Microbiology

Sterilization and Disinfection

Definitions

- Sterilization
  - Removal or destruction of all microbial life forms
    - Heat
    - Ethylene oxide (Gas)
    - Filtration
- Commercial sterilization
  - Heat required would degrade food
  - Heated to kill *Clostridium botulinum* endospores
  - Non-pathogens may survive, but do not grow

Definitions

- Commercial sterilization
  - Heat required would degrade food
  - Heated to kill *Clostridium botulinum* endospores
  - Non-pathogens may survive
    - Do not grow at storage temperature
    - Could grow if incubated

Definitions

- Disinfection
  - Destroying harmful microorganisms
  - Implies inert surface or substance
  - Destruction of vegetative (non – endospre forming) pathogens
  - Forms
    - Chemicals
    - Ultraviolet radiation
    - Boiling water or steam

Definitions

- Antisepsis
  - Disinfection directed towards living tissue
  - Antiseptic
    - Listerine
      - Antiseptic in the mouth or cut
      - Disinfectant on the table
  - Chemical must not be caustic to tissue

Definitions

- Degerming (degermation)
  - Mechanical removal of microbes in a limited area
    - Alcohol swab
- Sanitization
  - Lower microbe counts to safe public health levels
  - Minimize chance of disease spread
    - Bar glasses

Definitions

- - cide
  - Causes death of organism
    - Homicide
    - Virucide
    - Fungicide
    - Germicide
      - May not kill endospores
  - Bactericidal
    - Penicillin

Definitions

- - stat or –stasis
  - Inhibit growth and multiplication of microorganism
    - Bacteristatic antibiotics
      - Tetracycline

Definitions

- Sepsis
  - Indicates bacterial contamination
    - Septic tanks
    - Septicemia
- Asepsis
  - Absence of significant contamination
    - Aseptic technique

Definitions

TABLE 7.1	Terminology Relating to the Control of Microbial Growth	
	Definition	**Comments**
Sterilization	Destruction or removal of all forms of microbial life, including endospores.	Usually done by steam under pressure or a sterilizing gas such as ethylene oxide.
Commercial Sterilization	Sufficient heat treatment to kill endospores of *Clostridium botulinum* in canned food.	More-resistant endospores of thermophilic bacteria may survive, but they will not germinate and grow under normal storage conditions.
Disinfection	Destruction of vegetative pathogens.	May make use of physical or chemical methods.
Antisepsis	Destruction of vegetative pathogens on living tissue.	Treatment is almost always by chemical antimicrobials.
Degerming	Removal of microbes from a limited area, such as the skin around an injec tion site.	Mostly a mechanical removal by an alcohol-soaked swab.
Sanitization	Treatment intended to lower microbial counts on eating and drinking utensils to safe public health levels.	May be done with high-temperature washing or by dipping into a chemical disinfectant.

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Rate of Microbial Death

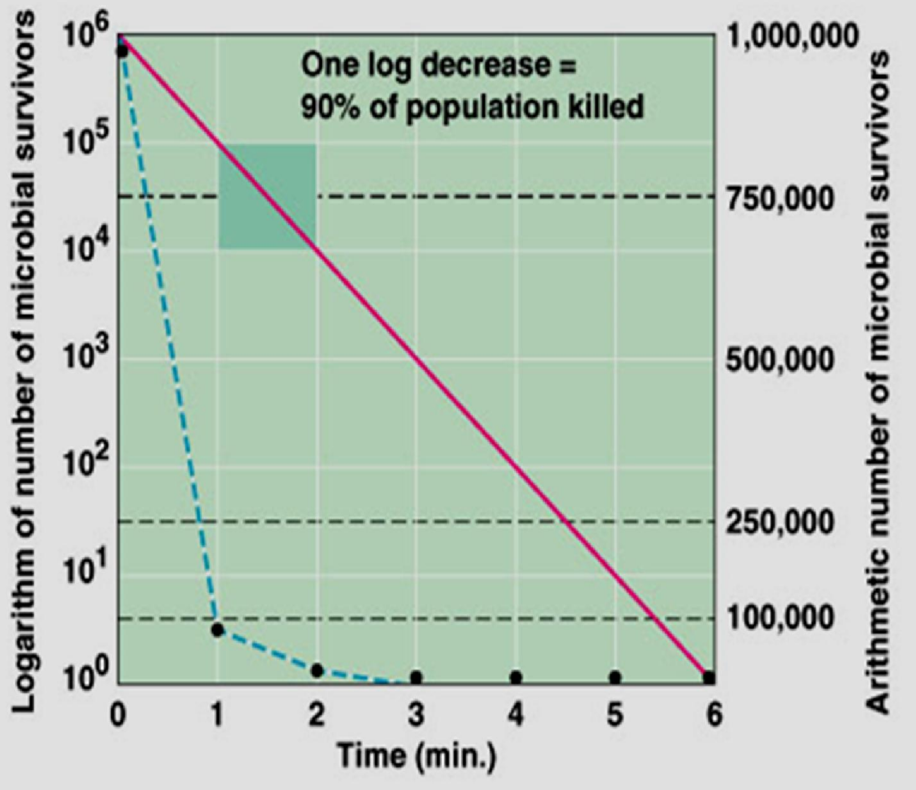
- Bacterial death occurs at a constant rate
  - I.e. 90% killed every minute of contact

TABLE 7.2 Microbial Death Rate: An Example

Time (mm)	Deaths per Minute	Number of Survivors
0	0	1,000,000
1	900,000	100,000
2	90,000	10,000
3	9000	1000
4	900	100
5	90	10
6	9	1

