Current and emergent strategies for disinfection of hospital environments

Ana C. Abreu1, Rafaela R. Tavares2, Anabela Borges1, Filipe Mergulhão1 and Manuel Simões1*

1LEPAE, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal; 2Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal

*Corresponding author. Tel: +351225081654; Fax: +351225081449; E-mail: mvs@fe.up.pt

A significant number of hospital-acquired infections occur due to inefficient disinfection of hospital surfaces, instruments and rooms. The emergence and wide spread of multiresistant forms of several microorganisms has led to a situation where few compounds are able to inhibit or kill the infectious agents. Several strategies to disinfect both clinical and medical equipment and the environment are available, often involving the use of antimicrobial chemicals. More recently, investigations into gas plasma, antimicrobial surfaces and vapour systems have gained interest as promising alternatives to conventional disinfectants. This review provides updated information on the current and emergent disinfection strategies for clinical environments.

Keywords: antimicrobial resistance, cross-contamination, disinfection, hospital-acquired infections

Introduction

The number of hospital-acquired infections (HAIs) has been growing exponentially worldwide since 1980, especially due to the emergence and wide spread of multidrug-resistant (MDR) bacteria. Multidrug resistance is an intrinsic and inevitable aspect of microbial survival and has been a major problem in the treatment of bacterial infections.1–6 The evolution of bacterial resistance is a consequence of the indiscriminate use of antibiotics and of the transmission of resistance within and between individuals.5–8 Also, the lack of new clinically relevant classes of antibiotics constitutes a major threat to public health.

HAIs are among the major causes of death and increased morbidity among hospitalized patients, with a minimum of 175,000 deaths every year in industrialized countries.5–12 Several investigations showed that >60% of worldwide HAIs have been linked to the attachment of different pathogens to medical implants and devices, such as venous and urinary catheters, arthroprostheses, fracture-fixation devices and heart valves.13–18 As a direct consequence, the replacement of implants, which entails significant costs and suffering for patients, often remains the only efficient therapy.19 Additionally, it has been demonstrated that the increased incidence of HAIs is related to cross-infections from patient to patient or hospital staff to patient and to the presence of pathogenic microorganisms that are selected and maintained within the hospital environment (including equipment).1,11,20–23 Poor infection control practices may facilitate patient-to-patient transmission of pathogens; for instance, in the accommodation of multiple patients in the same room. However, failure of the immune system due to illness and/or the use of immunosuppressors and other therapeutic drugs can increase the patient’s susceptibility to infections. Moreover, the use of antibiotics can inadvertently select antibiotic-resistant microorganisms.21

Since the environment serves as an important reservoir for infectious organisms, the control of hospital infections is a matter of great concern and a major challenge. The introduction of optimized disinfection products and processes is critical to control and prevent the spread of nosocomial infections, cross-resistance and persister cells.24 Within recent decades, requirements regarding the antimicrobial activity of disinfectants in the medical field have been defined in various European standards.25 Also, guidelines have been developed by the CDC, which recommend hospitals to thoroughly clean and disinfect environmental and medical equipment surfaces on a regular basis.26 However, there is a variety of products available on the market with moderate or even insufficient antimicrobial action.25 New products and technologies with ‘permanent’ antimicrobial activity without the risk of generating resistant microorganisms are needed. Hence, this manuscript provides information on the main pathogens causing HAI and the relevant in-use and emergent strategies for their control.

Main hospital pathogens

The increase in HAI is associated with the higher capacity of bacteria to resist and adapt to harsh environmental conditions, including the presence of antimicrobial agents. Deadly pathogens can survive for long periods of time on hospital surfaces, making the environment a continuous reservoir of infectious agents. The adhesion of pathogens to a surface followed by biofilm formation in <24 h is a critical microbiological problem for healthcare
Influence of clinical environment on HAI propagation

Pathogens can spread from patient to patient through contact with inanimate surfaces, including medical equipment and the immediate patient environment. There is clinical evidence suggesting an association between poor environmental hygiene and the transmission of microorganisms causing HAI. Cheng et al. found a strong correlation between environmental contamination by MRSA and hospital infection rates. Drees et al. demonstrated an increase in VRE infection risk for an occupant of a room where a patient with this infection was previously treated. It was also demonstrated that nosocomial transmission of norovirus, C. difficile and Acinetobacter spp. was correlated with contaminated environmental surfaces. In another study, a positive and measurable effect on the clinical environment was demonstrated with the introduction of one extra cleaner, which apparently protected the patients against MRSA infection. The potential for contaminated environmental surfaces to contribute to pathogen transmission depends on two important factors: the pathogens must survive on dry surfaces and the contamination has to occur on surfaces commonly touched by patients and healthcare staff at a sufficiently high level to enable transmission to patients. Moreover, pathogen transmission will also depend on the infectious dose and route of transmission, along with host susceptibility.

Disinfectant selection

Maintenance of a good hospital environment requires the implementation of adequate strategies. Such strategies are described in guidelines proposed by several committees, particularly the Healthcare Infection Control Practice Advisory Committee. For instance, in the case of surfaces with blood contamination, a disinfectant with activity against tuberculosis and hepatitis B virus (HBV)/HIV or a 5.25% bleach solution, at a final dilution of 1:10, can be used. These documents describe procedures to be implemented in healthcare facilities in order to achieve efficient cleaning and disinfection and also review the main uses of disinfectants.
### Table 1. Examples of clinically relevant nosocomial pathogens

