Health Protection Scotland

NHS National Services Scotland

Literature Review and Practice Recommendations: Existing and emerging technologies used for decontamination of the healthcare environment

Ozone

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Contents

Topic

The use of ozone for decontamination of the healthcare environment and reusable non-invasive patient care equipment.

Background

There is strong scientific evidence that contaminated environmental surfaces contribute to the transmission of pathogens in healthcare settings.[1-4] As such, environmental decontamination has an important role to play in the prevention and control of healthcare associated infections.[1-4]

The National Infection Prevention and Control (IP&C) Manual[4] for NHSScotland currently outlines the following recommendations on agents for **routine environmental decontamination** within the Standard Infection Control Precautions (SICPs chapter 1), which are the basic measures intended to be applied by all staff, in all care settings, at all times, for all patients:

> *A fresh solution of general purpose neutral detergent in warm water is recommended for routine cleaning. This should be changed when dirty or at 15 minutes intervals or when changing tasks.*
>
> *Routine disinfection of the environment is not recommended. However, 1,000 ppm available chlorine should be used routinely on sanitary fittings*.[4]

The National IP&C Manual also makes recommendations on agents for environmental decontamination in the chapter outlining Transmission Based Precautions (TBPs), which are intended to be applied when caring for patients who are known to have or are suspected of having an infection.[4] The following recommendations are made in relation to **routine environmental decontamination** when applying TBPs:

> *Patient isolation/cohort rooms/area must be decontaminated **at least daily** using either:*
>
> - *a combined detergent/disinfectant solution at a dilution of 1,000 parts per million available chlorine (ppm available chlorine (av.cl.)); or*
> - *a general purpose neutral detergent in a solution of warm water followed by disinfection solution of 1,000ppm av.cl.*[4]

In addition, the following recommendations are made in relation to **terminal cleaning** when applying TBPs:

The room should be decontaminated using either:

- *a combined detergent disinfectant solution at a dilution (1,000 ppm av.cl.); or*
- *a general purpose neutral detergent in a solution of warm water followed by disinfection solution of 1,000 ppm av.cl.*[4]

Chlorine releasing agents are recommended for decontamination of sanitary fittings and for environmental decontamination under TBPs based on substantial evidence of their effectiveness against pathogens of HAI significance including norovirus and *C. difficile.*[5]

However, several issues and problems associated with the use of chlorine releasing agents such as corrosion of equipment and furnishings, release of toxic gas and respiratory irritation, has encouraged interest in alternative methods of decontamination.[6] There are numerous other existing technologies such as steam cleaners, and a growing list of novel technologies becoming available for decontamination of the healthcare environment.[7-9] Currently, these technologies have not been sufficiently assessed to advocate their use for environmental decontamination in NHSScotland. A review is required to assess the effectiveness of technologies of interest to the infection control community, to consider any practical and safety considerations related to them, and to explore the associated costs.

Aim

To review the evidence for using ozone for decontamination of the healthcare environment and reusable non-invasive patient care equipment.

Objectives

- To provide a generic description of ozone, including the proposed or actual mechanism of action and the procedure for use.
- To assess the scientific evidence for effectiveness of ozone.
- To explore practical and safety considerations related to the use of ozone.
- To explore the costs associated with ozone.
- To produce a concise evidence sheet for ozone to assist the Environmental Decontamination Steering Group in making practical recommendations on the use of ozone for NHSScotland.

Research questions

The following research questions will be addressed for ozone:

1. Is ozone currently in use in UK healthcare settings?
2. What is the actual or proposed mechanism of action of ozone?
3. What is the procedure for using ozone?
4. What is the scientific evidence for effectiveness of ozone for decontamination of the healthcare environment?
5. Are there any safety considerations associated with using ozone in the healthcare setting?
6. Are there any practical or logistical considerations associated with using ozone in the healthcare setting?
7. What costs are associated with using ozone in the healthcare setting?
8. Has ozone been assessed by the Rapid Review Panel?

Methodology

Search Strategy

The following databases and websites were searched to identify relevant academic and grey literature:

- MEDLINE
- CINAHL
- EMBASE
- NHS Evidence (http://www.evidence.nhs.uk/)
- Health Technology Assessment (HTA) Database (http://www.crd.york.ac.uk/CRDWeb/)
- Database of Abstracts of Reviews of Effects (DARE) (http://www.crd.york.ac.uk/CRDWeb/)
- National Patient Safety Agency (http://www.npsa.nhs.uk/)
- NICE (http://www.nice.org.uk/)
- MHRA (http://www.mhra.gov.uk/)
- Rapid Review Panel Reports Archive (http://www.hpa.org.uk/ProductsServices/MicrobiologyPathology/RapidReviewPanel/ReportsArchive/)

Search terms were developed and adapted to suit each database or website. Literature searches were run on 24/06/2014, 25/06/2014 and updated on 11/12/2015. See Appendix 1 for an example search run in the Medline database.

