

Control and Sterilization

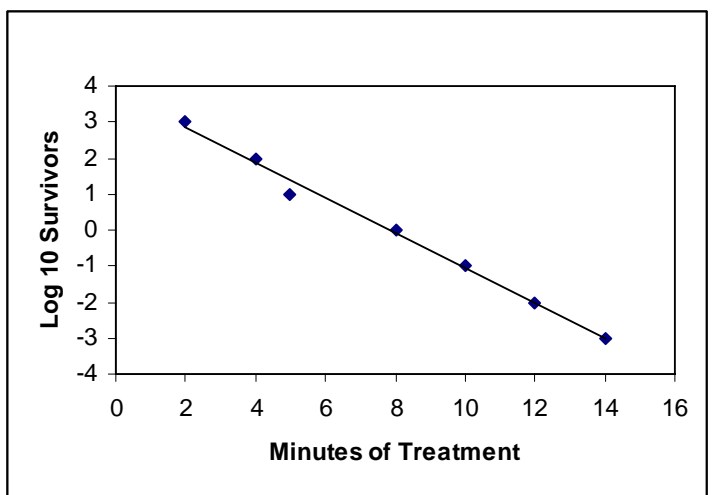
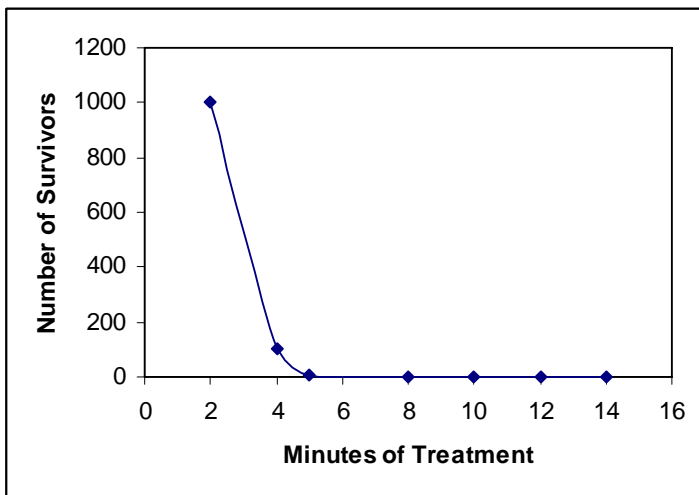
(see pages 671-698)

Some definitions:

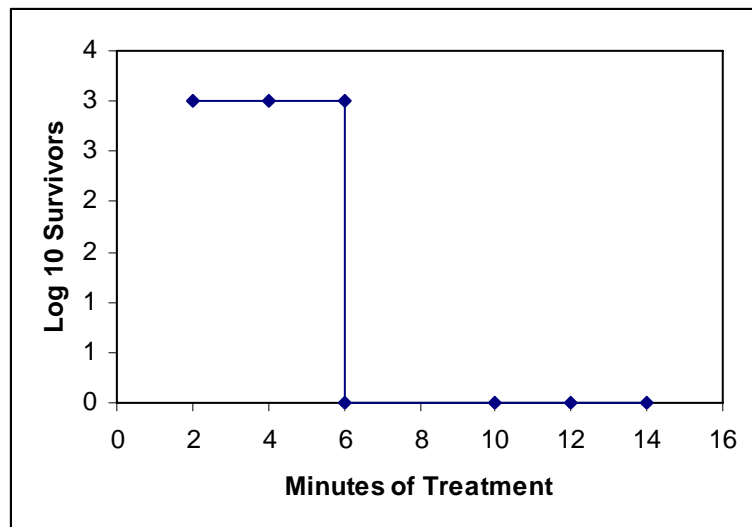
- Sterilization: Killing of all life. No cells living or capable of becoming metabolically active
- Disinfection: A clinical term. Removal of a potentially hazardous organism to the point where it does not pose a threat on surfaces...living or inanimate
- Germicide: A general term for killing microbes. Can be made specific as a fungicide, bactericide, viricide, etc...
- Bacteriostatic: as it is...an agent used to maintain the population level where it is.
- Death: Irreversible loss of reproductive ability PLUS the cessation of all metabolic activity permanently.

What do we know about death in cultures?

