The Spaulding Classification, Disinfection and Sterilization: Is it Time to Reconsider?

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STERIS Corporation

Hosted by
Prof. Jean-Yves Maillard
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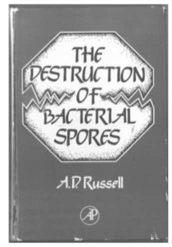
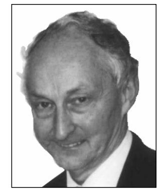
April 28, 2011

The A. Denver Russell Memorial Lecture (2011)





Professor Allan Denver Russell
(1936-2004)

Clinical Microbiology Reviews, Jan. 1999, p. 147–179 Vol. 12, No. 1
0893-8512/99/$04.00+0

Antiseptics and Disinfectants: Activity, Action, and Resistance

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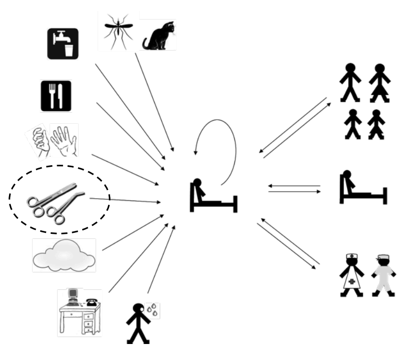
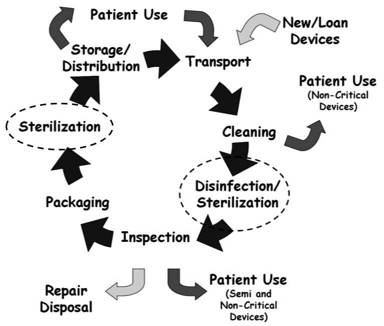
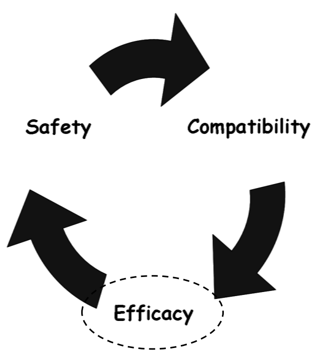
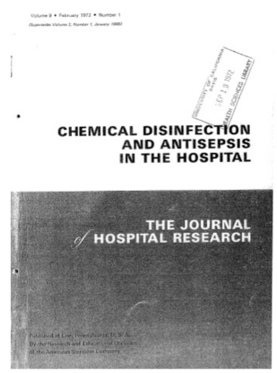




Definitions

- **Cleaning**
  - The removal of contamination from a surface to the extent necessary for further processing or for intended use.
- **Disinfection**
  - The process of reduction of the number of viable microorganisms to a level previously specified
  - Other terms may be used such as 'sanitization', 'pasteurization' and various levels of disinfection (high, intermediate and low)
- **Sterilization**
  - Validated process used to render a product/surface free from viable microorganisms, including bacterial spores.





CHEMICAL DISINFECTION AND ANTISEPSIS IN THE HOSPITAL

Earle H. Spaulding, Ph.D.

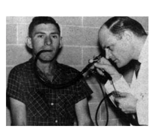
THE JOURNAL OF HOSPITAL RESEARCH

References

[1] Lawrence, C. A., and Block, S. S.: *Disinfection, Sterilization and Preservation*, 1968, Lea & Febiger, Philadelphia.

[2] Sykes, G.: *Disinfection and Sterilization*, 2nd ed., 1965, Lippincott, Philadelphia.

[3] Spaulding, E. H.: Chemical Disinfection in the Hospital, *Jour. Hosp. Research, 3* (No. 1):5-25, 1965.



1957: Hirschowitz, University of Michigan
First Fiberoptic Endoscopes

Spaulding (1972)

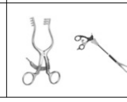
- **Sterilization is the destruction of all microbial forms**
  - **Disinfection is something less than sterilization**
    - **Can destroy most-and often all-microorganisms**
- **Microbial resistance (3 groups)**
  - **Most vegetative bacteria/fungi, large/medium lipid viruses**
  - **Tubercle bacilli, small non-lipid viruses**
  - **Bacterial spores**
- **Levels of germicidal (disinfection) action (3 groups)**
  - **Low level**
  - **Intermediate level**
  - **High level**

