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Review

Use of Hydrogen Peroxide Vapour for Microbiological Disinfection in Hospital Environments: A Review

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Abstract: Disinfection of nosocomial pathogens in hospitals is crucial to combat healthcare-acquired infections which can be acquired by patients, visitors, and health care workers. However, the presence of a wide range of pathogens and biofilms, combined with the indiscriminate use of antibiotics, present infection control teams in healthcare facilities with ongoing challenges in the selection of biocides and application methods. This necessitates the development of biocides and innovative disinfection methods that overcome the shortcomings of conventional methods. This comprehensive review finds the use of hydrogen peroxide vapour as a superior alternative to conventional methods. Motivated by these observations, herein we provide a comprehensive overview on the utilisation of hydrogen peroxide vapour as a superior high-level disinfection alternative in hospital settings. This review finds hydrogen peroxide vapour very close to an ideal disinfectant due to its proven efficacy against a wide range of microorganisms, safe to use, lack of toxicity concerns, and good material compatibility. The superiority of hydrogen peroxide vapour was recently demonstrated in the case of decontamination of N95/FFP2 masks for reuse to address the critical shortage caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) during the Covid-19 pandemic. Despite the significant number of studies demonstrating antimicrobial activity, there remains a need to critically understand the mechanism by performing studies which simultaneously measure the damage to all bacterial cell components, and a correlation of this damage with a reduction in viable cell count. This can lead to improvement in antimicrobial efficacy and foster the realisation of superior approaches.

Keywords: High Level Disinfection; Decontamination; Hospital Acquired Infections; Biocides; Hydrogen Peroxide Vapour; SARS-CoV-2; N95 respirators; FFP2 masks; Covid19.

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1. Introduction

Disinfection is described as a process that eliminates many or all pathogenic microorganisms on inanimate objects with the exception of bacterial endospores [1]. Disinfection is usually carried by chemical or physical means [1]. Among other settings, disinfection is of utmost importance in hospital environments due to pathogens living on hospital surfaces being the direct cause for hospital-acquired infections (HAIs). HAIs, also referred to as health care acquired infections [2], are infections that are not present or are not incubating at the time of hospital admission [3]; these can be acquired by patients, visitors and health care staff. HAIs also include infections acquired in healthcare settings outside hospitals; such settings include ambulatory care, care homes and family clinics [4]. These infections can appear 48 hours after hospital admission or within 30 days of receiving care [3]. HAIs have been one of the major causes of increases in deaths among patients receiving care in health care settings [2]. HAIs are a major risk not only to patient health but also

to occupational care staff and hospital visitors. The number of patients acquiring HAIs when in hospital care within the National Health Service in the United Kingdom is estimated to be 300,000 per annum [5]. Infections acquired in hospitals have an adverse effect on patient outcomes (increase in the duration of hospital stay and exposure to new infections) and increase the mortality rate and the costs associated with patient care [2, 6]. In the United States alone, HAIs are estimated to impact two million patients each year resulting in around 90,000 deaths per year and an estimated direct cost of US$28 to US$45 billion [6]. An exponential growth in the number of HAIs has been observed since the 1980s mainly due to the emergence of multi-drug resistant bacteria [7-9]. The indiscriminate use of antibiotics is a major contributing factor to this as it has led to some bacteria acquiring drug resistance. The increasing number of HAIs is a matter of serious concern as they can lead to severe illness, death and high health care costs.

Pathogens causing HAIs can spread through the touch of infected surfaces. Some studies have shown that pathogens can infect and survive on an inanimate surface from a period of a few hours to years [10, 11]. For example, *Escherichia coli (E. coli)* can survive on dry inanimate surfaces from 1.5 hours to 16 months, while *Clostridium difficile (C. difficile)* can survive on dry inanimate surfaces and hospital floors for a period of up to 5 months [10, 12]. *E. coli* spreads through ingestion of contaminated food, milk or water as well as through person-to-person transmission leading to blood and urinary tract infections [13]. *C. difficile* spreads through extensive surface contamination and causes diarrhoea and colitis [12, 14]. The ability of these clinically relevant nosocomial pathogens to survive on hospital surfaces has led to the need for disinfection or in simple terms to the need to kill these disease-causing micro-organisms. Indeed clinical studies have shown that poor environmental hygiene can lead to transmission of the pathogens [15]. Pathogens living on surfaces or on shared and non-shared equipment in hospitals can lead to hand contamination (upon contact) and to further transmission to equipment, patients and high-touch surfaces [16]. Pathogen transmission occurs via patients and healthcare staff by coming into contact with high-touch surfaces such as door handles, beds, taps and telephone receivers [16]. Further transmission can also occur by commonly shared clinical equipment like stethoscopes which come into contact with intact skin. In addition, during the procedure of urinary catheterization and gastrointestinal endoscopy, medical instruments come into contact with sterile or mucous tissues and thus both of these can lead to increasing infections. This is of particular concern because 25% of hospitalised patients in the United States need catheterization, while 10 million gastrointestinal endoscopies are carried out every year [17]. Records show that 30% of patients receiving urinary catheterization show systemic symptoms which relate to catheter-associated urinary tract infections caused by HAIs [18]. Urinary tract infections (UTIs) are the second most common type of HAIs in the United Kingdom accounting for 17.2 % of all HAIs, while pneumonia and other respiratory tract infections account for 22.8% of the total [5].