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Mode of transmission</th>
<th>Length of survival</th>
<th>Disease/symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>extensive environmental contamination(^\text{13})</td>
<td>33 days on plastic laminate surface;(^\text{33}) 3 days to 5 months on dry inanimate surfaces(^\text{12})</td>
<td>pneumonia and bloodstream infection(^\text{33})</td>
</tr>
<tr>
<td><em>Bordetella pertussis</em></td>
<td>airborne droplet infection (person-to-person transmission)(^\text{109})</td>
<td>3 – 5 days on dry inanimate surfaces(^\text{12})</td>
<td>mild whooping cough syndrome(^\text{110})</td>
</tr>
<tr>
<td><em>Clostridium difficile</em></td>
<td>extensive environmental contamination(^\text{13})</td>
<td>5 months on dry inanimate surfaces and hospital floors(^\text{32,33})</td>
<td>diarrhoea and colitis(^\text{111})</td>
</tr>
<tr>
<td><em>Chlamydia pneumoniae</em></td>
<td>transmission from asymptomatic carriers(^\text{112})</td>
<td>(\leq 30) h on dry inanimate surfaces(^\text{32})</td>
<td>acute respiratory disease, bronchitis, sinusitis, pneumonia, otitis media and chronic obstructive pulmonary disease, asthma, reactive airway disease, Reiter’s syndrome and sarcoidosis(^\text{113})</td>
</tr>
<tr>
<td><em>Corynebacterium diphtheriae</em></td>
<td>mainly by infected droplet spread through contact with an infected person(^\text{115})</td>
<td>7 days to 6 months on dry inanimate surfaces(^\text{12})</td>
<td>diphtheria(^\text{115})</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>ingestion of contaminated food, water or milk; person-to-person transmission(^\text{21})</td>
<td>1.5 h to 16 months on dry inanimate surfaces(^\text{12})</td>
<td>blood and urinary tract infection(^\text{30})</td>
</tr>
<tr>
<td><em>Enterococcus spp., including VRE</em></td>
<td>nosocomial and person-to-person transmission; also by transmission on food products(^\text{116})</td>
<td>5 days to 4 months on dry inanimate surfaces(^\text{32}); (\leq 58) days on counter tops(^\text{13})</td>
<td>blood, skin and respiratory tract infections(^\text{10})</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>person-to-person transmission through contact with discharges or droplets from the nose or throat of an infected person(^\text{21})</td>
<td>12 days on dry inanimate surfaces(^\text{32})</td>
<td>acute and chronic respiratory tract infections, meningitis(^\text{117})</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>contact with contaminated surfaces and objects, medical equipment and blood products(^\text{118})</td>
<td>2 h to (&gt; 30) months on dry inanimate surfaces(^\text{12})</td>
<td>urinary tract infections, pneumonia, septicaemias and soft tissue infections(^\text{118})</td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em></td>
<td>sputum droplets (exhaled through a cough or sneeze) of a person with active disease(^\text{21})</td>
<td>1 day to 4 months on dry inanimate surfaces(^\text{12})</td>
<td>lung infection(^\text{10})</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>contamination from tap water and different medical devices(^\text{119})</td>
<td>6 h to 16 months on dry inanimate surface; 5 weeks on dry floor;(^\text{32}) 7 h on glass slides(^\text{33})</td>
<td>lung and urinary tract infection(^\text{30})</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>direct hand–to–hand transmission; with contaminated invasive medical devices, work surfaces, intravenous and topical solutions(^\text{120})</td>
<td>3 days—2 months on dry inanimate surfaces; 5 weeks on dry floor(^\text{12})</td>
<td>urinary tract infections and pneumonia(^\text{121})</td>
</tr>
<tr>
<td><em>Staphylococcus aureus, including MRSA</em></td>
<td>contact with the organism in a purulent lesion or on the hands; burn units extensively contaminated(^\text{21,33})</td>
<td>(S.) aureus can remain virulent for 10 days on dry surfaces(^\text{122}); MRSA can survive for 7 days to 9 weeks on dry inanimate surfaces and 2 days on plastic laminate surfaces(^\text{32,33})</td>
<td>blood, skin and respiratory tract infection, septicaemia and death(^\text{23})</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>person to person through close contact via respiratory droplets; illness among casual contacts and attendants is infrequent(^\text{123})</td>
<td>1 – 20 days on dry inanimate surfaces(^\text{12})</td>
<td>blood, lung and ear infections(^\text{30})</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>respiratory droplets and skin contact with impetigo lesions(^\text{124})</td>
<td>3 days to 6.5 months on dry inanimate surfaces(^\text{12})</td>
<td>rheumatic fever, sepsis, severe soft-tissue invasion and toxic-shock-like syndrome (TSLS)(^\text{125})</td>
</tr>
</tbody>
</table>

*Continued*
disinfectants as well as their mechanism of action and activity. Rutala and Weber\textsuperscript{58} developed a set of guidelines for hospital environment cleaning. Cleaning is related to the clearance of foreign material from a surface or equipment, allowing the removal of some organic material and microorganisms by detergents.\textsuperscript{11,33} However, this process does not kill bacteria, which, under favourable conditions, can redeposit elsewhere and form biofilms.\textsuperscript{31} Consequently, cleaning must always precede disinfection and sterilization in order to eliminate infectious microorganisms.\textsuperscript{59} A significant amount of microorganisms is destroyed by the disinfection process, which involves the use of chemical agents such as quaternary ammonium compounds, aldehydes, alcohols and halogens, or radiation and heat.\textsuperscript{11,31,33,60} The control of hospital infection must involve both disinfection and sterilization processes and sometimes the use of aerosols to clean the air.\textsuperscript{11,33}

### Table 1. Characterization of disinfectants according to their class\textsuperscript{11,59,65,68,83}

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Spectrum of action</th>
<th>Required for</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterilants</td>
<td>all microorganisms, including bacterial spores\textsuperscript{65}</td>
<td>critical instruments that penetrate tissue or present a high risk if non-sterile (e.g. implants, needles and other surgical instruments)</td>
<td>heat, steam, higher concentrations of hydrogen peroxide and peracetic acid, glutaraldehyde (in 6 – 10 h)</td>
</tr>
<tr>
<td>High-level disinfectants</td>
<td>almost all microorganisms, but not spores</td>
<td>semi-critical items that do not penetrate tissues or contact mucous membranes (except dental) (such as endoscopes, respiratory therapy equipment and diaphragms)</td>
<td>hydrogen peroxide, glutaraldehyde, formaldehyde, ortho-phthalaldehyde, peracetic acid</td>
</tr>
<tr>
<td>Intermediate-level disinfectants</td>
<td>almost all vegetative bacteria, fungi, tubercle bacilli and enveloped and lipid viruses</td>
<td>non-critical items that touch intact skin (e.g. thermometers and hydrotherapy tanks)</td>
<td>alcohols, hypochlorites, iodine and iodophor disinfectants</td>
</tr>
<tr>
<td>Low-level disinfectants</td>
<td>not efficient for most bacteria, tubercle bacilli, spores, fungi and viruses</td>
<td>non-critical items: items such as stethoscopes, beds, blood pressure cuffs and bedside tables</td>
<td>phenolics, quaternary ammonium compounds</td>
</tr>
</tbody>
</table>

