

Limit growth by inhibition - **bacteriostatic**  
or Kill by sterilization - **bactericidal**

Control of microbes is obtained through :

1. Physical antimicrobial control  
heat, filtration and radiation
2. Chemical antimicrobial control      disinfection  
Each approach reduces the microbial load
3. Antimicrobial agents used *in vivo*
4. Control of viruses & eukaryotic pathogens
5. Antimicrobial Drug Resistance

**Section 20.1 Heat Sterilization**

Sterilization is the complete killing of all organisms, routinely through the application of heat. Macromolecules lose their structure - *denaturation*. The temperature/ time combination for heat sterilization is selected to kill the most heat-resistant organisms, bacterial endospores. For routine sterilization, an autoclave (pressure cooker) is used yielding steam heat under pressure at temperatures above the boiling point of water. Pasteurization is set to kill pathogenic microbes, and reduces load of spoilage microorganisms to prolong storage life.

The more rapid thermal death time is used

Total killing of ALL cell      cf. numbers, medium ,  
volume of medium -  
so here **Thermal Death Time (TDT)**  
depends on the size of the population

**Spores and Heat Sterilization**

Autoclave: pressure allows temperatures above boiling,  
e.g. 121°C. [15 pounds/square inch (lb/in<sup>2</sup>)= 121° C)

Sensitivity:- vegetative cells killed at 0.1-0.5 min at 65°C  
Endospores – 121°C for 4-5 min (routinely 15 min.)  
For dry heat 180 °C for 2 hr (glass, metal)  
Microbial death is more rapid at acidic pH  
e.g. tomatoes, fruits and pickles  
High concentrations of sugars, or proteins can decrease the heat penetration.

Heat resistance of bacterial endospores due to:  
low internal water  
Ca<sup>2+</sup>  
small acid-soluble spore proteins (SASPs)  
synthesis of **dipicolinic acid** all leads to a gel-like core.

Spore water content (10-30%) plus water content of the concentration of SASPs determines the heat resistance

Moist heat has better penetrating power  
= faster reduction in population numbers.

Kinetics of heat sterilization

At very high temperatures most macromolecules denature (**Fig. 20.1**)

Death by heating is an exponential  
(first order) (straight line) function

**DECIMAL REDUCTION TIME - D**

= time for 10-fold reduction of population Fig. 20.1

- Thus the rate of death is proportional **only** to the concentration of organisms.
- But, the time for a definite fraction (90%) to be killed is independent of initial cell concentration.
- The logarithm of D plotted against temperature yields a straight line (**Fig. 20.2**)

The slope is a measure of sensitivity. Use to predict processing temperatures and times.

Determination of the **Decimal Reduction Time (D)**  
is fairly lengthy.

**The Autoclave** (Fig. 20.3)

Routinely 1.1 kilograms/square centimeter (kg/cm<sup>2</sup>)

- (Temperature kills, not the pressure)
- With bulky objects heat transfer is slow .  
Hospital beds, 30,000 gallon fermentor ??

Salk (dead) vs live Sabine polio vaccines

**PASTEURIZATION** :Based initially on preventing spoilage of French army wine (55 °C did not disflavor the wine see the box - p.699).

Soxhlet suggested use for milk.  
Diseases: tuberculosis (and also *Mycobacterium bovis*), brucellosis, Q fever (*Coxiella burnetti*) and typhoid fever. Today also to *Salmonella* species and *E. coli* (O157:H7)

Milk Pasteurization - In bulk large vats 63-66 C for 35 min.  
Continuous flash pasteurization via a heat exchanger 71C for 15 sec Less alteration of flavor

**Section 20.2 Radiation sterilization** (p.700)

Under appropriate conditions, electromagnetic radiation [microwaves, ultraviolet (UV) radiation (T-T cross bonds p. 273), (hospital entry vents, motel toilets), X-rays, gamma rays (γ-rays)] and electrons effectively control microbial growth.