Critical evaluation of the available literature on HAIs highlights the need for effective and efficient disinfection methods and biocides followed by the correct use of disinfection techniques/methods. The literature contains a significant number of studies which document the efficacy of disinfectants and their antimicrobial action. However, not all biocides are effective against all types of pathogens. Furthermore, not all disinfection methods were seen to be effective [19-21]; in fact in one investigation, ready-to-use antibacterial wipes were observed to act as pathogen spreaders instead of eradicating the pathogens [22]. Hence both the selection of the biocide and the method of disinfection are important in determining the correct disinfection strategy. New and efficient approaches are therefore required particularly in the case of some multi-drug resistant organisms that are found on hospital surfaces in communities known as biofilms. Biofilms act as a reservoir of pathogens and their intrinsic properties make them resilient against disinfectants. Biofilms are multicellular communities held together by a self-produced extracellular matrix [23]. Such bacterial biofilms have been shown to be 1500 times more resistant to biocides than planktonic bacteria growing in liquid cultures [24]. Along these lines,

Vickery et al. [25] carried out a study on bacterial biofilms by disinfecting clinical samples using chlorine-based disinfectants and noticed the presence of biofilms for periods of 12 months even after routine cleaning. These observations refer to the consideration that it is not just the types of organisms present but the form they are in (e.g., in biofilms) that can impact the efficacy of a biocide. In addition to antimicrobial activity, infection control teams must assess a biocide for selection based on its safety to the user and the environment and consider the compatibility of the biocide with the treated materials. Among the large variety of biocides used and reported to date, hydrogen peroxide (H_2O_2) denotes an attractive option due to its demonstrated wide-range sterilant activity, its surface material compatibility and safety to the end user [26].

This review critically discusses the use of H_2O_2 as a biocide in hospital environments, its antimicrobial activity against clinically relevant pathogens, mechanism of action as well as its recent use for the decontamination of N95/FFP2 face masks for reuse to address the critical shortage caused due to the occurrence of severe acute respiratory syndrome coronavirus 2 (SARS -CoV-2).

2. Hydrogen peroxide vapour as a biocide

Hydrogen peroxide is used as a disinfectant/sterilant by being applied directly in the form of aqueous solution at concentrations ranging from 3 to 9 %(w/w) [27], formulated with different chemicals in water or gas, aerosolized or in vapour form [28]. The use of H_2O_2 as a biocide is found in multiple industries including the food and beverage sectors, agriculture, hospitals, the pharmaceuticals and cosmetics sector, the water supply industry and the public and commercial disinfection industry [4, 29]. Its use in the food and beverage sector in liquid form is targeted for disinfection and sterilisation of food contact surfaces that are used for milk and juice storage and for the preservation of water, milk and juices [29]. The use of H_2O_2 in the pharmaceutical and cosmetic industry takes place in liquid formulations at concentrations ranging from 3 to 9%(v/v) in products including wound applicants, oral disinfection in dentistry, contact lens disinfection and as a preservative in cosmetics [30-32]. Furthermore, higher concentrations of hydrogen peroxide solutions are used in the manufacturing of foam rubber, organic compounds, rocket fuel, and bleach for paper and textiles [32]. Examples of use in commercial sterilisation and in the water industry include industrial effluent treatment, algae control in water and wastewater deodorisation. The use of H_2O_2 in vapour form is found widely in the health care sector for disinfection and sterilisation [26]. In addition to its use against bacteria, hydrogen peroxide in vapour form is shown to be effective against a variety of organisms including certain types of hard-to-kill nematode worms and prions, thus finding use in animal husbandry [28, 33]. This widespread use of H_2O_2 in multiple industries is due to it being considered as an "ideal" biocide depending on how it is used [4]. An "ideal" biocide as defined by McDonnell [28] must be safe to use, easy to store, easy to apply and have a long-lasting effect, be environmentally-friendly and be chemically compatible with the surface it is applied on. An assessment of hydrogen peroxide vapour (HPV) against the attributes of an ideal biocide can be outlined as follows:

i) **Efficacy**: A significant number of *in-vitro* and *in-vivo* studies have demonstrated the efficiency of H_2O_2 both in liquid and vapour phases against organisms ranging from highly resistant bacterial endospores to enveloped viruses [19, 26, 34-37]. According to these studies, antimicrobial activities depend on the concentration of H_2O_2, exposure time and the method of application.

ii) **Safety**: Hydrogen peroxide is applied to the skin for wound disinfection and in acne products in liquid form at low concentrations of less than 3% w/w; this concentration level is considered very safe for use on human skin [31, 38]. However, with increase in concentration a decreased tissue compatibility has been reported [31, 38]. The safety of H_2O_2 is entirely dependent on how it is used. Owing to the absence (or to low toxicity effects), H_2O_2 is seen

as an excellent option for replacing more toxic chemicals like formaldehyde which is known to be carcinogenic and ethylene oxide which has high toxicity and carcinogenicity concerns [39-41]. A major advantage of modern hydrogen peroxide vapour systems is that they can be easily set up and operated remotely thus eliminating contact with the operator and thus reducing risk. The permissible exposure limit time weighted over 8 hours by OSHA (Occupational Safety and Health Administration) in United States is 1 ppm whereas the immediate danger to life or health is considered at 75 ppm [42].