Exclusion criteria

Academic and grey literature was excluded from the review on the basis of the following exclusion criteria:

- Item was published before 2005
- Item was not in English
- Item does not concern ozone (off topic)
- Item is an opinion piece or non-systematic review

- Item does not present evidence compatible with the McDonald-Arduino evidentiary hierarchy[10]
- Study does not have a comparison in the form of standard cleaning methods
  N.B. If the study has used rigorous methodology and includes comparisons in the form of positive and negative controls or has been conducted as a before and after study it may be considered for inclusion. If these studies are included, then these limitations must be highlighted in the report.

Manufacturer information was not subject to the exclusion criteria outlined above, as it was sought primarily for information about the procedure for using the technology in question.

Screening

There was a two-stage process for screening the items returned from the literature searches. In the first stage, the title and abstract were screened against the exclusion criteria by the lead reviewer. Items that were not excluded at the screening stage progressed to the second screening stage. In the second stage of the screening process, the full text of remaining items was screened against the exclusion criteria by the lead reviewer. Items that were not excluded at the second screening stage were included in the review.

Critical appraisal

Critical appraisal of the studies included in this review and considered judgement of the evidence was carried out by the lead reviewer using SIGN methodology.[11] The McDonald-Arduino evidentiary hierarchy[10] was used as the framework for assessing the evidence, and was integrated into the critical appraisal process.

Results

The search found 218 articles. After the first stage of screening using the title and abstract this was reduced to 22 full text articles to read. After stage two screening there were 10 articles that fulfilled the inclusion criteria and were critically appraised for inclusion in this review. All of these were experimental or observational analytical studies classed as **level 3 evidence**. Of these, one took place in a hospital setting[12] and nine took place in laboratory settings.[13-20]

Three of the studies took place in the UK,[12;14;17] three took place in Canada,[13;15;16] two took place in USA[18;19] and two took place in China.[20;21] The studies included in this review used a range of methodologies to investigate the effectiveness of ozone with different study aims, measures of outcome and test organisms. There was only one hospital based study in this review[12] and it investigated the clinical effectiveness and cost effectiveness of ozone against vegetative cells and spores of *Clostridium difficile* in isolation rooms following occupation by *C. difficile* positive patients and compared these to other methods of disinfection. It would be reasonable to generalise from the results of this study,[12] however it may not be reasonable to generalise from the laboratory based studies[13-20] as a controlled laboratory based setting may not be an accurate representation of real-world conditions.

It is difficult to assess the potential impact of the use of ozone as there was one hospital based study that used environmental surface contamination as an outcome measures and it is not possible to quantify the link between environmental contamination and healthcare associated infection. The impact of the intervention in healthcare settings cannot be assessed based on the studies that took place in laboratories.

Research Questions

1. Is ozone currently in use in UK healthcare settings?

There is no mention of ozone in the NHSScotland National Cleaning Services Specification,[22] the NHSScotland National Infection Prevention and Control Manual,[4] the HPS Standard Infection Control Precautions Literature Review of Routine Cleaning in the Environment in the Hospital Setting,[23] the Association of Healthcare Cleaning Professionals (AHCP) Revised Healthcare Cleaning Manual,[24] or the National Patient Safety Agency (NPSA) Revised Healthcare Cleaning Manual.[25]

2. What is the actual or proposed mechanism of action of ozone?

Ozone is a form of oxygen. It is a colourless gas with a distinctive odour.[13;17;26] It is unstable and highly reactive with a very high oxidation potential which stems from its ability to extract electrons from other molecules and release one of its own oxygen atoms in the process.[13;17;26] Its rate of decomposition back to oxygen depends on temperature and humidity.[26]

The mechanism of action of ozone against **bacteria** is not completely defined, with some studies suggesting that ozone oxidises the bacterial cell wall and cytoplasmic membrane, leading to cell lysis and death.[13;27] Oxygen may also disrupt cellular activity by targeting membrane glycoproteins, glycolipids or amino acids and by modifying bases in nucleic acids.[13;16;17]

The mechanism by which ozone inactivates **viruses** is also not well understood. It is possible that viruses react directly with molecular ozone, but it is also possible that viruses react indirectly with the products formed when ozone decomposes. Ozone may also react with viral amino acids, proteins, protein functional groups, and nucleic acids as with bacterial inactivation. Viral inactivation may therefore be the result of ozone acting on protein structures of virus capsids or on viral nucleic acids.[21] Both lipid-enveloped and non-lipid-enveloped viruses are susceptible to damage by ozone,[13;17] and it is thought that this apparent indiscriminate antiviral and antibacterial activity is a reflection of multiple oxidation effects.[16;17]

3. What is the procedure for using ozone?

Ozone is generated in gas form from pure oxygen or air, and due to its **instability** and short half life of approximately 20 minutes it needs to be produced and used on site at its point of use.[14;17;19]

