Microbial growth control

Nov 13, 2006

Ch. 20

Outline

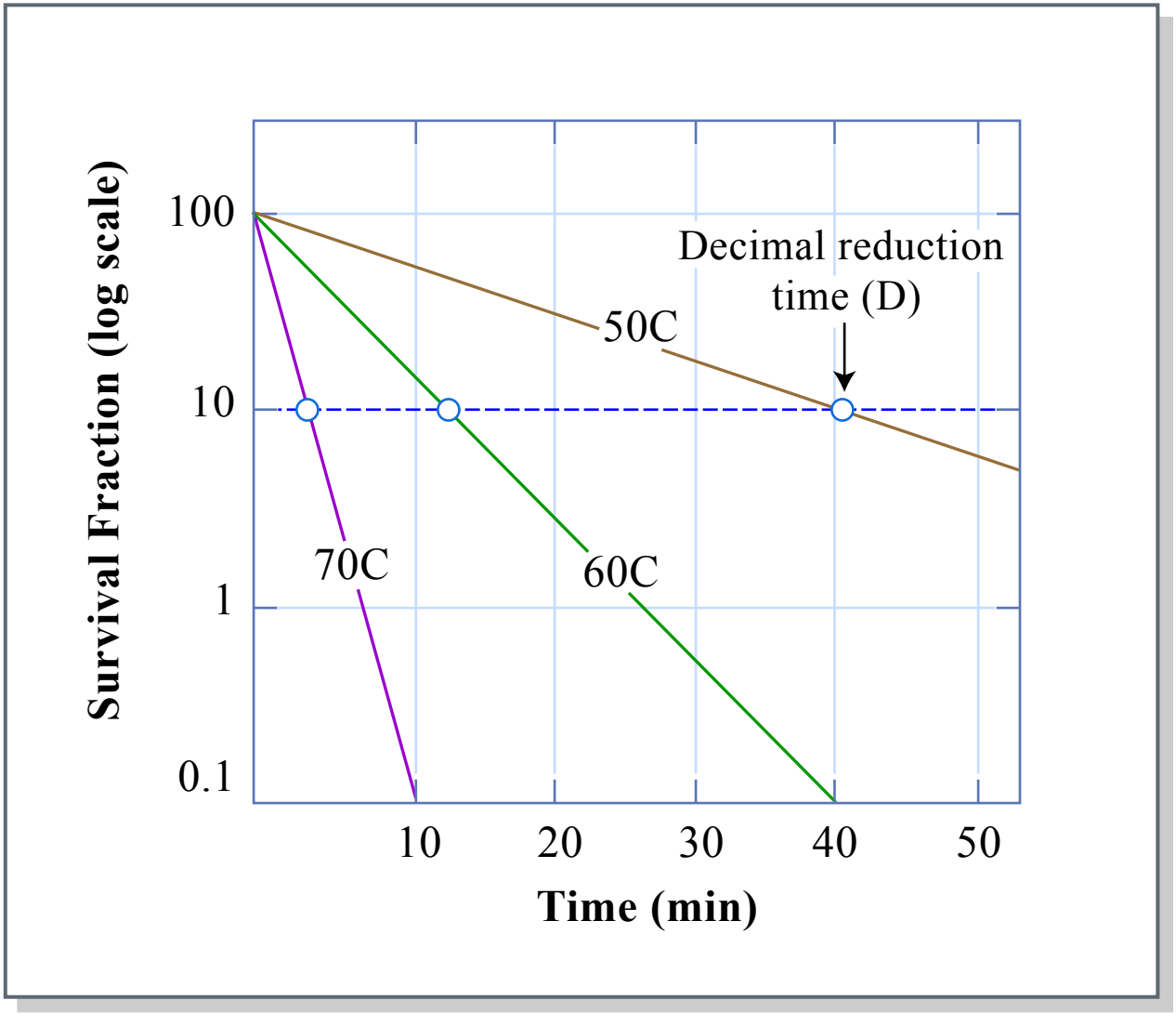
1. Physical antimicrobial control
2. Chemical antimicrobial control
3. Antimicrobial agents used in vitro
4. Control of viruses and eukaryotic pathogens
5. Antimicrobial drug resistance and drug discovery