Cleaning is related to the clearance of foreign material from a surface or equipment, allowing the removal of some organic material and microorganisms by detergents.\textsuperscript{11,33} However, this process does not kill bacteria, which, under favourable conditions, can redeposit elsewhere and form biofilms.\textsuperscript{31} Consequently, cleaning must always precede disinfection and sterilization in order to eliminate infectious microorganisms.\textsuperscript{59}
A biocide can target different locations on a cell as it may interact with the surface, the bacterial cell wall and the outer membrane, or it may penetrate the cell, where it can cause reversible or irreversible changes by interacting with nucleic acids, inhibiting enzymes and cell growth.24,60 When choosing a disinfectant, several factors must be taken into account, such as its efficiency, compliance with regulations, user acceptability, instrument compatibility, the types of surfaces and medical equipment, and the pathogenicity, infection rates and persistence of the microorganisms.61 A disinfectant must be safe, easy to use and effective against a wide range of pathogenic microorganisms and should not leave toxic residues.31,61 The efficiency of the disinfectant depends on several factors, mainly surface characteristics (hydrophobicity, charge and roughness), the amount of organic and inorganic matter, temperature, pH, the chemical structure of the biocidal agent and the type of infection and pathogen.11,24,31

The mode of action of the disinfectant and the route of entry into the cell (porin channels in the case of hydrophilic disinfectants and the hydrophobic path for hydrophobic disinfectants) also play significant roles.24 The risk of infection of the room/surface/equipment must also be considered in the choice of the disinfectant, as well as its concentration and exposure time.24,61 Hospital areas should be defined according to the risk of infection in order to establish and promote proper cleaning/disinfection. Areas where contamination is expected, e.g., laboratories, operating theatres, ambulatory surgical units, labour and delivery rooms, areas with blood or body fluid spills, and neonatal and burn units, must be cleaned and disinfected frequently (often several times per day). Areas of low risk, such as administration and waiting rooms, only require a daily cleaning.

According to its efficiency and ability to kill bacterial spores, an antimicrobial product can belong to one of four distinct groups: sterilants or high-, intermediate- and low-level disinfectants (Table 2). Given the increased resistance to antimicrobial agents displayed by bacteria upon biofilm formation, this hierarchy is only a rough guide.60,63,64 A disinfectant is almost never 100% effective due to the resistance of some bacteria to specific compounds and due to inefficient cleaning protocols.4 Once a disinfectant is removed, the surviving bacterial population can potentially regrow. Moreover, viable spores still attached to various materials can remain undetected by current sporidical tests, resulting in overestimation of the sporidical activity of sterilizing agents.65

The efficacy of diverse chemical disinfectants in inhibiting and killing some of the most clinically relevant bacteria, pathogenic fungi and yeasts has been evaluated by several authors. The activity of a disinfectant is generally analysed in terms of its MIC and MBC. However, the most suitable measure is the log$_{10}$ reduction of the number of cfu. The time taken to obtain a 5 log$_{10}$ reduction is also a reference to assess disinfection efficacy.61

**Traditional disinfection strategies**

The use of biocides has evolved over time. Alcohols such as ethanol have a long history of antiseptic use; around the 19th and 20th centuries phenolics and hypochlorites started to be employed and, later, quaternary ammonium compounds.66 More recently, the most common products have been chlorhexidine and silver salts, peroxyls, glutaraldehyde and ortho-phthalaldehyde.56,67

Alcohol disinfectants cause protein denaturation and are effective against vegetative bacteria, fungi and viruses, but have no effect on spores.63,68 Chlorine-releasing agents can oxidize membrane proteins and are very effective in removing biofilms from surfaces, requiring short exposure times for growth inhibition.57,61,68 However, these chemical agents are corrosive to metals and can be inactivated by the presence of organic matter.57,61,69 Moreover, in the last few years the use of chlorine has been associated with the formation of carcinogenic compounds and some pathogens have been shown to be resistant to chlorine.70 The aldehyde-based disinfectants disrupt proteins and nucleic acids by alkylation and have antimicrobial activity against spores, bacteria, viruses and fungi.61,64 Quaternary ammonium compounds and phenols solubilize the membrane and the cell wall.68 Hydrogen peroxide and peracetic acid promote protein denaturation, and are active against several groups of microorganisms and pathogens implicated in nosocomial infections.76,57,61,64 The mechanism of action of different disinfectant categories has been presented elsewhere in greater detail.54,71,72

