqueous O3 reduced ~100% of the bacterial load within 2 weeks of exposure **(Figure 3)**.

Table 2. Total isolate growth loads in CFU affected by O3 gas treatment

Bacterial Isolates	O3 Treatment Effect		
	Before O3	After 1 week	After 2 weeks
		***	***
Gram-positive Bacteria	X10^3 CFU/100 cm^2	X10^3 CFU/100 cm^2	X10^3 CFU/100 cm^2
Gm+ve Bacilli	72.3	12.0	2.0
CONS	55.3	8.7	1.2
Bacillus	45.0	10.0	1.5
Aerobic spores-forming	59.3	7.1	1.4
Actinomycetes	38.5	4.9	0.6
Diphtheroids Spp	60.0	5.0	1.0
Nocardia	40.0	0.3	0.0
Filamentous bacteria	100.0	5.0	0.0
MRSA	30.0	5.8	0.0
Gram-negative Bacteria			
Pseudomonas aeruginosa	24.5	3.5	0.0
Roseomonas species	10.0	1.0	0.0
Vibrio species group	5.0	1.0	0.0
Spirochetes	45.0	1.5	0.0
Empedobacter brevis	41.5	1.0	0.0
Klebsiella pneumoniae	14.0	4.7	0.0
Moulds			
Fungus SPP	24.3	2.0	0.1
Aspergillus SPP	16.7	1.3	0.0
Aspergillus Niger	15.0	1.5	0.0
Total	696.4	76.4	7.8
***	P<0.0005		

Table 3. The effectiveness of O3 gas in eliminating bacteria on emergency room surfaces

Sample Place	O3 treatment by weeks		
	Before O3	After 1 week	After 2 weeks
	X10^3 CFU/100 cm^2	X10^3 CFU/100 cm^2	X10^3 CFU/100 cm^2
Bed surfaces	49.1	9.2	1.5
Under the beds	52.0	9.3	1.8
Siderail upper surface	40.9	6.3	1.0
Curtain	57.0	21.9	2.0
Trollies	45.9	7.6	1.2
Drawers	69.5	12.6	1.8
Solution fusion stands	26.3	4.6	1.1
O2 cylinders	58.8	7.1	1.1
Blood pressure devices	51.4	3.9	0.9
Stethoscope	5.0	1.0	0.0
Door knobs	80.0	6.0	1.0
Floor	40.5	6.3	1.3
Walls	62.2	14.4	0.9
Electrical plugs	65.4	6.3	0.9
Door knobs	50.0	2.0	0.0
Waste cans	61.3	5.6	0.8
Chairs	6.7	1.3	0.3
Ventilation holes	66.1	5.6	1.2
Sink	80.0	31.0	1.5

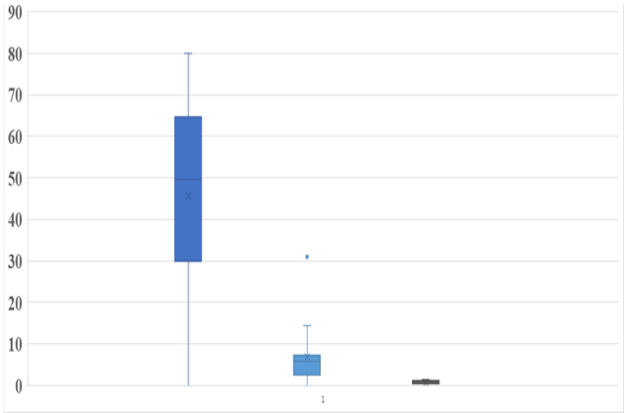

Figure 2. Effectiveness of gaseous O3 upon bacteria on different surfaces in an emergency room

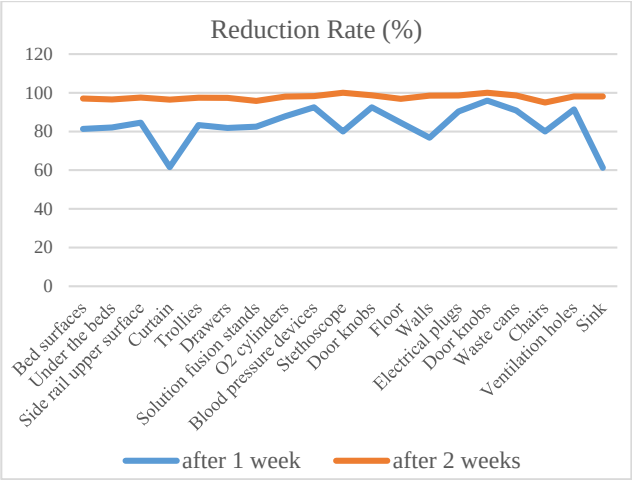

Figure 3. The reduction rate of bacterial count on emergency room surfaces

O3 Gas Effectively Controlled Nosocomial Fungal Pathogens

O3 gas application acts as a disinfectant on nosocomial fungal pathogens collectively, including Aspergillus SPP, Aspergillus Niger, and fungus SPP **(Table 2)**, as reported in **Table 4**.