Physical antimicrobial control

- Heat sterilization
  - Autoclave
  - Pasteurization
- Radiation sterilization
  - Ionizing radiation
- Filter sterilization
  - Depth filters
  - Membrane filters

Measuring heat sterilization

- Death is a first order function
- Time required for a 10-fold reduction in population density at a given temp. is the decimal reduction time (D)
- Thermal death time can also be used



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Autoclave

- 15 psi yields 121°C
- Kills bacterial spores within 15 min
- Larger volumes of liquid require extended cycle times

Autoclave images removed due to copyright restrictions.
See Figures 20-3a and 20-3c in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

Pasteurization

- Reduces microbial population in milk and other heat-sensitive liquids
- Uses a heat exchanger
- Controlled flow rate and temp.
- Flash pasteurization is 71°C for 15 seconds
- Controls *Listeria*, *Campylobacter*, *Salmonella*, *E. coli*, etc.

Photograph of jugs of milk being pasteurized removed due to copyright restrictions.

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Radiation sterilization

- UV limited to disinfection of exposed surfaces and some water applications
- Ionizing radiation used for medical supplies and food
- Typically x-rays or γ-rays from ^{60}Co or ^{137}Cs source

Table showing the radiation sensitivity of microorganisms and biological functions removed due to copyright restrictions. See Table 20-1 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

Filter sterilization

- Depth filters trap particles within the layers of the mat
  - Pre-filters
  - High-efficiency particulate air (HEPA) filters
- Membrane filters are used for heat-sensitive liquids

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See Figure 20-6b in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

Chemical growth control

- Selective toxicity
- Bacteriostatic
  - Agents often binds reversibly to ribosomes
- Bacteriocidal
- Bacteriolytic
  - Detergents and cell wall synthesis inhibitors

