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Abstract

As an increased challenge to hospitals, antimicrobial pathogens pose an ongoing issue, during clinical treatment of patients, due to causing healthcare associated infections (HAIs). Prevention of cross transmission and contamination are some of the difficult challenges posed by these pathogens. Existing methods to address this challenge have included aggressive sanitizers or "bleach" treatment and deployment of UV-C technology. Ultraviolet light with wavelengths between 200 - 280 nanometers (nm) is often referred to as UV-C. Limitations are seen with both methods. The effectiveness of sanitizer treatments is limited to the thoroughness of application process, often limited by time constraints between patient room turnover and the effectiveness of the cleaning staff. The effectiveness of UV-C technology is limited to line-of-sight and physical distance from the device, as well as by steady degradation of treatment efficacy with bulb life. In each case, the solution is a one- time treatment without continuous cleaning or ongoing prophylaxis, and neither solution addresses airborne pathogens.

Naturally occurring microorganisms along with HIAs is fostering a concern that constitute a global catastrophic biological risk (GCBR) are a growing topic of concern. The recent interest in understanding the broader pandemic threat landscape was generated due to the emergence of severe infectious diseases with pandemic potential. A considerable amount of infectious disease organizational activities have, up to now, centered on a chronological list-based tactic developed around biological warfare agents and on new occurrences, e.g., severe acute respiratory syndrome (SARS). However, this approach is not anticipatory or preventive and will fundamentally not account for causes not presently identified or those without historic instance. Actions that are exclusively restrained to list-based methods may substitute an inert foreign-born approach to the difficulty, hinders readiness, and reduces flexibility. Unfortunately, the United States, and numerous nations have accepted this approach. Recently conducted studies by Johns Hopkins Center for Health Security to illuminate the types of inherently occurring microorganisms that create a GCBR. Biological sanitization using a non-thermal gas release at atmospheric pressure in air is the topic of substantial investigation at this time. The process for bacterial inactivation endures much conjecture, principally with respect to the function of ions and volatile gas species. Two processes have been suggested: electrostatic disturbance of cell membranes and fatal oxidation of sheath or cytoplasmic constituents. Final outcomes show that death is accompanied by cell lysis and disintegration in Gram-negative bacteria but not in Gram-positive species, while cytoplasmic "leakage" is often noted. A source of charged particles, can be from gas discharges, yielding ions, reactive gas species, radicals, and radiation (visible, ultraviolet and infrared), many of which have recognized biocidal characteristics. The discrete roles participated by these decontamination processes are not well comprehended or enumerated. Conversely, the reactions of some species with biomolecules or any of numerous substances that are produced by cells and living organisms are documented in the literature. Oxidative stress is somewhat well examined, and it is likely that exposure to gas releases in air causes sever oxidative difficulty.

This study evaluates the effectiveness of an advanced oxidation technology developed by airPHX Health in addressing airborne and surface HAI organisms in common hospital facilities including stainless steel, plastic and linoleum floors. Nonetheless, among the different classes of microbes, many have some or all of the characteristics required to be identified as a GCBR. Viruses have several features that make this group of microbial agents the most likely source of GCBRs. Genetic mutability has a higher capacity in viruses due to both the structure of their genomes and the origination time for replication in which large numbers of offspring virus that are created each day. Also, the failure of a virus to be contested with a broad-spectrum antiviral contrasted with bacteria, fungi, and parasites makes viruses the plausible cause of a GCBR. RNA viruses, within the viral class warrant special concern mainly due to their greater mutability contrasted to DNA viruses. Treatment via an airPHX system assaults both virus and bacterial cell walls affecting a total destruction with no further RNA/DNA resistance mutation. These actions will be further delineated in subsequent publications aimed at GCBRs. It should be noted that airPHX Health technology eliminates airborne pathogens and distributes into the treatment space oxygen-based oxidizing molecules that sanitize ambient air and hospital surfaces. Using airPHX technology, treatment is not limited to line-of-sight or physical distance, can be scaled to any size treatment space without sacrificing treatment efficacy, and is continuous and highly effective.