iii) **Environmental Impact**: The environmental impact of hydrogen peroxide is entirely dependent on how it is used. HPV slowly decomposes into water and oxygen and because of this, it is considered safe for the environment [43]. As a result, no harmful residues are left on surfaces. The relatively unstable peroxide bond leads to its natural decomposition.

iv) **Ease of use**: Factors which impact the ease of use of H_2O_2 are its concentration and method of application. For example, hydrogen peroxide is highly effective when used in vapour form as it can easily reach crevices and other hard-to-reach areas. This can also be ideal for large area decontamination as multiple machines can be used at the same time. Modern, no-touch HPV systems reduce the number of labour hours when compared with traditional decontamination methods leading to a reduction in labour costs.

v) **Stability**: Hydrogen peroxide is stable in water and other formulations depend on the purity and its storage conditions. It is important that hydrogen peroxide is stored under conditions recommended by the manufacturer. Dissociation of hydrogen peroxide can take place if stored incorrectly. This will reduce the concentration of hydrogen peroxide in the solution and that will have an impact on the antimicrobial efficacy.

vi) **Compatibility with surface materials**: Hydrogen peroxide can be safe to surfaces depending on how it is used. Being an oxidising agent, it can oxidise certain metallic and plastic surfaces when in higher concentrations in liquid form [26]. However, these effects can be prevented when H_2O_2 is used in vapour form which is seen to be gentle to surfaces and to electrical equipment that are key parts of hospital environments. Boyce et al. [44] studied the impact of microcondensation HPV room decontamination on hospital physiological monitors over an 8-year period and observed that there was no increase in maintenance service calls; in fact a rather unexplained decrease in maintenance was apparent. Furthermore, a recent study by Sher and Mulder [45] on the use of vapour phase and aerosolized hydrogen peroxide for disinfection of dental surgery areas found no damage to any surface in the surgery. The effect of HPV on three metallic materials was characterised by Gale *et al.* [46] and no systematic effects were seen on the tensile strength or post HPV treated corrosion resistance of the alloys tested. The microstructural changes were seen confined to the areas adjacent to exposed surface and were considered to be relatively small [46].

Commercial disinfection systems commonly generate hydrogen peroxide vapour by controlled heating of 35% w/w aqueous solution [47]. The solution is continuously refilled onto the evaporator as the phase change from liquid to vapour takes place [48]. Commercial systems can use a hot plate to flash-evaporate from a 35% (w/w) hydrogen peroxide solution [49]. The resulting vapour is continuously fed to the room and some suggest that microcondensation can be formed at ~3μm thickness on the surfaces [47]. The hydrogen peroxide vapour can then be made to decompose into water vapour and oxygen upon catalysis by an active aeration system [50, 51]. A number of studies [52, 53] have shown that hydrogen peroxide in vapour form even at low concentrations is highly efficient when compared to liquid hydrogen peroxide. This has been attributed to the higher level of interaction with macromolecules (molecules considerably larger than an ordinary

molecule containing larger number of atoms) where greater oxidation has seen observed when the peroxide is in vapour form [53]. It is important to recognise that there are various commercially available hydrogen peroxide vapour systems and these can use significantly different methods [54]. Due to the fundamental differences in the delivery methods used by these processes, it is well-known that they yield noticeably different disinfection results [55, 56]. The term vaporised hydrogen peroxide® (VHP®) refers to a process that lowers the relative humidity (RH) of the room before adding peroxide to avoid reaching the dew point and condensation, and then regulates to a predetermined concentration by removing the vapour and adjusting the hydrogen peroxide injection rate to avoid reaching the dew point. In contrast, HPV is the term used for the process where vapour is purposefully delivered to reach the dew point and condensation by recirculating the peroxide and adding more vapour [47]. Whilst, VHP and HPV have previously been used indistinguishably during a study on decontamination of N95 respirators, [56] one must note the difference between the two. Additionally, the neutral term vapour phase hydrogen peroxide (VPHP) is employed to refer to both the HPV and VHP procedures as well as other similar processes. The ISO term for all these systems is vaporized hydrogen peroxide (VH_2O_2) [57].

3. Application of Hydrogen Peroxide Vapour against clinically relevant pathogens

The decontamination of health care environments using hydrogen peroxide vapour has been extensively studied because of its excellent antimicrobial efficacy. HPV systems have demonstrated antimicrobial efficacy ranging from highly-resistant bacterial endospores to the least resistant enveloped viruses as classified by Spaulding [58]. A significant number of *in-vitro* studies have demonstrated the microbial efficacy of HPV against frequently reported clinically relevant pathogens. HPV systems have achieved a greater than 6 $\log_{10}$ (greater than 99.9999 %) reduction of pathogens as validated by using *Geobacillus stearothermophilus* ATCC 7953 biological indicator spores [19, 35]. A greater than 6 $\log_{10}$ reduction has also been achieved against clinically relevant pathogens such as *C. difficle spores, methicillin-resistant Staphylococcus aureus (MRSA), Vancomycin-resistant enterococci (VRE), norovirus surrogates* and *Acinetobacter baumannii (A. baumannii)* [55, 59-64]. The use of hydrogen peroxide vapour has been effective in removing from environmental reservoirs of *C. difficle* [65], *MRSA* and *methicillin-susceptible Staphylococcus aureus (MSSA)* [19, 66], *multi-resistant gram-negative bacteria* [35, 67] and others [36].