Countless studies have been performed regarding the antimicrobial action and efficacy of different disinfectants. Rutala and Weber65 reviewed the use of inorganic hypochlorite (bleach) in healthcare facilities for disinfection of medical devices and environmental surfaces, and concluded that the many advantages of chlorine (e.g. fast microbicidal activity, cost-effectiveness and good track record) are likely to support its continued use in healthcare settings. Griffiths et al.73 evaluated the efficacy of several disinfectants (sodium dichloroisocyanurate, chlorine dioxide, 70% industrial methylated spirits, 2% alkaline glutaraldehyde, 10% succinidialdehyde and formaldehyde mixture, 0.35% peracetic acid and a peroxygen compound at 1% and 3%) against different strains of mycobacteria and showed that disinfectants based on sodium dichloroisocyanurate were more effective. Moreover, they concluded that clinical strains were more resistant to biocides than laboratory type strains.73 Other studies have shown that chlorhexidine at 0.5% concentration is the best choice, among several antiseptics and surface disinfectants (including betadine, hydrogen peroxide, sodium hypochlorite, alcohol and ultraviolet radiation), to kill clinical yeast isolates, either in planktonic cultures or in biofilm.74 Oie et al.75 analysed the effects of four different chemical treatments (0.2% alkyltriminoethol glycin, 0.01% or 0.1% sodium hypochlorite and 80% ethyl alcohol) on the disinfection of porous and smooth surfaces contaminated by S. aureus in a university hospital. The results demonstrated that the disinfection of porous surfaces was more difficult and none of the disinfectants was effective, highlighting the need for more frequent disinfection and the use of high-level disinfectants on these surfaces.73 More recently, Speight et al.76 evaluated the effect of 32 disinfectants on spores of *C. difficile* by a suspension test and only eight products gave $\geq 3$ log$_{10}$ reduction in viability within 1 min (to have a more realistic simulation of probable real-life exposures) under dirty conditions (3% BSA). These results underscore the importance of carefully selecting the disinfectant to eliminate spores of this particular microorganism.76 Kim et al.77 assessed the effectiveness of 13 disinfectants used in hospitals, day-care centres and food service kitchens (ZEP FS Amine Z, ZEP DZ-7, Lemonex, ZEP Micronex, T.B.Q., ZEP FS Formula 386 L, Perosan Liquid Sanitizer, LpH se, Vesphene I,se, Coverage Spray HB Plus, Coverage Spray TB, ZEP Kitchen Surface Sanitizer and ZEP FS RTU-D2) against *Enterobacter sakazakii* in suspension, dried on stainless steel and in biofilm, and
concluded that not all biocides used in hospitals can kill this microorganism. Also, the efficacy of disinfectants was higher for planktonic cells (reduction to undetectable levels). Bridier et al. studied the effects of three common disinfectants (peracetic acid, benzalkonium chloride and ortho-phthalaldehyde) on 77 bacterial strains and found that mycobacteria demonstrated a marked resistance to all the biocides. Benzalkonium chloride was inefficient even at very high concentrations. Also, resistance was dependent on the strain within the same species. Gutierrez-Martín et al. analysed the activity of 16 active compounds and 11 commercial disinfectants against Campylobacter jejuni by performing a suspension test in the presence and absence of serum. High levels of reduction (≥6 log10) for some disinfectants [chloramine-T, povidone iodine (1% available iodine), cetylpyridinium chloride, ethanol, isopropanol, chlorhexidine digluconate, formaldehyde, phenol and 10 of the 11 commercial formulations, especially those based on quaternary ammonium compounds] were obtained, regardless of the presence or absence of organic material. Table 3 compiles some of the available information regarding hospital disinfectants.

Disinfection methods employed in many intensive therapy units and other healthcare facilities include the use of antimicrobial wipes. Such products might be efficient in removing a microbial biofilm from a surface. The use of alcohol wipes was also demonstrated to generally decrease the mean daily bacterial load on toilets where wipes were made available. However, in most cases, antibacterial wipes used in hospitals were found to spread germs rather than eradicating them. Wipes can act as sources of cross-contamination when they are used on surfaces next to patients or on those commonly touched by staff and patients (e.g. tables, keypads). Moreover, many hospital personnel use a single wipe several times to clean and disinfect multiple surfaces before discarding it. Instead, they should use a wipe on a single discrete surface that requires only low-level disinfection.

A description of methods for hospital equipment disinfection with an analysis of the exposure time and the type of disinfectant and a summary of advantages and disadvantages of some chemical sterilants can be found elsewhere. Nevertheless, it has to be taken into account that some of these studies were performed with culture collection strains, whose responses to the presence of these disinfectants may differ from those of clinical isolates. Furthermore, some studies evaluated only a single strain of the species. Therefore, the results obtained may not always be representative of what occurs in clinical practice. Most of the presented investigations were performed on a laboratory scale, which may not truly reflect the complexity of a hospital scenario.

Alternatives to traditional disinfection

In this context, new disinfection strategies must be developed and their efficiencies must be evaluated in terms of their potential applications in hospital settings. This need for novel control methods has been emphasized by the increased resistance of bacterial species to some disinfectants, mainly as a consequence of biofilm formation.

One of these alternative strategies is steam vapour disinfection, which has been evaluated by different groups. Tanner demonstrated a reduction of 7 log10 in MRSA, VRE and P. aeruginosa to undetectable values within 5 s of application of a steam vapour system. Sexton et al. applied a steam vapour system to combat MRSA, total coliform bacteria and C. difficile cells, attaining a 90% reduction in bacterial levels. Hydrogen peroxide vapour (HPV) is also used for decontamination of clinical surfaces and equipment. Otter and French found that vegetative bacteria and spores (6–7 log10 cfu) survived on surfaces for >5 weeks, but were inactivated within 90 min of exposure to HPV in a 100 m3 test room. Initial inocula of M. tuberculosis (~3 log10) and Geobacillus stearothermophilus (6 log10) were exposed to HPV at 10 locations during room experiments and both microorganisms were inactivated in all locations within 90 min of HPV exposure. Falagas et al. reviewed other studies of disinfection with HPV against MRSA, C. difficile and other pathogens in several sampled hospital locations (including surgical wards, ward side rooms, single isolation rooms, multiple-bed ward bays and bathrooms); complete or almost complete disinfection of the sampled hospital sites was achieved with airborne hydrogen peroxide. HPV appears to have low toxicity and has good compatibility with most inanimate materials.

UV light exposure has also been applied for room decontamination. It has been used in air-handling systems and upper-room air-purifying systems to destroy microorganisms and can also inactive microorganisms on surfaces. UV-C was demonstrated to be effective in eliminating vegetative bacteria on contaminated surfaces (both in the line of sight and behind objects) within 15 min and in eliminating C. difficile spores within 50 min. In other study, a mobile UV-C light unit significantly reduced aerobic colony counts and spores of C. difficile on contaminated surfaces in patient rooms. It was also demonstrated that ceiling-mounted UV germicidal irradiation lamps were effective in reducing the viability of both Bacillus cereus and Bacillus anthracis vegetative cells and spores after a minimum exposure time of 1 h at an intensity as low as 8 μW/cm².

Both the HPV and UV light methods have demonstrated good results. However, they require the removal of patients and healthcare personnel from the room, have a high acquisition cost and increase room turnover time. Moreover, they do not replace standard cleaning and disinfection.

