I. Introduction
   A. Importance of disease
      1. historical
         a. cholera pandemic after each *hajj* (Moslem pilgrimage)
         b. crusaders brought back cholera and leprosy (1100 - 1300)
         c. plague (20% of European population died) (1300)
         d. syphilis brought back by Columbus
            (1) spread around world by other explorers
            (2) Charles VII of France died of syphilis, all heirs born dead of syphilis, end of dynasty
            (3) almost 80,000 Union soldiers during Civil War, 3 million during WWI, 1 million during WWII
      2. economic
      3. cultural/social
   B. Brief history of disease in the U.S.
      1. top 10 causes of death
         a. shift from infectious to chronic disease
         b. public health guidelines, including better sanitation and personal hygiene, are responsible for the increased life spans and overall better health we have today
         c. lifestyle
      2. less harmful diseases
      3. most common diseases (severity vs. frequency)
      4. emerging diseases/problems
      5. Darwinian medicine

II. Principles of Disease Occurrence
   A. Theories of disease causation
      1. *germ theory*
         a. infectious disease is caused by the transfer of infectious agents
         b. didn’t explain why some people got sick but others didn’t
      2. *multiple-causation* (Box 2-1)
         a. several factors contribute to development of disease
            (1) disease agents
            (2) host factors
            (3) environment
         b. accounts for noninfectious diseases, too
   B. Terminology
      1. *morbidity rate* = number of ill people per susceptible population during a specific time
      2. *mortality rate* = number of deaths per total number of disease cases
      3. *incidence rate*
         a. number of new cases occurring during a specific time
         b. number of new cases divided by population at risk (x 1000)
   C. Levels of prevention
      1. *primary prevention* = measures taken before disease occurs to reduce susceptibility
2. **secondary prevention** = diagnosis of disease already present
3. **tertiary prevention** = treatment of disease (return host to normal health)

D. Disease agents for infectious disease
1. viruses
   a. acellular (protein coat around genetic material)
      (1) DNA or RNA as genetic material
      (2) single or double stranded
   b. obligate parasites
2. bacteria (Table 2-2)
   a. prokaryotic (no membrane-bound organelles)
   b. normal flora helps protect against infection
3. fungi
   a. eukaryotic
   b. secrete hydrolytic enzymes (breaks down tissues)
   c. diseases can arise if antibiotics alter bacterial normal flora
4. protozoa
   a. animal-like eukaryotes
   b. release toxins and enzymes
5. metazoa
   a. tapeworms, roundworms, flukes
   b. can migrate throughout the body

III. The History of Infectious Disease
A. Birth of modern microbiology
1. Robert Hooke first person to see cells (but not bacteria) with a microscope (1665)
2. microorganisms first observed and described by Antony van Leeuwenhoek (1676)
   a. simple, single lens microscopes (50 - 300x magnification)
   b. huge curiosity, observed anything he could
      (1) animalcules (intestinal organisms, including Giardia)
      (2) bacteria from overnight pepper infusions
      (3) seeds and plant embryos
      (4) small invertebrates
      (5) spermatozoa
      (6) red blood cells
      (7) essentially all main unicellular organisms we know today
         (a) yeasts
         (b) protozoa
         (c) algae
         (d) bacteria
   c. "I have had several gentlewomen in my house, who were keen on seeing the little eels in vinegar; but some of them were so disgusted at the spectacle, that they vowed they'd never use vinegar again. But what if one should tell such people in the future that there are more animals living in the scum on the teeth in a man's mouth, than there are in a whole kingdom?"
   d. superior observation skills but did not allow others to copy his techniques and verify his results
3. Edward Jenner observed that milkmaids didn't get smallpox (1800)
   a. prior exposure to cowpox seemed to protect against smallpox
   b. began inoculation with cowpox
   c. Pasteur continued work along this line with vaccines against anthrax and rabies
4. Ignaz Semmelweiss improved hospital sanitary practices
   a. observed mortality rates lower with midwives than doctors
      (1) strep infection = child bed fever or puerperal sepsis
      (2) got fired
   b. friend died of P.S. from cut during autopsy of P.S. victim
   c. hypothesized an invisible agent responsible for disease
   d. sanitized hospitals by required handwashing and changing lab coats (fired again)
   e. died in insane asylum of P.S.
5. Pasteur termed the microbial spoilage of wine and beer "diseases"
   a. considered that microorganisms could act as agents of infectious disease
      (1) already known that fungi could cause disease in wheat and rye
      (2) a fungus was responsible for the great Potato Blight of Ireland
   b. observed foreign organisms (bacteria) in contaminated wine
6. John Lister sterilized instruments with heat and used phenol on dressings and sometimes on wounds
   (1) phenol kills bacteria
   (2) less wounds became infected
   (3) indirect evidence for role of bacteria in infection
7. Direct evidence for role of bacteria in disease was by Robert Koch, working with anthrax
   (1) injected a series of 20 healthy mice with anthrax bacilli
   (2) inoculated broth with spleen from infected mouse
   (3) isolated anthrax bacilli spores
   (4) injection of spores into mice resulted in anthrax
8. Koch's Postulates
   a. The organism should be present in every case of the disease, but absent in healthy individuals
   b. The suspected microorganism must be isolated and grown in pure culture
   c. The disease must result when the isolated microorganism is inoculated into a healthy host
   d. The same microorganism must be isolated again from the diseased host
9. Vaccination developed by Pasteur, working with chickens and cholera
10. The germ theory of disease (from Pasteur's and Koch's work) describes the demonstration that microbes can be the agents of disease
    a. general belief was that epidemics were penalties of God
    b. greatest impetus for development of microbiology
    c. contagious diseases spread through populations by contagions
    d. after discovery of microbes, contagions = microorganisms
B. Principles of microbial disease
   1. most important technological application of microbiology is medical
a. impetus for development of microbiology
b. microbes cause disease in man, animals, plants, and each other
c. not the only cause of disease
   (1) schistosomiasis
   (2) lung cancer
   (3) hemophilia or sickle cell anemia
2. disease is a form of parasitism (host supplies food)
3. disease occurs when a microbe infects a host to which it is imperfectly adapted but can grow and flourish
   a. biological defense processes brought into play
   b. if defenses overstrained or unsuccessful, host sickens and may die
      (1) if host dies, parasite dies
      (2) well-adapted parasites cause little or no damage
      (3) poorly-adapted or inadvertent parasites are dangerous and sometimes lethal
4. humans (and other higher organisms) have developed a balanced microbial flora, as bacteria are passed around between community members
   a. new-borns are almost sterile, carrying (inside and out) bacteria derived from the mother's vagina
      (1) soon pick up lactobacilli
      (2) gradually develops the adult population of mixed microbes
5. normal equilibrium can be upset by travel to another area (country) and exposure to new strains of bacteria