Keywords: Healthcare-Associated Infections (HAIs); Johns Hopkins Center for Health Security; Severe Acute Respiratory Syndrome (SARS); Global Catastrophic Biological Risk (GCBR); UV-C Technology; Oxygen-Based Oxidizing Molecules

Introduction

"Tubular Corona" Technology

Tubular corona technology has the ability of producing a constant coronal ionic emission (plasma) using a patented electro-mechanical device. The plasma field is created next to the inside and outside of a cylindrical dielectric, which is made, of pairs of tube-shaped wire mesh electrodes (anode and cathode) placed inside and outside of a canular insulator. Applying electrical power to these electrodes, a stable, non-thermal corona is generated lengthwise along the entire extent of the tubular dielectric which produces plasma. This plasma can be generated at changing external temperatures, with various levels of relative humidity, without a vacuum or a noble gas, and with contrasting low and high energy dielectric tubes in the same reaction chamber, are all a special feature of airPHX technology [1].

A stable, non-collapsing, non-drifting "tubular corona discharge" is generated inside the reaction chamber of the airPHX unit (Figure 1). Other systems create an enormous amount of heat due to the ineffectiveness in production of the plasma field. The airPHX technology does not increase the ambient temperature thus producing a "non-thermal" plasma.



Figure 1: "Tubular Corona" Technology.

A number of the oxygen molecules present are converted into oxygen based oxidizing molecules that are referred to as reactive oxygen species (ROS) as air is drawn through the plasma in the reaction chamber. These ROS include oxygen ions, free radicals and peroxides that are highly reactive due to the presence of an unpaired valence shell electrons [2-4].

Measurable levels of gaseous "dry" hydrogen peroxide and other ROS are produced within the chamber and given their half-lives, can be allowed to enter the surrounding environment when this is desired [5-6]. Gas-phase "dry" hydrogen peroxide generated in the reaction chamber is different than vaporized or aerosolized hydrogen peroxide. Gas-phase hydrogen peroxide has a more acute bond angle. An acute bond angle of 60° puts strain on the other atoms involved. Each atom in the molecule would like to be as far away from one another but close enough to share electrons. Thus gas-phased hydrogen peroxide and is considerably shorter lived than the more stable liquid or vaporized products. In addition to gas-phase hydrogen peroxide, the ROS includes hydroxyl radicals, superoxide, singlet oxygen and ozone. Slight amounts of ozone is released from the reaction chamber, it interacts with airborne contaminants and is consequently at very low dissolved levels [7].

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Adaptive technology

This technology has a wide range of applications due of its ability to produce a highly oxidative products within its reaction chamber and subsequently discharge these molecules that have an oxidizing capacity into the environment at levels that are safe for human exposure.

The application array is greater due to the fact that the equipment can be adapted or further scaled to meet the particular needs by:

- 1. Changing the number and size of the tubular dielectrics within a reaction chamber.
- 2. Power supply modifications to change the output of the ROS created.
- 3. The option of passing the discharge ROS through a catalyst or catalyst array to preclude or greatly reduce the output of specific ROS items from the unit.

Safety considerations

The airPHX technology ROS output is monitored through the use of an integrated Aeroqual sensor (Aeroqual Limited, Auckland, New Zealand) examining ozone (O_3) as the measure of ROS production. The low level O_3 byproduct of the clean process that takes place within an airPHX unit is safe according to the OSHA Hazard Communication Standard 29 CFR 1910.1200. The airPHX technology relies on electricity and the oxygen present in ambient air to produce marginal levels of reactive oxygen species where O_3 is stabilized (average less than 0.03 ppm) within a treated area or space. This level is lower than limits established by the Occupational Safety and Health Administration of 0.10 ppm and the Center for Disease Control through The National Institute for Occupational Safety and Health (NIOSH) of 0.10 ppm.

The "dry" hydrogen peroxide produced (previously discussed) is different from vaporized or aerosolized hydrogen peroxide (H_2O_2) . This by- product is from the clean process that takes place within an airPHX unit is not hazardous according to the OSHA Hazard Communication Standard 29 CFR 1910.1200. The airPHX technology relies on electricity and the oxygen present in ambient air to produce marginal levels of H_2O_2 where it is stabilized (average less than 0.07 ppm) within a treated area or space. Such treated area(s) should have consistent/ constant airflow to provide a uniform distribution of the sanitizer. This level is lower than limits established by the Occupational Safety and Health Administration for General Industry: 29 CFR 1910.1000 and the Center for Disease Control through The National. Institute for Occupational Safety and Health (NIOSH) of 1 ppm.

Mechanism of action, lethality

A large range of microbiological organisms are susceptible to plasma gas exposure, including Gram-negative, Gram-positive bacteria, bacterial spores, yeasts, viruses [8], and biofilms [9]. Reductions in bacteria viability of over 6-log are reported from exposures of less than 30s [10-12], and total eradication is seen after longer exposures.