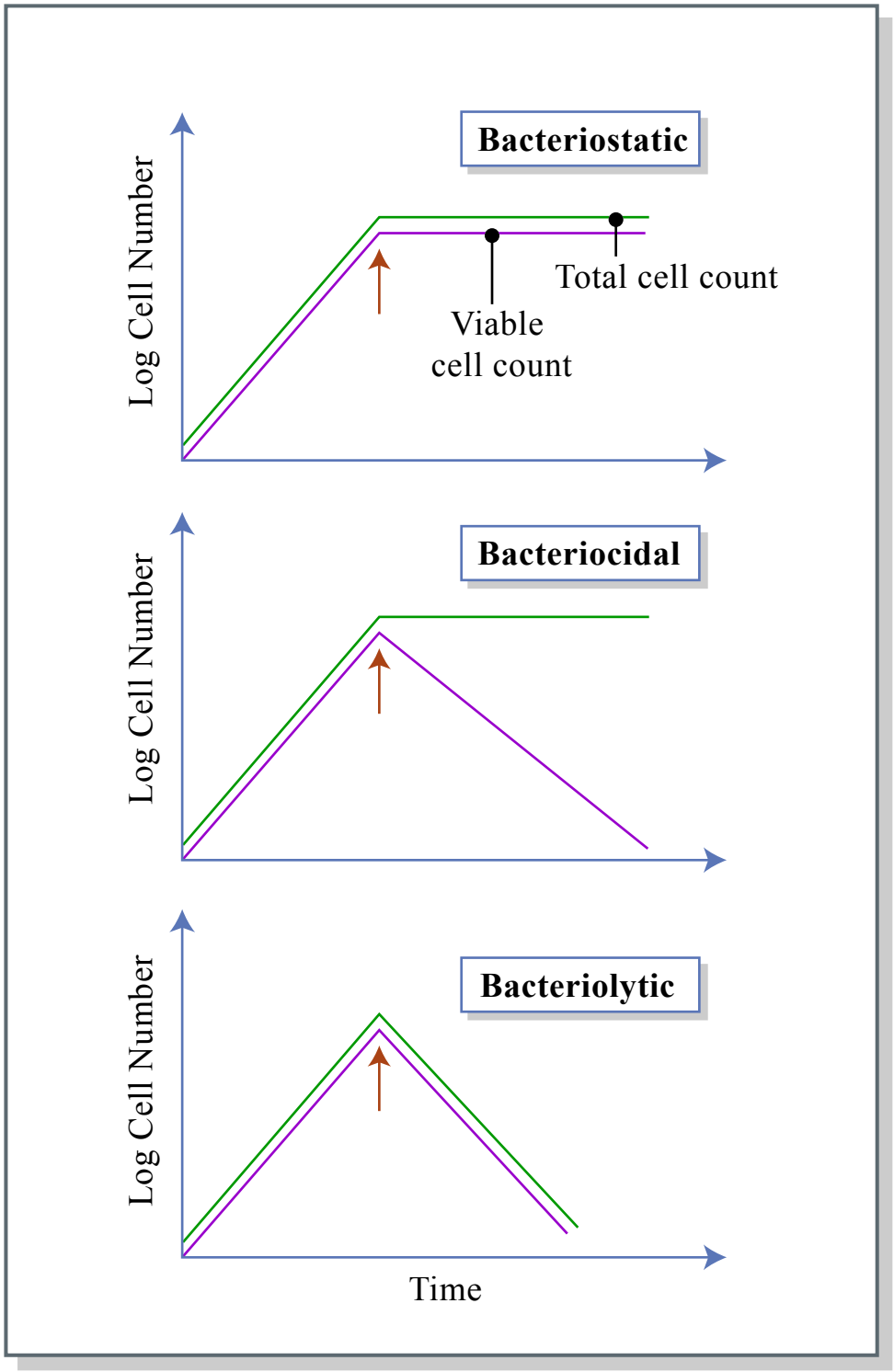
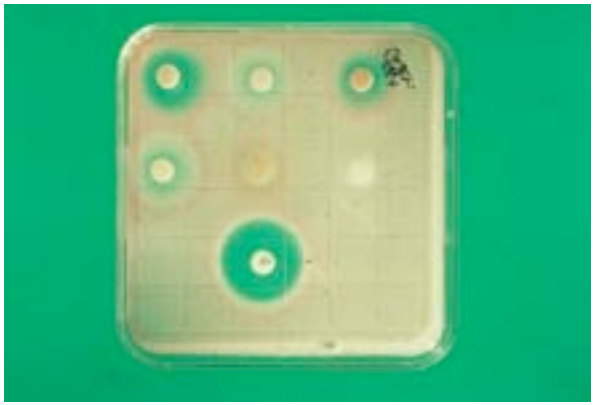


Figure by MIT OCW.

Measuring antimicrobial activity

- Minimum inhibitory concentration (MIC)
- Tube dilution technique
- Agar disc diffusion
- Both require standardization

Photograph showing the minimum inhibitory concentration in a series of increasingly diluted test tubes removed due to copyright restrictions. See Figure 20-10 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.



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Chemical agents used externally

- Control of non-pathogenic microbes
- Control of pathogenic microbes in the environment
  - Sterilants
    - Cold sterilization
    - Ethylene oxide gas, sodium chlorite solution
  - Disinfectants
    - Decontaminate floors, tables, etc.
    - Antiseptics can be used topically

Table of antiseptics, sterilants, disinfectants, and sanitizers removed due to copyright restrictions.
See Table 20-4 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms.* 11th ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

Antimicrobials used in vivo

- Chemotherapeutics
- Synthetic drugs
- Growth factor analogs
  - Sulfa drugs
    - Sulfanilamide is an analog of para aminobenzoic acid, a precursor for folic acid
  - Bacteria synthesize folic acid
  - Sulfamethoxazole plus trimethoprim is used clinically

