Journal of Hospital Infection 78 (2011) 163–170

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevierhealth.com/journals/jhin

Review

Disinfection: is it time to reconsider Spaulding?

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ARTICLE INFO

Article history:
Received 17 April 2011
Accepted 6 May 2011
by J.A. Child
Available online 8 June 2011

Keywords:
Disinfection
Sterilization
Resistance
Spaulding
Devices

SUMMARY

The Spaulding classification, originally proposed in 1957, is a widely used system for matching the disinfection and sterilization of surfaces, particularly those of re-usable medical/surgical devices, with available processes. It presents a ranking, from simple disinfection through to sterilization, that should be considered in the reprocessing of devices, based on the risks associated with their use, ranging from 'critical' (presenting a high risk), through 'semi-critical' to 'non-critical' (presenting a low risk). The different levels of disinfection are based on demonstrating antimicrobial activity against established marker micro-organisms representing a range of pathogens. Although this classification system is probably as valid today as it was in 1957, the understanding of microbiology and micro-organisms has changed. This article discusses some examples of disinfection studies with viruses, bacteria, protozoa and prions that challenge the current definitions and expectations of high-, intermediate- and low-level disinfection. In many of these examples, the test micro-organisms demonstrate atypical tolerance or resistance profiles to disinfection processes. In addition to laboratory-based studies, there is now clinical evidence for at least some of these micro-organisms that biocide resistance can lead to infection outbreaks due to unexpected disinfection failure. These reports should encourage the reader to challenge current dogma, and reconsider the expectations of disinfection and sterilization practices.

Introduction

Disinfection is one of the cornerstones of infection prevention and control, defined as the antimicrobial reduction of micro-organisms to a level previously specified as appropriate. This definition is intentionally broad to cover a variety of applications, including medical/surgical device reprocessing, liquid/gas treatment and general environmental surface disinfection. It encompasses other processes, such as pasteurization, sanitization, antisepsis, fumigation and preservation. Disinfection methods can be classified as being physical or chemical in antimicrobial activity.[1] Physical methods include radiation and heat, while chemical methods are based on the use of biocides such as alcohols, aldehydes, halogens and quaternary ammonium compounds. Given the range of disinfection methods available and their clinical applications, classification systems are used to aid healthcare workers to choose the correct method to safely reduce patient risks. One such system is the Spaulding classification for surgical or medical devices, which has been in use since 1957.[2] Spaulding defined the minimum levels of disinfection to be employed according to the infection risk associated with a device when used with a patient.

Critical devices present the highest risk as they enter a normally 'sterile' area of the body, such as the bloodstream. Sterilization of these devices is recommended. Sterilization is distinct from but encompasses disinfection, being defined as a process used to render a surface or product free from viable micro-organisms, including bacterial spores. A sterile device is free from viable organisms, while disinfected devices or surfaces can only be presumed to have reduced microbial levels. Typical sterilization processes use steam, ethylene oxide, liquid peracetic acid and hydrogen peroxide gas.

Semi-critical devices pose a lower risk as they may only contact mucous membranes or non-intact (broken) skin. In the past, many of these devices (such as flexible endoscopes) could not be sterilized in a reasonable time frame for practical clinical use. The compromise was to recommend high-level disinfection, thereby inactivating most pathogenic micro-organisms such as viruses, bacteria (including mycobacteria), fungi and, if possible, bacterial spores (in these cases, generally requiring longer exposure times). High-level disinfectants, such as those based on heat (hot water for some devices), glutaraldehyde, ortho-phthaldehyde (OPA), hydrogen

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doi:10.1016/j.jhin.2011.05.002

peroxide and peracetic acid, could provide rapid turnaround times for these devices.

Non-critical devices present the lowest risk to patients, as they may only contact intact skin. In these cases, low- or intermediate-level disinfection is often recommended, encompassing certain types of viruses [especially enveloped viruses such as influenza and human immunodeficiency virus (HIV)], most bacteria and some fungi. Intermediate-level disinfectants should also provide efficacy against a broader group of viruses (non-enveloped) and some mycobacteria. Efficacy claims of disinfectants vary between products. Examples include alcohol-, aldehyde-, phenolic- and quaternary-ammonium-compound-based disinfectants.