Rate of Microbial Death

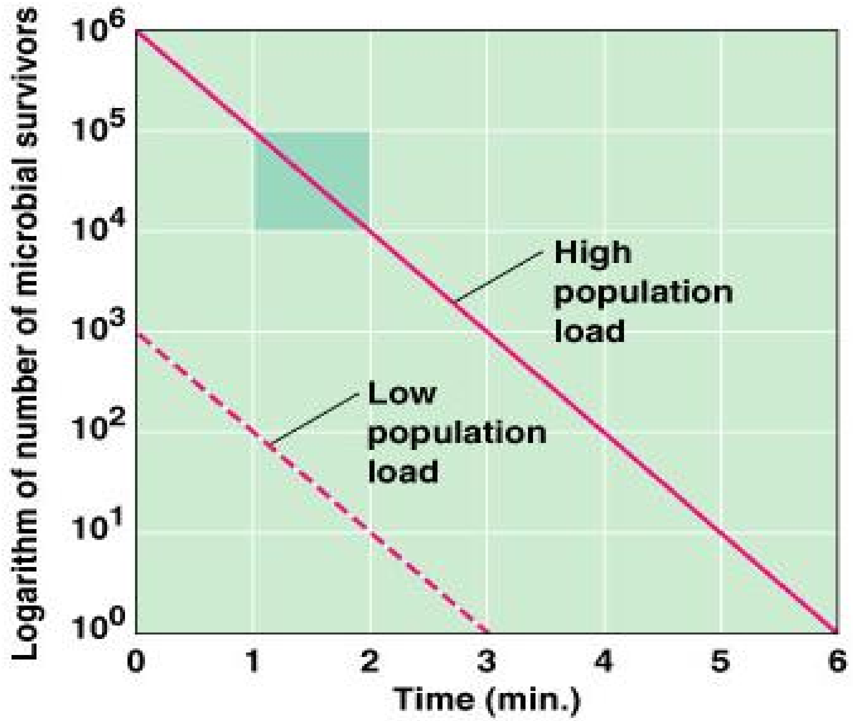
- Plotting microbial death
  - Death curve
    - Logarithmically
      - Linear
    - Arithmetically
      - Exponential curve



(a) The curve is plotted logarithmically (solid line) and arithmetically (broken line). In this case, the cells are dying at a rate of 90% each minute.

Factors Affecting Antimicrobials

- 1- Number of microbes present
  - More microbes = longer time to kill
  - Also called load



(b) The effect of high or low initial load of microbes. If the rate of killing is the same, it will take longer to kill all members of a larger population than a smaller one. This is true for both heat and chemical treatments.

Factors Affecting Antimicrobials

- 2 – Environmental factors
  - Organic matter
    - Often inhibits antimicrobials
      - Feces
      - Vomit
      - Blood
  - Temperature
    - Temperature dependent reactions
    - Warm temperatures are preferred

Factors Affecting Antimicrobials

- 3-Time of exposure
  - “contact time”
  - Extended times for endospores
  - Longer times can offset lower temperatures
    - Milk pasteurization
- 4-Microbial characteristics
  - Virus vs gram+ vs gram -

Actions of Microbial Agents

- Actions of microbial agents
  - Alter membrane permeability
    - Damage phospholipids or proteins in plasma membrane
    - Cellular contents leak out
    - Interferes with growth

Actions of Microbial Agents

- Damage to proteins and nucleic acids
  - Denatures proteins
  - Enzyme
    - Proteins necessary for bacteria metabolism
    - Shape necessary for function
      - Hydrogen bonds broken shape changes
      - Covalent bonds are broken
      - Sulfhydryl bonds – SH
      - All can be broken
  - Nucleic Acids
    - DNA and RNA
    - Can no longer replicate or synthesize proteins

Physical Methods of Microbial Control

- Heat
- Filtration
- Low temperatures
- High pressure
- Desiccation
- Osmotic pressure
- Radiation

Physical Methods of Microbial Control

- Heat
  - Common food preservation
  - Denatures protein
    - Changes shape
  - TDP – thermal death point
    - Lowest temp all microorganisms in a particular liquid suspension will be killed in 10 minutes
  - TDT – thermal death time
    - Minimal length of time required to kill all microorganisms in a suspension at a given temperature
  - DRT – decimal reduction time
    - Time in minutes in which 90% of bacteria at a given temperature will be killed

Physical Methods of Microbial Control

- Heat (cont)
  - Moist heat
    - Coagulation (denaturing) of proteins
    - Hydrogen bonds are broken
      - Egg white
    - Boiling
      - Kills most vegetative pathogens, viruses, fungi and spores within 10 minutes

Physical Methods of Microbial Control

- Boiling
  - some microbes resistant to boiling
    - Endospores (20 Hours)
    - Hepatitis viruses (30 minutes)
  - Not always effective
  - Kills most pathogens

Physical Methods of Microbial Control

- Heat (cont)
  - Autoclave
    - Moist heat (steam) and pressure
    - Preferred method
    - Limitations
      - Material must be able withstand heat and moisture
    - Steam under pressure increases temperature
      - Makes steam more effective
      - 15 psi (121° C) for 15 minutes will kill all organisms