These studies demonstrate both antimicrobial efficacy and repeatability which provides confidence in the use of HPV for decontamination. Furthermore, commercially available automated HPV disinfection systems can deliver the concentrations of fumigant required to achieve the criteria as stated by the EN17272 standard [68]. EN17272 is the European standard for determining the disinfectant activity of airborne room disinfection by automated processes and covers vegetative bacteria, mycobacteria, bacterial spores, yeasts, fungal spores, viruses and bacteriophages [69].

The use of HPV as a decontaminant has demonstrated significant potential in combating multi-drug resistant organisms (MDROs) in healthcare settings. HPV as a decontaminant provides an effective and practical method for reducing the environmental load of microorganisms resistant to several antibiotics, such as MRSA and VRE. Studies [70, 71] have shown that HPV may significantly decrease the amount of these bacteria on various hospital surfaces, lowering the risk of healthcare-associated illnesses (HAIs). Khandelwal *et al*.[70] conducted a study in a critical care setting and found that hybrid hydrogen peroxide fogging could lower bacterial counts on crucial surfaces, implying that this strategy is more effective than regular cleaning practices and ultraviolet light use in removing MDROs. Furthermore, a comprehensive review and meta-analysis by Marra *et al*.[71] confirmed the efficacy of no-touch disinfection technologies like HPV, revealing a statistically significant reduction in infections caused by particular MDROs such as C. difficile and VRE. These findings highlight the necessity of implementing modern disinfection technologies, such as HPV, into infection control regimens to improve patient safety and combat the spread of resistant pathogens.

HPV systems are known to have positive impact on the reduction of infections in clinical settings as demonstrated by three major studies [34, 72, 73]. A quasi-experimental study involving a 900-bed community hospital was conducted by Manian et al. [34]. Enhanced cleaning was performed using bleach followed by HPV disinfection of rooms vacated by patients with *C. difficile*-associated diarrhoea. The rate of *C. difficile*-associated diarrhoea infection dropped hospital-wide by 37% with the authors being able to demonstrate the safe use of HPV in a large hospital. Similar results were observed by Boyce *et al.* [74] who conducted a before-and-after intervention study in a hospital affected by an epidemic strain of *C. difficile*. HPV disinfection was reported to be efficacious in removing *C. difficile* from contaminated surfaces and the incidence of *C. difficile* associated infections post-HPV intervention was reduced to 0.88 cases/1000 patients from 1.89 cases /1000 patients pre-HPV intervention [74]. Furthermore, Passaretti *et al*. [73] demonstrated that the risk of a patient acquiring infection (with multidrug resistant organisms) was 64% less likely after the room has been sterilised with HPV compared to rooms cleaned using "traditional" processes. These studies demonstrated a reduction in the incidence of new infections and a lower risk to patients.

4. Effect on Bacteria

Some of the initial attention using HPV as a decontaminant targeted *E. coli*, a clinically relevant pathogen which is the most frequently reported pathogen and accounts for 17.5% of the total pathogens reported from over 5,626 health care facilities in the United States for the period 2015-2017 [75]. Back *et al*. [76] performed a study subjecting three strains of *E. coli* inoculated on lettuce to 10% HPV for 10 minutes. The authors [76] reported that the treatment led to reduction levels of 3.15 $\log_{10}$ CFU/g (colony forming unit per gram) for *E. Coli* O157:H7. In another study by Benga *et al*. [77], similar results were reported after treatment with HPV demonstrating complete disinfection of *E. coli* and other bacterial species (inoculated on bedding pieces housed in a mouse facility) in the presence of water and bovine serum albumin solutions (BSA).

The effectiveness of HPV was further demonstrated by a study by Otter *et al*. [64] who investigated the surface survival of commonly found spores and vegetative bacteria such as *Staphylococcus aureus (S. aureus)*, the second most commonly found nosocomial pathogen in health care settings [75]. While most vegetative bacteria and spores with inocula of 6 $\log_{10}$ CFU to 7 $\log_{10}$ CFU survived on surfaces for more than 5 weeks in a 100m^3 test room, they were inactivated within 90 min of exposure to HPV even in the presence of 0.3% bovine serum albumin that was used to simulate biological soiling. In another investigation, Lemmen *et al*. [78] evaluated the performance of HPV on the disinfection of organisms such as *MRSA, vancomycin resistant Enterococcus (VRE)* and *Acinetobacter baumannii (A. baumannii)* that were located on porous and non-porous surfaces using cotton and stainless steel as carriers in an operating room. The experiment was repeated three times and at each instance no pathogens were found on either porous or non-porous carriers after being subjected to automated HPV disinfection [78]. *Klebsiella pneumoniae (K. pneumoniae)*, the third most common nosocomial organism that is also found in health care settings [75] are known to cause urinary tract infections, pneumonia, septicemias and soft tissue infections [79] and can survive on inanimate surfaces from 2 hours to 30 months [10]. The work of Ali *et al*. [80] compared the efficacy between two different HPV systems using significantly different hydrogen peroxide concentrations in single isolation rooms using aerobically inoculated sterile broth of centrifuged *K. pneumoniae* suspended in 0.03% BSA (w/v) and 10% BSA (w/v) to simulate low and heavy soil loading. It was shown that enhanced cleaning with HPV reduced the risk of cross-contamination by killing the left-over surface contamination that was present after manual terminal cleaning. A study [77] has also been conducted on *Klebsiella oxytoca (K. Oxytoca)* which are known to cause HAIs in adults and have developed resistance to commonly used antibiotics [81]. The application of HPV by Benga *et al.*[77] on bacteria of laboratory animal origin showed that *K. oxytoca* and other bacterial species were readily disinfected upon being treated. Similar