**Ultraviolet radiation** is useful for decontaminating surfaces, and materials that do not absorb light (air and water). Most effective wavelength range is normally considered to be between 200 and 300 nm (esp. 260 nm) acts by cross linking of pyrimidines. The "near-visible light" is useful for disinfecting surfaces. Germicidal UV light to decontaminate the surface after use e.g. in transfer hood (see Fig. 20.4)

**Ionizing radiation** is necessary to penetrate solid or light-absorbing materials. Energetic and causes breaks in DNA. Ionizing radiation is very effective for sterilization and decontamination (letter in mail; *Bacillus anthracis*).

**Ionizing Radiation**

e<sup>-</sup>, hydroxyl radicals, OH•, and hydride radical, H•,  
Roentgen - radiation energy output from a source

Absorbed dose is the **rad** (100 ergs/gray)  
(one gray = 1 Gy-100 **rad**)

Microorganisms are much more resistant to ionizing radiation than higher organisms (Table. 20.1).Fig. 20.5

Radiation Sensitivity D10 (90% reduction) in Gy

For any bacterium at least 200 Gy  
Human dosage lethal at 10 or less

**Radiation:** Several sources of ionizing radiation, X-ray machines, cathode ray tubes and radioactive nuclides (gamma radiation)(<sup>60</sup>Co and <sup>137</sup>Cs cheap by products of nuclear fission)

Radiation is approved by the Food and Drug Administration for sterilization of: Petri dishes; surgical supplies; disposable lab-ware; drugs and even heart valves and tissue grafts (Table 20.2).

.There are installation costs and hazards of radiation equipment. Cleaning and preservation of food is practiced world wide but in the USA is more limited to sterilization of spices (alternate ethylene oxide is losing popularity). Recently use for hamburg and chicken approved in the USA.

Critiques: radioactive contamination (?), alteration in nutritional value, production of toxic or carcinogenic compounds and the production of "off" tastes. But in wold wide use (IAEA, Vienna)

)  
By adjusting the dose, irradiation can be used for sterilization, pasteurization and insect deinfestation.

**Section 20.3 Filter Sterilization p. 702**

Filter sterilization involves the removal of living microorganisms from liquids or gases. Membrane filters are widely used for sterilization of heat-sensitive liquids.

**TABLE 20.1 Radiation Sensitivity**

Species	Type of Microorganism	D10 <sup>a</sup> (Gy)
<i>Clostridium botulinum</i> **	Gram-positive anaerobic sporulating	3300
<i>Bacillus subtilis</i>	Gram-positive anaerobic sporulating	600
<i>Lactobacillus brevis</i>	Gram-positive	1200
<i>Deinococcus</i> ** <i>radiodurans</i>	Gram-negative	2200
<i>Aspergillus niger</i>	Mold	500
Foot-and-Mouth**	Virus	13,000
Insect deinfestation	-----	1000-5000

*Clostridium botulinum* ..... 3,300  
12D10 use. Here = 39,600 Gy  
*Deinococcus radiodurans* 2,200  
*Salmonella typhimurium* 200  
Yeast ..... 500

Filtration is and effective approach to sterilize heat-sensitive liquids or gases. The early definitions of viruses were based on filtration - infection particle 28-200nm.

**TYPES OF FILTERS**

(Fig. 20.6) Filter range: -depth, membrane and Nucleopore)

DEPTH FILTER: one of the oldest types. Random array of layers of overlapping paper (anthrax spores?), asbestos, or glass fibers

Perhaps prefilter - sand, Celite

MEMBRANE FILTERS are the most common type cellulose acetate, cellulose nitrate, or polysulfone contain a large number of tiny holes. Hole size can be adjusted via the polymerization conditions.

Membranes can be open structures, about 80-85% = occupied by space (Fig. 20.6b)

NUCLEATION TRACK (NUCLEOPORE) FILTER

Polycarbonate films (10 μm) uniform holes via nuclear radiation followed by chemical etching. chemical enlarge locations into holes. Use for microscopy - Fig. 20.7.

Adaptable for small or large-volumes. Wide use in food and pharmaceutical industries. Available pre-sterilized. See small lab. kit Fig. 20.8.