Autoclaving

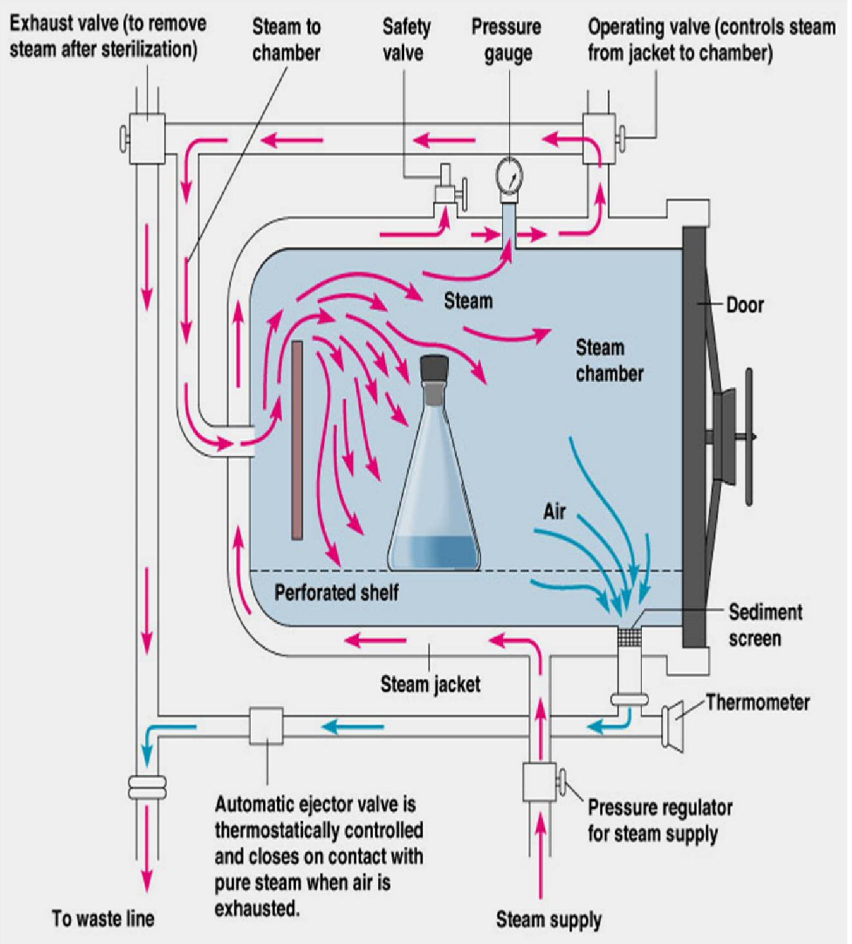


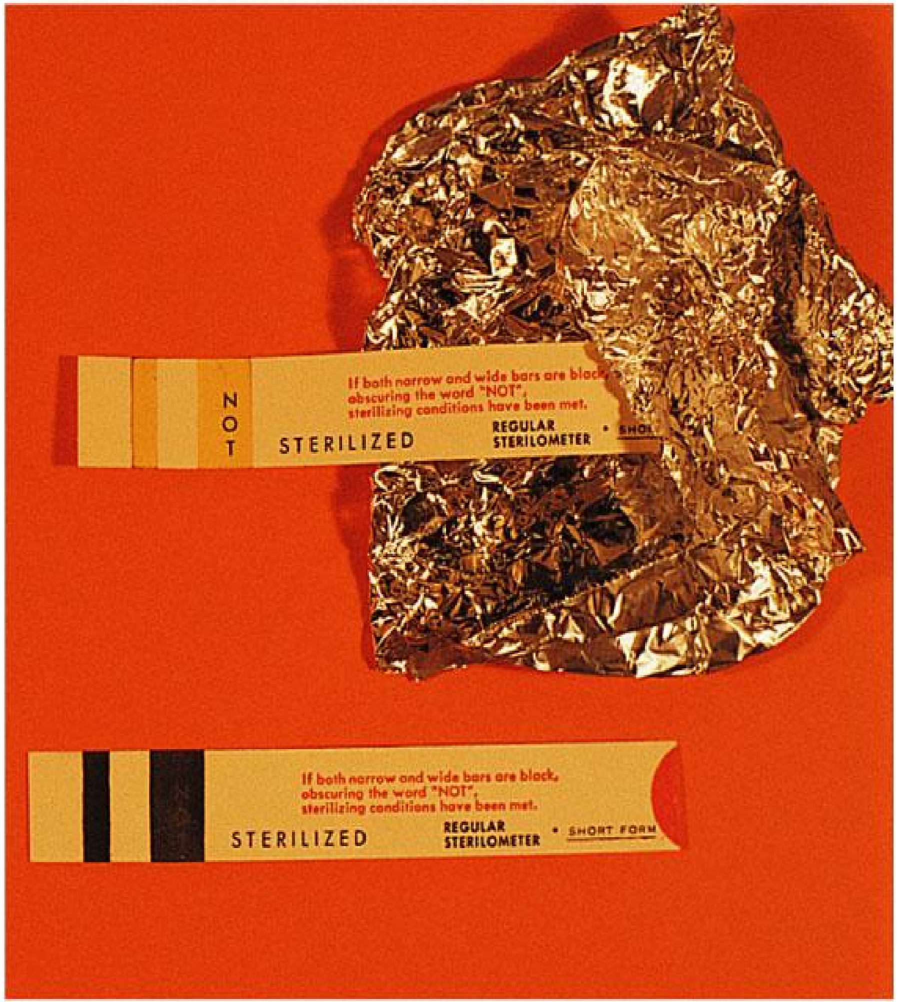
TABLE 7.3 The Relationship Between the Pressure and Temperature of Steam at Sea Level*

Pressure (psi in excess of atmospheric pressure)	Temperature (°C)
0 psi	100
5 psi	110
10 psi	116
15 psi	121
20 psi	126
30 psi	135

*At higher altitudes the atmospheric pressure is less, which must be taken into account in operation of an autoclave. For example, in order to reach sterilizing temperatures (121°C) in Denver, Colorado, whose altitude is 5280 feet (1600 meters), the pressure shown on the autoclave gauge would need to be higher than the 15 psi shown in the table.

Autoclaving

- Autoclaving
  - Extra time to reach center of solids
  - Paper should be used to wrap instruments
  - Indicators
    - Strips
    - Tape



Pasteurization

- Pasteurization
  - Louis Pasteur
  - Mild heating
    - Kills most pathogens
    - Kills bacteria that cause spoilage
    - Preserves taste of product
    - Lowers bacterial numbers
  - Phosphatase test
    - Enzyme inactivated if properly pasteurized

Pasteurization

- Pasteurization
  - Initially 63° C for 30 minutes
  - High – temperature short – time pasteurization (HTST)
    - Kills pathogens
    - Lowers bacterial numbers, milk keeps while refrigerated
  - Ultra – high – temperature pasteurization (UHT)
    - Milk can be stored with no refrigeration
    - Super heated steam (temp goes 74° to 140° to 74° in 5 sec)

Pasteurization

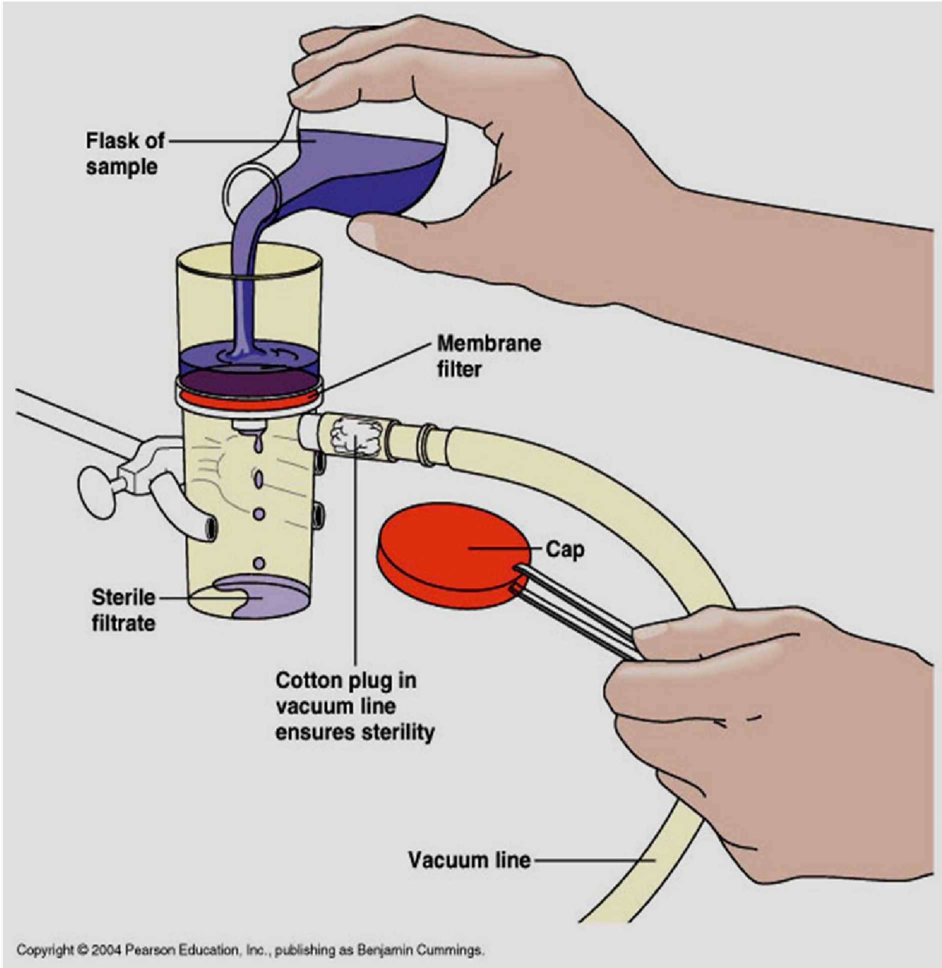
- Equivalent treatments
  - Different time and temperature combination end with same results
    - I.e. endospores killed at
      - 70 minutes of 115° C = 7 minutes at 125° C
    - Classic pasteurization = HTST = UHT