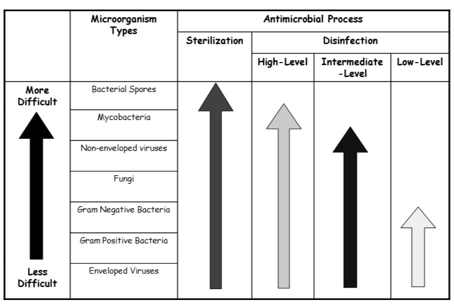
TABLE III – LEVELS OF DISINFECTANT ACTION

	BACTERIA			FUNGI[2]	VIRUSES	
	Vege-tative[1]	Tubercle Bacillus	Spores		Lipid and Medium Size	Nonlipid and Small
HIGH	+*	+	+	+	+	+
INTERMEDIATE	+	+	–	+	+	+
LOW	+	–	–	+	+	–

* + indicates that a cidal effect can be expected when use-concentrations of available disinfectants are properly employed. (1) Common forms of bacterial cells, e.g., staphylococcus. (2) Includes usual asexual "spores," but not necessarily dried chlamydospores and sexual spores.

Spaulding, 1972

Patient Contact	Examples	Device Classification	Minimum Inactivation Level
Intact skin		Non-Critical	Cleaning and/or Low/Intermediate Level Disinfection
Mucous membranes or non-intact skin		Semi-Critical	High Level Disinfection
Sterile areas of the body, including blood contact		Critical	Sterilization

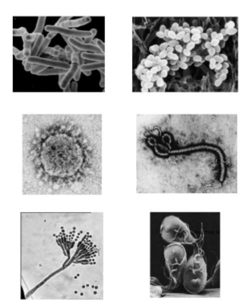


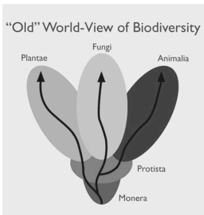
Inactivation Method	Examples
Sterilization	Physical: Steam (e.g., at 121C for 15 mins or 134C for 3 minutes) Chemical: Ethylene oxide or Hydrogen peroxide gas
High-level disinfection	Physical: Hot water (e.g., 93C for 3 minutes), UV light Chemical: Glutaraldehyde, Peracteic acid, Ortho-phathalaldehyde (OPA), sodium hypochlorite (chlorine), hydrogen peroxide
Intermediate-level disinfection	Physical: Hot water (e.g., 90C for 1 minute), UV light Chemical: Phenolics, sodium hypochlorite (chlorine), iodophor
Low-level disinfection	Chemical: Quaternary ammonium compounds (QACs/QUATs), alcohols

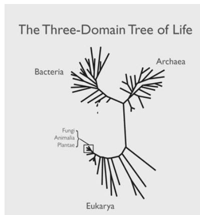
*

Microbiology

- The study of microscopic 'life' ('micro-organisms')
- Microorganisms
  - Bacteria
  - Viruses
  - Fungi
  - Protozoa
  - Helminths ('worms')
- They are not 'simple'
  - Complex, diverse, adaptable…..







'Life on Earth is overwhelmingly microbial. In fact, the extent of microbial diversity is so great that scientists have difficulties estimating its actual size. Some estimates place the number of microbial species in the range of billions, exceeding the number of species of "large" organisms by several orders of magnitude.'

Harvard Magazine, 2007

	Microorganism	Examples
Greater Resistance	Prions	Creutzfeld-Jakob disease
	Bacterial Spores	*Bacillus, Geobacillus, Clostridium*
	Protozoal cysts/Helminth Eggs	*Cryptosporidium, Acanthamoeba, Schistosoma*
	Mycobacteria	*Mycobacterium tuberculosis*
	Small, Non-Enveloped Viruses	Poliovirus, Parvoviruses, Papilloma viruses
	Fungal Spores	*Aspergillus, Penicillium*
	Gram negative bacteria	*Pseudomonas, Escherichia*
	Vegetative Fungi and Algae	*Aspergillus, Trichophyton, Candida,*
	Vegetative Helminths and Protozoa	*Ascaris, Cryptosporidium, Giardia*
	Large, non-enveloped viruses	Adenoviruses, Rotaviruses
	Gram positive bacteria	*Staphylococcus, Streptococcus, Enterococcus*
Less Resistance	Enveloped viruses	HIV, Hepatitis B virus, Herpes Simplex virus