disinfection results was observed when BSA was smeared on smooth surfaces to simulate soiling [77].

A study conducted by Watson *et al*. [82] on the effect of HPV on *Pseudomonas aeruginosa (P. aeruginosa)* used bacterial biofilms generated by a drip flow reactor. These biofilm samples were subjected to HPV treatment in an enclosure using a commercial vapour generator. The results after 100 minutes of exposure to HPV resulted in a reduction greater than 6 $\log_{10}$ in the enclosed room-based scenario. Microscopy results after the HPV treatment revealed a noticeable impact on the disruption of microcolony formation. To further compare HPV decontamination with conventional terminal cleaning, Otter *et al*. [83] compared hydrogen peroxide vapour decontamination with conventional terminal cleaning. Their work involved reservoirs of multidrug-resistant Gram-negative rods (MDR-GNR) such as *Enterobacter cloacae* in a 1389 m^3 intensive care unit (ICU) room using samples from different locations and putting 40 Tyvek-pouched 6 $\log_{10}$ *Geobacillus stearothermophilus* ATCC 7953 biological indicators along the periphery. The results suggested that HPV decontamination was more efficacious than conventional terminal cleaning. The removal of the environmental reservoirs of MDR-GNR could also have stopped the cycle of transmission of these organisms.

The results of these studies have demonstrated the effectiveness of HPV as a disinfectant and biocide against the most common bacteria. Impressive results were generally achieved quickly within about 100 minutes (dependent on room size) of exposure to HPV. This is interesting in that the treatment can potentially be applied in hospitals leading to a reduction in operational disturbance. An example of studies demonstrating the efficacy of hydrogen peroxide vapour against the clinically relevant bacteria commonly found in hospitals can be found in Table 1.

Table 1. Examples of studies demonstrating efficacy of hydrogen peroxide vapour against the clinically relevant bacteria commonly found in hospitals.

Microorganism	Associated Diseases/ Symptoms	HPV Studies
Escherichia coli	Blood and urinary tract infection [84].	[76, 77, 85]
Staphylococcus aureus	Blood, skin and respiratory tract infection, septicaemia and death [86].	[50], [21, 78, 87-92]
Klebsiella pneumoniae	Urinary tract infections, pneumonia, septicaemia and soft tissue infections [79].	[50], [85], [80], [82]
Klebsiella oxytoca	Urinary tract infection, Pneumonia [81]	[77]
Pseudomonas aeruginosa	Lung and urinary tract infection [13].	[77], [82], [93]
Enterococcus faecalis/faecium	Blood, skin and respiratory tract Infection [13].	[50], [87], [78], [82], [93], [94]
Enterobacter cloacae	Urinary tract infections, respiratory tract infection [95]	[83]

5. Effect on Fungi

Candida spp., a well-known fungus that can cause HAIs in the gastrointestinal tract, in the vagina and oral cavity [96], is known to survive from 1-120 days on dry inanimate surfaces [10]. Due to its clinical relevance, an *in-situ* study was carried out on samples purposefully collected from a *Candida auris (C. auris*) outbreak at the Royal Brompton Hospital in London with Infection control methods documented [97]. The authors [97] demonstrated a successful outcome through the use of a new infection control method by applying a high-strength chlorine-based agent followed by hydrogen peroxide vaporisation. An example of studies demonstrating the efficacy of hydrogen peroxide vapour against the clinically relevant fungi commonly found in hospitals can be found in Table 2.

Table 2. Examples of studies demonstrating efficacy of hydrogen peroxide vapour against the clinically relevant fungi commonly found in hospitals.

Microorganism	Associated Diseases/ Symptoms	HPV Studies
Candida spp.	Infections of the gastrointestinal tract, vagina and oral cavity [96].	[93], [98], [97]