Dry Heat Sterilization

- Dry heat sterilization
  - Flaming
  - Incineration
  - Hot air sterilization
    - Placed in oven
    - 170 ° C for 2 hours

Filtration

- Filtration
  - Liquids
  - Heat – sensitive materials
  - Vacuum assists gravity
  - Small pores prevent passage of bacteria
  - HEPA
    - High efficiency particulate air filters
    - Operating rooms
    - Masks



Filtration

- Filtration (cont)
  - Filters composed of cellulose or plastic polymers
  - Sizes of pores
    - .01μm - .1mm
    - .01μm can filter some viruses and proteins

Low Temperatures

- Refrigeration
  - Bacteriostatic
  - Psychrotrophs still present and grow
  - Slow freezing more harmful to bacteria than rapid
    - Ice disrupts the cell structure
  - Thawing damages bacteria as well

High Pressure

- Applied to liquid suspensions
- Alters protein shape
- Endospores are resistant
  - Can be killed by altering pressure cycles
  - Endospores germinate then exposed to pressure again

Desiccation

- Absence of water
- Microorganisms cannot grow but still survive
- Re – introduce water microorganisms resume growth and division
- Effectiveness varies between organisms
  - *Neisseria* withstand dryness for one hour
  - *Mycoplasma* withstand dryness for months
  - Endospores remain for centuries

Osmotic Pressure

- High concentrations of salt and sugar
- Creates hypertonic environment
- Water leaves microbes cell
- Molds and yeasts can grow better than bacteria in high osmotic pressure or low moisture

Radiation

- Ionizing radiation (gamma rays)
  - High energy short wavelength
  - Radioactive elements
  - X- rays
  - Penetrate deeply
  - Require longer times
  - Ionizes water to form hydroxyl radicals
  - Food preservation in other countries
  - USA ?
    - Spices, some meats and vegetables

Radiation

- Non- ionizing radiation (UV light)
  - Ultraviolet light
  - Damages DNA
    - Adjacent thymines (pyrimidine base) form bonds
    - Forms thymine dimers
    - Inhibits correct replication of DNA
  - UV lamps
    - Germicidal lamps
  - Disadvantage
    - Rays do not penetrate, microbes on surfaces
    - Cannot penetrate paper
    - Prolonged exposure
      - Eyes damage, burns, and skin cancer

Microwaves

- Little effect on microorganisms
- Heat will not kill bacteria
- Pockets of heat due to moisture content
- Microwaved pork
  - Trichinosis

TABLE 7.5	Physical Methods Used to Control Microbial Growth		
Method	**Mechanism of Action**	**Comment**	**Preferred Use**
Heat			
1. Moist heat			
a. Boiling or flowing steam	Protein denaturation	Kills vegetative bacterial and fungal pathogens and almost all viruses within 10 min; less effective on endospores.	Dishes, basins, pitchers, various equipment
b. Autoclaving	Protein denaturation	Very effective method of sterilization; at about 15 psi of pressure (121°C), all vegetative cells and their endospores are killed in about 15 min.	Microbiological media, solutions, linens, utensils, dressings, equipment, and other items that can withstand temperature and pressure
2. Pasteurization	Protein denaturation	Heat treatment for milk (72°C for about 15 sec) that kills all pathogens and most nonpathogens.	Milk, cream, and certain alcoholic beverages (beer and wine)
3. Dry heat			
a. Direct flaming	Burning contaminants to ashes	Very effective method of sterilization.	Inoculating loops
b. Incineration	Burning to ashes	Very effective method of sterilization.	Paper cups, contaminated dressings, animal carcasses, bags, and wipes
c. Hot-air sterilization	Oxidation	Very effective method of sterilization, but requires temperature of 170°C for about 2 hr.	Empty glassware, instruments, needles, and glass syringes
Filtration	Separation of bacteria from suspending liquid	Removes microbes by passage of a liquid or gas through a screenlike material. Most filters in use consist of cellulose acetate or nitrocellulose.	Useful for sterilizing liquids (enzymes, vaccines) that are destroyed by heat

Chemical Methods of Microbial Control

- Effective disinfection
  - Class of organisms
  - Substance disinfecting
  - Proper dilution
  - Presence organic material
  - Contact time

Evaluating a Disinfectant

- Phenol coefficient test
  - Compared activity to phenol
  - Older test
- Use – dilution test
  - Current standard of AOAC
    - American Official Analytical Chemist