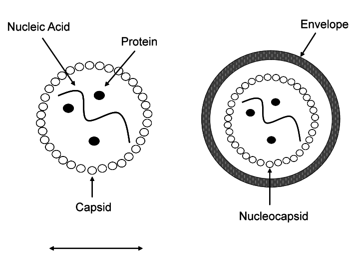
Extreme Resistance

Microorganism	Resistance
Thiobacillus	Arsenic/Copper Resistance
Pseudomonas	Biofilm Formation
Deinococcus	Radiation
Pyrolobus	Temperature (>85^{o}C)
Helicobacter	Acidic pH (1-2)
Geobacillus	All Biocides

Pathogen Surprises

- Viruses
- Bacteria
  - Mycobacteria
- Protozoa
- Prions

Virus Structure



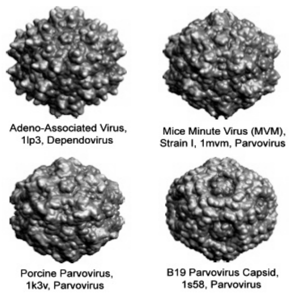
Non-Enveloped Viruses

Table 2. Examples of nonenveloped viruses discovered as new and/or emerging since 1968

Virus	Year of discovery	Virus family (approximate particle size in nm)	Associated disease(s)
Enterovirus 70	1968	Picornaviridae (30)	Acute hemorrhagic conjunctivitis; rare cases of paralysis
Coxsackievirus A24 (variant)	1970	Picornaviridae (30)	Acute hemorrhagic conjunctivitis
Enterovirus 71	1969	Picornaviridae (30)	Aseptic meningitis; hand-foot-mouth disease
Norovirus (Norwalk agent)	1972	Caliciviridae (30)	Acute gastroenteritis
Rotavirus	1973	Reoviridae (70)	Acute gastroenteritis
Parvovirus BI9	1975	Parvoviridae (25)	Aplastic anemia
Hepatitis E	1988	Unclassified (30)	Hepatitis
Anellovirus	1997	Circoviridae (17)	Hepatitis
Bocavirus	2005	Parvoviridae (25)	Respiratory infections

Examples: Parvoviruses

- Non-enveloped, hydrophilic
- Small; 18-26nm
- Single stranded, DNA virus
- Highly resistant to disinfection



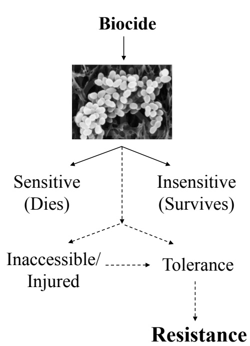
Heat exposure conditions	Time (mins)	Porcine parvovirus	Minute virus of mice	Poliovirus strain Sabin	Adenovirus type 5	Vaccinia virus
Dry Heat 80°C	10	0.5 ± 0.4	0.3 ± 0.1	> 4.6	3.8 ± 0.1	> 4.6
Dry Heat 90°C	1	0.4 ± 0.2	0.4 ± 0.3	> 4.6	3.9 ± 0.2	> 4.6
Moist heat 70°C	10	0.7 ± 0.1	0.6 ± 0.2	> 4.6	> 4.1	> 4.6
Moist heat 80°C	10	5.9 ± 0.1	4.0 ± 0.3	nd	nd	nd
Moist heat 90°C	10	> 6.1	> 4.4	nd	nd	nd
Moist heat 90°C	1	6.0 ± 0.2	3.7 ± 0.4	> 5.0	> 4.1	> 4.6

Eterpi *et al* (2009). *J. Hosp. Infection*

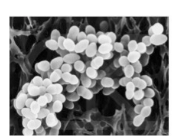
Disinfectant	Contact Time	Disinfectant Reduction (Log_{10})			
		Parvoviruses	Polio	Adeno	Vaccina
Alcohol (70%)	10 mins	<1	2	>4	>4
QUAT (0.05%)	10 mins	<1	<1	1	3
Bleach (1/10)	10 mins	0.6 to 3	3	>4	>4
2% Glutaraldehyde	20 mins	3 to 4	>4	>4	>4
0.55% OPA	10 mins	3 to 4	>4	>4	>4
0.2% PAA (at 20°C)	10 mins	>4	>4	>4	>4

Eterpi *et al* (2009). *J. Hosp. Infection*

Resistance…..is futile?