As with most sterilization procedures, the degree and magnitudes of reduction is in response to a particular treatment system which differ for various species and strains of bacteria. Most resistant are spores followed by vegetative or actively growing bacteria and the relative susceptibility of Gram-negative or Gram-positive bacteria is not fully discerned. Inactivation of bacteria is accomplished by leak-age of proteins, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) from the cellular cytoplasm [13]. Specifically, macromolecules are detectible in the supernatant of a cell suspensions of Gram-negative E. coli treated with atmospheric cold plasma after 10s, and 15s.

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Observations of the physical damage on Gram-negative and Gram-positive bacteria yield unique differences. Damage of certain metabolic functions, not resulting in cell death, have been reported from near-lethal exposures, suggesting changes in enzyme activity [14-15]. The method of cell death produced by gas discharge has two main hypotheses. Each involves lethal damage to cell membrane constituents and ultimately leading to loss of cytoplasmic fillings or lysis. Electrostatic breakage includes oxidation of membrane components. The electrostatic breakage mechanism [16] suggests that the total electric force caused by the buildup of surface charge could exceed the total tensile force on the membrane (Gram-negative). Where surface irregularities give regions of higher local curvature have a greater probability of failing. The tensile strength of the membrane is conferred by a murein layer, with Gram-positive bacteria which is thicker (~15-18 nm) than Gram-negative bacteria (~2 nm), meaning a lower collected charge would be required for lysis of Gram-negative bacteria than Gram-positive. In the second mechanism, the energetic ions, radicals, and reactive species generated by gas discharge and oxidation damage of membrane or cellular components are suggested to be the cause. Plasma directly creates active radicals which are generated and diffuse to the cell surface, with secondary radicals produced while reaction chemistry in a moisture layer on the cell surface. It is well documented that ROS have significant damaging effects on cells through reactions with numerous biomacromolecules [17-20]. The participation of superoxide in the bactericidal effects of a corona discharge is suggested to by the protective effects verified by super oxide dismutase (SOD) enzymes [21-22].

Objective

The objective of this study was to evaluate the effectiveness of ROS exposure in reducing bacterial air and surface populations in an active oncology wing of a hospital, the Oncology Wing Test (OW) and the waiting area in a hospital Emergency Room (ER) Waiting Area Test.

Material and Methods

The tests were conducted in a 600-bed hospital in the Mid-Atlantic states. The senior executives of the hospital system in the areas of Safety and Industrial Hygiene and Facilities Operations conducted direct oversight of the testing. The Chief Physician Officer of the hospital system reviewed and validated the trial results.

Volumetric air sampling

Air sampling utilized a MicroBio MB1 volumetric air sampler, Cantium Scientific, Clarendon Gardens, Dartford UK. Scientific Air (do not hyphenate) So- lutions is the (still need to remove "the") a North American Distributer for the MB1 and MB2 volumetric air samplers. All air samples were taken via the MB-1 air sampler, 30 liters per sample throughout the various locations. Results normalized to colony forming units per cubic meter of air (cfu/ m3) Calculation: CFU/m3 = 1,000 x nc / vs where nc is the corrected number of colonies counted and vs is the sample volume in liters.

Air samples were impinged on 15x100mm potato dextrose agar plates acquired from Hardy Diagnostics, Santa Maria, California. Air sample morphology and enumeration was completed by Scientific Air Solutions, Turlock, California.

Surface testing

All surface contact swab samples were taken with a Romer Sponge Handle Sampling System, Romer Labs, Inc, Newark, Deleware. Swab dimensions are 10 cm x 10 cm = 100 cm² (4" x 4") and normalized to CFU/cm². Calculation: $CFU/cm^2 = n_c / s_s$. Where n_c is the number of colonies counted and s_s is the surface sample in square centimeters, our standard is 10 cm x 10 cm = 100 cm². All swab sponges were forwarded to Scientific Air Solutions for enumeration. All swab samples were examined for the number of organisms and recorded as colony forming units per square centimeter, CFU/cm^2 .