Death is exponential

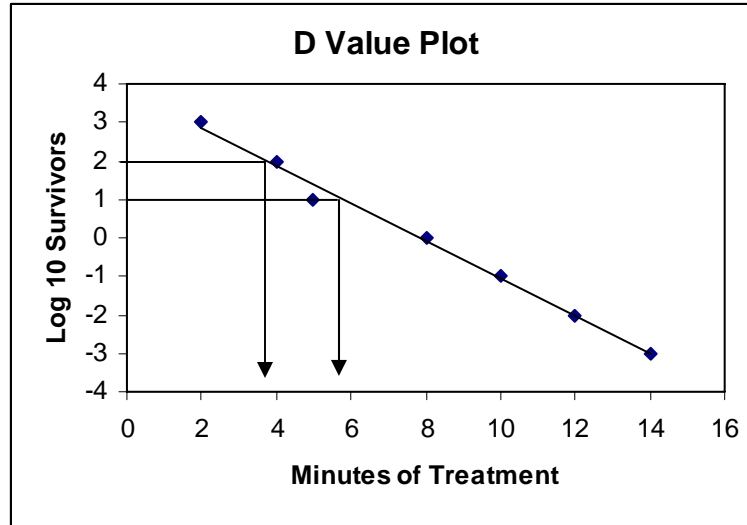


Death is never instantaneous. First order kinetics (define) indicated that only one particular effect causes death



Desirable to know death rate which is simple the slope of a death curve

- D-value: a metric that can be used to compare various types of killing agents. It is defined as the time necessary to cause a 10-fold decrease in the number of living cells.



- When sterilizing....you wish to assure the number of survivors is infinitesimally small....less than 1 in a million survive treatment

Things that affect sterilization treatments

- The organism itself
- Initial population size or the Bioburden
- The D value for the organism in combination with the treatment method
 - Generally...for mixed cultures or unknown organisms....you should treat to assure death of the most resistant of microbes.
- Condition of cell
 - Spores
 - Age: easier to kill young growing cells than old crotchety cells
- Environment
 - Organic matter or particulate matter may protect cells by
 - Chemical reactions with the agent uses it up
 - Creates a non-toxic material
 - Simply encapsulating to organism preventing contact with the agent

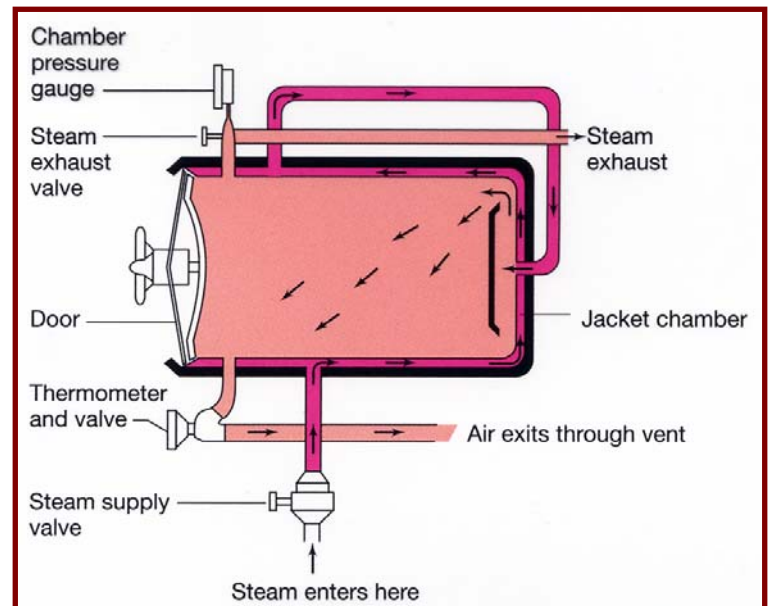
A tour of killing agents: (Review Table 20.4, pg. 680)

- Chemicals (Acids, alkalis, hypochlorite, chloramines and halogens (F1, Cl, Br, I))
 - all oxidize organic matter
- Metals
 - Precipitate proteins
- Wetting agents (soaps and detergents)
- Cause membranes to become wetted

- Physical agents
 - Dry Heat
 - Oxidize proteins
 - Cells have a high survivability
 - Not good for liquids, some plastics, some paper
 - Typical treatment times in circulated air

