Principals of Disinfection, Antisepsis, and Chemical Sterilization
Jason A. Tetro, University of Ottawa
A Webber Training Teleclass

PRINCIPLES OF DISINFECTION, ANTISEPSIS
AND CHEMICAL STERILIZATION:
CONCEPTS AND CONTROVERSIES

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Hosted by Paul Webber
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CURRENT STATUS OF PATHOGENS

• OVER 1,400 KNOWN PATHOGENS FOR HUMANS
• SOME 200 PATHOGENS ARE STILL UNCHARACTERIZED
• IN THE LAST 30 YEARS, SOME 300 NEW AND EMERGING INFECTIOUS DISEASE EVENTS HAVE OCCURRED
• MANY HAVE HAD IMPLICATIONS ON A GLOBAL SCALE
  - SARS
  - AVIAN INFLUENZA
  - C. DIFFICILE
  - VRE
  - MDR/XDR TB

BREAKDOWN OF KNOWN HUMAN PATHOGENS

518 BACTERIA (35%)
217 VIRUSES & PRIONS (15%)
287 HELMINTHS (20%)
397 FUNGI (22%)
66 PROTOZOA (5%)

BACTERIA

• UBQUITOUS IN MANY ENVIRONMENTAL SETTINGS
  - BIOFILMS ARE A PRIMARY CONCERN
• SEVERAL ENVIRONMENTAL PATHOGENS
  - GRAM POSITIVE BACTERIA
    - STAPHYLOCOCCUS, STREPTOCOCCUS, BACILLUS
  - GRAM NEGATIVE
    - ESCHERICHIA, PSEUDOMONAS, SALMONELLA
• GENERALLY EASY TO KILL WITH DISINFECTION AND ANTISEPSIS
  - BIOFILMS MAY PROVIDE INCREASED RESISTANCE

MYCOBACTERIA

Mycobacterium tuberculosis
• CAUSE DISEASES SUCH AS TUBERCULOSIS
• MANY NON-TUBERCULOUS (ENVIRONMENTAL) TYPES CAUSE DISEASE IN THE IMMUNO-SUPPRESSED
• MANY ARE SLOW & HARD TO GROW IN THE LAB
• EMERGING FOOD- & WATERBORNE PATHOGENS
• BIOFILMS HARBOUR NUMEROUS MYCOBACTERIAL SPECIES
• HIGHER RESISTANCE THAN NORMAL BACTERIA TO INACTIVATION

BACTERIAL SPORES

• PRODUCED BY CERTAIN TYPES OF GRAM-POSITIVE BACTERIA (BACILLUS, CLOSTRIDIUM)
• SPORES SURVIVE WELL IN THE ENVIRONMENT
  - ABLE TO WITHSTAND pH, HEAT, ACID AND OTHER STRESSORS
• CLOSTRIDIUM DIFFICILE IS AN IMPORTANT PATHOGEN
• TETANUS & ANTHRAX ALSO CAUSED BY SPORE-FORMERS
• ONE OF THE MOST DIFFICULT MICROBIAL FORMS TO KILL
• COMMONLY USED AS BIOINDICATORS
  - GOLD STANDARD FOR STERILIZATION TESTING

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**VIRUSES**

- **Two major forms of viruses**
  - Enveloped viruses
    - Influenza, Coronavirus and HIV
  - Non-enveloped viruses
    - Norovirus, Poliovirus, Adenovirus, Rotavirus
- Enveloped viruses are generally easy to inactivate due to their lipid envelope
- Non-enveloped viruses are more resistant as they contain tightly organized protein that may be difficult to disrupt or denature

**FUNGI**

- **Uni-cellular or multi-cellular plants**
- Cause many diseases
- A serious problem for the immuno-suppressed
  - Organ transplant, cancer treatment etc.
- Fungi associated with publically known problems
  - Mold and mildew
  - Sick building syndrome
  - Hypersensitivity pneumonitis

**PROTOZOA**

- **Microscopic, single-celled animals**
- Many have complex life cycles
  - E.g. cysts, trophozoites
- Emerging as water- & foodborne pathogens
  - Giardia & Cryptosporidium
  - Toxoplasma gondii
  - Acanthamoeba spp.
- Gastrointestinal, eye & infections
- "Oocysts" are very difficult to inactivate
- Resist numerous environmental factors

**PRIONS**

- Cause many slowly progressive diseases of the central nervous system
  - Kuru
  - Creutzfeldt-Jakob Disease (CJD)
  - Iatrogenic cases
  - New variant CJD
- Once thought to be highly resistant to disinfection but evidence suggests otherwise
  - 134 degrees C for 18 min or 121 degrees C for 30 min
  - 1N Sodium Hydroxide for 15 min
  - Enzymatic cleaners
  - Phenolic disinfectant