**Section 20.4 Chemical growth control** p.703

Chemicals can be used to control microbial growth (Lister, phenol). Usage includes defining the minimum concentration necessary to kill or inhibit microbial growth.

An antimicrobial agent is a chemical that kills or inhibits.

Inhibit = -static agents fungistatic (Fig. 20.9)

Killing = -cidal agents, bacteriocidal  
e.g. quaternary ammonium compounds

Kill and lyse = bacteriolytic (penicillin)

**MEASURING ANTIMICROBIAL ACTIVITY**

Assess minimum inhibitory concentration (MIC) or the minimum lethal concentration (MIL)

Assessment: lowest conc. of agent that inhibits growth  
Tube dilution technique (Fig. 20.10).  
(to assess killing it is necessary to plate out afterwards to determine the number of live surviving cells)

Or use the agar diffusion method (Fig. 20.11) S. Katz  
Antimicrobial agent are added to filter paper discs  
incubation → a zone of inhibition

Plot concentration vs diameter<sup>2</sup> = straight response

**Section 20.5 Antiseptics, Disinfectants, Sterilants**

p.705

**Antiseptics** are used to decontaminate living tissues.  
hand washing; surface wounds (non-toxic to humans)

**Disinfectants** are used to decontaminate or sterilize nonliving material. Diverse use: in the home, health care, and industrial sites.

**Sterilants** can kill all microbial life  
e.g. germicides used widely in situations where it is impractical to use heat or radiation  
Bench tops, hospital floors.

Chemical sterilization of heat-sensitive materials:  
from thermometers to polyethylene tubing to space ships!

Cold sterilization gases such as ethylene oxide but volatile, explosive and highly toxic.

Gas/ liquids include:  
formaldehyde, per-acetic acid or hydrogen peroxide

Water is treated with chlorine  
see later Chapter 28 (p.941)

Diversity of inhibitory chemicals is reviewed in Table 20.3

**TABLE 20.3 Antiseptics, Disinfectants, and Sterilants**

Agent	Use	Mode of action
<b>Antiseptics</b>	-----	-----
Alcohol (60-85% ethanol)	Skin	Lipid solvent -& protein denaturant
Phenol-containing compounds (hexachlorophene, triclosan)	Soaps	Disrupts cell membrane
Cationic detergents (quaternary ammonium)	Soaps, lotions	Interact with phospholipids of membrane
Hydrogen peroxide (3% solution)	Skin	Oxidizing agent
Iodine + iodophor compounds	Skin	Iodates tyrosine

Silver nitrate	Eyes of newborn to prevent blindness ( <i>Neisseria gonorrhoeae</i> )	Protein precipitant
<b>Disinfectants and Sterilants</b>	-----	-----
Alcohol (60-85% ethanol)	-----	-----
Cationic detergents	-----	-----
Chlorine compounds (sodium hypochlorite)	-----	-----
Copper sulfate	Algicide in swimming pools	Protein precipitant
Ethylene oxide (gas)	-----	alkylating agent
Iodine-containing iodophor	Disinfectant for medical instruments	-----



Sulfanilamide is active in bacteria but not in higher animal because only the bacteria synthesize their own folic acid.

Note Prontosil is not active against bacteria, but is converted in the animal model to sulfanilamide, which is active. Animal models were part of the discovery.

#### Other growth factor analogs:

e.g. for vitamins, amino acids, purines, pyrimidines  
Isoniazid - interferes with mycolic acid synthesis of the cell wall of Mycobacteria - narrow activity spectrum (Fig. 20.15) (Details of attack of TB by Isoniazid (p.884 Fig. 26.9 = nicotinamide - see p.106 and Fig. 5.10, p.116) and F. Ryan The Forgotten Plague - Lehmann + Schatz and Waksman)

Other examples (Fig. 20.17) : Fluorouracil replaces uracil  
Bromouracil replaces thymine used more as mutagens.  
Also can be used as anti-virals - 20.10 and 9.3

**QUINOLONES** chemical inhibitors not growth factor analogs  
Interacts with bacterial DNA gyrase → prevents supercoiling (Fig. 20.13) Nalidixic acid is a prototype (Fig. 20.12 )