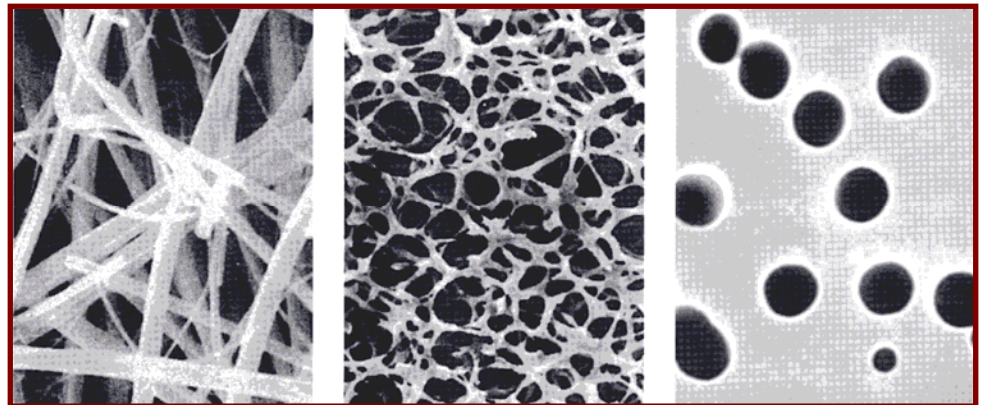
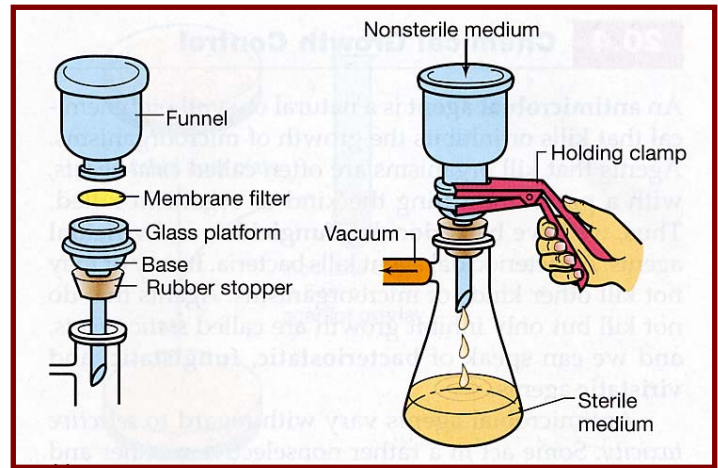
| Temperature (C) | Time (h) |
|---|----------|
| 170 | 1 |
| 160 | 2 |
| 150 | 2.5 |
| 140 | 3 |
| RECOMMENDED TREATMENT CONDITIONS | |
| 170 | 2 |

- Wet Heat
 - 'Penetrating heat' coagulates proteins
 - primarily for liquids that are not heat sensitive
 - Autoclave (Figure from Madigan et al. 2002)
 - A device for increasing pressure and temperature so high heat may be maintained but boiling prevented
 - Time Temperature Pressure relationships
 - 121 C @ 15 psi for 15 minutes
 - This relationship is volume dependent
 - The inner most object must be maintained at these points to assure sterilization



- Pasteurization
 - Reduces populations in liquids
 - Originally aimed at pathogens but also controls the growth of carbohydrate metabolizing organisms (milk rots!)
 - Process
 - Heating to 63-66 C for 30 minutes (alters flavor)
 - Flash Pasteurization, 71 C for 15 sec, followed by rapid cooling

- Filtration (figures from Madigan et al. 2002)
 - Primarily for heat sensitive liquids
 - Miller genuine draft
 - Filter types
 - Torturous, glass fiber, and plastic (polycarbonate)