As ozone is a gas, it is able to **diffuse** and spread throughout, unlike non gaseous disinfectants which are restricted to the surfaces they are applied to. This allows it to better access organisms to inactivate. In addition, as ozone decomposes to oxygen naturally after a few hours, there is no need for a post-treatment clean up to take place. However, there are catalytic destruction units (scrubbers/quench gas) available to speed up the decomposition process if required.[19] The use of quench gas or scrubber can be used to quickly and efficiently **remove** any ozone gas from a room after decontamination has taken place, enabling the use of higher ozone concentrations.[14]

As ozone is a potentially harmful gas, the area being decontaminated needs to be **sealed** off to make it air tight, ensuring that all windows and doors are sealed shut and that any ventilation systems are turned off.[13]

Ozone gas has been used as a **deodorising** agent after fires in homes and offices to remove the odour of smoke. In these instances, the affected areas are **sealed** before setting up an ozone generator. After the ozone oxidises the organic residues, the ozone generator can be turned off and the sealed area can be opened up to allow fresh air to circulate.[19]

Hudson *et al.*[15] used a prototype ozone generator as a portable module containing multiple discharge units, a circulating fan and an efficient catalytic converter (scrubber) to reconvert ozone to oxygen at the end of the exposure period. A portable rapid humidifying device (RHD) was used to provide a burst of water vapour when required, as the effectiveness of ozone appears to increase with higher levels of humidity. Vents, windows, and doors were sealed with tape, and the unit was controlled remotely from outside the test room.[15] Sharma *et al.*[16] had a similar set up to Hudson *et al.*,[15] and used an ozone generator and rapid humidifying device to decontaminate the test room, followed by a scrubber to remove all the ozone gas. They also sealed all windows and vents and operated the ozone units remotely from outside the room.

4. What is the scientific evidence for effectiveness of ozone for decontamination of the healthcare environment?

As detailed in the protocol, the McDonald-Arduino evidentiary hierarchy was used as the framework for assessing the evidence, and has been integrated into the critical appraisal process.[28]

Level V – Demonstration of reduced microbial pathogen acquisition (colonisation or infection) by patients via *non-outbreak* surveillance testing and clinical incidence:

No evidence identified.

Level IV – Demonstration of reduced microbial pathogen acquisition (colonisation or infection) by patients via *outbreak* surveillance testing and clinical incidence:

No evidence identified.

Level III – Demonstration of in-use bioburden reduction that may be clinically relevant:

No evidence identified.

Level II – Demonstration of in-use bioburden reduction effectiveness:

Doan *et al.*[12] conducted a hospital based randomised prospective study to investigate and compare the clinical and cost effectiveness of novel disinfection methods for terminal disinfection of hospital isolation rooms contaminated with *C. difficile*. The study had 3 stages: pre-cleaning followed by contamination and then disinfection. Eight disinfection methods were tested, with each method randomly allocated to one of eight side rooms in a non functional hospital. The only methods discussed here are dry ozone at 25 ppm and 1,000 ppm chlorine-releasing agent. The clinical effectiveness was measured by the standardized median $\log_{10}$ reduction in colony count from the contamination phase to the disinfection phase. The chlorine releasing agent was able to reduce *C. difficile* spores and vegetative bacteria by 2.301 $\log_{10}$ compared to ozone which showed a reduction of 1.347 $\log_{10}$ and this difference was statistically significant ($p<0.05$)

Summary: ozone was *less effective* at reducing *C. difficile* spores and vegetative bacteria than a chlorine releasing agent used at a concentration of 1,000ppm in a hospital isolation room.

Level I – Laboratory demonstration of bioburden reduction efficacy:

Moat *et al.*[14] conducted an experimental study that investigated the effect of ozone gas for environmental and laboratory based decontamination using a laboratory and small test room and surfaces with bacteria associated with healthcare associated infection or contamination in clinical or food preparation areas. The organisms tested had different levels of sensitivity to ozone gas, with *E.coli* appearing to be the most sensitive organism both in the laboratory and test room. The most resistant vegetative organisms in the laboratory were *E. faecalis* and MRSA, and spores of *B. cereus* were more resistant to ozone gas than the vegetative cells of all other organisms tested. Overall, the use of ozone in the laboratory had a mean treatment effect equivalent to a $\log_{10}$ reduction factor of 2.92 for the vegetative species falling to 2.81 when *B. cereus* was included. In the small test room, all the test organisms

showed reduction factors of greater than 3 log_{10}. In addition, increasing the concentrations of ozone generally led to greater treatment effects. Spores of *C. difficile* were reduced by 3.20 log_{10} when ozone was used at 25 ppm for 75 min, supporting the suggestion that bacterial spores could be targeted using ozone gas.

Summary: ozone gas reduced surface organism contamination by 2.92 log_{10} in a laboratory setting. Ozone gas reduced surface organism contamination by more than 3 log_{10} in a small test room. Ozone gas reduced spores of *C. difficile* by 3.20 log_{10}.