6. Effect on Viruses

Viruses spread via respiratory droplets or by direct contact [99] and aerosolisation after sweeping and via fomites [10] and account for 90% of all respiratory diseases. According to an annual report published by Public Health England on the surveillance of influenza and other respiratory viruses in the UK for the winter of 2018-2019, 26408 deaths were attributed to influenza viruses. 84.2% of those deaths occurred in the age group of 65+ years. The transmission of influenza results in high impact on the health services in terms of an increase in the number of hospitalisations, ICU admissions and to a significantly higher mortality rate [99]. Some of the early attention explored the effect of HPV on common viruses including the *Influenza virus, Avian Influenza virus, Influenza A (H1N1)* and *Swine Influenza Virus (H3N2)* [37]. Heckert *et al*. [37] studied the effect of HPV on inactivation of equipment and inanimate materials potentially contaminated with a variety of animal and mammalian species viral agents belonging to the *Orthomyxoviridae, Reoviridae, Flaviviridae, Paramyxoviridae, Herpesviridae, Picornaviridae, Caliciviridae* and *Rhabdoviridae* virus families. The authors reported the high efficacy of HPV; for all the viruses tested under all conditions (except one) the virus titre was reduced to 0 embryo-lethal doses for all the avian viruses and to less than 10 tissue culture infective doses for the mammalian viruses [37]. Furthermore, the authors recommended the use of HPV for inactivation of potentially exotic animal disease virus contaminated objects from biocontainment level III laboratories. Similar effects were reported by Rudnick *et al.* [100] in as study where HPV was applied to influenza viruses which were deposited on stainless steel surface coupons and exposed to HPV at different concentrations ranging from 10-90 ppm. It was reported that 99% inactivation of influenza was achieved after only 2.5 minutes of exposure at the lowest studied concentration of 10 ppm. Even better results were achieved at higher HPV concentrations. This outcome was further supported by a different study by Goyal *et al.*[101] where SARS-COV-2 surrogates such as *feline calicivirus, human adenovirus type 1, transmissible gastroenteritis coronavirus of pigs and influenza viruses* were subjected to HPV exposure and no viable viruses were observed with the treatment achieving greater than 4 $\log_{10}$ reduction post-treatment. Such results provide confidence in the efficacy of the use of HPV for surface inactivation of viruses. An example of studies demonstrating the efficacy of hydrogen peroxide vapour against the clinically relevant viruses commonly found in hospitals can be found in Table 3.

Table 3. Examples of studies demonstrating efficacy of hydrogen peroxide vapour against the clinically relevant fungi commonly found in hospitals.

Microorganism	Associated Diseases/ Symptoms	HPV Studies
Influenza virus *Avian Influenza Virus* *Influenza A (H1N1)* *Swine Influenza Virus* *(H3N2)*	Influenza [96].	[37, 100, 101]

7. Mechanism of biocidal action.

Hydrogen peroxide in liquid and gaseous form has been shown to provide excellent antimicrobial activity against a broad spectrum of organisms. However, there is lack of knowledge of the mechanism of biocidal action which is still not fully understood; in spite of its demonstrated effectiveness in destroying infectious micro-organisms, there remains a need to critically understand the mechanism by performing studies which simultaneous measure the damage to all bacterial cell components, and a correlation of this damage with a reduction in viable cell count [53]. The main mechanism leading to decontamination through the use of hydrogen peroxide has been thought to be the deactivation of microorganisms by oxidation of macromolecules that form viral and cellular structure/function such as lipids, carbohydrates, protein and nucleic acids [26, 28]. However, a study by Linley *et al*. [102] on the mechanism of cytotoxicity and genotoxicity of H_2O_2, it was proposed that the mechanism is due to localised formation of short-lived hydroxyl radicals by intracellular reaction between Fe^{2+} ions and H_2O_2 (known as the Fenton reaction). Evidence for the Fenton reaction leading to the biocidal action of H_2O_2 on bacterial cells was sought by Repine et al. [103] who grew *S. aureus* bacteria in a nutrient broth with increased concentration of iron. This approach effectively increased the iron content in the *S. aureus* cells and this was associated with significant enhancement in the killing of the cells when they were exposed to H_2O_2. The death of cell walls in bacteria is dependent on the overall extent of peroxide-induced damage and on the effect on target cells which have the ability to repair DNA damage. This implies that bacteria strains that are exposed to H_2O_2 have reduced ability to repair DNA damage and are therefore more susceptible to be killed from exposure to H_2O_2 [103]. Since viruses have no repair mechanisms and McDonell [26] has suggested that excessive damage to viral nucleic acids should therefore be considered important in the overall virucidal effect. However, there is no evidence to support this. Indirect evidence of DNA damage in *E. coli* following exposure to H_2O_2 was provided by Imlay and Linn [104] who also proposed two kinetically distinguishable modes of killing of the bacteria. The killing of cells at lower H_2O_2 concentrations was referred to as mode-one and was reported to take place by DNA damage. Mode-one killing was observed to be maximal at concentrations between 1 to 2 mM of H_2O_2 [104]. Exposure to H_2O_2 was observed to lead to damage in a dose-dependent manner; this damage could undergo repair during a growth lag, but while cell growth occurred there was no evidence of septation. The failure to successfully complete the repair of cells would lead to mode-two killing which was evident at higher H_2O_2 concentrations. The authors [104] thought that mode-one killing was probably internal, while mode-two killing could be external. If that were indeed the case, mode-one killing would be expected to be diffusion-controlled. However, an earlier investigation by Schwartz et al. [105] had suggested otherwise.