Use – dilution test

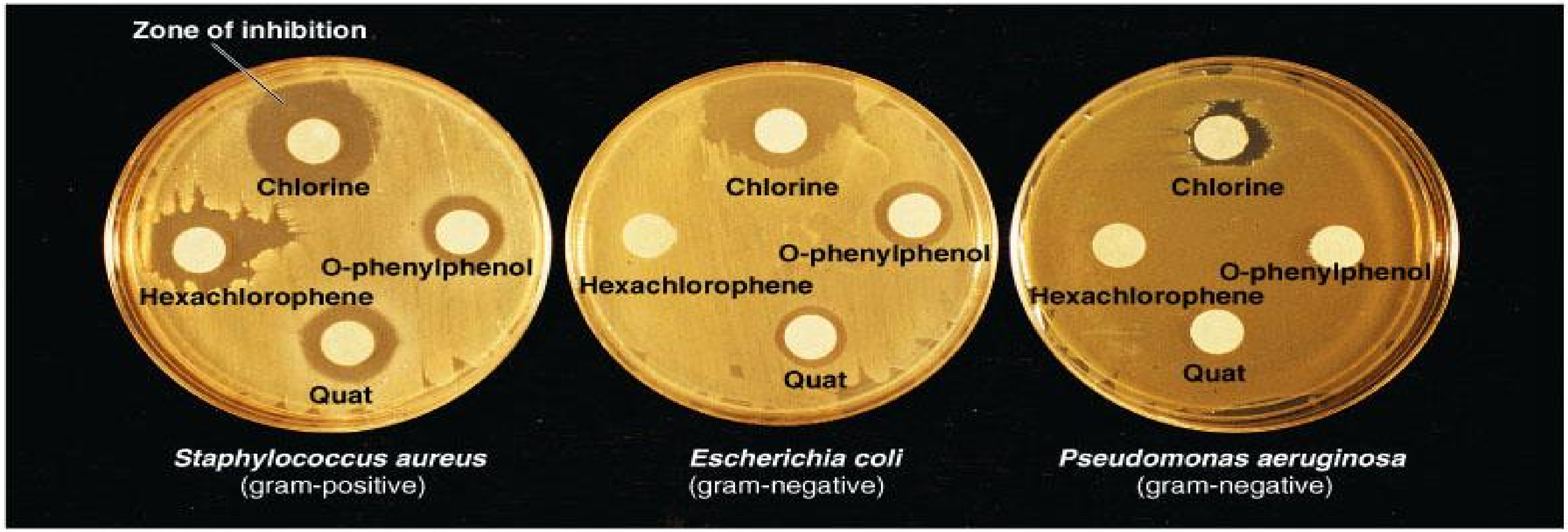
- Use – dilution test
  - Utilize 3 bacteria
    - *Salmonella choleraesuis*
    - *Staphylococcus aureus*
    - *Pseudomonas aeruginosa*
  - Metal rings dipped in pure culture
  - Dried at 37° C
  - Rings placed in disinfectant
    - 10 minutes
    - 20° C temperature
  - Rings placed on media
    - # of colonies grown counted

Virucides

- Viruses
  - Usually use Newcastle virus
  - Exposed to disinfectant
  - Cultures injected into embryonated chick embryos
  - Presence of virus would kill embryos

Disk Diffusion Method

- Agar plate covered with bacteria
- Disk soaked with disinfectant placed on agar
- Incubated
- Clear zone represents inhibition of bacterial growth
- Antibiotic sensitivity done similarly



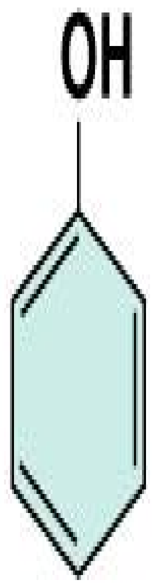
Phenols

- Phenols
  - First used by Lister
  - Rarely used now
  - Irritates skin
  - Throat sprays and lozenges
    - 1 % solution
    - Antibacterial

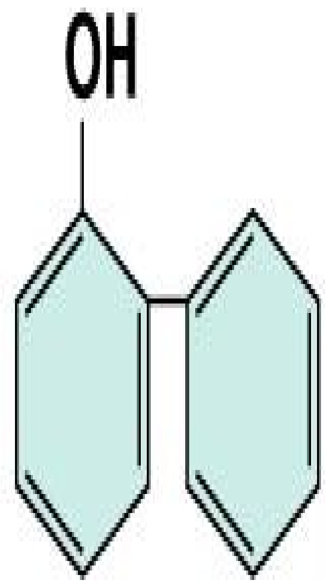
(a) Phenol

Phenolics

- Phenolics
  - Derivatives of phenol
  - Increased antibacterial activity
  - Decrease irritation to tissue
  - Often with soap or detergent
  - Injure plasma membrane
  - Active in presence of organic material



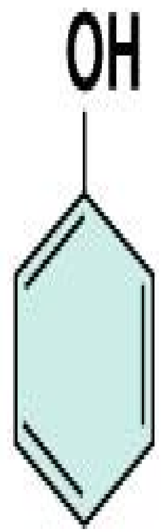
(a) Phenol



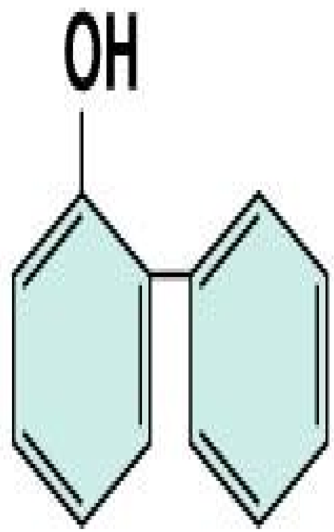
(b) O-phenylphenol

Phenolics

- Phenolics
  - Good for disinfecting pus, saliva and feces
  - Effective against *Mycobacterium*
    - Cell wall high lipid content
    - Very effective
  - Cresols
    - O – phenylphenol
      - Lysol



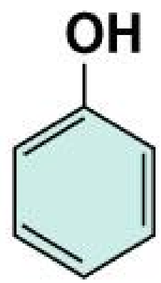
(a) Phenol



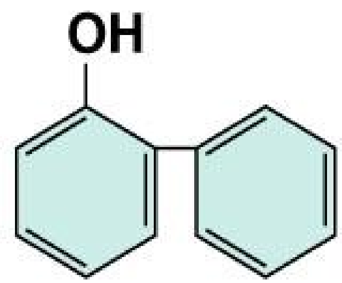
(b) O-phenylphenol

Bisphenols

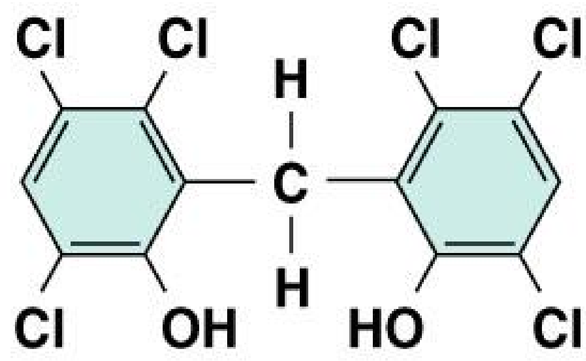
- Phenol derivatives
- Hexachlorophene
  - pHisoHex
    - Prescription antibacterial lotion
    - Gram + in newborns
      - <u>*Staph*</u>
      - <u>*Strep*</u>