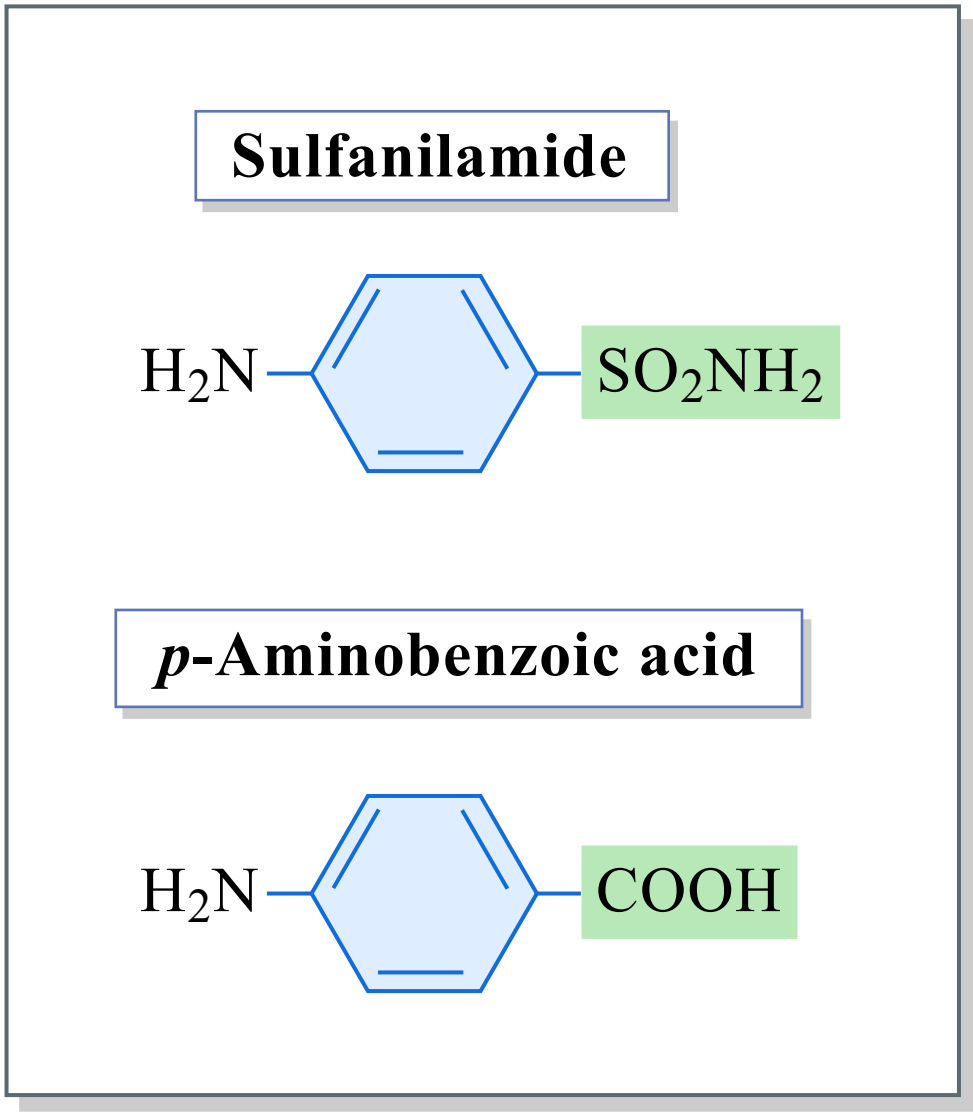
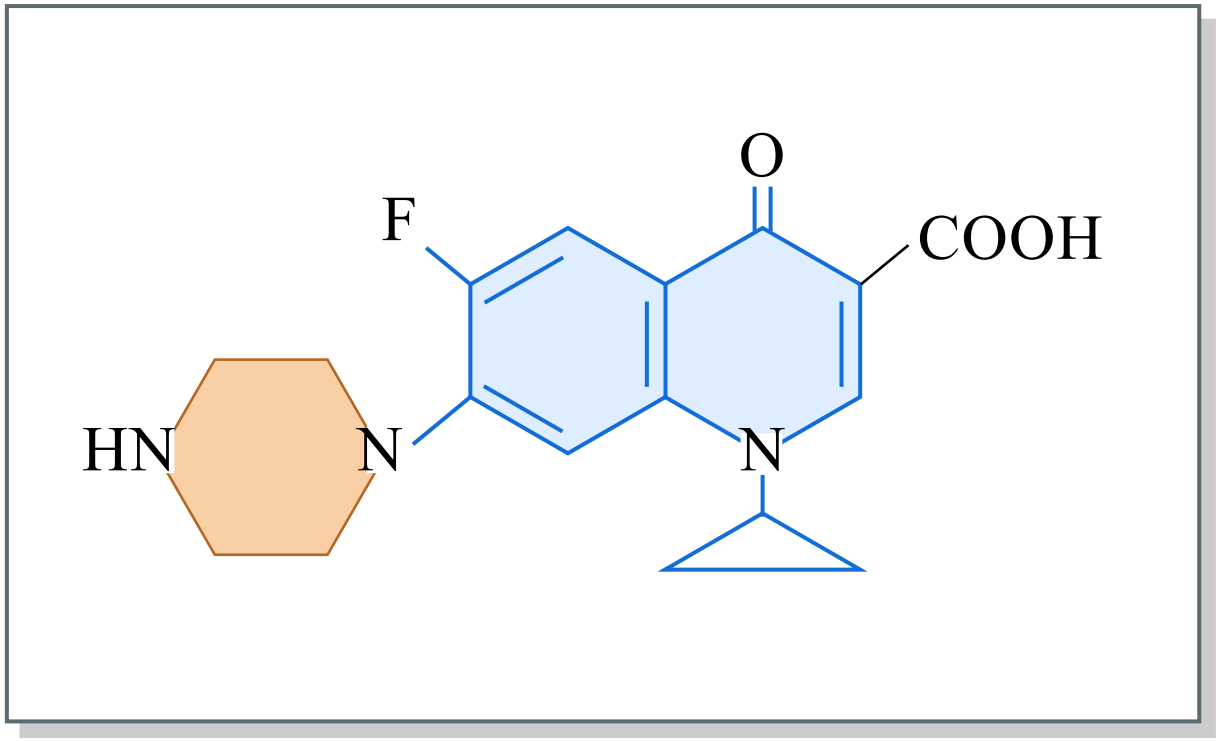