**DEFINITIONS**

- **Chemical sterilant (cold sterilant)** – A chemical in gaseous form that kills all life – validated process
- Decontamination – physical or chemical procedure to remove or inactivate microbes to safe levels
  - Usually a process that includes disinfection and/or sterilization
- Load – interfering material to challenge an active
  - Soil load – organic and inorganic material
  - Bioburden – only organisms
- Microbicidal – general term for a disinfectant or an antiseptic
  - Moving away from ‘germicidal’
- Test protocol – a specific method designed to challenge an antimicrobial agent against a specific microbe
## Principles of Microbicide Use

- **Disinfectants vs Antiseptics**
  - Difference is target
  - Disinfection for decontamination of surfaces
  - Antisepsis for decontamination of skin & mucous membranes
- **Issues for Consideration are the Same**
  - Claims
  - Physical Environment
  - Microbial Resistance
- **Application Environment**
  - Objects
  - Frequently touched surfaces
  - Skin

## Choosing a Product

- Extensive information in a limited space
- Can be difficult to interpret
- Knowledge of what's on the label is not enough
- Take a closer look at some of the more important aspects of what is on the label
- Center for Food Security and Public Health, Iowa State University

## Looking at and Past the Labels

- A disinfectant or antiseptic label is highly regulated
- The breakdown of a label is strictly adhered based on extensive testing of the active in laboratory environments
- Labels may cause confusion
  - Water as a diluent (means distilled water)
  - Tap water is normally used in the field
  - Contact time
    - A few seconds in application vs. a few minutes on the label
  - Different dilutions
    - Separate dilutions for different classes of pathogens (target unknown in the field)

## Claims

- Claims are validated using various testing methods
  - Tests methods are standardized (AOAC, ASTM, EU)
  - Which method used depends on location (Europe, U.S., Canada)
  - Actives must meet reduction levels to validate a claim
- Sporicidal, mycobactericidal & bactericidal activity
  - Product required to reduce the viability titre of the test organisms by at least 6 Log₁₀ (one million fold)
- Virucidal activity
  - Product required to reduce the viability titre of the test organisms by at least 3 Log₁₀ is needed
- Fungicidal activity
  - A minimum reduction of 5 Log₁₀ is required

## Issues with Testing Claims

- Levels of active
  - Different concentrations offer different claims
  - Hypochlorite, peroxide, ethanol
- Protocol Issues
  - Suspension tests
  - Carrier tests
  - Skin-based tests (in vivo)
- Contact times
  - Requires "wet contact" between active and organism
  - Most contact times are not in line with realities of use
  - Several seconds vs. several minutes on label
- Residual/Persistent Activity
  - How is the activity measured over several hours?
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PHYSICAL FACTORS

- **pH**
  - Microbicides have specific pH optimal levels
  - Bleach is best active at low pH (~5.0)
  - Aldehydes are best at higher pH (~8.0)

- **Temperature**
  - Activity should be optimal at room temperature
  - For Antiseptics, activity should be optimal at skin temperature (~33°C)

- **Relative Humidity**
  - Enveloped viruses prefer lower RH
  - Non-enveloped viruses prefer higher RH
  - Bacteria & fungi prefer higher RH
  - Spores can survive well under a wide range of RH
  - Optimal range of use should be 40-60%

MICROBIAL RESISTANCE

- **Resistance is a major issue to consider**
  - Need a good knowledge of the target organisms
  - Need to determine the highest level of an active
  - Always err on the side of caution

- **Resistance can be generalized based on the nature of the organism**
  - Bacteria and enveloped viruses are easiest to kill
  - Non-enveloped viruses are mid-range
  - Protozoan cysts and spores have high resistance
  - Prions are the most difficult to inactivate

RELATIVE MICROBIAL RESISTANCE

- **Cold Sterilant**
  - Kills all organisms and ensures sterility

- **High-Level Disinfector**
  - Kills all organisms, except high levels of bacterial spores

- **Intermediate-Level Disinfector**
  - Kills Mycobacteria, most viruses, and bacteria with a chemical microbicide registered as a “tuberculocide”

- **Low-Level Disinfector**
  - Kills some viruses and bacteria with a chemical microbicide

DISINFECTION LEVELS

- **Materials Compatibility**
  - Plastics, stainless steel, metal alloys, skin, etc.