Sharma and Hudson[16] conducted a laboratory based experimental study to test the effect of ozone gas against various Gram positive and Gram negative bacteria in a test chamber and a test room. The effects on wet and dry samples were also compared. These results demonstrate that ozone at 25 ppm and RH 90% is bactericidal with reductions of > 3 log_{10} in bacterial CFU/ml to strains of bacteria that commonly cause nosocomial infection, and the bactericidal effect was accomplished with an exposure time of 20 minutes. The results don't state whether there was a difference between the effect of ozone gas on Gram positive and Gram negative bacteria. However, it is useful to note that the inactivation of bacterial samples dried onto soft surfaces (e.g. fabrics, cotton, and filter paper) was similar to that found for samples dried onto plastic, indicating that ozone gas could be used to decontaminate curtains, linen, furniture, and walls in health care facilities.

Summary: ozone gas at 25 ppm and RH 90% tested in a laboratory and a test room was able to reduce the numbers of Gram positive and Gram negative bacteria tested by > 3 log_{10} in an exposure time of 20 minutes. Ozone was able to inactivate bacteria on soft surfaces suggesting it can be used to decontaminate soft furnishings in health care settings.

Hudson *et al.*[15] conducted an experimental study to evaluate the ability of ozone gas to inactivate norovirus and its animal surrogate feline calicivirus (FCV) in dried samples placed at various locations within a hotel room, a cruise liner cabin and an office. An ozone level of 25 ppm, at high relative humidity (in excess of 70%) was maintained for 20 minutes. The results demonstrated that ozone was able to inactivate FCV, and by extension norovirus, by a factor of more than 10^3. Virus samples dried onto soft furnishings were vulnerable to ozone, indicating that ozone should be able to inactivate viruses present on soft furnishings such as curtains, bedding etc. Virus samples in less accessible sites under tables or on fabric taped to windows were also inactivated by ozone.

Summary: ozone gas at 25 ppm and RH > 70% tested in a hotel room, cruise liner cabin and an office was able to inactivate FCV, and by extension norovirus, by a factor of more than 10^3. Viruses on soft furnishings and in less accessible sites were also inactivated.

Nicholas *et al.*[17] conducted a laboratory based experimental study investigating the effects of gaseous ozone and open air factor (OAF) on environmental samples of *Listeria monocytogenes*. The effects on surface attached bacteria and bacterial biofilms were also compared. A biofilm is an effective defence mechanism in protecting cells against environmental stresses including antimicrobial agents such as biocides. This study demonstrated that 10 ppm ozone gave a 1 log reduction in bacterial samples and 45 ppm ozone gave a 3 log reduction. Ozone was more effective against surface-attached bacteria than OAF at 45 ppm, however the authors recommend not using ozone at these concentrations on a large scale due to the associated toxicity to humans. OAF was significantly better than ozone at reducing the number of biofilm organisms.

Summary: ozone gas at 10 ppm gave a 1 log reduction and 45 ppm ozone gave a 3 log reduction against environmental samples of *Listeria monocytogenes* in a laboratory based study.

Zoutman *et al.*[13] conducted an experimental study in a laboratory and in a test room to evaluate the conditions for optimal effectiveness of ozone in combination with hydrogen peroxide vapour to disinfect surfaces and materials against common healthcare associated pathogens. For the purpose of this review only results from the use of ozone are considered. At a concentration of 50-180 ppm ozone with 80% RH and an exposure time of 90 minutes, inactivation rates of MRSA were negligible; however, at 500 ppm ozone, there was a >6 $\log_{10}$ reduction. This demonstrated the effects of increasing the concentration of ozone. A >7 $\log_{10}$ reduction in MRSA was achieved after 90 minutes of exposure to 80 ppm ozone and 0.2% hydrogen peroxide at 80% humidity, however under the same conditions but without the use of hydrogen peroxide, bacterial reduction was minimal, suggesting that hydrogen peroxide had a synergistic effect when used with ozone gas. This synergistic effect was noted for MRSA, VRE, *E. coli,* and *P. aeruginosa* and was able to achieve the same levels of inactivation but with a shorter exposure time of 30 minutes.

Summary: ozone gas at a concentration of 500ppm, 80%RH and an exposure time of 90 minutes reduced MRSA by >6 log_{10}. The addition of hydrogen peroxide vapour had a synergistic effect on bacterial inactivation and was able to either increase the levels of bacterial inactivation or reduce the exposure time required when compared to inactivation levels from using just ozone.