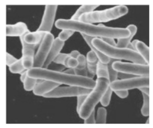
Bacteria Resistance



- Intrinsic (Natural)
  - Cell wall surface
  - Spore formation
  - Biofilm formation
- Acquired
  - Mutations
  - Plasmid/transposon acquisition

Mycobacterium

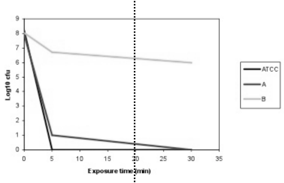
- Slow to very slow growing bacteria, acid-fast, generally Gram^{+}, aerobic, rod-shaped
- Typical pathogens
  - *M. tuberculosis, M. leprae, M. avium*
- Atypical
  - *M. chelonae, M. gordonae, M. fortuitum*
  - Commonly found in water



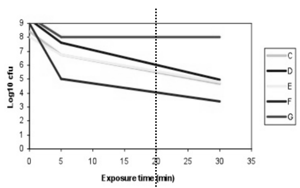
Glutaraldehyde-Resistance

- vanKlingeren & Pullen (1993)
  - Repeated isolation from a washer-disinfector
    - Netherlands
    - Used 2% glutaraldehyde
  - Isolated *Mycobacterium chelonae*
  - Not inactivated at 60min exposure to 2% GTA
- Griffiths *et al* (1997)
  - Isolated *Mycobacterium chelonae*
    - From multiple washer-disinfectors in the UK
    - Used 2% glutaraldehyde
  - Misidentification and iatrogenic infections
  - Not inactivated at 60min exposure to 2% GTA

M. chelonae strains



"Hybrid" mycobacteria strains



2% Glutaraldehyde

McDonnell *et al*, 2009

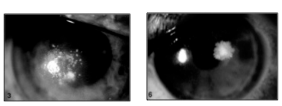
J Clin Microbiol. 2009 Jul;47(7):2149-55. Epub 2009 Apr 29.

Epidemic of postsurgical infections caused by Mycobacterium massiliense.

Duarte RS, Lourenço MC, Fonseca Lde S, Leão SC, Amorim Ede L, Rocha IL, Coelho FS, Viana-Niero C, Gomes KM, da Silva MG, Lorena NS, Pitombo MB, Ferreira RM, Garcia MH, de Oliveira GP, Lupi O, Vilaça BR, Serradas LR, Chebabo A, Marques EA, Teixeira LM, Dalcolmo M, Senna SG, Sampaio JL.

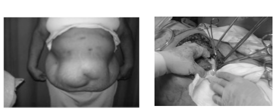
Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil. rsduarte@ufrj.br

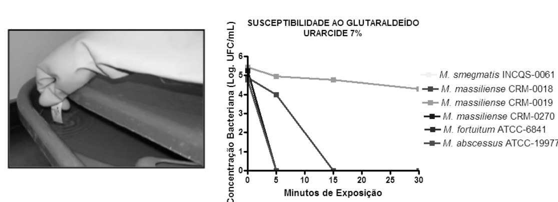
An epidemic of infections after video-assisted surgery (1,051 possible cases) caused by rapidly growing mycobacteria (RGM) and involving 63 hospitals in the state of Rio de Janeiro, Brazil, occurred between August 2006 and July 2007. One hundred ninety-seven cases were confirmed by positive acid-fast staining and/or culture techniques. Thirty-eight hospitals had cases confirmed by mycobacterial culture, with a total of 148 available isolates recovered from 146 patients. Most (n = 144; 97.2%) isolates presented a PRA-hsp65 restriction pattern suggestive of Mycobacterium bolletii or Mycobacterium massiliense. Seventy-four of these isolates were further identified by hsp65 or rpoB partial sequencing, confirming the species identification as M. massiliense. Epidemic isolates showed susceptibility to amikacin (MIC at which 90% of the tested isolates are inhibited [MIC(90)], 8 microg/ml) and clarithromycin (MIC(90), 0.25 microg/ml) but resistance to ciprofloxacin (MIC(90), >or=32 microg/ml), cefoxitin (MIC(90), 128 microg/ml), and doxycycline (MIC(90), >or=64 microg/ml). Representative epidemic M. massiliense isolates that were randomly selected, including at least one isolate from each hospital where confirmed cases were detected, belonged to a single clone, as indicated by the analysis of pulsed-field gel electrophoresis (PFGE) patterns. They also had the same PFGE pattern as that previously observed in two outbreaks that occurred in other Brazilian cities; we designated this clone BRA100. All five BRA100 M. massiliense isolates tested presented consistent tolerance to 2% glutaraldehyde. This is the largest epidemic of postsurgical infections caused by RGM reported in the literature to date in Brazil.