Brandi *et al*. [106] noted a similar pattern of bimodal killing in their study on the effect of HPV on *E. coli*. suggesting that cell membrane damage leading to reduction in cell volume is the major component of mode-two killing, whereas no effect was seen in mode-one killing. This observation actually strengthens the proposal that the biocidal mechanism upon exposure to H_2O_2 is due to the Fenton's reaction by mode-two killing and is dependant on the presence of hydroxyl radicals, unlike mode-one. Furthermore, it is

important to note that oxidation reduction potential (ORP) of hydrogen peroxide in a solution plays a crucial role in the mechanism of antimicrobial action. The extent and rate of Fenton's reaction in a solution will be directly affected by the ORP of the solution. Higher ORP indicates a more oxidizing environment implying a greater tendency for H_2O_2 to donate electrons and form hydroxyl radicals hence a more efficient and potent antimicrobial action could be expected.

According to Finnegan *et al*. [53] vaporised hydrogen peroxide interacted differently against amino acids when compared to liquid hydrogen peroxide. These authors [53] observed that liquid hydrogen peroxide at different concentrations was able to oxidise amino acids like cysteine, methionine, lysine, histidine and glycine whereas vaporised hydrogen peroxide was unable to oxidise amino acids [53]. However, vapour phase hydrogen peroxide was able to degrade aldolase and BSA completely whereas no impact was observed when hydrogen peroxide was used in the liquid phase. The damage to the various macromolecular cell targets upon treatment of *E.coli* with liquid and vapour phase hydrogen peroxide studied by Linley [107] has been depicted in Figure 1. Similar results of vapour-phase hydrogen peroxide being able to degrade protein oxidatively in comparison to liquid-phase hydrogen peroxide were also reported by McDonnell [108] in his studies on the neutralisation of bacterial protein toxins. These studies serve to highlight the difference in efficacy between vapour and liquid phase hydrogen peroxide. The difficulty with most of these studies on understanding the mechanism of killing of bacteria through the use of hydrogen peroxide vapour is that entire cells are exposed to hydrogen peroxide, and this results in a variety of direct and indirect effects as the causes for cell death.

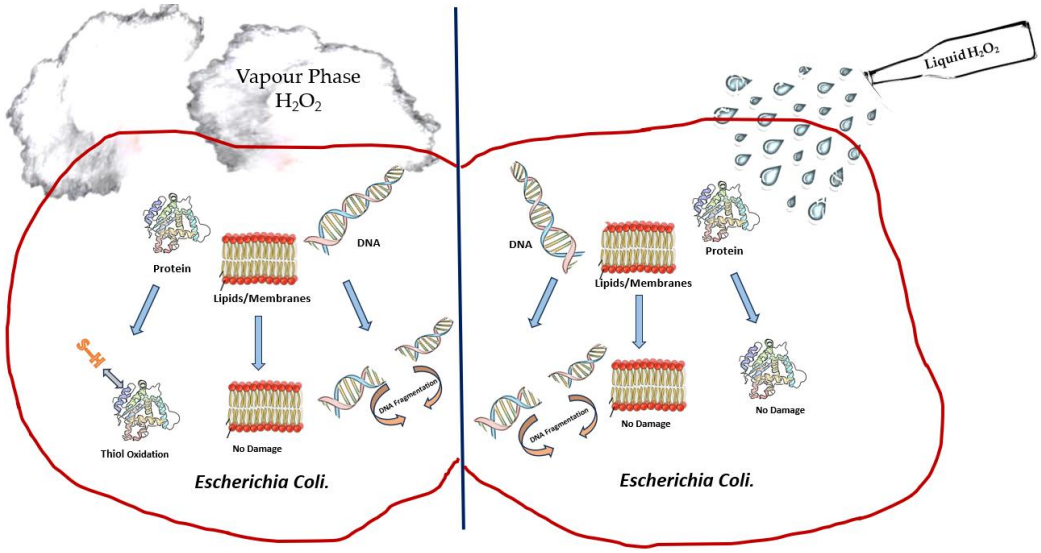

Figure 1. Depiction of the damage to cell components of *E. coli* upon treatment with with liquid phase hydrogen peroxide and vapour phase hydrogen peroxide.

There is still a need for further work to be carried out to improve the understanding of the exact killing mechanism of vapour phase hydrogen peroxide. Earlier studies from 1990s such as that of Klapes and Vesley [41] consider the application of vapour-phase hydrogen peroxide as a sterilant to be "clearly still in its infancy" due to the lack of understanding of the mechanism of action and the factors influencing it. Almost twenty years later, Hall *et al*. [109], in their study on using hydrogen peroxide vapour to deactivate *Mycobacterium tuberculosis*, stated that "the exact mechanism of action of HPV remains to be fully elucidated".