Fluoroquinolones are used in beef and poultry for respiratory diseases, and norfloxacin and Ciprofloxacin for human urinary tract infections - gram positive and gram negative bacterial infections (Fig. 20.15, and Fig. 20.18) .One of the choices for treatment of penicillin resistant anthrax (attacks DNA

#### Inhibitors of Protein Synthesis: Fig. 20.13

streptomycin ..... protein chain initiation  
  
chloramphenicol ..... protein elongation  
tetracycline  
cycloheximide

#### Inhibitors of RNA Polymerase Fig. 20.13

rifamycins .....  $\beta$  subunit of RNA polymerase  
streptovaricins (bacteria, chloroplasts and mitochondria)  
  
actinomycin ..... binds to DNA and stop RNA production

#### Section 20.8 $\beta$ -Lactams Penicillins and Cephalosporins

Note Pie chart Fig. 20.14

The  $\beta$ -lactams are the most important clinical antibiotics - the penicillins, cephalosporins and cephamycins. Specific for the bacterial cell wall synthesis enzymes and hence have low mammalian toxicity - see Fig. 20.19 top),

They account for over one-half (!) of all used antibiotics  
*Penicillium chrysogenum* - penicillin  
*Cephalosporium* sp -- cephalosporin.

#### Types of Penicillin (Fig. 20.19)

Variants of the basic penicillin G (benzyl-penicillin)  
methicillin (acid stable, --- lactamase resistant);  
carbenicillin (broad; injection)

synthesis, and thus can be used against gram negatives and gram positives. (Respiratory diseases in beef and chickens.

#### SECTION 20.7 ANTIBIOTICS (Pie Chart 20.14)

Antibiotics are a chemically diverse group of -static or -cidal compounds produced by microorganisms. They can act by a variety of mechanisms to disrupt microbial metabolism. Most known "antibiotics" have no clinical applications.

Production and discovery see Chapter 30. p.972 and the Waksman Laboratory in the Martin Hall basement

#### Targets of antibiotics

Definition p.712? Production by micro-organisms to inhibit or kill other microorganisms at low concentration

Plants, animals Magins (?frog skin inhibitory protein)  
Chemical synthesis - chloramphenicol, monobactam  
Chemical modifications → semisynthetic antibiotics

Range of effectiveness (or bacterial sensitivity - Fig. 20.15)  
Gram-positive bacteria are usually more sensitive  
If effective against Gram-positive & Gram-negative bacteria  
= a **broad-spectrum antibiotic**

Narrow-spectrum antibiotics have specialized and very useful applications - vancomycin acts against the cell wall but only of *Staphylococcus*, *Bacillus* and *Clostridium* and **Enterococcus**.

Acyl side groups of ampicillin and carbenicillin facilitate transport through the gram negative outer membrane.

#### Mechanisms of action

1. Penicillins: inhibit transpeptidation  
- cross-linking of two glycan-linked peptide chains

Transpeptidases = termed penicillin binding proteins (PBPs)

No cross linking - the wall becomes progressively weaker

BUT ALSO the antibiotic-PBP complex stimulates release autolysins

**Vancomycin** -glycopeptide acts differently on D-ala-D-ala

7% of humans have serious antibody-mediated allergies

#### **Cephalosporins** six-member dihydrothiazine ring

Similar to penicillins - prevent cross-linking of peptidoglycan

**Ceftriaxone** is highly resistant  $\beta$ -lactamase and of major use in treatment of *Neisseria gonorrhoeae*

#### Section 20.9 Antibiotics from Prokaryotes (Section?)

The aminoglycosides, macrolides, and tetracycline antibiotics are structurally complex molecules produced by prokaryotes, and are active against other prokaryotes. Erythromycin and the various tetracyclines are used widely in clinical medicine.