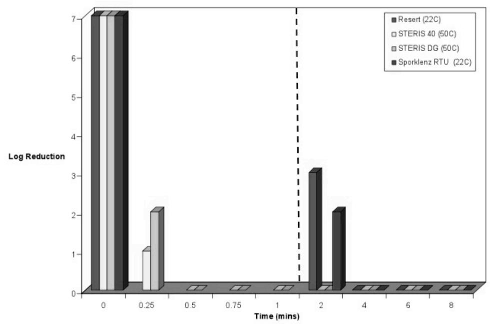
Keratitis
-*M. abscessus*
-*M. chelonae*

Surgical Site Infections
-*M. abscessus*
-*M. chelonae*





M. massiliense BRA-100



USA Study

- Washer-Disinfectors (USA)
  - 3 tested
  - Post cleaning-disinfection-rinsing cycles
  - Disinfectants
    - 2.5% glutaraldehyde at 25C
    - 2.3% glutaraldehyde at 35C
    - 0.55% OPA at 25C
- Microbiology
  - Rinse water (100mL) and swab sites (10)
  - Culturing
    - Mesophilic, aerobic bacteria: TSA agar, 30C, 7 days
    - Mycobacteria: 7H11 agar, 30C, 7-14 days
  - Analysis
    - Identification
    - Biocide sensitivity
    - Resistance investigations

Results

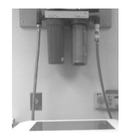
- All washer-disinfectors contaminated post-disinfection cycles
- Range of bacteria identified
  - Many could not be sub-cultured
  - Identifications
    - ***Mycobacterium***
    - ***Methylobacterium***

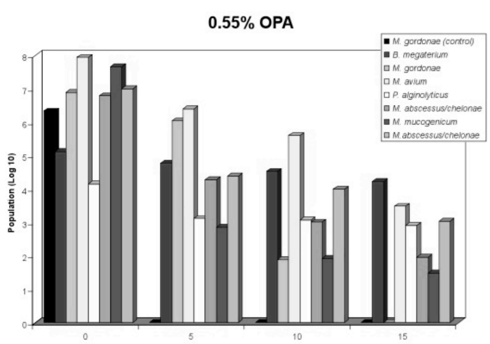
Table 1. Bacterial strains identified from washer-disinfectors

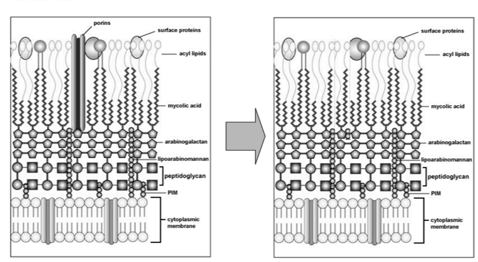
Bacterial Strains	Locations
Mycobacterium neoaurium	**Water Port**
Mycobacterium chelonae	**Final Rinse**
Mycobacterium gordonae	**Cold Tap Spigot**
Mycobacterium gordonae	**Hot Tap Spigot**
Mycobacterium gordonae	**Post-Clean Rinse**
Mycobacterium gordonae	**Rinse 2**
Mycobacterium avium	**Rinse 2**
Mycobacterium mucogenicum	**Post Wash-Rinse Suction Line**
Mycobacterium mucogenicum	**Fluid Sample-Tap Water**
Mycobacterium mucogenicum	**Detergent/Prewash**
Mycobacterium mucogenicum	**Rinse 3**
Mycobacterium abscessus/chelonae	**Post Wash-Rinse Suction Line**
Mycobacterium abscessus/chelonae	**Detergent/Prewash**
Mycobacterium abscessus/chelonae	**Rinse 2**
Mycobacterium abscessus/chelonae	**Rinse 3**
Methylobacterium fujisawaense	**Final Rinse**
Methylobacterium rhodesianum	**Feed Water Hose**
Methylobacterium radiotolerans	**Hot Tap Spigot**
Methylobacterium extorquens	**Glutaraldehyde Line**
Bacillus megaterium	**Feed Water Hose**
Paenibacillus alginolyticus	**Lid**