Simple as this classification may appear, it can often be difficult to make a decision regarding the risk to a patient. Examples include flexible endoscopes and similar devices that are considered to be semi-critical devices. Does the semi-critical or critical definition not depend on how and why the device is used on a patient? For simple investigational purposes, they may be considered semi-critical, but are they also critical in the case of an internal bleed, taking a biopsy during a procedure or in the surgical use of such devices (e.g. for natural orifice transluminal endoscopic surgery)?[3] As a general point, it is itself a paradox that these devices – being difficult to reprocess, frequently associated with infection outbreaks (published and unpublished), and of such complexity – are not subjected to greater scrutiny from an infection control point of view.[4]

In addition to the practical difficulties, there is growing scientific debate on the practices and expectations of disinfection. Disinfection/sterilization methods are classified and labelled for use based on an understanding of the hierarchy of microbial resistance to such processes. The traditional hierarchy considered by Spaulding is still widely used today, and was essentially based on microbial knowledge in 1957 (Figure 1). Demonstration of efficacy against different

a

Disinfection level	Bacteria			Fungi	Viruses	
	Vegetative	Mycobacteria	Spores		Enveloped/ lipid	Non-enveloped/ non-lipid
High	+	+	+	+	+	+
Intermediate	+	+	-	+	+	+
Low	+	-	-	+	+	-

Source: Spaulding EH. Chemical disinfection and antisepsis in the hospital. *J Hosp Res* 1957;**9**:5–31.

b

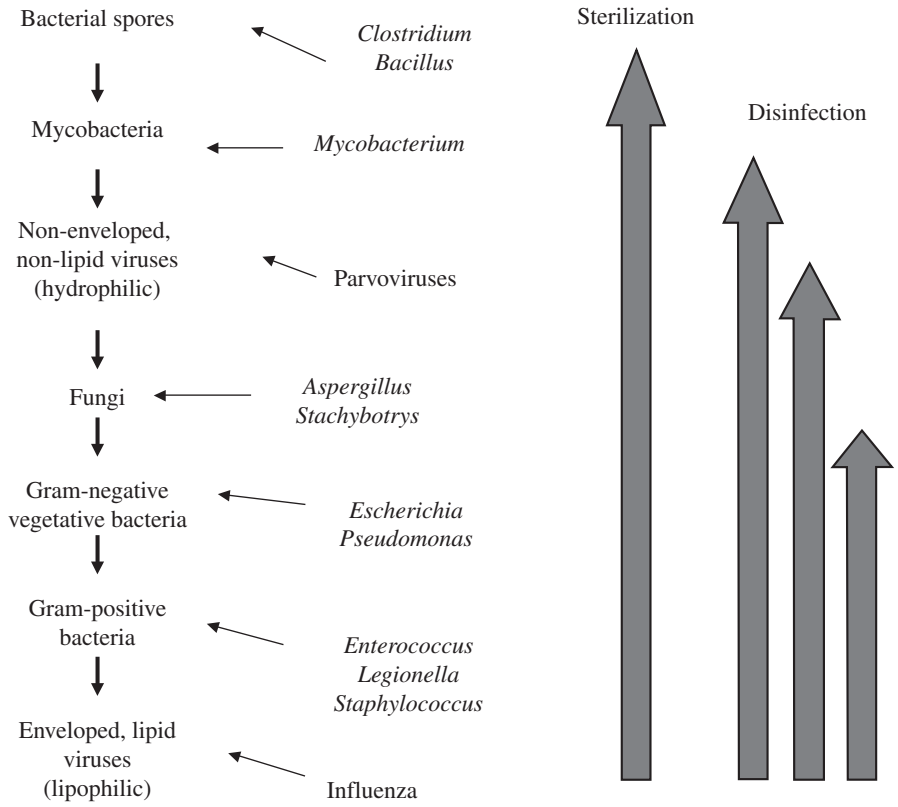

Figure 1. The Spaulding classification for device disinfection. (a) Based on Spaulding (1957). (b) As typically used today in healthcare facilities, with the decreasing level of resistance shown on the left, with examples of micro-organism types that are typical of each grouping and the equivalent levels of sterilization/disinfection.

types of micro-organisms was sufficient to define low-, intermediate- or high-level disinfection, where high-level disinfection was considered to be effective against most known pathogens. Today, depending on local registration requirements (if they exist in a given country/region), various strains of marker organisms representative of the different classes of micro-organisms in this hierarchy are used to test the efficacy of disinfectants/sterilants. For example, mycobactericidal activity demonstrated by passing a disinfectant test with marker strains of *Mycobacterium bovis* or *M. terrae* is considered as evidence of activity against all mycobacteria and other types of micro-organisms that are considered to be less resistant (e.g. other bacteria, viruses and fungi). The understanding of micro-organisms, particularly pathogens, has evolved and a more updated summary can be proposed as a guide (Table I). It is important to note that these hierarchical scales are only given as guides to microbial resistance, as they can vary depending on the type of micro-organism, how they are presented for disinfection, and the antimicrobial process under investigation. This may often be related to the test method. As an example, greater levels of resistance to disinfection can be shown with vegetative bacteria (often tested in the presence of interfering soils) compared with 'clean' suspensions of bacterial spores. Such comparisons are artefacts of test methods rather than a true resistance concern. In contrast, fungal spores (particularly *Aspergillus*) are more resistant to ultraviolet (UV) irradiation than bacterial spores, and certain strains of mycobacteria demonstrate extreme resistance to aldehydes at concentrations that are effective against bacterial spores.[5,6] Such reports highlight that although these hierarchy and classification lists may be useful, they may also be misleading. This article discusses some examples of inactivation studies with viruses, bacteria, protozoa and prions that challenge the current definitions and expectations of disinfection.