Overall results show that O3 gas controlled fungal growth, as it decreased to a minimal level on some swabbed surfaces or even to no growth on most of the swabbed medical devices and work surfaces. For instance, nosocomial fungal growth was significantly controlled on medical wastebaskets from ~100.0X10^3 CFU/100 cm^2 to ~0.2 X10^3 CFU/100 cm^2, before and 2 weeks after O3 application, respectively. Moreover, fungal growth significantly declined from ~50.0 X10^3 CFU/100 cm^2 to ~0.0X10^3 CFU/100 cm^2 on a swabbed hand sterilizing dispenser before O3 gas application and 2 weeks after. The wall isolates were affected significantly by O3 gas application, as the mould growth loads were ~30.0X10^3 CFU/100 cm^2 before O3 gas application, reducing to ~0.0X10^3 CFU/ 100 cm^2 by 2 weeks after application. The drawers were contaminated with ~25.0X10^3 CFU/100 cm^2 before O3 gas application, while mould growth was ~0.25X10^3 CFU/100 cm^2 at 2 weeks after application. While mould growth on medical trollies was high before O3 gas application (~20.0X10^3 CFU/100 cm^2), it was reduced to no growth (~0.0X10^3 CFU/100 cm^2) 2 weeks after application. Blood pressure devices and their components were contaminated with ~18.0X10^3 CFU/ 100 cm^2 before O3 gas application, and mould growth on the same devices was ~0.2X10^3 CFU/ m^2 at 2 weeks after application. Mould growth from swabbed electrical blogs was reported as ~24.0X10^3 CFU/100 cm^2 before O3 gas application, while 2 weeks after application, the growth was ~0.0X10^3 CFU/m^2. Bed surfaces were contaminated with ~15.0 X10^3 CFU/100 cm^2 before O3 gas application, and fungal growth declined to ~0.0 X10^3 CFU/100 cm^2 by 2 weeks after application. The surfaces under beds were contaminated with ~10.0 X10^3 CFU/100 cm^2 of fungal growth before O3 gas application, and the growth was controlled by 2 weeks after application, as it was ~0.0 X10^3 CFU/100 cm^2. Rails were contaminated with ~12.7 X10^3 CFU/100 cm^2 before O3 application, but this declined to ~0.0 X10^3 CFU/100 cm^2 by 2 weeks after application. Fungal growth on drug fusion stands and chairs before O3 application was ~2.0 X10^3 CFU/100 cm^2 and ~1.0 X10^3 CFU/100 cm^2, respectively; however, this was controlled by 2 weeks after O3 gas application, as they were each at ~0.0 X10^3 CFU/ m^2. The aqueous O3 reduced ~100% of the bacterial load within 2 weeks of exposure **(Figure 4)**.

Table 4. The effect of O3 on nosocomial fungal pathogen growth on swabbed emergency room surfaces

Sample place	O3 treatment By Weeks		
	Before O3 CFU/100 cm^2	After 1 Week CFU/100 cm^2 ***	After 2 weeks CFU/100 cm^2 ***
Bed surfaces	15.0	4.5	0.0
Under the beds	10.0	1.0	0.0
Siderail upper surface	12.7	1.3	0.0
Solution fusion stands	1.0	1.0	0.0
Trollies	20.0	2.0	0.0
Drawers	25.0	1.8	0.3
Blood pressure devices	18.0	1.8	0.2
Walls	30.0	2.0	0.0
Electrical plugs	24.0	1.5	0.0
Waste cans	10.0	7.0	0.2
Chairs	2.0	1.0	0.0
Steriliser dispenser	50.0	2.0	0.0
***	*P* <0.0005		