Contamination Sources

- Contaminated rinse water
  - Same organisms found in the rinse water/lines
- Inadequate disinfection
  - Biofilm development
    - Organisms sensitive to disinfectant when isolated
  - Disinfectant resistance
    - *Mycobacterium*

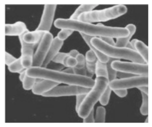






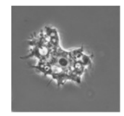
Summary

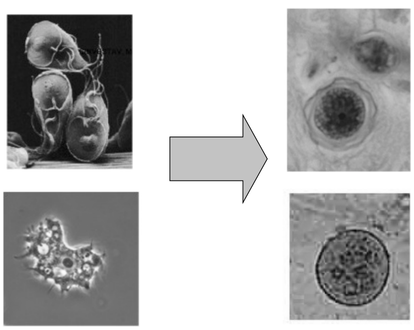
- Mycobacteria can develop high resistance to aldehyde (glutaraldehyde and OPA) disinfectants
  - Can survive normal high level disinfection
- Resistance appears to be due to changes in cell wall structure
  - e.g., deficient in porin proteins (MspA, MspA/C)
- Cross-resistance observed to antibiotics
  - e.g., rifampicin, vancomycin, tetracycline, clarithromycin
- Other impacts
  - Increased pathogenicity



Protozoa

- One of the most abundant forms of microorganisms
- Often difficult to cultivate and diagnose under laboratory conditions
- Pathogen examples
  - *Giardia*
  - *Cryptosporidium*
  - *Plasmodium*
  - *Acanthamoeba*





Oocyst Disinfection

Biocide	*C. parvum* activity
Steam	+
ETO	+
VHP	+
Gas Plasma	+
SYSTEM 1	+
Liquid Peroxide	+/-*
Liquid PAA	+/-*
2% Glutaraldehyde	-
0.55% OPA	-

Cryptosporidium parvum

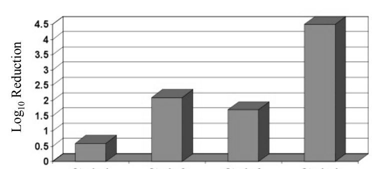
*Depends on temperature, concentration and contact time
Barbee *et al*, 1999
Sell *et al*, 1999
Quilez *et al*, 2005

Acantameoba Cyst

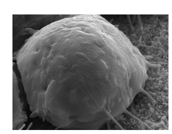
Disinfectant	Contact Time	Disinfectant Reduction (Log_{10})	
		Collection Strains	Hospital Isolates
Hot water (55°C)	10 mins	<1	<1
Hot water (65°C)	10 mins	>5	>5
Bleach (1/10)	10 mins 20 mins	2 to >5 >5	0 to >3.5 1 to >5
2% Glutaraldehyde	20 mins	3 to >5	0 to 4
0.55% OPA	10 mins	2 to 3	1 to 4
2% Hydrogen Peroxide	10 mins	>5	>5
0.2% PAA (at 55°C)	10 mins	>5	>5

Coulon et al, 2010. *Journal of Clinical Microbiology*, *48*, 2689-2697.

Acantameoba Trophozoites

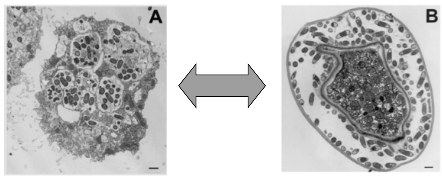


Coulon *et al*, 2010. 2% glutaraldehyde, 20 mins



Trophozoites in suspension fixed in 2.5% glutaraldehyde for 3 days at +4°C, stained and observed

More that just Protozoa



Amoebal Trophozoite | Amoebal Cysts

REVIEW ARTICLE

Free-living amoebae and their intracellular pathogenic microorganisms: risks for water quality