Viral resistance

In general, viral resistance to disinfection is not as well studied as bacterial resistance. Viruses have been classified into three groups based on their structure and lipophilicity: enveloped viruses [e.g. HIV and hepatitis B virus (HBV); very sensitive to disinfectants], large non-enveloped viruses (e.g. adenoviruses and rotaviruses; intermediate resistance to disinfectants) and small non-enveloped viruses (e.g. poliovirus and papilloma viruses; highest resistance to disinfectants).[1,7] Internationally, test methods to demonstrate the viricidal efficacy of disinfectants can vary considerably. It is expected that representative viruses from each of these three groups should be tested, otherwise the claim can be misleading. Due to their notoriety, enveloped viruses such as HIV, HBV and influenza are often used, but these viruses are considered relatively susceptible to most disinfectants due to disruption of their outer envelope being sufficient to render them non-infectious. A note of caution is warranted; although some viruses may be very sensitive to inactivation, other factors such as virus clumping and the presence of protective organic materials can increase their resistance profile to disinfection. This is not only true for viruses but for all micro-organisms. Non-enveloped viruses display higher intrinsic resistance based on their structure. In the past, the marker virus for this group was poliovirus (an enterovirus in the *Picornaviridae* family, a group of RNA non-enveloped viruses); however, this is under review in different countries, primarily due to efforts to eradicate poliovirus from the human population.

The resistance profiles of other non-enveloped viruses (human and animal pathogens) have been investigated recently. These include parvoviruses, coxsackieviruses, other enteroviruses, hepatitis A virus and noroviruses. Disinfection studies have shown that some of these viruses are distinctly more resistant than the poliovirus marker, including thermal and chemical disinfection methods.[8–10] The most significant to date are the parvoviruses. Parvoviruses are small (18–26 nm), non-enveloped, hydrophilic, single-stranded DNA viruses. Although they have been widely associated with animal infections, until recently, they were not frequently associated with human infections.[11] The first human parvovirus was described in the 1970s, when B19 (B19V) was reported to be a cause of aplastic anaemia in children. In recent years, at least two new groups have been described, including human bocaviruses (HuBoV) and the Parv4 viruses.[11] HuBoV is now considered to be a significant cause of lower respiratory tract infections in children, being both community- and hospital-associated.[12] Parv4 has been isolated from patients with acute viral infection syndrome, and has been suggested to have a possible role in liver and heart disease.[13–15] It has been known for some time that parvoviruses present unique resistance to disinfection. In a German study in 1979, bovine parvovirus was found to be highly resistant to chemico-physical disinfection methods.[16] It is interesting to note from this report that other non-enveloped viruses, such as a reovirus and three enterovirus strains, also showed high-resistance profiles. Bovine parvovirus was found to be highly resistant to thermal inactivation in the 75–90 °C range typically used for disinfection (including pasteurization) applications.[17] More recent reports have highlighted this concern in hospital disinfection practices.[8,9,18] There is no standardized test method, but each study investigated the resistance of enveloped and non-enveloped viruses using their specified test methods. In all cases, the parvoviruses were the most resistant viruses. In one study, two different parvovirus strains (porcine and minute virus of mouse) were compared with other marker viruses.[9] The parvoviruses demonstrated the highest level of resistance to chemical and heat-based disinfection, with porcine parvovirus being particularly tolerant. This included a lack of significant activity with some intermediate- and high-level disinfectants (particularly aldehydes), despite rapid activity against the reference poliovirus. Interestingly, minute virus of mouse demonstrated greater resistance to acid-based disinfectants, and porcine parvovirus was more resistant to alkaline-based disinfectants.[9,19] Studies with parvovirus B19, as a human pathogen, also suggested high resistance.[20] Some parvoviruses used in industrial settings have even higher resistance to disinfectants (Eterpi and Thomas, personal

Table I
Hierarchy of microbial resistance to disinfectants and sterilants, based on McDonnell (2007). Micro-organisms are listed in order (highest to lowest) of known resistance to disinfectant inactivation, but this will vary depending on the disinfectant. It cannot be taken for granted that efficacy against micro-organisms with higher resistance will be effective against micro-organisms lower in the list