(a) Phenol



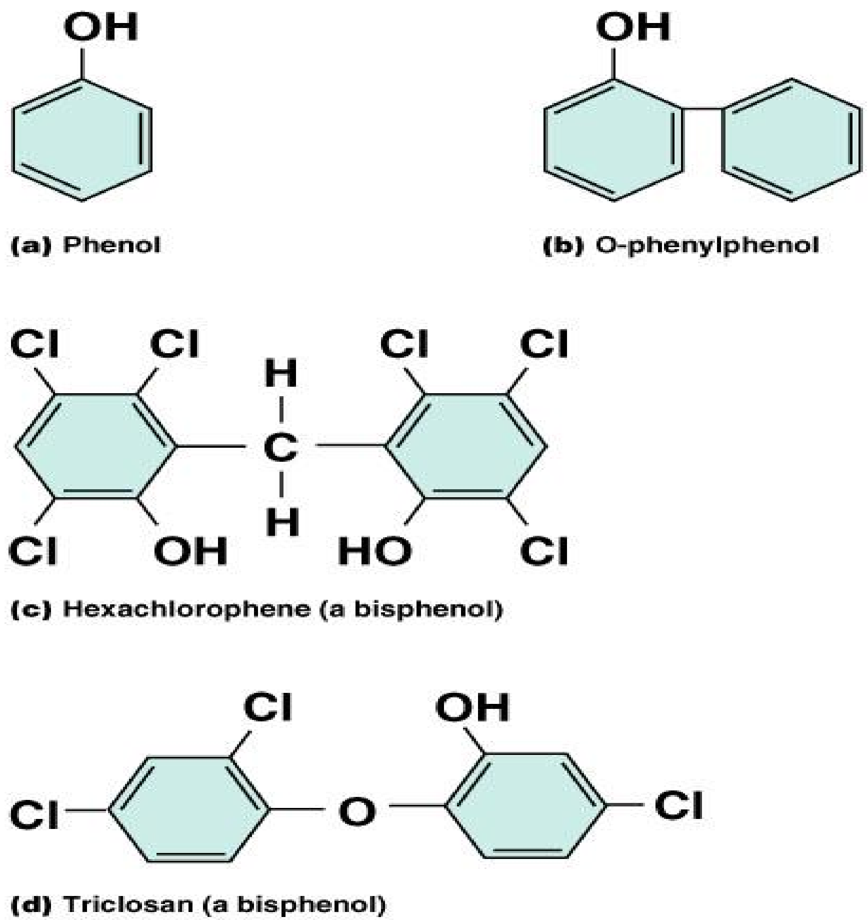
(b) O-phenylphenol



(c) Hexachlorophene (a bisphenol)

Bisphenol

- Triclosan
  - Anti-bacterial soaps
  - Kitchen cutting boards
  - Some cases of resistance
  - Inhibits synthesis of fatty acids
  - Effective against g+ and g-
  - <u>*Pseudomonas aeruginosa*</u>



Biguanides

- Chlorhexidine
  - Broad spectrum
  - Used on skin and mucus membranes
    - Scrubs
    - Washes
  - Low toxicity
  - Damaging to eyes
    - Damages plasma membrane
  - *Mycobacteria,* endospores and protozoa are resistant
  - Effective on some viruses
    - Lipohilic viruses

Halogens

- Iodine (I_2)
  - Oldest
  - Very effective
    - Bacteria, endospores, various fungi, some viruses
  - Exact mode is unknown
    - Possibly combines with amino acids
  - Tincture
    - Iodine in an aqueous alcohol solution
  - Iodophor
    - Iodine attached to an organic molecule, slowly releases Iodine
    - Do not stain like tinctures

Iodine

- Povidone iodine
  - Betadine
  - Increases wetting action of water
  - Acts as reservoir of free iodine

Chlorine

- Gas (Cl_2) or in combination
- Hypochlorous acid (HOCl) forms in water
- Unknown mechanism of action
- NaOCl – sodium hypochlorite
  - Clorox = bleach
- Used to disinfect drinking water, swimming pools, sewage

Chlorine

- Chlorine dioxide (ClO_2)
  - Can kill endospores
    - Anthrax
- Chloramines
  - Chlorine and ammonia
  - Release chlorine over long periods
  - Slow acting
  - Toxic to fish

Alcohols

- Kill bacteria and fungi
- Ineffective on endospores and nonenveloped viruses
- Denature proteins and dissolve lipids
- Evaporate
- Not good for wounds
- Ethanol and isopropranol (preferred)
- Often added to other disinfectants to increase effectiveness
  - Tinctures

Heavy Metals

- Denature proteins at – SH bonds
- Oligodynamic action
  - Small amount of ions diffuse away from metal
- Silver, mercury, copper
  - Silver nitrate sticks
  - Silver sulfadiazine (Silvadene)
  - Surfacine – silver, iodine, and biguanide

Heavy Metals

- Inorganic mercury
  - Mercuric chloride
  - Mercurochrome
  - Control mildew in paint
  - Bacteriostatic
  - Toxicity