- **Dosage & Rate of Application**
  - Least amount of active as infrequent as possible

- **Training and Ease of Use**
  - Personal protection, open vs. closed environment, dilution considerations

- **Safety of Personnel & Environment**
  - Odor, environmental pollution, exposure limits

LIST OF ACTIVES

- **Many actives known and available commercially**
  - Only a few are relevant to this scope

- **Focus will be on the mechanism and the claims**
  - Chemical structure
  - General concepts on activity
  - Claims that are known for the active

- **Issues regarding any controversies will be mentioned but not thoroughly discussed**

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CHLORINE: GAS, LIQUID OR POWDER

- MOST WIDELY USED MICROBICIDE
- EFFECTIVE AGAINST MOST PATHOGENS
  - DISRUPTION OF CELL WALLS, MEMBRANES AND PROTEINS
  - AMOUNT OF CHLORINE NEEDED DEPENDS ON
    - NATURE OF THE PATHOGEN
    - ORGANIC DEMAND FOR CHLORINE
    - pH OF THE ENVIRONMENT (LOWER = MORE ACTIVE)
- SODIUM HYPOCHLORITE (BLEACH) IS MOST COMMON
  - CHLORINE GAS USED FOR WATER & SEWAGE DISINFECTION
  - CHLORINE DIOXIDE & MONOCHLORAMINE ALSO USED FOR WATER DISINFECTION

PHENOLIC COMPOUNDS

- LONG-STANDING MICROBICIDAL AGENTS
  - VARIETY OF USES DEPENDING ON MODIFICATIONS
    - RESORCINOL – LOZENGES
    - P-CHLORO-M-XYLENOL (PCMX) – DISINFECTANT (DETTOL)
    - TRICLOSAN - ANTIBACTERIAL
  - USED FOR LIPID AND PROTEIN DISRUPTION
    - LIPID NATURE OF PHENOLICS DISRUPT MEMBRANES
  - GOOD BACTERICIDES & MYCOBACTERICIDES BUT LIMITED ACTIVITY AGAINST NON-ENVELOPED VIRUSES
  - TOXICITY NEEDS TO BE FULLY UNDERSTOOD
    - PHENOL IS A HUMAN TOXIN IN NATURAL FORM

SURFACHTANTS

- POSSESS ONE HYDROPHOBIC AND ONE HYDROPHILIC TAIL
- MOST COMMON ARE QUATERNARY AMMONIUM COMPOUNDS
  - CAUSE MEMBRANE DISRUPTION
  - EXCELLENT AGAINST BACTERIA AND ENVELOPED VIRUSES
  - POOR AGAINST NON-ENVELOPED VIRUSES, MYCOBACTERIA AND SPORES
  - ENVIRONMENTAL CONCERNS BECAUSE OF LOW BIODEGRADABILITY

ALDEHYDES

- ALKYLATING AGENTS
  - FORMALDEHYDE
  - GLUTARALDEHYDE
  - OPA (ORTHO-PHTALDEHYDE)
  - USED IN LOW CONCENTRATIONS TO ‘FIX’ PROTEINS
    - REQUIRES HIGH pH (≥8) FOR OPTIMAL ACTIVITY
    - HIGHLY LIPOPHILIC
  - GOOD AGAINST MOST PATHOGENS EXCEPT PRIONS AND SOME PROTOZOA CYSTS
  - FORMALDEHYDE AND GLUTARALDEHYDE ARE TOXIC
    - OPA IS LESS SO BUT STILLRequires CARE

METALS

- DOSE-DEPENDENT ACTION
  - TOXIC AT FOR ALL ORGANISMS IN HIGH DOSES
  - AT LOWER DOSES, SOME DEMONSTRATE ANTIMICROBIAL PROPERTIES
  - HEAVY METALS ARE BACTERICIDAL AND FUNGICIDAL
  - HAVE LITTLE EFFECT ON VIRUSES AND SPORES
  - COPPER IS NOW A REGISTERED ANTIMICROBIAL IN THE U.S.
    - COPPER SURFACES SHOWN TO KILL AND RESIST MICROBIAL GROWTH
    - CAUSES DAMAGE TO THIO (-SH) GROUPS PREVENTING GROWTH
    - COMPATIBILITY WITH OXIDATION DISINFECTANTS AN ISSUE
  - SILVER HAS BEEN USED AS AN ANTIMICROBIAL FOR CENTURIES
    - SILVER NITRATE (AgNO₃) INTERACTS WITH MEMBRANES AND DNA
    - USED AS AN ANTISEPTIC
    - NOW CONSIDERED AS A BACTEROIDSTATIC AGENT IN TEXTILES
    - SILVER RESISTANCE IN BACTERIA HAS BEEN KNOWN TO EXIST