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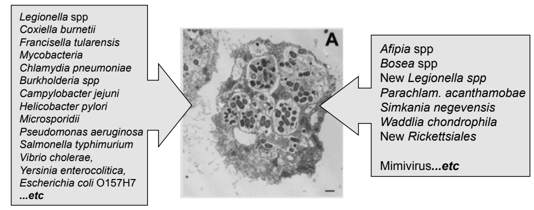
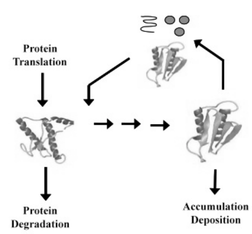


Prion Diseases

- **What are 'Prions'?**
  - ***Still debated!***
  - **Proteins**
  - **Appear to be devoid of nucleic acid**
  - **Identified as the causative agents for a group of central nervous system diseases**
    - **TSEs**
    - **CJD, vCJD**



McDonnell G. (2010). Managing Medical Devices Contaminated with Prions: What's New? In, W.A. Rutala (ed.) Disinfection, Sterilization and Antisepsis in Healthcare.

Infection Control Concerns

- **100% fatal**
- **Transmissible**
  - **Medical/surgical devices**
  - **Tissues, including blood**
  - **Environment**
- **'Resistance'**
  - **Cleaning**
  - **Disinfection/Sterilization**

Cleaning

Method	'Log' Reduction
Water Washing + Steam Sterilization[1]	~5.5
Klenzyme	~4.5
Enzyme Cleaner 2	~1
Klenzyme + Steam Sterilization[2]	~6.5
Enzyme Cleaner 2 + Steam Sterilization[1]	~3.0

[1]134ºC x 18 mins
[2]121ºC x 20 mins

Fichet *et al* (2004) Lancet
McDonnell (2007) J. Periop Practice

Alkaline Cleaning

Method	'Log' Reduction
Hamo100 (1.6%; 43ºC; 15 mins) (0.8%; 43ºC; 7.5 mins) (0.8%; 43ºC; 7.5 mins); Plastic wires (0.2%; 55ºC; 5 mins + steam sterilization 134ºC; 4 mins)	>5 >5 >5 >5
Prolystica Alkaline 2x (0.4%, 5 mins, 65C) + steam sterilization (134ºC; 4 mins)	>5
Prolystica Alkaline 10x (0.04%, 5 mins, 65C) + steam sterilization (134ºC; 4 mins)	>5

Chemical Sterilization

Method	Test Parameters	'Log' Reduction
V-Pro 1	1, 3 or 6 pulses at ~1.6mg/L gas under vacuum	>5
Gas Plasma (STERRAD NX)	1 or 2 Advanced Cycles at ~ 8mg/L gas under vacuum	>5
Gas Plasma (STERRAD 100S)	2 or 4 pulses at ~ 6mg/L gas under vacuum	~1

Fichet *et al* (2007) J Hosp Infect 47: 279-287
Yan *et al* (2008) Cent Steril 16: 26-34

PRIONS AND POTENTIAL PRIONOIDS

Disease	Protein	Molecular transmissibility	Infectious life cycle
Prion diseases	PrP^{Sc}	Yes	Yes
Alzheimer's disease	Amyloid-β	Yes	Not shown
Tauopathies	Tau	Yes	Not shown
Parkinson's disease	α-Synuclein	Host-to-graft	Not shown
AA amyloidosis	Amyloid A	Yes	Possible
Huntington's disease	Polyglutamine	Yes	Not shown
Phenotype	**Protein**	**Molecular transmissibility**	**Infectious life cycle**
Suppressed translational termination (yeast)	Sup35	Yes	Not shown
Heterokaryon incompatibility (filamentous fungi)	Het-s	Yes	Not shown
Biofilm promotion (bacteria)	CsgA	Yes	Not shown

In humans and animals, infectious prion diseases are caused by PrP^{Sc}, which spreads by recruiting its monomeric precursor PrP^{C} into aggregates. Aggregates then multiply by breakage, a process that is termed molecular transmissibility. Other proteins involved in disease and in phenotypes of fungi and bacteria, can also undergo self-sustaining aggregation, but none of these 'prionoid' proteins behaves like typical infectious agents, nor do any of them enact a complete infectious life cycle — with the possible exception of AA amyloid.

Aguzzi, 2009. *Nature* 459: 924-925

Conclusions

The A. Denver Russell Memorial Lecture