Micro-organism	Examples
Prions	Scrapie, Creutzfeld–Jakob disease, chronic wasting disease
Bacterial spores	*Bacillus, Geobacillus, Clostridium*
Protozoal oocysts	*Cryptosporidium*
Helminth eggs	*Ascaris, Enterobius*
Mycobacteria	*Mycobacterium tuberculosis, M. terrae, M. chelonae*
Small, non-enveloped viruses	Poliovirus, parvoviruses, papilloma viruses
Protozoal cysts	*Giardia, Acanthamoeba*
Fungal spores	*Aspergillus, Penicillium*
Gram-negative bacteria	*Pseudomonas, Providencia, Escherichia*
Vegetative fungi and algae	*Aspergillus, Trichophyton, Candida, Chlamydomonas*
Vegetative helminths and protozoa	*Ascaris, Cryptosporidium, Giardia*
Large, non-enveloped viruses	Adenoviruses, rotaviruses
Gram-positive bacteria	*Staphylococcus, Streptococcus, Enterococcus*
Enveloped viruses	Human immunodeficiency virus, hepatitis B virus, herpes simplex virus

Source: McDonnell G. *Antisepsis, disinfection and sterilization: types, action and resistance*. Washington, DC: ASM Press; 2007.

communication). The resistance of parvoviruses may be due to the nature of the capsid structure and the stability of the internal DNA molecule. In some cases, only the nucleic acid molecule is required to initiate viral infection; therefore, biocidal effects that do not directly disrupt these structures presumably do not eliminate infectivity.[1]

Although less research has been undertaken, similar non-enveloped enteroviruses (also of the *Picornaviridae* family), such as coxsackieviruses and echoviruses, have been the subject of some water disinfection investigations due to their tolerance to chlorine and known pathogenicity.[8,10,21,22] Coxsackieviruses are often associated with mild influenza-like illness, but can also cause more severe infections such as meningitis, particularly in children. Other enteroviruses cause a similar range of infections, and have recently been linked with other health effects such as chronic fatigue syndrome.[24] An example is enterovirus 71, closely related to poliovirus, that has been identified in large outbreaks.[25] To date, little has been reported regarding the effectiveness of disinfectants used on medical devices against these viruses. At least one report has suggested marked variation in the efficacy of surface disinfectants, particularly aldehyde-based disinfectants.[26] Hepatitis A virus, a non-enveloped virus, is a concern as a leading cause of acute liver disease. Disinfectant studies to date have shown mixed results depending on the strains and disinfectants tested.[10,18,27] Finally, there is ongoing debate regarding the innate resistance of, and disinfectant efficacy against, noroviruses.[28,29] Noroviruses (also referred to as 'Norwalk agents') are members of the *Caliciviridae* family, and are leading causes of viral gastroenteritis. They are a particular concern in hospitals due to their highly infectious and persistent nature.[30] Noroviruses are non-enveloped viruses, and, to date, it has not been possible to routinely culture human strains in laboratory conditions. For this reason, surrogate strains (such as feline calicivirus) have been used in disinfection studies, and resistance appears to be equivalent to that of poliovirus.[31,32] Recent studies with murine and human norovirus strains reported higher resistance to chlorine disinfection (in aquatic studies) compared with poliovirus.[33]

The innate resistance of a virus, as with other micro-organisms, can be disinfectant-specific. This has been shown, for example, in UV water disinfection studies with adenoviruses. Adenoviruses are enteric, medium-sized viruses that are typically described as having resistance to disinfection that is intermediate between enveloped and non-enveloped viruses. Studies have shown that adenoviruses actually have greater resistance to UV light than enteroviruses such as poliovirus, coxsackieviruses and echoviruses.[34] In another report, chlorine dioxide was particularly effective against a coxsackievirus, but calicivirus and hepatitis A virus demonstrated greater resistance.[35] Overall, many enveloped and non-enveloped viruses demonstrate surprising resistance to widely used chemical and physical methods of disinfection, and further investigation is warranted to understand the implications for hospital disinfection practices. In some cases, as highlighted with parvoviruses, currently used high-level disinfectants may not provide the expected level of efficacy based on efficacy claims for poliovirus inactivation. It is also true to conclude that efficacy against poliovirus may not be sufficient to assume efficacy against other non-enveloped and pathogenic viruses.