Baumann *et al.*[18] conducted a laboratory based experimental study to determine the efficacy of power ultrasound and ozone gas used individually and in conjunction for the removal of biofilms produced by *Listeria monocytogenes*. The results for power ultrasound used individually are not included in this review. There was a significant increase in efficacy of ozone as the concentration was increased, and at the maximum texted concentration of 1.0 ppm with an exposure time of 60 seconds there was a 4.2 log CFU/ml reduction in *L. monocytogenes*. The reduction of cell numbers due to ultrasound sonication was greater at both 30 and 60 seconds than any of the ozone concentrations or times used, and this difference was statistically significant. However, the simultaneous use of low ozone concentrations with sonication appeared to have additive effects on bacterial reduction. At 60 seconds of exposure, higher concentrations of ozone combined with power ultrasound led to synergistic effects that were not seen at 30 seconds of exposure. These results suggest that a combination of power ultrasound and ozone may be an effective treatment for *L. monocytogenes* biofilm removal from stainless steel surfaces.

Summary: ozone gas at a concentration of 1.0ppm with an exposure time of 60 seconds led to a 4.2 log CFU/ml reduction of *L. monocytogenes* biofilms. Used individually, power ultrasound was more effective than ozone, and the use of power ultrasound in conjunction with ozone led to synergistic effects.

Tseng *et al.*[21] conducted a laboratory based experimental study to investigate the effects of ozone concentration, contact time, different capsid (protein shell) architecture of viruses, and relative humidity (RH) of inactivating viruses on surfaces by ozone. Bacteriophages with different capsid protein structures were used because ozone is known to mainly damage viral capsid proteins. The results showed that the survival rates of all four bacteriophages decreased exponentially with increasing ozone dose, indicating that the inactivation of surface viruses depends on ozone dose.

The required ozone doses at 85% RH were one to two times lower than those found at 55% RH for the same 90% and 99% reduction of bacteriophages. In addition, the required ozone

contact time at 85% RH was 1.2 to 2.4 times shorter than at 55% RH for a 90% and 99% reduction in bacteriophages.

The authors also found that bacteriophages with simple capsid structures were more susceptible to ozone than bacteriophages with a more complex capsid structure. The authors also suggest that since the survival fraction of viruses declined exponentially with ozone dose increase, disinfection of surface viruses should be performed for longer exposure time and at lower ozone levels.

Summary:

- **At 55%RH for a 90%viral reduction:**
  - **ozone concentration of 0.6 ppm needed a contact time of 22-100minutes**
  - **ozone concentration of 0.9ppm needed a contact time of 17min**
  - **ozone concentration of 1.2ppm needed a contact time of 7 min**
  - **For 99%viral reduction at the ozone concentration of 0.9 and 1.2 ppm, the required contact time was twice as long as for 90%reduction of all four viruses**
- **At 85%RH for a 90%viral reduction, an ozone concentration of 0.6 ppm needed a contact time of 18-70 minutes**
- **For 99%viral reduction at 0.6ppm ozone, the bacteriophages needed a contact time of 36-126 minutes.**
- **Bacteriophages with simple capsid architecture were more susceptible to ozone than bacteriophages with a more complex capsid structure.**
- **Disinfection of surface viruses should be performed for longer exposure time and at lower ozone levels since the survival fraction of viruses declined exponentially with ozone dose increase.**

Aydogan *et al.*[19] conducted a laboratory based experimental study to assess the effectiveness of gaseous ozone against *Bacillus subtilis* spores, which share the same physiological characteristics as *Bacillus anthracis* spores that cause anthrax. Ozone at 3 mg/l (1500 ppm) produced a 3 log reduction within 4 hr at 90% RH and no additional benefit was observed in terms of increased inactivation rate at higher ozone concentrations. Higher humidity levels and 3 hours of prehydration of the spores increased the rate of inactivation. Spores were tested on a variety of surfaces and the type of surface had an impact on the rate of inactivation.

Summary: ozone gas at 1500 ppm produced a 3 log reduction of *Bacillus subtilis* spores within 4 hr at 90%RH. Increasing the ozone concentration showed no increase in the rate of inactivation. Higher humidity levels and 3 hours of prehydration of the spores increased the rate of inactivation. Spores were tested on a variety of surfaces and the type of surface had an impact on the rate of inactivation.

Liu *et al.*[20] conducted a laboratory based experimental study to investigate the effect of UV-C light and ozone individually and together on the conidia (fungal spores) of two strains of *Aspergillus niger*. Treatments were split into four groups based on whether UV irradiation was used and the presence or absence of ozone. Only the results of using ozone are included here. The strain with melanin was more resistant to the effects of ozone than the strain without melanin. Fungal spore survival was lower when UV-C and ozone were used together compared to exposure to UV-C, demonstrating that ozone induced more inactivation in the presence of UV-C. Ozone causes cell injury by inducing generation of cytotoxic free radicals, such as hydroxyl radicals. Melanin protects fungal spores (conidia) against UV damage and free radical scavenging from the use of ozone. Results showed that increasing the exposure time of ozone didn't reduce survival rates of the fungal spores. Based on this study, ozone appeared to be a less powerful agent for conidial inactivation than UV –C light, indicating that a combination of ozone and UV irradiation was necessary for optimal disinfection efficiency.

Summary: ozone was less effective at reducing fungal spores than UV-C light, but the combination of ozone and UV irradiation showed the greatest reduction in fungal spores.