Bacterial adhesion to stainless steel surfaces (the most commonly used material in industry and hospitals) is one of the major reasons for cross-contamination in many scenarios. Thus, more recently, different strategies for the production of antimicrobial surfaces with the purpose of reducing HAIs have been extensively investigated and developed. Researchers have focused on the development of surfaces with antimicrobial coatings in order to inhibit biofilm formation by either killing the bacteria or preventing their adhesion. Bacterial growth control can then occur by three different modes of action: biocide leaching, which involves the release of a cytotoxic compound to kill attached microorganisms; adhesion prevention, which uses a super-hydrophobic
<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Microorganism</th>
<th>Method/test</th>
<th>Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nu-Cidex® (0.35% peracetic acid)</td>
<td>Mycobacterium (5 strains)</td>
<td>suspension and surface test</td>
<td>complete inactivation after 5 min of exposure&lt;sup&gt;129&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nu-Cidex® (0.35% peracetic acid)</td>
<td>Bacillus subtilis spores</td>
<td>suspension test</td>
<td>&gt;5 log&lt;sub&gt;10&lt;/sub&gt; reduction in 5 min with 10% serum&lt;sup&gt;130&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cidex Long-Life® (2% glutaraldehyde)</td>
<td>B. subtilis spores</td>
<td>suspension test</td>
<td>&gt;5 log&lt;sub&gt;10&lt;/sub&gt; reduction in 2 h with 4% blood&lt;sup&gt;130&lt;/sup&gt;</td>
</tr>
<tr>
<td>Titan Sanitizer® (sodium dichloroisocyanurate with Cl content of 2.2%)</td>
<td>B. subtilis spores</td>
<td>suspension test</td>
<td>&gt;5 log&lt;sub&gt;10&lt;/sub&gt; reduction (disinfectant at 5% in 3 h); no reduction in the presence of 2% blood&lt;sup&gt;130&lt;/sup&gt;</td>
</tr>
<tr>
<td>Presept® disinfectant granules (sodium dichloroisocyanurate with Cl content of around 30%)</td>
<td>B. subtilis spores</td>
<td>suspension test</td>
<td>&gt;5 log&lt;sub&gt;10&lt;/sub&gt; reduction (disinfectant at 1% in 1 h in the absence of blood and in 2 h in the presence of 2% blood)&lt;sup&gt;130&lt;/sup&gt;</td>
</tr>
<tr>
<td>Haz-Tab® disinfectant granules (sodium dichloroisocyanurate with Cl content of around 60%)</td>
<td>B. subtilis spores</td>
<td>suspension test</td>
<td>5 log&lt;sub&gt;10&lt;/sub&gt; reduction (disinfectant at 1% in 2 – 3 h in the absence of blood, but little kill in 3 h in the presence of 2% blood)&lt;sup&gt;130&lt;/sup&gt;</td>
</tr>
<tr>
<td>Virkon® (peroxygen)</td>
<td>B. subtilis spores</td>
<td>suspension test</td>
<td>4 log&lt;sub&gt;10&lt;/sub&gt; reduction in 10 h and 2 log&lt;sub&gt;10&lt;/sub&gt; reduction in 10 h in the presence of 2% blood&lt;sup&gt;130&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sporicidin® (2% glutaraldehyde, 7.05% phenol, 1.2% sodium phenate)</td>
<td>B. subtilis spores</td>
<td>suspension test</td>
<td>&gt;5 log&lt;sub&gt;10&lt;/sub&gt; reduction in 2 min with 5% horse serum (except for C. difficile spores since the disinfectant activity diminished in the presence of organic load)&lt;sup&gt;131&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sterilox® (9:1 v/v) (hypochlorous acid at a concentration of 144 mg/L and free chlorine radicals)</td>
<td>C. difficile spores, Helicobacter pylori, VRE, C. albicans and 4 Mycobacterium species</td>
<td>suspension test</td>
<td>complete inactivation except for Mycobacterium and spores; resistance to inactivation after repeated inoculation; did not corrode clean instruments; when organic matter was added, it cleaned without corrosion&lt;sup&gt;135&lt;/sup&gt;</td>
</tr>
<tr>
<td>10% Povidone iodine</td>
<td>10 MRSA, 10 methicillin-susceptible S. aureus (MSSA), 9 VRE and 10 vancomycin-susceptible Enterococcus faecalis</td>
<td>European surface test method&lt;sup&gt;132&lt;/sup&gt;</td>
<td>3.14, 3.49, 3.47 and 3.78 log&lt;sub&gt;10&lt;/sub&gt; reduction, after 1.5 min for VRE, VSE, MRSA and MSSA, respectively&lt;sup&gt;133&lt;/sup&gt;</td>
</tr>
<tr>
<td>Perasafe® (peroxygen system equivalent to peracetic acid at 0.26%)</td>
<td>C. difficile and Bacillus atrophaeus spores</td>
<td>surface test on stainless steel and polyvinyl chloride floor covering</td>
<td>5.5 – 6 log&lt;sub&gt;10&lt;/sub&gt; reduction in 10 min&lt;sup&gt;134&lt;/sup&gt;</td>
</tr>
<tr>
<td>Perasafe®</td>
<td>S. aureus ATCC 25923, MRSA, MSSA, E. faecalis, Enterobacter cloacae, K. pneumoniae, P. aeruginosa, Acinetobacter anitratus, C. albicans, Mycobacterium fortuitum ATCC 609 and B. subtilis (spore strips).</td>
<td>suspension, surface, sporicidal, endoscope model test, capacity and corrosion tests</td>
<td>complete inactivation except for Mycobacterium and spores; resistance to inactivation after repeated inoculation; did not corrode clean instruments; when organic matter was added, it cleaned without corrosion&lt;sup&gt;135&lt;/sup&gt;</td>
</tr>
<tr>
<td>2% Glutaraldehyde</td>
<td>S. aureus ATCC 25923, MRSA, MSSA, E. faecalis, E. cloacae, K. pneumoniae, P. aeruginosa, A. anitratus, C. albicans, M. fortuitum ATCC 609 and B. subtilis (spore strips)</td>
<td>suspension, surface, sporicidal, endoscope model test, capacity and corrosion tests</td>