Figure by MIT OCW.

More synthetic antimicobials

- Isoniazid
  - Only effective against Mycobacterium tuberculosis
  - Nicotinamide analog
  - Inhibits mycolic acid synthesis
- Quinolones
  - Inhibit DNA gyrase
    - Nalidixic acid
    - Fluoroquinolone derivatives

      Ciprofloxacin



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Antibiotics

- Broad-spectrum vs. narrow spectrum
- Targets include ribosomes, the cell wall, the cytoplasmic membrane, and RNA Pol
  1. Penicillins and cephalosporins
  2. Aminoglycosides
  3. Macrolide antibiotics
  4. Tetracyclines

β-lactam antibiotics

- Penicillin G first antibiotic discovered
  - Highly selective
  - Primarily active against gram positive bacteria
  - Many semisynthetic pencillins are effective against gram negative bacteria
  - Sensitive to β-lactamase
- Bind irreversibly to penicillin-binding proteins (transpeptidases) and prevent cross-linking of peptidoglycan chains
- Cephalosporins have broader spectrum and are resistant to β-lactamases

Aminoglycosides

- Produced by bacteria and active against bacteria
- Inhibit protein sythesis (30S subunit)
- Useful clinically against gram negative bacteria
- Neurotoxicity and nephrotoxicity
- Used as fallback; not initial drugs of choice

Macrolides and tetracyclines

- Large lactone rings
- Important group (11% of all antibiotics produced worldwide)
- Erythromycin is commonly used in patients allergic to penicillin
- Inhibit 50S subunit of the ribosome

- Tetracyclines
  - Inhibits 30S subunit of the ribosome
  - Still widely used; growing problem of resistance