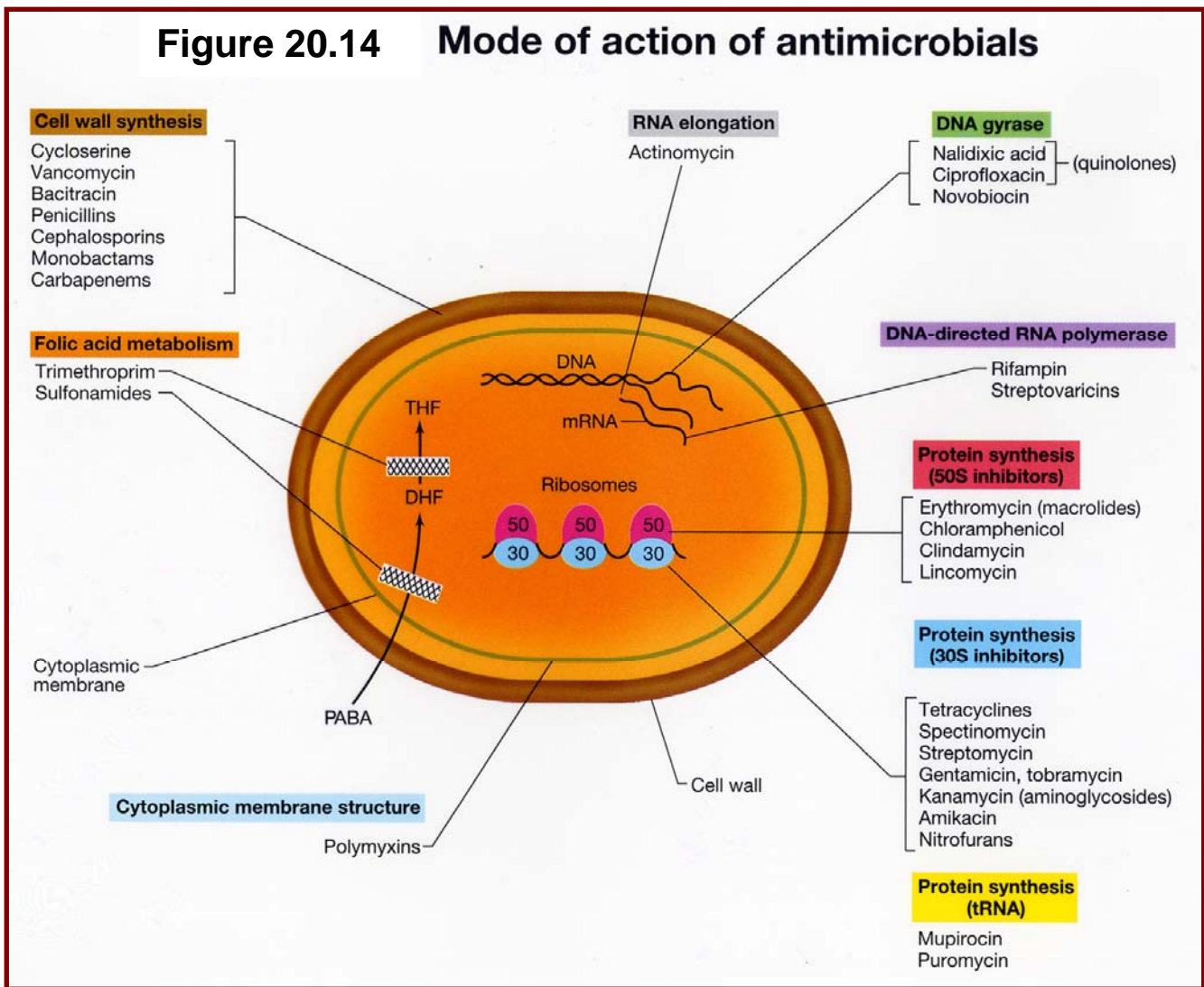


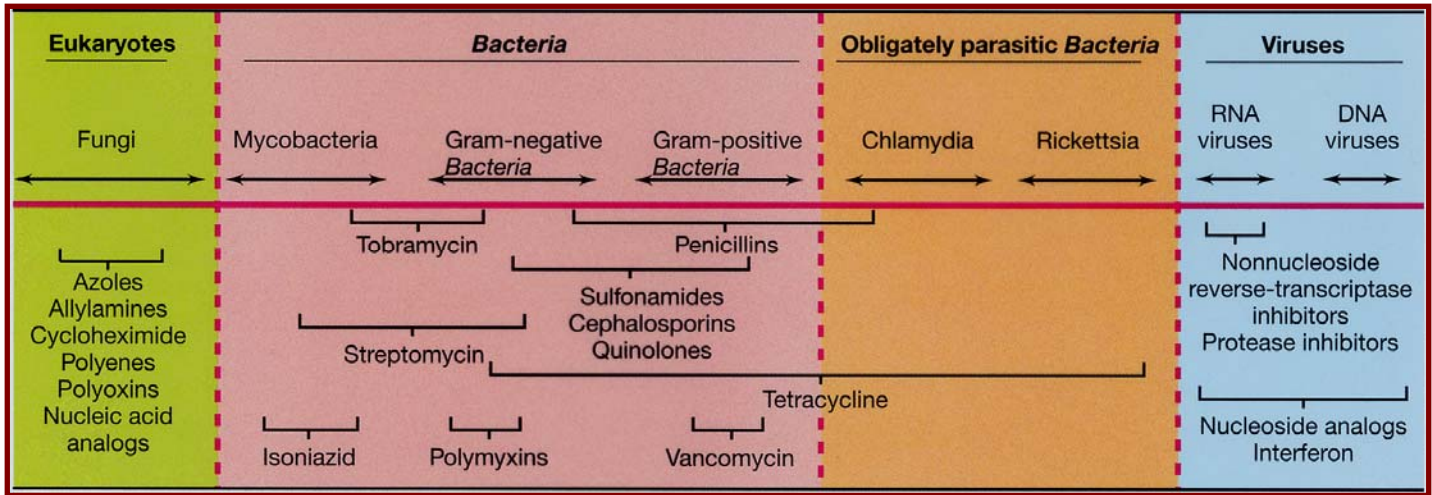
- Gas
 - Primarily for large heat sensitive materials
 - Agent is ethylene oxide
 - Very reactive with organic material but has no penetrability
 - Not locally practical
 - Ether odor
 - Colorless
 - Boils at 10.8 C
 - Flash point is -17 C
 - Explosive at 3-100% in air
- Radiation
 - Non-ionizing
 - Ultraviolet
 - UV created thymine-thymine dimers, low penetrability
 - Infrared
 - Infrared and radiofrequency (microwave) kill by localized heating
 - Ultrasonic
 - Ultrasound by sonic disruption
 - Radiofrequency
 - radiofrequency (microwave) kill by localized heating
 - Bioburden is very important