Bacterial resistance

Intrinsic and acquired bacterial resistance to disinfection have been well documented.[1] Intrinsic resistance is defined as any mechanism that is a natural property of bacteria. Examples include bacterial cell wall structures (particularly the mycobacterial cell wall), biofilm development and sporulation (with *Bacillus, Geobacillus* and *Clostridium*). A recent review discussed the implications of chemical disinfectant resistance mechanisms for infection prevention and control, and suggested that the current risks to healthcare disinfection practices were low.[23] This viewpoint requires closer examination. Acquired resistance, although 'tolerance' may be a more correct term in most of these cases, is defined as resistance due to mutations (developed environmentally and under laboratory conditions) and/or acquisitions of plasmids/transposons. Examples include increased minimum inhibitory concentration (MIC) levels to biocides such as chlorhexidine, triclosan and quaternary ammonium compounds.[36–38] Increased MICs did not translate to clinical failure as the biocides remained active at higher concentrations, and the minimum bactericidal concentration or biocidal effects of disinfectants remained the same, as in the case of triclosan.[39] In this sense, 'tolerance' refers to an increased MIC but not 'resistance' that would relate to clinical failure. A greater concern has been the indirect impact on antibiotic resistance, where the induction of biocide tolerance could lead to antibiotic resistance and *vice versa*. This topic has been discussed elsewhere.[23,40] Overall, it does not appear that such reports in the literature should affect the clinical use of disinfectants, apart from highlighting the importance of using such products prudently and correctly. However, there has been an increase in the number of reports suggesting that the development of true disinfectant resistance with clinical implications is a reality.

The development of glutaraldehyde resistance in mycobacteria associated with outbreaks has been reported in many countries since the 1990s.[6,41–43] Mycobacteria, such as *M. tuberculosis, M. avium* and *M. abcessus*, are known pathogens that are widely distributed in the environment, including water. Despite their intrinsic resistance to disinfection, high-level disinfectants are considered to be effective against mycobacteria and other micro-organisms, with the exception of large numbers of bacterial spores.[44] 'Pseudo-outbreaks' with mycobacteria strains of *M. chelonae var. absessus, M. chelonae var. chelonae* and *M. gordonae* have been associated with the use of flexible endoscopes and automated washer-disinfectors. Such strains were found to have developed high-level resistance to glutaraldehyde-based disinfectants. It does not appear, from these reports, that these strains caused serious infections in patients, but they were clinically highlighted as being acid-fast mycobacteria and presumptively attributed to *M. tuberculosis*. Investigations on these strains have shown significant resistance to glutaraldehyde disinfection at recommended or extended contact times, but sensitivity to other types of disinfectants. Since these reports, there has been growing concern regarding mycobacterial infections associated with re-usable medical devices, chemical-based washer-disinfectors and contaminated rinse water.[45,46] Surgical site infections with strains of *M. avium, M. chelonae* and *M. fortuitum* followed the use of such devices in ophthalmology procedures, mesotherapy, implants, arthroscopy, laparoscopy, cholecystectomy and even bronchoscopy. Other mycobacteria outbreaks have been reported, but the source of contamination was not investigated.[47,48] Due to the slow-growing nature of these micro-organisms, infections do not appear for many months following surgery, which presents significant difficulties in investigating their sources. The most significant outbreak to date was reported in Brazil, and affected over 3000 patients following surgical or bronchoscopic procedures. This outbreak was predominantly due to one clonal strain of *M. massiliense* (Duarte, personal communication).[43] Although under continued study, initial investigations confirmed that *M. massiliense* strains are highly resistant to glutaraldehyde (up to 8% for >1 h), but are rapidly susceptible to alternative oxidizing-agent-based disinfectants (Jackson and Duarte, personal communication). Biochemical and genetic investigations have been conducted with a *M. massiliense* outbreak strain (known as BRA-100),[43] as well as similar investigations to understand the mechanism(s) of resistance in *M. chelonae* and *M. smegmatis*.[49] These studies concluded that the cell surface structure has been modified to protect it from the activity of glutaraldehyde. At a minimum, the mechanism

of resistance includes the deletion or change in surface availability of porins.[49] Porins are proteins associated with the cell wall surface that allow the transport of chemicals into and out of the cell. They are also a major protein component of the outer mycobacteria cell wall.[50] Glutaraldehyde and other aldehydes are surface-acting molecules that act by cross-linking proteins.[1] The lack of reactivity in resistant strains appears to be due to a lack of or unavailability of porins, and therefore reactive proteins, at the cell surface.[49] These significant structural changes at the cell surface have also raised concern about cross-resistance to antibiotics, which was tested by MIC analysis.[49] One *M. chelonae* strain was found to have increased resistance (five- to 100-fold) to large and/or hydrophobic antibiotics such as rifampicin, vancomycin, ciprofloxacin, clarithromycin and erythromycin.[49] The strain was also four- to >10-fold more resistant to linezolid and tetracycline. Cross-resistance has also been described in the *M. massiliense* outbreak strains. As a preliminary note, in-vivo studies in mice have shown that the *M. massiliense* BRA-100 strain can persist in tissues such as the lungs and spleen, therefore demonstrating increased virulence factors in addition to biocide/antibiotic resistance (Ordway and Jackson, personal communication). It is acceptable to speculate, based on the mode of action of glutaraldehyde,[1] that the lack of available surface or reactive protein on the cell wall surface of these bacteria is sufficient to provide resistance to glutaraldehyde as a biocide (Figure 2); this conclusion is supported by genetic and biochemical studies, but may not be the only mechanism of resistance in some strains.[49] A further collection of strains (including *Mycobacterium*, *Methylobacterium* and other bacterial strains) isolated from washer-disinfectors using glutaraldehyde or OPA for disinfection have shown particularly high-level resistance to OPA as a disinfectant but not glutaraldehyde and *vice versa*, suggesting that there are differences in the phenotypic expression of aldehyde resistance.[51]