<td>complete inactivation except for Mycobacterium and spores; resistance to inactivation after repeated inoculation; did not corrode clean instruments; when organic matter was added it fixed the matter to the scalpel, causing corrosion within 2 h&lt;sup&gt;135&lt;/sup&gt;</td>
</tr>
<tr>
<td>Disinfectant</td>
<td>Microorganism</td>
<td>Method/test</td>
<td>Efficiency</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-----------------------------------------</td>
<td>----------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Sodium dichloroisocyanurate</td>
<td><em>C. difficile</em> and <em>B. atrophaeus</em> spores</td>
<td>surface test on stainless steel and polyvinyl chloride floor covering</td>
<td>0.7 – 1.5 log_{10} reduction in 10 min(^{134})</td>
</tr>
<tr>
<td>Monopercitric acid (peroxy-acid-based disinfectant)</td>
<td><em>Clostridium</em> spores</td>
<td>suspension test</td>
<td>0.5% disinfectant is sporicidal within 5 min(^{136})</td>
</tr>
<tr>
<td>0.2% Alkyl-diaminoethylglycine and 80% (v/v) ethyl alcohol</td>
<td><em>S. aureus</em>, MRSA and MSSA</td>
<td>wiping and membrane filtration technique</td>
<td>reduction of bacteria to an undetectable level(^{75})</td>
</tr>
<tr>
<td>0.01% Sodium hypochlorite and 0.1% sodium hypochlorite</td>
<td><em>S. aureus</em>, MRSA and MSSA</td>
<td>wiping and membrane filtration technique</td>
<td>reduction of bacteria to a minimal detectable level(^{75})</td>
</tr>
<tr>
<td>Ortho-phthalaldehyde</td>
<td><em>Pseudomonas fluorescens</em> (planktonic)</td>
<td>respirometry; adenosine triphosphate release; outer membrane protein expression and bacterial colour changes</td>
<td>complete inactivation (MBC 0.5 mM)(^{137})</td>
</tr>
<tr>
<td>Ortho-phthalaldehyde</td>
<td><em>P. fluorescens</em> (planktonic)</td>
<td>respiratory activity, membrane permeabilization and integrity, and physico-chemical characterization</td>
<td></td>
</tr>
<tr>
<td>Ortho-phthalaldehyde</td>
<td><em>S. aureus</em>, <em>E. coli</em>, <em>P. aeruginosa</em></td>
<td>suspension and carrier tests</td>
<td>≥5 log reduction in viability within 1 min of exposure(^{138})</td>
</tr>
<tr>
<td>Ortho-phthalaldehyde</td>
<td>bacteria found in 100 endoscopes</td>
<td>surface test</td>
<td>5 log&lt;sub&gt;10&lt;/sub&gt; reduction of bacteria with an exposure time of 5 min(^{139})</td>
</tr>
<tr>
<td>Glutaraldehyde</td>
<td><em>P. fluorescens</em> (planktonic)</td>
<td>respirometry; adenosine triphosphate release; outer membrane protein expression and bacterial colour changes</td>
<td>no bacterial inactivation was detected(^{137})</td>
</tr>
<tr>
<td>Cetyltrimethyl ammonium bromide</td>
<td><em>P. fluorescens</em> (planktonic)</td>
<td>respirometry; adenosine triphosphate release; outer membrane protein expression and colony colour changes</td>
<td>complete inactivation (MBC 5 mM)(^{137})</td>
</tr>
<tr>
<td>Polyhexamethylene guanidine hydrochloride</td>
<td><em>S. aureus</em>, <em>P. aeruginosa</em>, <em>Salmonella choleraesuis</em>, MRSA and <em>E. coli</em></td>
<td>phenol coefficient (PC)&lt;sup&gt;3&lt;/sup&gt; value (for <em>S. aureus</em>, <em>P. aeruginosa</em> and <em>S. choleraesuis</em>); MIC and MBC (for MRSA and <em>E. coli</em>)</td>
<td>PC values for <em>S. aureus</em>, <em>S. choleraesuis</em> and <em>P. aeruginosa</em> were 7.5, 6.1 and 5, respectively; the MIC value for MRSA and <em>E. coli</em> was 0.04% and 0.005% (w/v), respectively, in 1.5 min(^{140})</td>
</tr>
<tr>
<td>Allrent&lt;sup&gt;®&lt;/sup&gt; (2-propanol: 1%–5% weight; tensides 1%–5% weight; 60%–100% weight water)</td>
<td><em>B. subtilis</em>, <em>S. aureus</em>, <em>C. albicans</em></td>
<td>colony counting in agar plates and bioluminescence detection of adenosine triphosphate</td>
<td>final residual cfu percentage of 35.7%(^{151})</td>
</tr>
<tr>
<td>Appeartex&lt;sup&gt;®&lt;/sup&gt; (active polymer A-200, polyhexamethylene biguanide and a surfactant solution)</td>
<td><em>S. aureus</em>, <em>Enterococcus hirae</em></td>
<td>contact agar plates; direct agar inoculation using swabs; swab rinse technique (on a laboratory scale and in a hospital ward)</td>
<td>reduction in magnitude of 10–10&lt;sup&gt;3&lt;/sup&gt;(^{142})</td>
</tr>
</tbody>
</table>
covering in order to prevent microbial adhesion; and contact killing, which consists of disruption of cell membranes in contact with the surface.13 Many of these techniques consist of surface modification by introduction of antimicrobial substances such as antibiotics, metals (such as copper and silver) and antiseptics.93–98 These surfaces can be obtained by different methods, including adsorption, ligand–receptor pairing and covalent binding.96 Surfaces that release antimicrobial products can work quite efficiently, although they eventually become exhausted and the diffusible antimicrobials pose the potential problem of inducing microbial resistance, since the surfaces are continually releasing active compounds to the environment for a long period of time.55,99 Alternatively, the design of surfaces that kill microbes on contact and do not release biocides may solve this problem. The covering of surfaces with transient metals, such as copper and silver as mentioned above, is a well-established strategy. Moreover, surfaces that catalytically produce biocides using externally applied chemical, electrical or optical energy are an interesting alternative.99