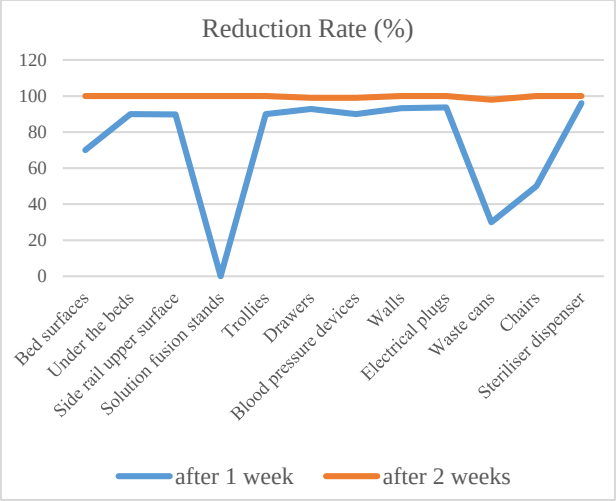

Figure 4. The reduction rate of fungal count on emergency room surfaces

The current study was conducted during the COVID-19 pandemic lockdown in a fairly crowded hospital in Makkah City, located in an area with a significant number of COVID-19 cases. In the present study, we demonstrated that disinfecting the ambient air, the medical equipment, and the surfaces of the surrounding workspaces in an open emergency room through the action of generated gaseous O3 served to eradicate and reduce the growth of different nosocomial bacterial and fungal pathogens.

This finding aligns with previous studies associated with high concentrations of generated gaseous O3 [1]. Both aqueous and gaseous O3 have been found to effectively reduce a wide variety of microbial (bacteria and fungi) growth on surfaces contaminated by dairy cattle manure [3]. It is common to find that the spread of a nosocomial infection may have originated in an emergency department. This mainly occurs via airborne droplet nuclei, large-particle droplets, or direct contact between patients and the surrounding medical devices [20]. Interestingly, the source of infection and airborne transmission of various severe and highly infectious diseases, such as tuberculosis, measles, and severe acute respiratory distress syndrome (SARS), is transmitted from the emergency room [21-23]. This indicates that there are challenges in fighting nosocomial pathogens, and emergency rooms remain the primary source of nosocomial infections [24]. Further, previous studies have suggested that a high

concentration of gaseous O3 is significantly effective at reducing microbial growth in a short period [1].

Our study demonstrates and argues that a low concentration of gaseous O3 that complies with the associated FDA standard for human exposure also provides significant and effective results in eradicating several types of microbial growth within just 2 weeks of exposure. The use of O3 gas at such concentration levels could be greatly beneficial because it would not only function as a good disinfectant and sterilizer but also refresh the air by removing unwanted odors and increasing the amount of fresh O2 available, which remains after O3 is used [25, 26].

According to the Healthcare Infection Control Practices Advisory Committee, environmental surfaces within an emergency department can be categorized into two types: the surfaces of medical equipment (such as O2 cylinders, blood pressure devices, etc.) and housekeeping surfaces (such as floors, walls, etc.) [27]. The latter can be further subcategorized into "high touch" surfaces (e.g., door handles, bed rails, and light switches) and "low touch" surfaces (e.g., floors and ceilings) [27]. Moreover, according to previous studies, the high-touch surfaces in healthcare facilities, especially in emergency departments, are not decontaminated by environmental service workers as thoroughly as the low-touch surfaces [28]. This could cause nosocomial infections to spread among patients, leading to serious diseases. Our findings regarding several nosocomial pathogens' isolation from high-touch, low-touch, and medical equipment surfaces in the emergency department are in alignment with those of previous studies. In addition, our suggested application of gaseous O3 in emergency departments as a sterilizer and disinfectant has shown to yield considerably significant growth reductions of common nosocomial bacterial pathogens even from difficult, hidden, and remote surfaces, such as the inside of drawers and the underside of beds and side rails. These areas are extremely difficult to clean and disinfect.

Most of the isolates identified in the current study, such as bacteria, have been previously identified [29, 30]. For instance, MRSA, as a multidrug-resistant bacteria and common nosocomial infection pathogen, was isolated in the current study, and its growth was downregulated successfully through O3 gas application. MRSA is widely known to be highly prevalent in emergency departments and healthcare facilities [31, 32]. Similarly, the diversity of Gram-positive bacteria was isolated in the current study, including Gram-positive bacilli, CONS, bacillus, aerobic spore-forming, actinomycetes, diphtheroids spp., Nocardia, and Filamentous bacteria. Most of these bacteria have been previously isolated from healthcare facilities, and Gram-positive bacteria distribution within the hospital environment is greater than that of Gram-negative bacteria [33, 34]. These bacteria were successfully eradicated using O3 gas during disinfection, even when the O3 gas was at a concentration low enough to be compatible with the FDA-approved volumes for human exposure.