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Treatment

Volumetric Air Samples, CFU/m ³ Sample Location	# Samples	Pre-Treatment	In-Treatment	% Reduction
Oncology Main Hallway	20	767	40	94.8
Nurse Station 1-3 Utility Hallway	5	1,113	80	92.8
Elevator Bay Floor 1-7	7	1,538	62	96.0
Elevator Bay Floor 8	4	1,742	33	98.1
Exterior	2	2,933	2,917	0.5
Surface "Contact" Swabs, CFU/cm ² Sample Location	# Samples	Pre-Treatment	In-Treatment	% Reduction
Treatment Area, see "S" samples	13	17	0.21	98.8
Negative control	1	0	0	0

Table 1: Summary of testing	results: OW Test.
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Volumetric Air Samples, CFU/m ³ Sample Location	# Samples	Pre-Treatment	In-Treatment	% Reduction
Waiting Room Interior	10	880	77	91.3
Exterior	2	2,417	2,400	0.7
Surface "Contact" Swabs, CFU/cm ² Sample Location	# Samples	Pre-Treatment	In-Treatment	% Reduction
Treatment Area, see "S" samples	7	41	1.9	95.4
Negative control	1	0	0	0

 Table 2: Summary of testing results: ER Waiting Room Test.

For both the OW Test and the ER Waiting Room Test sample locations were mapped and noted as either air sampling or surface swabbing. Upon completion of pre-treatment sampling, an airPHX PA2400P portable unit was placed in the treatment area and activated. The airPHX unit was allowed to operate continuously for eighteen days in the OW Test and thirty-six days in the ER Waiting Room Test. At the end of the treatment period, in-treatment volumetric air samples and surface swabbing were taken in the same locations as the pre-treatment sampling. External air samples were taken to understand the influence of the supplied air to the two test locations. Results are given in tables 1 and 2.

Results and Discussion

Oncology wing

The OW is on a single floor of the facility, and it includes patient rooms, three nurse stations and a utility hall. Fresh air is required in all patient spaces. Typically, inpatient areas have six (6) total air exchanges per hour with two being fresh or outdoor air. These figures can increase as most air handling units have an economizer, using 100% outdoor air when outside conditions permit. All air passes through 95% filters in the air handlers, and the patient rooms have HEPA terminal filters at the diffusers. A total of 36 air samples and 13 surface swabs were taken (Figure 2).

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Figure 1: Layout of oncology wing with volumetric air and surface sample locations, designated as S1 to S13, noted below.

Volumetric air samples

- Pre-treatment air samples ranged from 767 to 1,742 CFU/m^{3.}
- In-treatment results ranged from 33 to 80 CFU/m³, a reduction of 93-98% thus showing the airPHX system reduced the outside air bioburden and further sanitizes the locations examined.
- Exterior air samples showed relatively high organism counts, approximately 2,933 and 2,917 CFU/m³ respectively, indicating a very high bioburden being introduced into the OW.
- 4. Favorable treatment results extended beyond the treatment location, as sampling in the elevator bays on the seven (7) other floors were reduced from 1,538 to 62 CFU/m³ showing a 96% reduction.

Surface Contact "Swabs

- Pre-treatment surface swab results were 17 CFU/cm².
- 2. In-treatment surface testing results revealed 0.21 CFU/cm², yielding a 99% reduction.

Emergency waiting room

Upon completion of the OW test, the airPHX portable device was moved to the waiting room area of the emergency room. The ER waiting room includes three (3) counters, several administrative desks, and a patient waiting area. The testing was conducted in the midst of flu season, and the foot traffic and turnover of patients was elevated, including frequent exterior door openings and introduction of outside air and contaminants. The airPHX unit ran continuously; however, when conducting the in-treatment tests, it was discovered that the unit had been turned off, so the results may reflect only intermittent activation of the technology. A total of ten (10) air samples and seven (7) surface swabs were taken (Figure 3).



Figure 3: Layout of ER waiting room with volumetric air and surface sample locations designated as S-01 to S-07, noted below.

Volumetric air samples

- Pre-treatment air samples averaged 880 CFU/m³.
- In-treatment results averaged 77 CFU/m3, a 91% reduction.
- Exterior air samples showed relatively high bioburden, approximately
- 2,917 and 2,400 CFU/m³ respectively, indicating a very high bioburden being introduced into the ER waiting room.
- Favorable treatment results were seen, notwithstanding frequent door openings and high foot traffic in this area.

Surface Contact "Swabs"

- Pre-treatment surface swab results were 41 CFU/cm².
- In-treatment surface testing results revealed 1.9 CFU/cm², yielding a 95% reduction.

Conclusion

Exterior samples, an indication of the effectiveness of the airPHX treatment in overcoming the existing high bioburden from the outside environment. The in-treatment test results in both spaces showed excellent reductions in counts from both air and surface tests, indicating that the airPHX technology dramatically reduced the bioburden in the existing air and will definitely favorably impact infection control efforts in traditional HAI environments. The scalable and easily deployed nature of the airPHX technology appears to offer a solution to the large and unpredictable risks posed by GCBRs.

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