Protozoal resistance

Protozoa are an abundant group of micro-organisms, including various pathogens, which are not generally considered in device disinfection/sterilization discussions. This is surprising in that they include notable pathogens, such as *Giardia lamblia, Acanthamoeba castellanii, Plasmodium falciparum* and *Cryptosporidium parvum*. They are a particular challenge to inactivate as they have both vegetative and dormant (cyst or oocyst) forms during their life cycles (Figure 3).[1]

Protozoal cysts/oocysts are known to present greater resistance to environmental factors (such as drying and, to a limited extent, elevated temperatures compared with bacterial endospores) and chemical disinfection.[1,52] It has previously been estimated (but not confirmed) that the vegetative forms (known as trophozoites or sporozoites) demonstrate similar resistance to inactivation as vegetative fungi. This is an oversimplification given the range of structures, and does not always appear to be the case.[52–54] Many studies have focused on the effects of biocides used in liquid disinfection against *Acanthamoeba* trophozoites, such as biguanides (chlorhexidine and the polyhexamethylene biguanides), quaternary ammonium compounds, chlorine and oxidizing agents such as hydrogen peroxide.[52] Formulations containing hydrogen peroxide have been reported to have greater efficacy, although long contact times appear to be required for both trophozoites and (particularly) amoebal cysts. In contrast, low concentrations of hydrogen peroxide gas appear to be particularly effective.[55] Overall, there are many discrepancies in reports of antimicrobial efficacy against protozoal vegetative and dormant forms.

Dormant cyst forms (e.g. those produced by *Giardia* and *Acanthamoeba*) are often considered to present resistance at least as high as that of some fungal spores (e.g. *Aspergillus* ascospores), and oocysts demonstrate even greater resistance (e.g. *Cryptosporidium parvum*). In a recent example, disinfection efficacy against a variety of *Acanthamoeba* culture collection and environmental isolates (trophozoites and cysts) was studied.[53] Overall, a significant difference in disinfection efficacy was observed between strains, with higher resistance being observed for environmental (including hospital) isolates. The trophozoites of all strains were inactivated by all the chemical disinfectants tested (including 70% ethanol and various high-level disinfectants), with the notable exception of glutaraldehyde-based disinfectants; for the latter, survival was observed after 30-min exposures. Efficacy against cysts was more variable. Moist heat disinfection was consistently effective at 65 °C for 10 min, with little to no effect observed at 55 °C. Disinfection with chlorine (0.25% sodium hypochlorite, approximately equal to a 1/20 dilution of household bleach) for a 10-min contact time demonstrated a significant range in activity, from no effect to complete (>5 $\log_{10}$) reduction in cyst viability, depending on the strains tested. Some strains even survived exposure for 30 min, particularly those isolated from hospital sources. Surprisingly, 70% ethanol was more effective against cysts than a range of glutaraldehyde-based high-level disinfectants; neither disinfectant type was fully effective against all strains tested, with some strains demonstrating little to no effect to 30-min exposure to glutaraldehyde. Although formulation dependent, disinfectants based on hydrogen peroxide and peracetic acid displayed consistent disinfection efficacy against all strains. However, even with these biocides, the strains ranged in tolerance to inactivation. Overall, amoebal cysts can be highly resistant to some high-level disinfectants; this has potential implications for clinical practice. *C. parvum* oocysts have been the subject of a number of disinfection studies and are particularly resistant to most widely used high-level disinfectants, including those based on glutaraldehyde, OPA and, in some cases, peracetic acid.[56,57] With chemical disinfection, oocysts can be considered to have higher resistance