5. Are there any safety considerations associated with using ozone in the healthcare setting?

Chlorine releasing agents are considered the cheapest and easiest environmental disinfection method. However, they have some limitations such as the release of irritating vapours and toxic gases which may affect the eyes and respiratory tracts of healthcare workers at high concentrations (e.g. 10,000 ppm available chlorine) and for this reason personal protective equipment (PPE) is recommended. Hypochlorite based products can be corrosive to various materials. In addition, the disinfection process must be performed manually-which can be time consuming and the quality of disinfection depends on the staff

performing disinfection. This has led to an interest in alternative methods of decontamination.[6;29;30]

Ozone is **toxic** to humans at high concentrations, with adverse health effects found at sites of initial contact, typically the respiratory tract (nose, throat and airways). The principal health effects tend to be irritation and damage to the small airways of the lungs, but sensitivity to the gas can vary considerably.[26] This means that **respiratory protection** (RPE) is required for anyone working with ozone and it also means that ozone gas cannot be used to decontaminate areas where people are present. In practice this means that it can only be used in rooms that can be sealed off and quarantined for the duration of treatment.[15;27;31] In addition to **sealing** rooms or areas being treated in order to effectively contain the gas, monitoring for leakage and assessing safe levels before allowing re-entry is also vital.[13;27;31] Using ozone in rooms that are sealed off for the duration of the treatment and ensuring that no one is allowed entry into the room being decontaminated is important as it means no one is exposed to the toxic effects of the gas.[16]

Otter *et al.* report **safe exposure levels** of <0.1 ppm ozone gas in the UK and USA of (compared with 1 ppm for hydrogen peroxide),[31] whereas Davies *et al.* recommend an exposure limit of 0.2 ppm for a contact time of 15 minutes. They state that some people may still experience respiratory symptoms at this concentration.[32] Moat *et al.* report that the Health and Safety Executive recommended exposure levels are 0.1 ppm for 8 hours or 0.2 ppm for 15 minutes[26] and that the time taken to reduce ozone concentrations to safe levels in the small test room they used depended on the concentration of ozone used but was less than 20 minutes in all the exposure runs.[14]

Ozone decomposes into oxygen, meaning that no toxic residues are left behind after it is used.[16;17] The additional use of quench gas or a scrubber at the end of the treatment cycle can rapidly reduce ozone concentrations to safe levels.[14]

6. Are there any practical or logistical considerations associated with using ozone in the healthcare setting?

As ozone is a potent oxidiser known to **corrode** metals,[27;31;32] more research is needed to investigate its effect on materials in the healthcare settin [31;32] as its primary use in the healthcare setting has so far been in the decontamination of laundry.[32] Aydogan *et al.* report that they inspected the carrier materials used in their ozone experiments after 4 hours and found no damage or differences before and after the use of ozone. The carrier materials they used were glass, carpet, paper, vinyl floor material and hardwood as these are commonly used in offices and households.[19]

Ozone gas is able to **penetrate** every part of a room including any sites or equipment that may be difficult to access using manual cleaning methods or may be missed during standard cleaning.[12;15] Hudson *et al.* report that in their tests virus samples deposited under the table or on fabric taped to a window were just as susceptible to inactivation by ozone gas as virus samples placed in more accessible sites.[15] Doan *et al.* suggest that hospital equipment such as drip stands or commodes could be collected in a room and effectively decontaminated in conjunction with the room.[12]

One key consideration associated with using ozone is the need for the room being decontaminated to remain **empty** before it is safe for people to enter, which in a healthcare setting means that there may be fewer rooms available for patients while ozone gas is being used. This also means that ozone decontamination cannot take place in areas continuously occupied by people.[12;15] However, ozone release can be controlled from outside the room being decontaminated.[16]

Doan *et al.* also highlight the need for a team of specially **trained personnel** to operate the specialised machinery involved in ozone decontamination and the need for all surfaces to be **manually cleaned prior to use** of ozone to remove any visible dirt and also to allow effective penetration of the gas.[12;31]

One major difficulty in decontaminating healthcare settings is that environmental surfaces are very quickly **recontaminated**. The CDC does not recommend fumigation for disinfection in routine patient care areas because of the issue of recontamination and the paucity of evidence that these methods are able to effectively reduce healthcare associated infections.[33]

7. What costs are associated with using ozone in the healthcare setting?

There are several factors to take into account when considering the costs associated with using ozone in the healthcare setting. It is important to consider whether the ozone generating and decontamination system will be owned and operated by the hospital itself (leading to high capital costs) or whether the ozone system can be outsourced as a cleaning service. Another option is leasing the system which reduces the high capital costs. Other upfront costs of such a system include training and recruitment costs as well as storage costs. Running costs include paying personnel, consumables, depreciation, maintenance and electricity.[31;32] The cost of environmental monitoring should also be factored into the total costs.[33]