## 1. Aminoglycoside antibiotics p.714

*Streptomycin* (Fig. 20.12) and *Kanamycin* (Fig. 20.20)

Also *gentamicin* and *neomycin* (H. Lechevalier and S. Waksman, 1949)

Aminoglycosides inhibit protein synthesis binding to the 30S subunit of the ribosome (Fig. 20.13)

Active towards gram-negative Bacteria and Sm to TB.

*In toto* today account for 3% of production. (Fig. 20.14).

Several serious side effects (otic nerve - hearing) and they are susceptible to inactivation.

**Macrolide antibiotics** large lactone rings connect to sugar moieties (Fig. 20.21)

erythromycin, oleandomycin, spiramycin

11% of world use (Fig. 20.14)

Inhibit protein synthesis binding to the 50S ribosome subunit

Especially effective towards *Legionella pneumophila*

**Tetracyclines** broad-spectrum

Based on a naphthacene ring (Fig. 20.22)

Variations based on substitutions

*Chlortetracycline* chlorine atom

*Oxytetracycline* an extra hydroxyl

Diverse semisynthetic tetracyclines

Protein synthesis inhibitor -30S ribosomal subunit function

viral nucleic acid chain at the level host cell nucleic acid polymerase. Nearly always exhibit some level of host toxicity

**Nucleotide** analog *cidofovir* works in the same way (Table 20.5) (NRTI nucleotide reverse transcriptase inhibitors).

Influenza

Other inhibitors:

**Nevirapine** a nonnucleoside reverse transcriptase inhibitor binds directly to reverse transcriptase

**Phosphonoformic acid** is an analog of pyrophosphate inhibits inter-nucleotide linkage

**Rifamycin** (antibiotic) binds to RNA polymerase antiviral drugs

**Protease inhibitors** (Table 20.5) - prevent infection by binding up the active site of HIV protease, thereby inhibiting the processing of viral polypeptides

**INTERFERON** p.716

Interferons are antiviral substances produced by many animals in response to infection

Low-molecular weight proteins (17,000MW)

Interferons from virus-infected cells interact with receptors on noninfected cells promoting the synthesis of antiviral proteins

Tetracyclines used in veterinary medicine and also even as a nutritional supplement for poultry and swine. wide-spread antibiotic resistance

## Section 20.10 Viral control p.716

Viruses use host cell metabolic machinery to replicate, and thus inhibitors of viruses will often inhibit the host. Even so several viral enzymes and processes can be interrupted with chemotherapeutic agents to disrupt viral replication.

Many clinically effective antiviral agents are nucleoside analogs, and work by inhibiting viral nucleotide polymerases, Other agents including the protease inhibitors interfere with viral maturation steps. Host cells also produce antiviral proteins called interferons that stop viral replication. However, interferons are not yet available in clinically useful forms.

### Antiviral Chemotherapeutic Agents

AIDS has stimulated the search for viral inhibitors.

The most general are nucleoside analogs (Table 20.5)

The first was zidovudine, or azidothymidine (AZT) which inhibits retroviruses

It is a thymidine dideoxy derivative and AZT inhibits by blocking the synthesis of the DNA intermediate (reverse transcription) and successfully inhibits multiplication

Nearly all nucleoside analogs Table 20.5 inhibit elongation

that can prevent further virus infections

Three molecular types:

IFN- $\alpha$  produced by leukocytes

IFN- $\beta$  produced by fibroblasts

IFN- $\gamma$  produced by immune cells (lymphocytes)

All are effective

They were found by an observation that one virus interferes with subsequent infection with another virus, i.e. interferon.

Highly virulent viruses inhibit cell protein synthesis before any interferon can be produced

The inducer of interferon may be double stranded RNA (replicative form) as it does not exist in uninfected cells.

Interferons are not virus-specific but host specific, i.e.

an interferon from one animal inhibits multiplication of other viruses!!!!

Yet it has no affect on viruses in other animals. i.e. host specific.

Interferons have yet to be routinely developed - they must be present in relatively high local concentrations, meaning that for clinical utility one has to deliver interferon to local areas in the host. Perhaps this is through use of viral nucleotides or non-virulent viruses

**Section 20.11 Fungal control** p.718

Antifungal agents fall into a wide variety of chemical categories. As with viruses, selective toxicity is hard to achieve as fungi are Eukarya as are humans, animals and plants. Treatment of fungal infection is now an important human health issue.