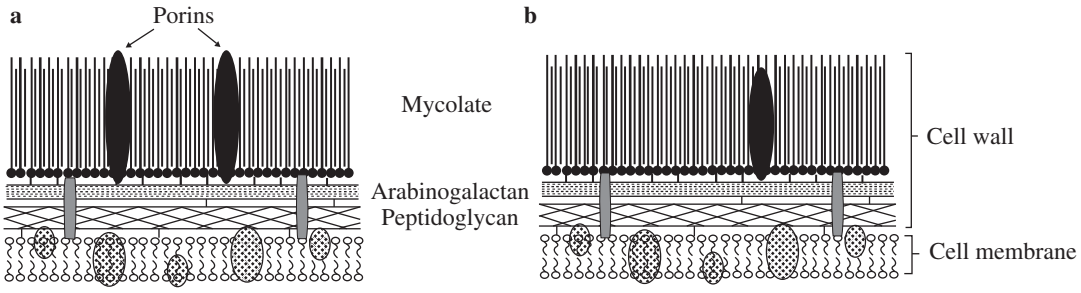

Figure 2. Proposed mechanism of high-level resistance to glutaraldehyde. (a) A simplified representation of the mycobacterial cell wall structure, showing the inner cell wall and tripartite cell wall structure (peptidoglycan, arabinogalactan and mycolate, including the long-chain mycolic acids). Porins represent a major, stable component of the outer surface, allowing chemical transport into and out of the cell. (b) A mutant cell wall structure is associated with the lack of or inaccessibility of porin proteins on the surface, significantly limiting the reactivity and therefore the antimicrobial activity of glutaraldehyde and other aldehydes.

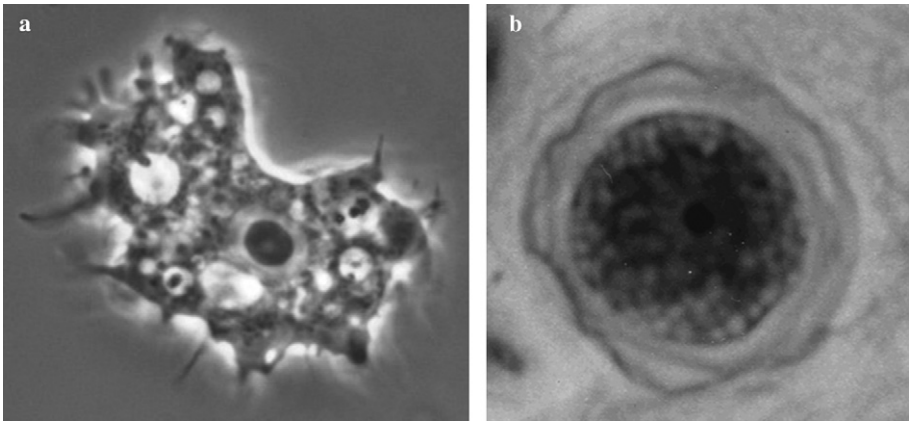

Figure 3. (a) *Acanthamoeba* trophozoite and (b) mature cyst.

than mycobacteria and bacterial endospores to some biocides. As pathogenic micro-organisms, protozoa should be considered as part of disinfection/sterilization practices, but are outside the scope of the existing Spaulding classification.

The significance of protozoa to human health can be debated based on their direct and indirect effects. It has been known for some time that free-living amoebae, in both vegetative and dormant forms, provide an internal environment for the survival and growth of a growing list of bacteria and viral pathogens.[52,58] Amoebae are found ubiquitously in aquatic environments, and can offer unique intracellular ecosystems for other micro-organisms. Notable bacterial pathogens that are known to survive and/or replicate in amoebae include *Acinetobacter*, *Campylobacter*, *Escherichia*, *Helicobacter*, *Legionella*, *Listeria*, *Mycobacterium*, *Pseudomonas* and *Staphylococcus*. Further interactions have been described with various viral (e.g. coxsackieviruses and adenoviruses) and fungal (e.g. *Cryptococcus*) pathogens. Many newly identified micro-organisms have also been described.[52,59] For this reason, amoebae, and other less studied protozoal strains, are considered to be the 'Trojan horses' of the microbial world. It is of concern that these pathogens can essentially escape disinfection/sterilization within protozoa by intracellular protection in both vegetative and dormant forms. The implications of such associations remain to be understood.