Heavy metals

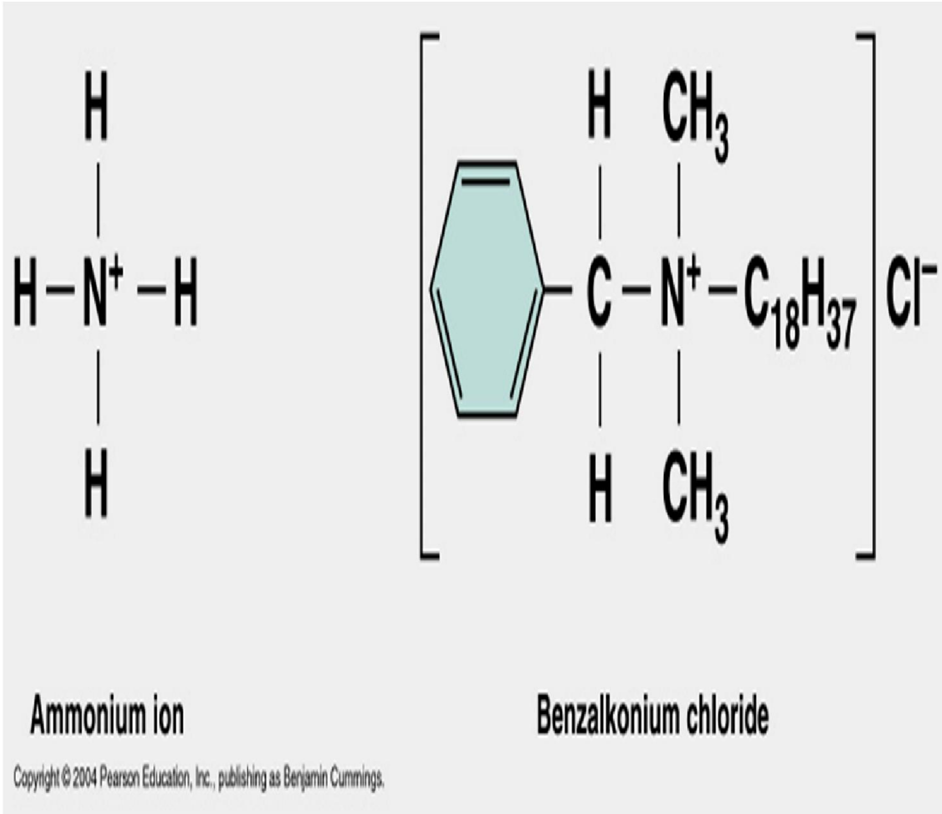
- Copper
  - Copper sulfate
    - Destroy algae
    - Control mildew in paint
- Zinc
  - Used to galvanize nails
  - Zinc chloride
    - Mouthwashes
  - Zinc oxide
    - Antifungal in paints, and adds pigment

Surfactants

- Decrease surface tension
- Soaps and detergents
- Soap breaks up oil film into tiny droplets
  - Emulsification
- Acid anionic surfactants
  - Used on dairy equipment

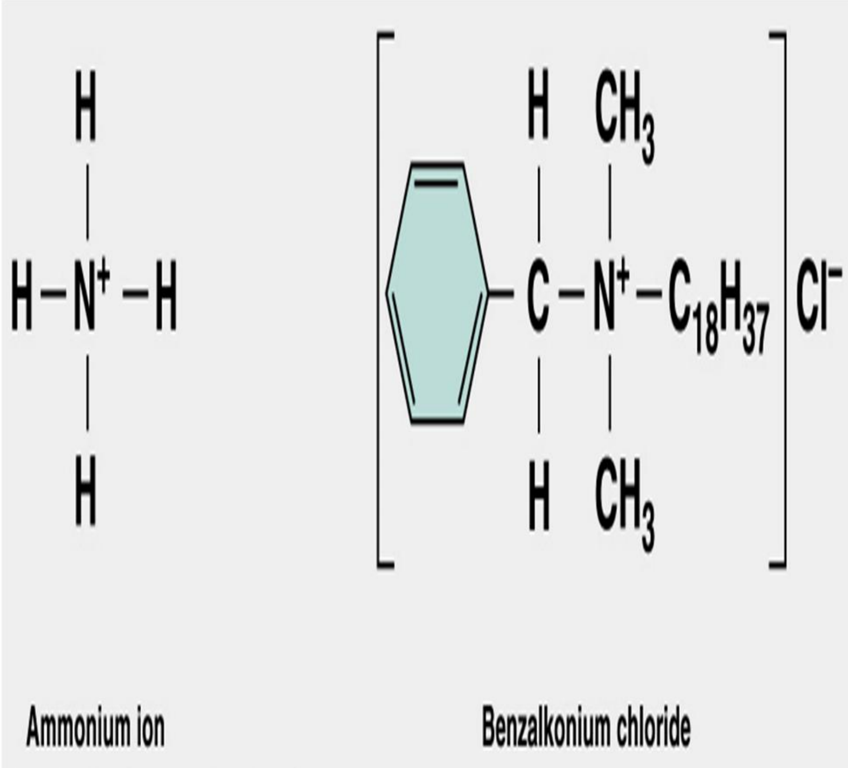
Quaternary Ammonium Compounds (Quats)

- Surfactants
  - Surface-active agents
- Cationic detergents
- Strong bactericidal (alter plasma membrane)
  - Gram +
  - Gram – (less effective)
- Fungicidal
- Amoebicidal
- Virucidal (enveloped)
- Do not kill
  - Endospores
  - mycobacteria



Quaternary Ammonium Compounds (Quats)

- Zephiran
  - Benzalkonium chloride
- Cepacol
  - Cetylphyridinium chloride
- Organic material interferes
- Rapidly broke down by soaps
- *Pseudomonas* can actually grow in quats



Chemical Food Preservatives

- Sulfur dioxide (SO_2)
  - Wine – making
- Sorbic acid
- Potassium sorbate
- Sodium benzoate
- Calcium propionate

Chemical food preservatives

- Sodium nitrate and Sodium nitrite
  - Bacon, meats,
  - Preserves red color of meat
  - Prevent germination of botulism spores
  - Can form nitrosamines
    - Carcinogen

Antibiotics

- Antibiotics
  - Non treatment
    - Nisin – added to cheese to prevent spoilage
      - Bacterocin – antibiotic produced by one bacteria and inhibits another
      - Naturally found in dairy products
    - Natamycin (pimaricin)
      - Antifungal, antibiotic used in cheeses

Aldehydes

- Aldehydes
  - Formaldehyde
    - Gas or solution
    - Preservative and in vaccinations
  - Glutaraldehyde
    - Less irritating than formaldehyde
    - Cidex
    - Sterilizing agent
  - Inactivate proteins
  - Very effective antimicrobials

Gaseous Chemosterilizers

- Ethylene oxide
- Propylene oxide
- Beta- propiolactone
- Used on medical instruments
- Suspected carcinogens

Oxidizing agents

- Peroxygens
  - Oxidize cellular components
  - Ozone
  - Hydrogen peroxides
    - May slow healing
  - Benzyl peroxides
    - Acne
  - Peracetic acid
    - Considered a sterilant

Microbial characteristics

- External lipopolysaccharide layer
- Porins
  - Highly selective
- Waxy cell wall
  - *Mycobacterium*
- Endospores
- Viruses
  - Lipid containing envelope
- Prions
  - Resistant to autoclaving
  - NaOH for one hour
    - Fairly effective