PEROXYGENS

- STRONG OXIDIZING AGENTS
  - PRODUCE FREE RADICALS THAT DAMAGE CELLS
  - HYDROGEN PEROXIDE, PERACETIC ACID
  - EFFECTIVE AGAINST MOST PATHOGENS INCLUDING SPORES AT HIGHER CONCENTRATIONS
    - CONC. DEPENDS ON ACTIVITY REQUIRED
    - DETERGENTS, ORGANIC ACIDS & ANTI-CORROSIVES ADDED TO H₂O₂
  - ISSUES WITH PEROXYGENS
    - PERACETIC ACID IS HIGHLY CORROSIVE AND ONLY USED IN CONTROLLED ENVIRONMENTS
    - PEROXIDE REQUIRES STABILIZATION FOR LONG-TERM USE
    - NO RESIDUE; ENVIRONMENTALLY SAFE
ALCOHOLS

- Fixative action through disruption of protein structure
- Two major alcohol types
  - Ethanol (usually 62-80%) – good wide-spectrum microbicidal
  - Isopropanol (60-95%) – only good bactericide
- Both are ineffective against spores
- Concerns over best concentration for use
  - 62%, 70%, 80%, 95%
  - Studies in our lab show that 62% may be the best as antiseptic

COLD STERILIZATION

- Only three are approved by the FDA
  - Ethylene oxide
  - Ozone
  - Hydrogen peroxide
- Must be used under highly controlled environments
  - More modern uses include ‘autoclave-like’ models that sterilize medical devices in hospitals
- Only trained individuals may work with cold sterilants
  - Working with gaseous agents brings higher risk
  - More controlled machinery helping

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OZONE

- Strong oxidizer
- Highly toxic
- Threshold for humans < 0.1 PPM/day
- Usually 1-3 PPM are used

ETHYLENE OXIDE

- Strong alkylator
- Highly toxic
- Threshold for humans is < 5 PPM/day
- Some companies use up to 1500 PPM
- Required aeration times may take hours

GASEOUS HYDROGEN PEROXIDE

- In gaseous/plasma or vaporized form
  - Oxidation is as potent as or better than in liquid form
  - Increased potential when charged (plasma)
  - Compatibility with almost all materials
  - Easily aerated
- Used at room temperature and RH between 35-80%
- Toxicity is low as only 10 PPM required for use
- Longer times needed over others
- Controlled settings also needed
- Number of models using hydrogen peroxide increasing

OVERVIEW OF ACTIVITIES

<table>
<thead>
<tr>
<th>Active Ingredient</th>
<th>Activity Against</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD. HYPPOCHLORITE (1 PPM)</td>
<td>B</td>
</tr>
<tr>
<td>SOD. HYPPOCHLORITE (1000 PPM)</td>
<td>B,EV,NEV,F,M</td>
</tr>
<tr>
<td>D-PHENYLPHENOL (200 PPM)</td>
<td>B,M,NEV</td>
</tr>
<tr>
<td>QUATERNARY AMMONIUM COMPOUNDS (100-300 PPM)</td>
<td>B, EV, F</td>
</tr>
<tr>
<td>ALKALINE GLUTARALDEHYDE (2%)</td>
<td>B, EV, NEV, M,F,S</td>
</tr>
<tr>
<td>HYDROGEN PEROXIDE (3%)</td>
<td>B</td>
</tr>
<tr>
<td>ACCELERATED HYDROGEN PEROXIDE (7.5%)</td>
<td>B, EV, NEV, M,F,S</td>
</tr>
<tr>
<td>PERACETIC ACID (1-1500 PPM)</td>
<td>B, EV, NEV, M,F,S</td>
</tr>
<tr>
<td>ETHANOL 62% (V/V)</td>
<td>B, EV, NEV, F</td>
</tr>
</tbody>
</table>

- B = Bacteria, EV = Enveloped Viruses, NEV = Non-Enveloped Viruses, M = Mycobacteria
- F = Fungi, S = Bacterial Spores

LAST THOUGHTS

- Infectious disease is one of the greatest problems facing humanity today
- Need to be diligent to prevent pathogen spread
  - Each of us needs to be informed and willing to comply
- Disinfection, antiseptic and sterilization are options but always remember that prevention of infectious disease spread is based more on human action than chemical activity
  - “Hygiene is two thirds of health” – Lebanese proverb
  - “Nine-tenths of our sickness can be prevented by right thinking plus right hygiene” – Henry Miller
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March 5
Fundamentals of Disinfection, Antisepsis, and Chemical Sterilization
Jason Tetro, University of Ottawa

March 10
Fundamentals of HAI Definitions
Robert Garcia, Brookdale University, New York

March 18
Basics of Steam Sterilization
Dr. Lynne Sehulster, CDC

March 26
Basics of Controlling Device-Related Infections
Loretta Litz Fauerbach, Shands Hospital, University of Florida

March is Novice Month

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