Infectious proteins: on the edge of microbiology

Prions, as infectious proteins and the causative agents in a group of diseases known as transmissible spongiform encephalopathies, are notable in their resistance to disinfection and sterilization.[1,60,61] Initial studies on the inactivation of prions indicated that aggressive physical (steam sterilization at 134 °C for 18 min) and chemical (1N NaOH or 2% available chlorine, in the form of sodium hypochlorite, for 1 h) methods were required, and this was recommended by the World Health Organization in 1999.[62] Since this time, these methods have been tested on contaminated surfaces and found to be effective (although associated infectivity is not always removed completely), but damage numerous types of devices.[61,63] At the same time, relatively simple cleaning processes can be effective against prions without broad-spectrum antimicrobial activity.[63] Removal of prion contamination is a complicated matter, with studies showing that cleaning chemistries can decrease or even increase the resistance of prion contamination to steam sterilization.[61,63] In terms of the efficacy of disinfection, moist heat and various disinfection methods are not considered to be effective against prions. Some peracetic acid, phenolic and biocide combination formulations, lower concentrations of alkali (NaOH and KOH), and hydrogen peroxide gas treatments have significant activity. Indeed, the activity of hydrogen peroxide gas in vacuum-based sterilization processes has been shown to be effective in some cases[64,65] but not others,[65,66] despite similarities in these processes, highlighting the complexity of prion inactivation. It has been suggested that prion decontamination can be considered as being addressed during normal, routine reprocessing of devices when appropriate cleaning formulations and reprocessing methods are used. Considering the long incubation times and often sporadic nature of prion diseases, this may be prudent but is still a topic of some discussion. Of further debate is the handling of devices that are known to be or are at high risk of being contaminated with prions, as reviewed elsewhere.[63] In some cases, removal of such devices from clinical use is recommended, while others suggest reprocessing these devices with cleaning and extended steam sterilization (at 134 °C for 18 min). Overall, prions may not be highly resistant to reprocessing methods, depending on the process used, and efficacy against prions does not imply that other micro-organisms have been inactivated.

Prion diseases are rare, with the most prevalent being Creutzfeldt-Jakob disease (1–3 cases per million population). However, prion diseases are considered to be representative of other protein-precipitation-associated diseases (Table II).

There is considerable debate about the transmissible nature of such diseases,[67,68] with growing evidence under experimental conditions for Alzheimer's disease.[69] This suggests, by a similar seeding mechanism to that described for prions, that such diseases could be transmissible under certain situations, including transfer via contaminated surfaces.[70] Further research is required to verify these reports, but if they are confirmed, the impact of current reprocessing standards, including effects of disinfection, will need to be reconsidered.

Table II
Diseases associated with protein precipitation

Disease	Associated protein precipitation	Evidence of transmissibility
Alzheimer's disease	Amyloid β-peptide and Tau	Experimental evidence
Parkinson's disease	α-Synuclein	No evidence
Cataracts	Crystallins	No evidence
Systemic amyloidosis	Amyloid-A and apolipoprotein AII amyloid	Experimental evidence suggests a potential transmissible/acceleration nature

Sources: Aguzzi A. *Nature* 2009;**459**:924–925. Scheibel T, Buchner J. *Handb Exp Pharmacol* 2006;**172**:199–219.

Conclusions

Dr Earle Spaulding defined a classification system for the safe reprocessing of surgical/medical devices to address the clinical needs of the day; these needs have changed little since 1957 and are equally applicable today. The expectations associated with various different levels of disinfection, and indeed sterilization, are based on the understanding of microbiology, particularly pathogens, and risks associated with device use on patients. Disinfection and sterilization claims are based on passing established test methods, with marker organisms chosen to represent various types of micro-organisms. With high-level disinfection, for example, the demonstration of mycobactericidal and some level of sporicidal activity are taken to indicate efficacy against most known types of pathogens. However, this is not always true. Various types of viruses, bacterial strains and protozoa have been shown to survive existing high-level disinfection/sterilant processes, outside of what would be expected from the Spaulding classification system. Protozoa are rarely considered, and marker strains of viruses and bacteria may not always reflect disinfection activity against groups of micro-organisms. The potential risks with atypical transmissible agents such as prions and other protein-precipitation-associated diseases are already considered completely outside of such classification systems. It is difficult to estimate the true clinical risks associated with many of these agents. Recent examples with large device-related outbreaks and atypical mycobacteria that are resistant to high-level aldehyde-based disinfectants should be considered as a warning sign. Such high-level disinfectants may be labelled as passing a series of tests, but may not be effective against many types of pathogens. It is expected that many other similar device-related infections may occur due to inadequate disinfection/sterilization but are not always identified, investigated or published. One suggestion is that the Spaulding classification remains the same, but that the required test methods to confirm the various levels of disinfection are changed to include many of the pathogens discussed in this review. As part of this, it is suggested that protozoa (vegetative and dormant forms) should be included for high-level disinfection and sterilization claims. Similarly, if certain types of existing high-level disinfectants/sterilants are not effective against certain types of viruses and mycobacteria, this should be recognized as they may not be applicable for use in certain (such as semi-critical) clinical applications. It should also be considered, especially for sterilization applications, that efficacy against prions should be required to provide a standard precaution against these agents. From this brief review, while the Spaulding classification system is as applicable today as it was in 1957, the expectations for the efficacy of various levels of disinfectants, and even sterilants, and how they are determined may need to be reconsidered.

Conflict of interest statement

Both authors are employed by disinfection/sterilization-related companies.

Funding sources

None.

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