Sharma *et al.* state that ozone can be generated cheaply,[16] however de Boer *et al.* report that using ozone disinfection cost € 2,000 in 2006[27] and a study by Doan *et al.* comparing

the cost effectiveness of eight disinfection methods found dry ozone to be the most expensive of the methods tested, with a cost per use of £ 116.29 and a monthly cost of £ 1,232.67 compared to a cost per use of £ 14.14 and a monthly cost of £ 149.65 for a chlorine releasing agent in 2012.[12]

Another cost associated with using ozone in the healthcare setting is the **time** taken to disinfect the room and the additional time for the ozone levels to return to safe levels for staff and patients to enter rooms again. This additional time could affect room turnover rates in a healthcare setting and potentially create a significant burden on the short supply of beds in hospitals.[33]

The cost of ozone decontamination appears to be substantially greater than the cost of standard terminal cleaning using housekeeping personnel, highlighting the need for additional studies to determine the cost effectiveness of ozone and to identify where and when it should be used. The need to pre-clean, the time required to empty and seal rooms or wards, the need to test for residual chemicals and the delays in reopening rooms should all be balanced against any additional microbial reduction ozone can offer.[15]

8. Has ozone been assessed by the Rapid Review Panel?

The Rapid Review Panel (RRP) is a panel of UK experts established by the Department of Health to review technologies with potential to help in the prevention and control of HAI.[34]

The RRP reviewed three ozone products to disinfect air and water in 2005 which are out with the remit for this review and therefore not included here. In 2014 the RRP reviewed an ozone product called “Lotus PRO stabilised aqueous ozone cleaning and sanitising system” produced by Green World Innovations Ltd and awarded it a level 4b recommendation, which means the following:

> *“Potentially useful product but insufficient evidence presented; further research and development with the product as intended to be used in the NHS is required to demonstrate improvements in infection prevention and control interventions to reduce healthcare associated infections before it is ready for in use evaluation within the NHS.”*

Discussion

There is evidence from one **hospital based** randomised prospective study[12] (**level 3 evidence**) that dry ozone was *less effective* at reducing *C. difficile* spores and vegetative bacteria than a chlorine releasing agent used at a concentration of 1,000 ppm. This difference was statistically significant ($p<0.05$).

There is evidence from a laboratory based experimental study[14] (**level 3 evidence**) that ozone gas reduced surface organism contamination by 2.92 $\log_{10}$ in a laboratory setting and by more than 3 $\log_{10}$ in a small test room. There is also evidence that ozone gas reduced spores of *C. difficile* by 3.20 $\log_{10}$.

There is evidence from a laboratory based experimental study[16] (**level 3 evidence**) that ozone gas at 25 ppm and 90% relative humidity was able to reduce the numbers of Gram positive and Gram negative bacteria tested by > 3 $\log_{10}$ in an exposure time of 20 minutes in a laboratory and a test room. There is also evidence that ozone inactivated bacteria on soft surfaces suggesting it can be used to decontaminate soft furnishings in health care settings.

There is evidence from an experimental study[15] (**level 3 evidence**) that ozone gas at 25 ppm and RH > 70% tested in a hotel room, cruise liner cabin and an office was able to inactivate feline calicivirus (FCV), and by extension norovirus, by a factor of more than 10^3. There was also evidence that viruses present on soft furnishings and in less accessible sites were inactivated by ozone.

There is evidence from a laboratory based experimental study[17] (**level 3 evidence**) that ozone gas at 10 ppm reduced environmental samples of *Listeria monocytogenes* by 1 $\log_{10}$ and 45 ppm ozone reduced environmental samples of *L. monocytogenes* by 3 $\log_{10}$.

There is evidence from a laboratory based experimental study[21] (**level 3 evidence**) that increasing ozone dose decreased the survival rate of bacteriophages exponentially. The evidence also demonstrated that increasing the relative humidity from 55% to 85% reduced the exposure time by 1.2-2.4 times and that the ozone doses required for viral reduction at 85% RH were one to two times lower than those found at 55% RH. Bacteriophages with more complex capsids (protein shell) were more resistant to the effects of ozone than bacteriophages with simple capsids.

There is evidence from a laboratory based experimental study[19] (**level 3 evidence**) that ozone gas at 1500 ppm produced a 3 $\log_{10}$ reduction of *Bacillus subtilis* spores within 4 hr at 90% RH. Increasing the ozone concentration showed no increase in the rate of inactivation.

Higher humidity levels and three hours of prehydration of the spores increased the rate of inactivation.

Three studies in this review used **ozone in conjunction with other cleaning methods**, and based on the results it would be useful to investigate these combinations of cleaning methods in future work. There is evidence from a laboratory based experimental study[13] (**level 3 evidence**) that ozone gas at a concentration of 500ppm, 80% RH and an exposure time of 90 minutes reduced MRSA by >6 log_{10}. Using ozone in conjunction with **hydrogen peroxide** vapour was able to either increase the levels of bacterial inactivation or reduce the exposure time required when compared to inactivation levels using ozone without hydrogen peroxide.