An example of biocide leaching is the use of Surfacine, which incorporates an antimicrobial compound (silver iodide) in a surface-immobilized coating (a modified polyhexamethylenebiguanide).83 This compound interacts with the microorganism by electrostatic attraction, penetrates the cell and finally kills it.83 Surfaces treated with silver iodide resulted in excellent elimination of VRE at challenge levels of 100 cfu/in² for at least 13 days.11 Furthermore, these surfaces retained the antimicrobial effect even after cleaning.11 Photocatalytic oxidation on surfaces coated with titanium dioxide (TiO₂) is also a possible alternative. In the presence of water and oxygen, highly reactive OH⁻ radicals generated by TiO₂ and mild UV-A are able to destroy bacteria, thus reducing bacterial contamination.100 Another example of a product that releases an organic antimicrobial is Microban®, which contains triclosan [5-chloro-2-(2,4-dichlorophenoxy)-phenol] as the antimicrobial agent, making the surface resistant to the growth of microorganisms.35 This antimicrobial technology can be found in hundreds of consumer, industrial and medical products around the world.

Covalent immobilization of bioactive compounds onto functionalized polymer surfaces has also seen rapid growth in the past decade in such industries as biomedical, textiles, microelectronics, bioprocessing and food packaging.101 Table 4 presents some examples of the application of these surfaces.

Furthermore, other kinds of surface are stimulating intensive research and some of them are already used, while others are promising candidates for practical application.102–104 Examples include materials for medical implants (such as catheters), devices and instruments that are in contact with patients, surgical gowns and other protective clothing (such as surgical masks, caps) and polymeric coatings on surfaces such as shower walls.102,103 Antimicrobial polymers provide a very suitable strategy for achieving this objective since they can be applied to diverse objects. Polyelectrolyte multilayers (PEMs) have also been investigated extensively as biomaterials and biomaterial interfaces and also for bacterial contamination prevention.104

Gas plasma is another promising alternative to sterilization that can be applied in healthcare services, although it is mainly targeted to equipment rather than to surfaces. Plasma consists of a mixture of photons, electrons, ions, atoms and radicals (such as atomic oxygen, ozone, nitrogen oxides, hydroxyl and superoxide). As a result of air plasma discharges, the gas enters an ionized state (by energetic transfer) and exhibits antimicrobial properties.9,20,105,106 Two types of plasma can be defined according to the conditions under which the plasma is formed: thermal and non-thermal plasmas. Compared with non-thermal plasmas, thermal plasmas require higher pressure and are characterized by higher temperatures and a local thermodynamic equilibrium.105,106 These systems have many advantages over more conventional disinfection techniques as they enable the disinfection of the interior of some types of equipment and materials, such as needles, at low cost and with easy handling.107,108 Furthermore, gas plasma does not require chemical products and it is not toxic to the skin.20 Shimizu et al.108 performed treatments with plasma using a surface micro-discharge device, under different temperature and humidity conditions. The antimicrobial effect

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Microorganism</th>
<th>Method/test</th>
<th>Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous chlorine dioxide solution</td>
<td><em>B. anthracis</em> spores</td>
<td>quantitative bacteriological culture methods</td>
<td>8 log₁₀ reduction in 3 min in sealed microfuge tubes and 1 log₁₀ reduction for spraying or spreading the disinfectant onto surfaces (when using the solution in 5% bleach—0.3% sodium hypochlorite—its full activity is restored)¹⁴¹</td>
</tr>
<tr>
<td>Dismozon® pur; Kohrsolin® extra; Kohrsolin® FF</td>
<td><em>C. difficile</em> ribotype 027</td>
<td>suspension test in different concentrations at various exposure times</td>
<td>≥4 log₁₀ (Dismozon pur at 1.5% and 2 h exposure time; Kohrsolin extra at 2% and 4 h exposure time; and Kohrsolin FF at 2% and 6 h exposure time)¹⁴⁴</td>
</tr>
</tbody>
</table>