- Ionizing radiation
 - Particulate (alpha or beta particles) or electromagnetic (x-ray, gamma rays)
 - Create ions that are toxic
 - Excellent penetrability
 - Good for large scale commercial applications.

Chemotherapeutic agents

- Control agents for use within living tissue
- Selective toxicity
- Diverse modes of action that, for the most part, take advantage of the uniqueness of bacterial metabolic pathways (Figures 20.14 and 20.15 from Madigan et al. 2005)
- Not many effective against eukaryotes....Why?





Two broad categories

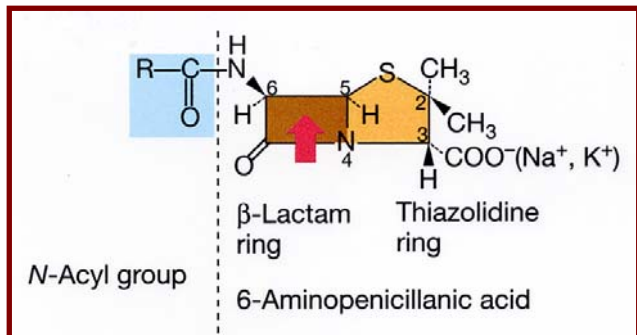
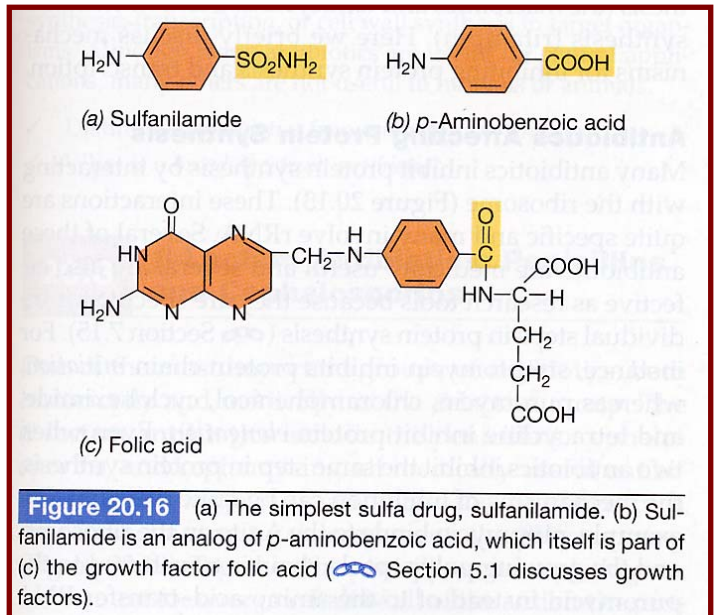
- Synthetic agents
- Antibiotics

Synthetics (figure 20.16 from Madigan et al. 2002, see 20.17 Madigan and Martinko 2005)

- Chemically synthesized materials that generally function as growth factor analogues
- Example and perhaps one of the best known: sulfanilamide
- An analogue for p-aminobenzoic acid which is a component of the essential vitamin Folic acid