There are a range of effective antifungal drugs, but most are limited to topical (surface) application.

**Ergosterol inhibitors**

Ergosterol replaces the cholesterol component of higher eukaryotic membranes, and inhibition of ergosterol synthesis or its function gives a lead to selective attack of fungi. ① polyenes ② azoles and allylamines

**Table 20.6**

**Polyenes** from Streptomyces bind to ergosterol and this disrupts membrane function

NYStatin (Rachel Brown & Elizabeth Hazen, 1949)

Hubert Lechevalier (candididin, 1952)

Amphotericin B Me Ester (Squibb, 1960)

**Azoles** inhibit ergosterol biosynthesis → membrane damage.

Fluconazole (oral) Clotrimazole (topical)

**Allylamines** also inhibit ergosterol biosynthesis are not taken up by animal cells and can only be used topically

Others inhibit folate biosynthesis

DNA topology (griseofulvin disrupts microtubule aggregation during mitosis)

5-fluorocytosine is a nucleic acid synthesis inhibitor

Others include vincristin, vinblastin and taxol are effective antifungal agents and have anti-cancer activity.

Also emergence of resistant and “new” fungal pathogens. for example *Candida* species

**20.12 Antimicrobial drug resistance** p.719

An important side effect of the use of antimicrobial chemotherapeutic agents is the development of resistance by the targeted microorganisms. In many cases, resistance results from the selection of existing resistance genes, enhanced by improper and indiscriminate uses of antimicrobial drugs. Many formerly useful antimicrobial agents are no longer useful cf. drug resistance. Some pathogens are super multi-drug resistant - prompting fears of a return to the pre-antibiotic era. New antimicrobial compounds are being sought, but progress is slow.

**TABLE 20.6 Antifungal Drugs**

Category	Target	Example	Use
Polyenes	Ergosterol synthesis	Amphotericin B	Oral
Nucleic acid analogs	DNA synthesis	5-Fluorocytosine	Oral
Polyoxin	Chitin synthesis	Polyoxin A	Agricultural
Azoles	Ergosterol synthesis	Fluconazole Clotrimazole	Oral Topical
Allylamines	Ergosterol synthesis	Terbinafine	Oral

Other antifungal agents:

Specific anti fungals - seek fungus-specific structures  
chitin, a polymer of -acetyl-glucosamine fungi and insects  
**polyoxins** inhibit cell wall synthesis, but no cell wall targeting drugs are used clinically

Some microorganisms are naturally resistant

1. lack structures with an antibiotic target  
Mycoplasmas, lack cell walls and thus are resistant to penicillins
2. impermeable: penicillins do not cross the outer membrane of gram-negative bacteria
3. inactivate: staphylococci β-lactamases cleave β-lactam ring (**Fig. 20.24**)
4. modify the antibiotic target e.g. ribosome shape
5. physiologic change: develop an alternate (and also resistant) biochemical pathway
6. pump out an antibiotic

Table 20.7

Mechanism of resistance may be chromosomally or plasmid encoded. The latter has resulted in mobile resistance plasmids R factors

**TABLE 20.7 Resistance to Antibiotics**

Mechanism	Antibiotic	Genetic Bases**
Reduced permeability	Penicillins	Chromosomal
Inactivation (penicillinase, acetylases, phosphorylases)	Penicillins Chloramphenicol	Plasmid and Chromosomal
Alteration of target (ribosomes)	Streptomycin	Chromosomal
Development of resistant biochemical pathway	Sulfonamides	Chromosomal
Efflux	Tetracyclines, Chloramphenicol	Plasmid Chromosomal

\*\*C = chromosomal

P = plasmid

#### Enzymatic Inactivation of antibiotics (Fig. 20.24)

Aminoglycoside antibiotics (streptomycin, neomycin, kanamycin and spectinomycin) modified by phosphorylation, acetylation or adenylation.