Concerning Gram-negative pathogens, we isolated *K. pneumoniae*. This achievement is comparable with previous reports that *K. pneumoniae* can also be found in emergency departments [35, 36]. In the United States, K. pneumoniae is reported to be one of the leading causes of nosocomial infection [37]. It has been described as an opportunistic pathogen, as it may cause infections in hospitalized or immunocompromised patients. K. pneumoniae causes serious infections, including pneumonia, UTIs, and bloodstream infections that have a mortality rate as high as 50% [37]. Further, other Gram-negative bacteria, such as *Pseudomonas aeruginosa (P aeruginosa)*, Roseomonas spp., vibrio spp. group, spirochetes, and Empedobacter brevis were isolated in this study.

Several studies have discussed one or more of the Gram-negative bacteria mentioned above [38]. Importantly, Gram-negative bacteria are known to be serious disease causatives in humans, and nosocomial infections caused by Gram-negative bacteria are considered the most threatening for infection control professionals, as they are antibiotic-resistant [39]. As demonstrated in the current study, gaseous O3 application greatly downregulates the growth of Gram-negative bacteria. Nosocomial fungal pathogens are considered one of the most virulent, causing illnesses in domestic patients. Moreover, invasive nosocomial filamentous fungal infections are usually associated with high morbidity and mortality, especially in immunocompromised patients. In this study, we identified the diversity of common nosocomial fungal SPP pathogens (12.7% of 213 isolates). These include Aspergillus spp. (about 1.4%) and Aspergillus niger (0.5%). The main aim of this study was to eliminate highly virulent nosocomial pathogens, including moulds, using gaseous O3. We have demonstrated that artificial O3 gas application in healthcare facilities can significantly reduce the growth of all hidden nosocomial fungal pathogens. This illustrates the relationship between Aspergillus niger and nosocomial infections. We demonstrate that the generated gaseous O3 effectively mitigates the growth of fungal spp. Several studies have recommended that healthcare facilities should be as safe and clean as possible concerning airborne fungal pathogens, especially those present in the air and surrounding surfaces [14]. This has also been previously achieved by using a high concentration of gaseous O3 to eliminate both bacterial and mould growth [1].

To our knowledge, this study could be the first in its field to employ O3 as a disinfectant and sterilizer in healthcare facilities and hospitals, specifically in the most crowded area: the emergency room. Although previous studies have shown that O3 gas can mitigate the growth of several types of pathogens, its application was tested within laboratories and with commercial bacterial strains. In contrast, we used O3 in a real hospital setting and an open emergency room.

Moreover, to our knowledge, we are the only ones who have used a low gaseous O3 concentration that matches the concentration levels recommended by the FDA for human safety and proved that this low concentration is indeed suitable for use as a microbial-pathogen disinfectant in both open and long-term applications (2 weeks). Conventional chemical disinfectants fail to eliminate nosocomial pathogens, have side effects, and do not affect difficult-to-reach, hidden areas, such as inside drawers and under beds; thus, O3 gas can be used to overcome these disadvantages, especially in reaching hidden areas. Importantly, it may be the right choice for healthcare providers to employ gaseous O3 as a disinfectant due to its cost-effectiveness, the use of portable devices, the quick disinfection results, and the smaller number of workers required. In contrast, conventional chemical disinfectants have high costs and require difficult and laborious applications.

Conclusion

The emergency department has been proven to be the most critical area in healthcare facilities due to crowding and the diversity of the patients who pass through it. This study identified the diversity of nosocomial infection pathogens in an emergency room. We have demonstrated that gaseous O3 can serve as an effective, safe, and cheap disinfectant. Moreover, O3 is capable of eradicating nosocomial pathogens present in hidden areas, even at low concentrations that match levels approved by the FDA for human exposure. O3 could effectively work to eliminate both nosocomial bacteria and fungi.

ACKNOWLEDGMENTS: We would like to thank the dean of scientific research at Najran University for supporting this research and to the Saudi ozone company for their technical help.

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: This research was funded by the dean scientist research at Najran University, in Saudi Arabia (NU/MID/18/028).

ETHICS STATEMENT: The research was approved by the research ethical committee of Najran University with reference no (442-42-37841-DS).

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