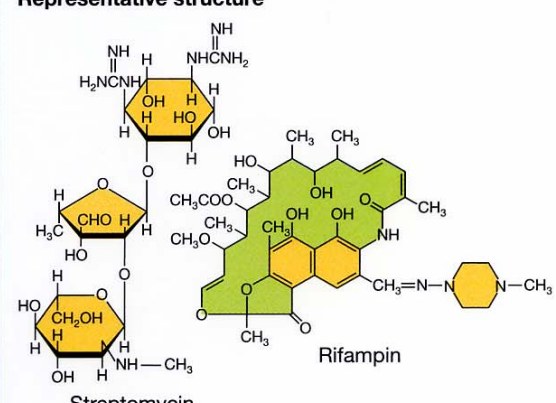
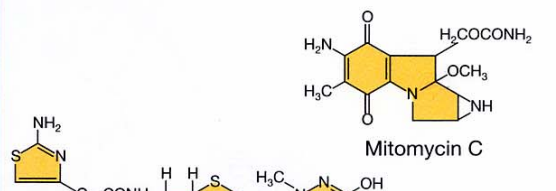
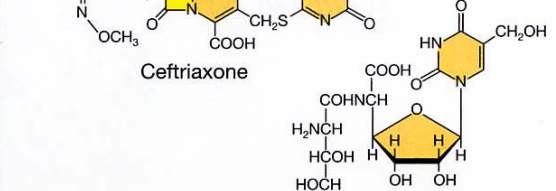
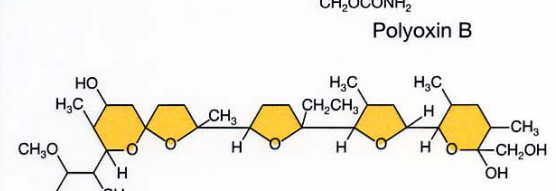
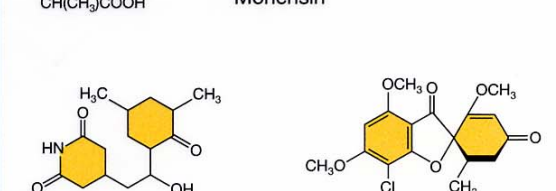
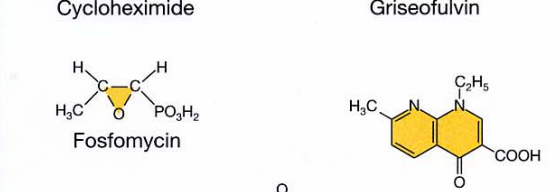
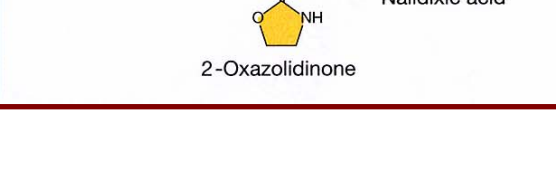

Antibiotics (figure 20.19 from Madigan et al 2002, see 20.20 Madigan and Martinko, 2005)

- Produced by organisms that inhibit or kill microorganisms
- Review the concept of broad-spectrum vs. narrow spectrum
- Chemical modification of the parent compound can alter
 - Its effectiveness
 - Its spectrum