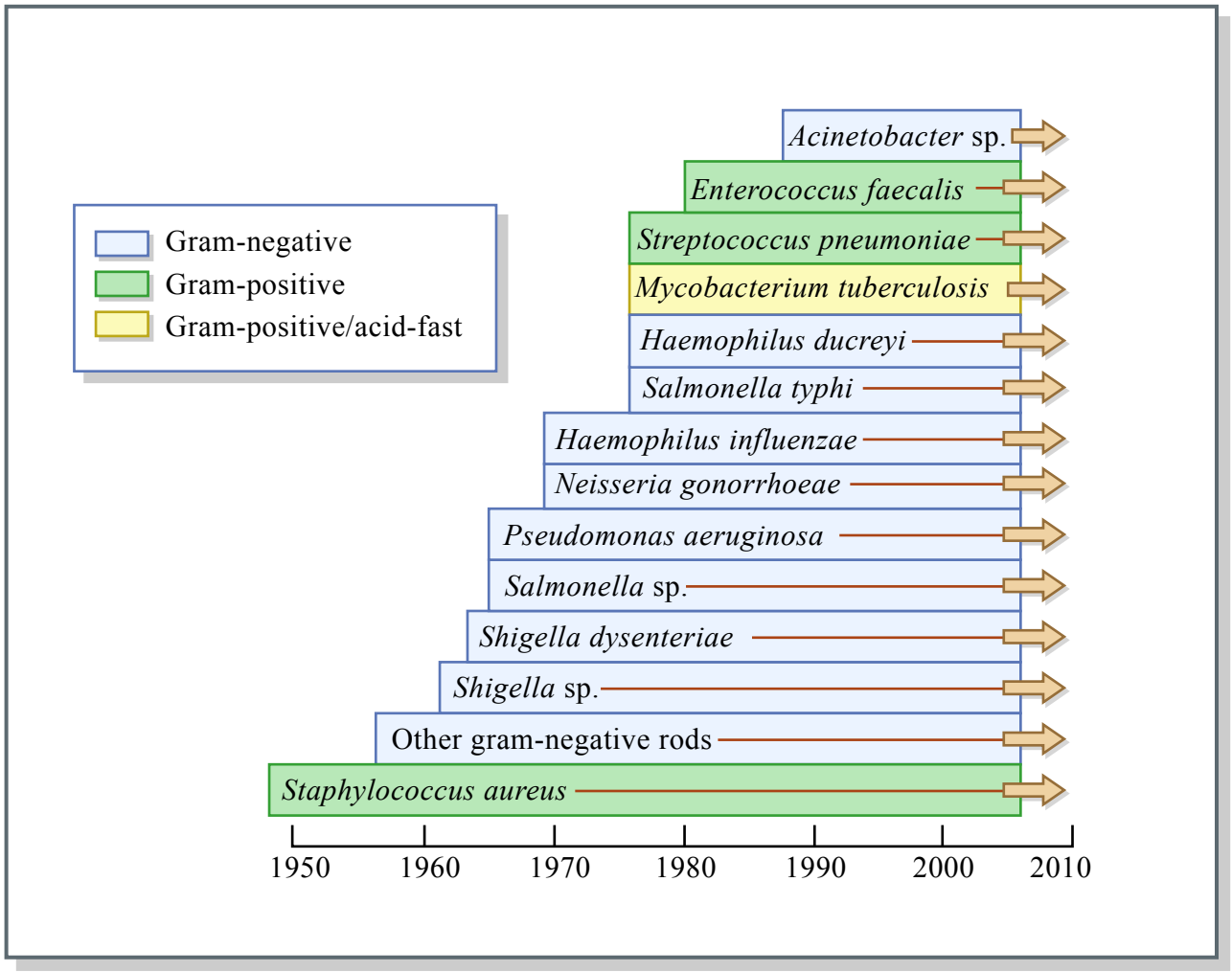
Antimicrobial drug resistance

- Acquired ability to resist effects of a chemotherapeutic to which it is normally susceptible
- Common mechanisms
  1. Lack structure drug targets
  2. May be impermeable to drug
  3. Organism may be able to modify drug to an inactive form
  4. Organism may modify the target itself
  5. Organism may develop a new pathway
  6. Organism may be able to pump out the drug

R plasmids

- Most drug resistant bacteria isolated from patients contain drug resistance genes on plasmids
- Many R plasmids encode enzymes that inactivate drugs
- R plasmids predate medical use of antibiotics
- Widespread emergence of multi-drug resistance

Resistance to all known drugs…



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- Methicillin-resistant *Staphylococcus aureus* (MRSA)
- Vancomycin-resistant *Enterococcus* (VRE)