8. Hydrogen peroxide vapour as a biocide for reuse of N95/FFP2 face masks during the Covid 19 pandemic

The current pandemic caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) epi-centred in Hubei province in the People's Republic of China has been overwhelming the healthcare systems and negatively impacting world economies [110, 111]. An extreme shortage of critical N95/FFP2 masks used as Personal Protective Equipment (PPE) in health care settings occurred at the beginning of the Covid 19 pandemic. N95/FFP3 masks are arguably the most critical part of PPE for health care workers due to the aerosol transmission of SARS -CoV-2. Even though the supply of N95/FFP2 masks improved with time, new rapidly spreading variants of severe SARS -CoV-2 still pose a threat of critical shortage due to increase in the demand and to the fast depletion of existing supply lines. To address such shortage, decontamination of face masks for reuse was investigated as a viable option. As HPV is widely used for surface disinfection in hospital environments and is effective against SARS-Coronavirus surrogates on surfaces [101], it was therefore considered as a method of inactivation. Since the beginning of Covid 19, numerous *in-vitro* studies have been published on the use of HPV for inactivation of N95/FFP2 face masks [112-115]. Wigginton *et al*. [112] studied the inactivation of 3M-1860 N95 masks, their filtration efficiency and integrity using a HPV whole room decontamination system and other methods. A 1.5 $\log_{10}$ to greater than 4 $\log_{10}$ inactivation of the tested viruses was measured using the HPV system. The integrity of the face seal and the filtration efficiency was observed not to be affected after 5 cycles. Decontamination with other methods like the use of ethylene oxide raised toxicity concerns, while the hydrogen peroxide gas plasma decontamination method led to a decrease in the filtration efficiency [112]. Further studies by Oral *et al*. [113] who used a HPV system found high level inactivation of viruses and biological indicators after one cycle and found no evidence of detrimental effects on the fit for use and the filtration efficiency. The authors [112] are conducting further testing to determine the number of times a mask can be reprocessed. However, the HPV systems by Battelle Memorial Institute with emergency use authorisation from United States Food and Drug Administration [116] for decontamination of N95 masks at the onset of the Covid-19 pandemic were approved to be used for 20 cycles. Similar results were observed by a study by Kumar *et al*. [114] where four different N95 mask model types were used for decontamination with HPV and other methods. Full inactivation of SARS-CoV-2 or *Vesicular stomatitis* by VHP was seen with no loss in the function and structural integrity of the mask up to a minimum of ten cycles. In another investigation, Kenney *et al*. [115] further demonstrated the effectiveness of HPV whole-room systems for inactivation of N95 masks inoculated with bacteriophages. The authors found high virucidal activity post-HPV treatment on N95 masks with acceptable limits for filtration efficiency (>99%) up to three cycles. The filtration efficiency was seen to fall below 95% after 5 cycles and hence the authors [115] recommended that the reuse after inactivation should be limited to three cycles only.

A study by Schwartz *et al*. [117] described the process used and demonstrated VHP as an efficacious method for the decontamination of N95 /FFP2 face masks in terms of both its ability to kill pathogens and preserved the structural integrity and functionality of the masks. Perkins et al. [118] highlighted the low toxicity of the HPV processes. From the results of these investigations, HPV can be concluded to lead to very low concerns about toxicity due to its mechanism of action. In addition, it must be noted that the entire processing workflow from collection to post-processing as demonstrated by Grossman *et al.* [119] allowing health care workers to keep their own N95 respirators in a large academic metropolitan health care system was accomplished in less than 24 hours. Bailey *et al*. [68] validated the decontamination of a specialist transport system for patients with high consequence infectious diseases "EpiShuttle" (a patient transport system designed to fit into air ambulance) using HPV fumigation. The authors [68], upon decontamination with HPV, achieved a complete kill for all the commercially available used Bioquell HPV 6 Log_{10} *Geobacillus stearothermophilus* ATCC 12980 endospores, alongside organic liquid

suspensions and dried surface samples of MS2 bacteriophage. However, it was important to note that HPV fumigation can be less efficient if larger amounts of biological fluids are present on the surface due to its limited penetration. Hence, the follow up cleaning with surface disinfectant wiping was recommended in such cases. The advantages of using HPV fumigation in comparison with manual disinfection methods include better penetration into hard-to-reach areas, no risk of cross infection by exposure to operators and reduction in deviation from the manufacturer's instructions [68]. Such accomplishments suggest that the HPV system would be ideal to investigate for use in medical emergencies involving new bacteria and viruses.

9. Conclusion

The repeatability in the efficacy of HPV against a broad range of clinically relevant pathogens, its clinical impact, positive environmental impact and ease of use with no-touch disinfection methods has been demonstrated by multiple *in-situ* and *in-vitro* studies. The findings of this review highlight HPV to be very close to an ideal high-level disinfectant for use in health care environments due to its reliability against a broad spectrum of organisms, good material compatibility and no-negative environmental impact. This makes HPV a biocide of choice in health care environments not only for traditional surface disinfection of high-touch areas but also for the decontamination of face masks and ambulances which are important parts of the health system. The promising results of HPV being able to decontaminate N95 masks during the ongoing SARS-CoV-2 pandemic without affecting their structural integrity and filtration efficiency demonstrate the potential for use in emergency situations where supply chains for single use PPE products are severely depleted. The use of HPV for mask decontamination provides a viable alternative to address the disadvantages of limited penetration due to shadowing effects and direct exposure which can be difficult to achieve in complex geometries such as that of masks using Ultraviolet Light (UV) disinfection another highly studies no touch disinfection method. These benefits demonstrate the potential of HPV for further development as a biocide. However, the review has also identified that whilst significant efforts have been devoted to the understanding of the underlying mechanism of action, additional work is required which will aid in optimising the antimicrobial activity of HPV. The authors recommend that further research should focus on performing studies on organisms where simultaneous damage to all bacterial cell components is investigated and correlated.

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