Chloramphenicol resistance via acetylation of the antibiotic

R plasmids confer multiple antibiotic resistance

#### Origin of resistance plasmids

Circumstantial evidence suggest that plasmids with multiple resistance (R plasmids), existed before the antibiotic era. A strain of *E. coli* freeze-dried in 1946, had plasmids conferring resistance to tetracycline and streptomycin, this being several years before clinical use.

R plasmids have also been detected in non-pathogenic gram-negative soil bacteria, and here the resistance plasmids may confer selective advantage. It seems that R plasmids are not a recent phenomenon

#### Nonmedical uses of antibiotics (BOX: pg. 721)

Should antibiotics be used in animal feed to reduce production costs?

25 milligrams (mg) per pound of chicken feed saves 2 billion lb (900 million kg) of feed due to more rapid weight gains and feeding efficiency.

Mechanism? probably inhibition microbes responsible for low grade infections - less inflammation, etc.

Molecular studies of resistant strains of *Salmonella* isolated from poultry indicate that the resistance resides on conjugative plasmids (transposons).

Animals previously fed antibiotics, when put on antibiotic-free rations do not lose the resistant bacteria.

Non medical use of antibiotic has reinforced a simple lesson "the environment selects the best adapted species".

#### MECHANISM OF RESISTANCE MEDIATED BY R PLASMIDS. p.720

Antibiotic resistance with modification of the target of e.g. a ribosome, tends to be on a chromosomal gene.

In contrast R plasmid sequester genes inactivating drugs.

#### Spread of antimicrobial drug resistance Figs. 20.25/26

In general, high levels of antibiotic use resulted in high levels of resistance.

Continued use has resulted in greater resistance, e.g. penicillin to inhibit *Neisseria gonorrhoeae* Fig. 20.25b.

Penicillin is no longer useful antibiotic for treatment of gonorrhea

Virtually all resistant strains have developed since 1980, in part through nonessential use

- ① growth-promoting substances in animal feeds
- ② prophylactics (to prevent the occurrence of disease rather than to treat an existing one)

e.g. fluoroquinolones have been extensively used for only 10 years. Even so, fluoroquinolone-resistant *Campylobacter jejuni* have already emerged presumably related to the practice of treating the whole flocks of poultry in order to prevent spread information

**Reversing Antibiotic Resistance:** In Hungary, in the 1980's, 50% of all cases children's *Streptococcus pneumoniae*, were penicillin resistant. Non-beta-lactam antibiotics were substituted. Resistance fell to 34%. Prudent use and careful monitoring may reverse the unfortunate consequences of prior over use.

**20.13 The search for new antimicrobial drugs** p.723

New analogs of existing anti-microbials are best "designed / screened" through rational design, e.g. solubility and affinity structural recognition. As opposed to finding novel compounds through blind screening of new microbial isolates, Best is defined in part by efficiency and cost.

Automated robotic methods (combinatorial chemistry) employ systematic modifications of known anti-microbials to generate numerous new analogs.

For tetracycline as a lead compound: 5 reagents being introduced to 4 different R group sites yields  $5 \times 5 \times 5 \times 5$  (five derivatives at each of four sites) → 725 different tetracycline derivatives.

and accomplished in a few hours though toxicity and efficacy  
Increases the number of lead compound X ten!!

But the rate of success for a new successful drug is:

1 per 7 million!

Trials in animals and humans will take years.

**COMPUTERIZED DRUG DESIGN** p.724

Increases in the efficiency and understanding of the structure/ function of biological molecules has allowed advances in rational design. Creation and testing in a computer environment.

Saquinavir, a new HIV protease inhibitor, was designed by computer to fit the active site of HIV protease.

This peptide analog displaces the HIV precursor protein, resulting in inhibition of virus maturation and slowing HIV development in the human host.

Indinavir a further protease inhibitor is

Illustrated in Fig. 20.27

.  
This example illustrates the practicality and the rapid and cost effective nature of computer design.

(Note the Protein Data base [www.pdb.bnl.gov](http://www.pdb.bnl.gov) has been moved to Rutgers).