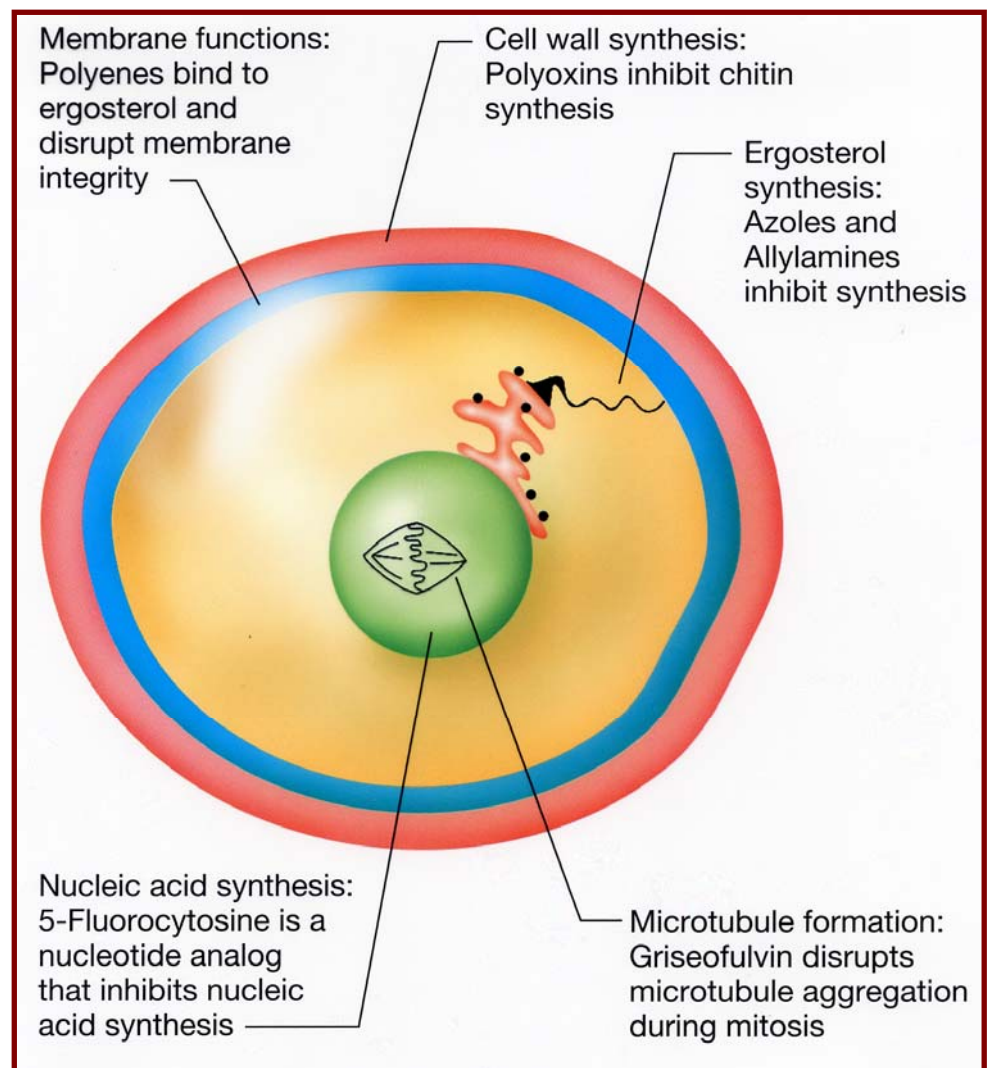
| Designation | N-Acyl group |
|---|--|
| NATURAL PENICILLIN Benzylpenicillin (penicillin G) Gram-positive activity β -lactamase-sensitive | <chem>c1ccc(cc1)CC(=O)O</chem> |
| SEMISYNTHETIC PENICILLINS Methicillin acid-stable, β -lactamase-resistant | <chem>COC1=CC=C(C(=O)O)C=C1C2=NC(=O)SC2C</chem> |
| Oxacillin acid-stable, β -lactamase-resistant | <chem>COC1=CC=C(C(=O)O)C=C1C2=NC(=O)SC2C(=O)O</chem> |
| Ampicillin broadened spectrum of activity (especially against gram-negative bacteria), acid-stable, β -lactamase-resistant | <chem>Nc1ccc(cc1)C(=O)O</chem> |
| Carbenicillin broadened spectrum of activity (especially against <i>Pseudomonas aeruginosa</i>), acid-stable but ineffective orally, β -lactamase-sensitive | <chem>OC(=O)c1ccc(cc1)C(=O)O</chem> |

A look at the classification and mode of action of common antibiotics. (Figure 20.13 from Madigan and Martinko 2005)

| Antibiotic classification | Subclassification | Example | Representative structure |
|---|--|--|---|
| I. Carbohydrate-containing compounds | Pure sugars Aminoglycosides Orthosomycins N-Glycosides C-Glycosides Glycolipids | Nojirimycin Streptomycin Evernimycin Streptothricin Vancomycin Moenomycin |  Streptomycin |
| II. Macrocyclic lactones | Macrolide antibiotics Polyene antibiotics Ansamycins Macrotetrolides | Erythromycin Candididin Rifampin Tetranactin |  Rifampin |
| III. Quinones and related compounds | Tetracyclines Anthracyclines Naphthoquinones Benzoquinones | Tetracycline Adriamycin Actinorhodin Mitomycin |  Mitomycin C |
| IV. Amino acid and peptide analogs | Amino acid derivatives β-Lactam antibiotics | Cycloserine Penicillin, ceftriaxone |  Ceftriaxone |
| | Peptide antibiotics Chromopeptides Depsipeptides Chelate-forming peptides | Bacitracin Actinomycin Valinomycin Bleomycin |  Polyoxin B |
| V. Heterocyclic compounds containing nitrogen | Nucleoside antibiotics | Polyoxins | |
| VI. Heterocyclic compounds containing oxygen | Polyether antibiotics | Monensin |  Monensin |
| VII. Alicyclic derivatives | Cycloalkane derivatives Steroid antibiotics | Cycloheximide Fusidic acid |  Cycloheximide |
| VIII. Aromatic compounds | Benzene derivatives Condensed aromatics Aromatic ether | Chloramphenicol Griseofulvin Novobiocin |  Griseofulvin |
| IX. Aliphatic compounds | Compounds containing phosphorus | Fosfomycin | Fosfomycin |
| X. Quinolone compounds | 4-Quinolone Fluoro-4-quinolones | Nalidixic acid Ciprofloxacin | Nalidixic acid |
| XI. Oxazolidinone | Cyclic lactone | 2-Oxazolidinone | 2-Oxazolidinone |