There is evidence from a laboratory based experimental study[18] (**level 3 evidence**) that ozone gas at a concentration of 1.0ppm with an exposure time of 60 seconds led to a 4.2 log_{10} CFU/ml reduction of *L. monocytogenes* biofilms. There was also evidence that ozone was *less effective* than power ultrasound and the use of **power ultrasound** in conjunction with ozone led to increased levels of inactivation.

There is evidence from a laboratory based experimental study[20] (**level 3 evidence**) that ozone was *less effective* at reducing fungal spores than UV-C light, but the combination of ozone and **UV irradiation** showed the greatest reduction in fungal spores.

Conclusion

There is evidence from one hospital based randomised prospective study[12] (level 3 evidence) that dry ozone was *less effective* at reducing *C. difficile* spores and vegetative bacteria than a chlorine releasing agent used at a concentration of 1,000ppm. This difference was statistically significant ($p<0.05$). However, this was the only one study in this review that compared the effectiveness of ozone with other cleaning methods, making it difficult to assess whether ozone is as effective/more effective/less effective than standard cleaning methods which has an impact on the recommendations that can be made based on this review of the literature.

The laboratory based studies all showed that ozone was effective against a range of bacteria (including MRSA), spores (including *C. difficile*) and viruses (including norovirus) tested at different concentrations with different exposure times.

Two laboratory based studies also stated that ozone was effective at decontaminating bacteria on soft surfaces suggesting it can be used to decontaminate soft furnishings in health care settings.[15;16]

Three laboratory based studies found that the effectiveness of ozone against bacteria, bacterial biofilms and fungal spores was increased when used in conjunction with hydrogen peroxide vapour,[13] power ultrasound [18] or UV-C light.[20]

The low level evidence on this topic – **all level 3** experimental or observational analytical studies – may reflect the fact that it is challenging to undertake well designed studies to explore the effectiveness of cleaning methodologies in the healthcare setting due to practical considerations. It may also reflect the fact that environmental decontamination in healthcare has not been considered a priority area for research.

The outcomes measured in the studies in this review only consider bioburden in-use or in a laboratory setting which is less useful than demonstrating reduced infections or clinical incidence. It would be useful if future studies included outcome measures that assessed the rates of healthcare associated infections present before and after the use of ozone in healthcare settings, keeping all other factors constant so any results seen would be the direct result of using ozone. Such studies would provide more relevant evidence to base recommendations on the use of ozone in these settings; however, they would also probably be more costly and difficult to conduct.

In order to determine the benefit of using ozone to decontaminate healthcare settings it is important to consider the severity of potential exposure to ozone and the probability of

exposure to the gas. The toxicity of ozone is also a key concern. Patients with pre-existing illness may be more severely affected by exposure to fumigants that have no obvious effects on healthy workers.[33]

The introduction of any novel decontamination technology should be used as part of a coordinated and structured infection control intervention and it is essential that recommendations by the local infection control team are followed. There may be circumstances where it is appropriate to use alternative decontamination technologies such as ozone gas to supplement but not replace standard cleaning and disinfection methods, such as fumigation of a ward following an outbreak.[35]

Implications for research

This review identified some gaps in the literature in relation to ozone. Although there were studies demonstrating a reduction in environmental contamination levels it would be useful to investigate the impact of ozone on colonisation and infection in patients in a healthcare setting. However studies such as this would be harder to conduct and this may explain the paucity of evidence in this field.[36]

Studies comparing the effectiveness of ozone gas compared to other cleaning methods, preferably conventional chlorine releasing agents and assessing clinical outcomes would be useful for evidence based decision making and reduce reliance on manufacturer claims.[31]

There are insufficient data on the cost of implementing these products to enable cost-benefit analyses to be undertaken to establish the feasibility of using ozone.

There is also currently a lack of consensus and guidance on the safe application protocols for the use of fumigants such as ozone in a healthcare setting. Methods to recognise and control hazards to protect staff, patients and visitors need to be developed, approved and implemented before the widespread use of fumigants such as ozone.[33]

Recommendations for practice

This review makes the following recommendations based on an assessment of the extant scientific literature on ozone.

If NHS boards use ozone products for decontamination of the healthcare environment and patient care equipment, the following must be considered:

- There is no evidence to support the use of ozone decontamination systems as an alternative to routine cleaning or disinfection within the healthcare environment. **(Good Practice Point)**

Appendix 1: Medline Search

Ovid MEDLINE(R) 1946 to present with daily update

AND

Ovid MEDLINE(R) In-process & other non-indexed citations

Search dates

24/06/2014, 25/06/2014 and updated on 11/12/2015

AND

1 (all "OR")	2 (all "OR")
Ozone/	Sterilization/ Decontamination/ Disinfection/ Housekeeping, Hospital/

Limits

English language

Publication Year 2005-current

Results: 178

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