¹A measure of the bactericidal activity of a chemical compound in relation to phenol.
<table>
<thead>
<tr>
<th>Surface coating</th>
<th>Method</th>
<th>Bacteria tested</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanoparticulate silver-coated titania thin films</td>
<td>sol-gel preparation</td>
<td>MRSA and E. coli</td>
<td>99.9% reduction due to the presence of the silver ion for E. coli and due to enhanced photocatalysis for MRSA (^{37})</td>
</tr>
<tr>
<td>Copolymer poly(butylmethacrylate)-co-poly(Boc-aminooethyl methacrylate) on silicon wafers and glass surfaces</td>
<td>atom transfer radical polymerization</td>
<td>E. coli and S. aureus</td>
<td>100% reduction in (&lt;5) min (^{22})</td>
</tr>
<tr>
<td>TiO(_2) film on medical grade AISI 304 stainless steel</td>
<td>arc ion plating</td>
<td>E. coli and S. aureus</td>
<td>log(_10) reductions of 3.0 and 2.5 for S. aureus and E. coli, respectively, due to photocatalysis action (^{145})</td>
</tr>
<tr>
<td>Perfluoroctylated quaternary ammonium silane coupling agent in cotton fabrics</td>
<td>pad-dry-cure</td>
<td>S. aureus</td>
<td>97.3% reduction and 95.6% reduction after 10 laundering cycles (^{146})</td>
</tr>
<tr>
<td>Ag and CuO layers on glass</td>
<td>flame-assisted chemical vapour deposition (FACVD) and overlaid with TiO(_2) using thermal CVD</td>
<td>E. coli, S. aureus and P. aeruginosa</td>
<td>95% - 99.9% reduction for hospital-related pathogens (^{147})</td>
</tr>
<tr>
<td>Magainin I and nisin peptides on stainless steel</td>
<td>covalent binding via an intermediate chitosan layer</td>
<td>Listeria ivanovii</td>
<td>reduction of bacteria adhesion by a factor of 2 - 3 (^{39})</td>
</tr>
<tr>
<td>Pseudo-polyelectrolytes (pPE) and poly(4-vinylphenol) (PVPh) into multilayer systems with poly(allylamine hydrochloride) (PAH) and poly(diallyldimethylammonium chloride) (PDADMAC)</td>
<td>layer by layer</td>
<td>S. epidermidis</td>
<td>60% and 70% growth inhibition for PAH/PVPh and PDADMAC/PVPh, respectively (^{13})</td>
</tr>
<tr>
<td>Molybdc acid (H(_2)MoO(_4)) and molybdenum trioxide (MoO(_3))</td>
<td>sol-gel</td>
<td>S. aureus and P. aeruginosa</td>
<td>surfaces almost without microorganisms after 6 h (^{12})</td>
</tr>
<tr>
<td>Stainless steel surfaces with different copper content (with a maximum of 7.1 wt % for a gas pressure of 60 Pa)</td>
<td>plasma surface alloying technique at various gas pressures</td>
<td>E. coli</td>
<td>reduction of 98% of cells within 1 h (^{93})</td>
</tr>
<tr>
<td>Copper-containing titanium nitride films on commercial stainless steel</td>
<td>hybrid processes combining dual magnetron sputtering</td>
<td>E. coli</td>
<td>very effective in killing the bacteria; longer TiN deposition time may lead to superior antibacterial capability, corrosion and wear resistance (^{96})</td>
</tr>
<tr>
<td>Duplex-treated plasma alloyed AISI 304 stainless steel with Ni with plasma alloying with Cu</td>
<td>double-glow plasma surface alloying technique</td>
<td>E. coli and S. aureus</td>
<td>reduction of 99.9% and 100% for E. coli and S. aureus, respectively (^{148})</td>
</tr>
<tr>
<td>Surfaces of titanium, Ti6Al4 V alloy or TiN, modified with SiO(_2) – TiO(_2) layer</td>
<td>glow-discharge nitriding, sol-gel and electrophoresis</td>
<td>S. epidermidis</td>
<td>formation of biofilms on polished and ground titanium and titanium alloy surfaces covered with TiN, but not on those modified with SiO(_2) – TiO(_2) nanofilm (^{149})</td>
</tr>
<tr>
<td>Medical grade poly(vinyl chloride) (PVC) chemically modified with the incorporation of monovalent silver</td>
<td>radio frequency oxygen (RF-O(_2)) glow discharge pre-functionalization and two-step wet treatment in sodium hydroxide and silver nitrate solutions</td>
<td>P. aeruginosa</td>
<td>100% reduction in initial bacterial adhesion (^{98})</td>
</tr>
<tr>
<td>Copolymer soft block containing trifluorethoxy (89 mol %) and C-12 alkylammonium (11 mol %) side chains</td>
<td>cationic ring opening polymerization; nucleophilic substitution</td>
<td>P. aeruginosa, E. coli and S. aureus</td>
<td>100% kill and 3.6 - 4.4 log reduction in 30 min (^{150})</td>
</tr>
<tr>
<td>Silicon wafers and glass surfaces functionalized with poly(butylmethacrylate)-co-poly(Boc-aminooethyl methacrylate) Poly electrolyte multilayers of poly(allylamine hydrochloride) (PAH) and poly(sodium 4-styrene sulfonate) (SPS)</td>
<td>surface-initiated atom transfer radical polymerization</td>
<td>S. aureus and E. coli</td>
<td>100% kill in (&lt;5) min (^{72})</td>
</tr>
<tr>
<td></td>
<td>SPS/PAH PEMs assembled on plain glass slides with poly(sodium 4-styrene sulfonate)</td>
<td>S. epidermidis, E. coli</td>
<td>viability of bacteria was effectively reduced on SPS/PAH multilayers displaying accessible cationic charge (^{104})</td>
</tr>
</tbody>
</table>
was tested on *Escherichia coli* and *Enterococcus mundtii* with a reduction of 5 log10 after 15 and 30 s of plasma treatment for *E. mundtii* and *E. coli*, respectively. Other researchers have developed a large-scale plasma dispenser and evaluated its effect on *E. coli* and *Candida albicans* in agar plates in the presence and absence of textiles. Their results demonstrated that the system was not affected by the presence of the textile, and in both cases a 15 s treatment caused a 5 log10 reduction and, after treatment for 5 s, the fungi were reduced by 4 log10. Joshi et al. studied the biocidal efficacy of non-thermal dielectric-barrier discharge plasma against *E. coli*, *S. aureus* and MRSA in biofilms and planktonic cells. The planktonic cells were completely eliminated after 120 s of treatment, whereas the MRSA growing in biofilms were killed by >60% within 15 s, suggesting that the effectiveness of a plasma system is highly dependent on exposure time and cell density. Recent investigations evaluated the effect of non-thermal gas plasma on biofilms of *Staphylococcus epidermidis* and MRSA on glass surfaces; a log10 reduction of 4 and 4.5 was observed after 1 h of exposure, respectively, and greater reductions could be attained with more prolonged exposure times. Burts et al. tested an atmospheric non-thermal plasma as a disinfectant for hospital pagers by analysis of MRSA reduction, and found complete disinfection within 30 s. Different cell densities and exposure times were also evaluated and a 4–5 log10 reduction was obtained within 10 min. More information on this disinfection strategy has been reviewed by Moisan et al. These novel disinfection techniques are important means of reducing the high numbers of nosocomial infections and are more efficient than conventional disinfection methods.

**Conclusions**

There is great concern about the growth and prevalence of HAI due to the increased incidence of resistant bacteria. Furthermore, the development of new antibiotics is a difficult task because of high research costs and regulatory issues. Conventional cleaning methods for the eradication of hospital environmental contamination seem to be inefficient. This manuscript reviews several new disinfection alternatives as attempts to overcome these problems. Most of the data currently available have been generated by the manufacturers and need to be validated by independent investigations. Studies concerning bacterial biology and physiology allied to genomics and computer analysis should be applied to identify and understand the pathogenesis associated with resistant bacteria and crucial targets for novel biocides. Thus, further evaluation and implementation of new measures and new disinfection methods are necessary, not forgetting their validation in terms of effectiveness, safety and disposal. Additionally, it is important always to evaluate the risk of emerging phenotypic resistance when developing new disinfection strategies.

**Funding**

This work was supported by Operational Programme for Competitiveness Factors—COMPETE, FCT/MEC (PIDDAC) and FEDER through Projects Biorest—PTDC/EBB-EBI/105085/2008 and Phyto Disinfectants—PTDC/DTP-SAP/1078/2012 (COMPETE: FCOMP-01–0124-FEDER-028765) and the PhD grants awarded to A. C. A. (SFRH/BD/84393/2012) and A. B. (SFRH/BD/63398/2009).

**References**

20. Burts ML, Alexeff I, Meek ET et al. Use of atmospheric non-thermal plasma as a disinfectant for objects contaminated with...


Review

70 Bermúdez-Aguirre D, Barbosa-Cánovas GV. Disinfection of selected vegetables under nonthermal treatments: chlorine, acid citric, ultraviolet light and ozone. Food Control 2013; 29: 82–90.


115 Murphy JR. Corynebacterium diphtheriae. In: Baron S, ed. Medical Microbiology. Galveston, TX: University of Texas Medical Branch at Galveston, 1996; Chapter 32.