- Some modes of action of chemotherapeutic agents affecting eukaryotes
 - Most antifungal (anti-eukaryotic) are topical treatments) (Table 20.6 and Figure 20.23 from Madigan et al. 2002 see figure 20.24 in Madigan and Martinko 2005)

| TABLE 20.6 Antifungal drugs (fungicides) | | | |
|--|----------------------|------------------|--------------|
| Category | Target | Examples | Use |
| Polyenes | Ergosterol synthesis | Amphotericin B | Oral |
| Nucleic acid analogs | DNA synthesis | 5-Fluorocytosine | Oral |
| Polyoxins | Chitin synthesis | Polyoxin A | Agricultural |
| | | Polyoxin B | Agricultural |
| Azoles | Ergosterol synthesis | Fluconazole | Oral |
| | | Itraconazole | Oral |
| | | Ketoconazole | Oral |
| | | Clotrimazole | Topical |
| | | Miconazole | Topical |
| | | Voriconazole | Oral |
| Allylamines | Ergosterol synthesis | Terbinafine | Oral |



- A look at some antiviral agents (Table 20.5 from Madigan et al. 2002)
 - Some of the same problems exist with controlling viruses as controlling eukaryotic pathogens
 - Cellular machinery used by the virus to grow and replicate is that of the host cell. So it becomes difficult to attack the virus without also attacking the host.
 - Almost all anti-virals exhibit some level of host toxicity.
 - Almost all antivirals are forms of nucleoside analogs that inhibit reverse transcriptase (review reverse transcriptase)

TABLE 20.5 Antiviral chemotherapeutic compounds

| Category/drug | Mechanism of action | Virus affected |
|--|---|---|
| Nucleoside analogs | | |
| Acyclovir | Viral polymerase inhibitors | Herpes viruses, <i>Varicella zoster</i> |
| Ganciclovir | | Cytomegalovirus |
| Trifluridine | | Herpesvirus |
| Valacyclovir | | Herpesvirus |
| Vidarabine | | Herpesvirus, vaccinia, hepatitis B virus |
| Didanosine (dideoxyinosine or ddI) | Reverse transcriptase inhibitors | HIV ^a |
| Lamivudine (3TC) | | HIV, hepatitis B virus |
| Stavudine (d4T) | | HIV |
| Zalcitabine (ddC) | | HIV |
| Zidovudine (AZT) (🔗 Figure 26.28) | | HIV |
| Ribavirin | Blocks capping of viral RNA | Respiratory syncytial virus, influenza A and B, Lassa fever |
| Synthetic amines | | |
| Amantadine | Block uncoating of virus | Influenza A |
| Rimantadine | | Influenza A |
| Nucleotide analog | | |
| Cidofovir | Viral polymerase inhibitor | Cytomegalovirus, herpesviruses |
| Pyrophosphate Analog | | |
| Phosphonoformic acid (Foscarnet) | Viral polymerase inhibitor | Herpesviruses, HIV, hepatitis B virus |
| Nonnucleoside reverse transcriptase inhibitor (NNRTI) | | |
| Nevirapine | Reverse transcriptase inhibitor | HIV |
| RNA polymerase inhibitor | | |
| Rifamycin | RNA polymerase inhibitor | Vaccinia, pox viruses |
| Protease inhibitors | | |
| Indinavir (Figure 20.27) | Protease inhibitors | HIV |
| Ritonavir | | HIV |
| Saquinavir (Figure 20.27) | | HIV |
| Nelfinavir | | HIV |
| Abacivir | | HIV |
| Lopinavir | | HIV |
| Interferons | | |
| Interferon α | Induces proteins that inhibit viral replication | Broad spectrum (host specific) |
| Interferon β | | |
| Interferon γ | | |

^a Human immunodeficiency virus

References:

Madigan, M. T., J.M. Martinko, and J. Parker. 2002. Brock Biology of Microorganisms 10th ed. Prentice Hall.