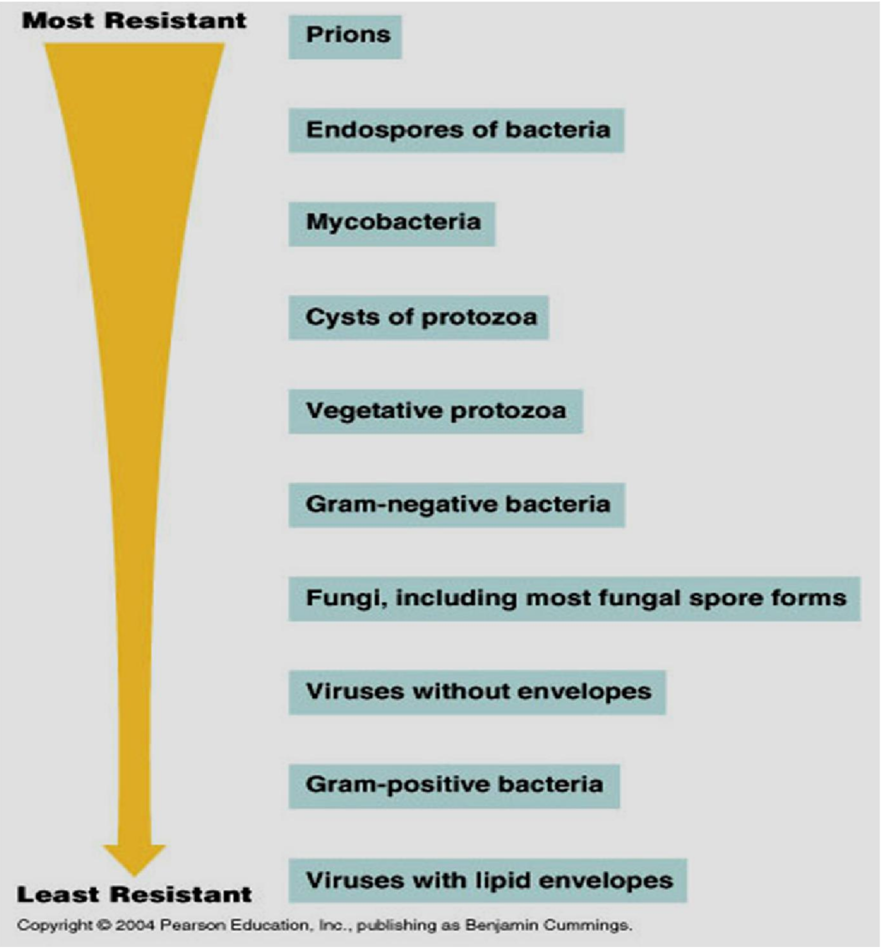


TABLE 7.8	Chemical Agents Used to Control Microbial Growth		
Chemical Agent	**Mechanism of Action**	**Preferred Use**	**Comment**
Phenol and Phenolics			
1. Phenol	Disruption of plasma membrane, denaturation of enzymes	Rarely used, except as a standard of comparison.	Seldom used as a disinfectant or antiseptic because of its irritating qualities and disagreeable odor.
2. Phenolics	Disruption of plasma membrane, denaturation of enzymes	Environmental surfaces, instruments, skin surfaces, and mucous membranes.	Derivatives of phenol that are reactive even in the presence of organic material; O-phenylphenol is an example.
3. Bisphenols	Probably disruption of plasma membrane	Disinfectant hand soaps and skin lotions.	Triclosan is an especially common example of a bisphenol. Broad spectrum, but most effective against gram-positives.
Biguanides (Chlorhexidine)	Disruption of plasma membrane	Skin disinfection, especially for surgical scrubs.	Bactericidal to gram-positives and gram-negatives; nontoxic, persistent.
Halogens	Iodine inhibits protein function and is a strong oxidizing agent; chlorine forms the strong oxidizing agent hypochlorous acid, which alters cellular components.	Iodine is an effective antiseptic available as a tincture and an iodophor; chlorine gas is used to disinfect water; chlorine compounds are used to disinfect dairy equipment, eating utensils, household items, and glassware.	Iodine and chlorine may act alone or as components of inorganic and organic compounds.

TABLE 7.8	**Chemical Agents Used to Control Microbial Growth** (continued)		
Chemical Agent	**Mechanism of Action**	**Preferred Use**	**Comment**
Alcohols	Protein denaturation and lipid dissolution.	Thermometers and other instruments; in swabbing the skin with alcohol before an injection, most of the disinfecting action probably comes from a simple wiping away (degerming) of dirt and some microbes.	Bactericidal and fungicidal, but not effective against endospores or nonenveloped viruses; commonly used alcohols are ethanol and isopropanol.
Heavy Metals and Their Compounds	Denaturation of enzymes and other essential proteins.	Silver nitrate may be used to prevent gonorrheal ophthalmia neonatorum; mercurochrome disinfects skin and mucous membranes; copper sulfate is an algicide.	Heavy metals such as silver and mercury are biocidal.
Surface-Active Agents			
1. Soaps and acid-anionic detergents	Mechanical removal of microbes through scrubbing.	Skin degerming and removal of debris.	Many antibacterial soaps contain antimicrobials.
2. Acid-anionic detergents	Not certain; may involve enzyme inactivation or disruption.	Sanitizers in dairy and food-processing industries.	Wide spectrum of activity; nontoxic, noncorrosive, fast-acting.

TABLE 7.8	**Chemical Agents Used to Control Microbial Growth** (continued)		
Chemical Agent	**Mechanism of Action**	**Preferred Use**	**Comment**
3. Cationic detergents (quaternary ammonium compounds)	Enzyme inhibition, protein denaturation, and disruption of plasma membranes.	Antiseptic for skin, instruments, utensils, rubber goods.	Bactericidal, bacteriostatic, fungicidal, and virucidal against enveloped viruses; examples of quats are Zephiran® and Cepacol®.
Organic Acids	Metabolic inhibition, mostly affecting molds; action not related to their acidity.	Sorbic acid and benzoic acid effective at low pH; parabens much used in cosmetics, shampoos; calcium propionate used in bread; all are mainly antifungals.	Widely used to control molds and some bacteria in foods and cosmetics.
Aldehydes	Protein denaturation.	Glutaraldehyde (Cidex™) is less irritating than formaldehyde and is used for disinfection of medical equipment.	Very effective antimicrobials.
Gaseous Sterilants	Protein denaturation.	Excellent sterilizing agent, especially for objects that would be damaged by heat.	Ethylene oxide is the most commonly used.
Peroxygens (Oxidizing Agents)	Oxidation.	Contaminated surfaces; some deep wounds, in which they are very effective against oxygen-sensitive anaerobes.	Ozone is widely used as a supplement for chlorination; hydrogen peroxide is a poor antiseptic but a good disinfectant. Peracetic acid is especially effective.