C. pathology = scientific study of disease
   1. etiology = the cause of disease
   2. pathogenesis = manner in which a disease develops
   3. structure and functional changes brought about by disease and its final effects on the body
   4. infection = invasion or colonization of the body by pathogens
      a. normal flora can be present in abnormal area (e.g., *E. coli* OK in intestine, pathogenic in urinary tract)
      b. most microbes are non-pathogenic
   5. disease = result of an infection that changes the state of health
      a. = abnormal state in which part or all of the body is not properly adjusted or carrying out normal functions
      b. infections can exist without disease (e.g., HIV)
   6. normal flora
      a. animals are germ-free in utero
      b. microbial populations begin to establish themselves at birth
      c. estimated $10^{13}$ body cells and $10^{14}$ bacterial cells
      d. normal flora = microorganisms that colonize the body but do not produce disease under normal conditions
      e. transient flora = microorganisms that are present for brief periods (days - months) and then disappear
   7. symbiosis = living together
a. **commensalism** = one organism benefits, the other is unaffected
   (1) many, if not most, of normal flora
   (2) especially common relationship on external skin
b. **mutualism** = both organisms benefit
   (1) common with intestinal bacteria
   (2) *E. coli* gets nutrients in large intestine, produces vitamin K and B vitamins
c. **parasitism** = one benefits, the other is harmed (typical pathogenic relationship)

8. **opportunists** = potentially pathogenic organisms that do not ordinarily cause disease in their normal habitat in a healthy person
   a. organisms that gain entrance to the bloodstream through broken skin
   b. compromised hosts are subject to diseases caused by normal flora
   c. often, normal flora includes pathogens kept in check

9. for determination of the etiological agent, recall Koch's postulates
   a. The organism should be present in every case of the disease, but absent in healthy individuals
   b. The suspected microorganism must be isolated and grown in pure culture
   c. The disease must result when the isolated microorganism is inoculated into a healthy host
   d. The same microorganism must be isolated again from the diseased host

10. some exceptions, where causative agent cannot be identified using Koch's postulates
    a. *Treponema pallidum*, the causative agent of syphilis, has never been cultured on artificial medium
    b. *Mycobacterium leprae*, the causative agent of leprosy, has never been cultured on artificial medium
    c. alternative steps can be used to modify Koch's postulates, for example inoculating test organisms with infected tissue instead of isolated microorganisms

D. classification of infectious diseases
1. **symptoms** = changes in body function
   a. e.g., pain and malaise
   b. often subjective (not apparent to an observer)
2. **signs** = objective changes that can be observed and measured by a physician
   a. lesions (changes in tissue caused by disease), swelling, fever, paralysis
   b. **syndrome** = specific group of symptoms that always accompany a particular disease
3. **communicable** = able to spread (directly or indirectly) from one host to another (chicken pox, measles, herpes, tuberculosis)
   a. **contagious** = easily communicable (chicken pox, measles)
   b. **noncommunicable** = not spread from one host to another (tetanus)
4. infection can be **localized** (invading MO limited to relatively small body area) or **systemic (generalized)** (spread throughout the body)
   a. localized = boils or abscesses
   b. systemic = measles
5. **reservoir of infection** = source of disease organisms
a. human body is primary reservoir
   (1) carriers = harbor and transmit pathogens without exhibiting illness themselves
   (2) latent diseases can be spread during incubation period (before symptoms appear) or during the convalescent period (during recovery)

b. animal reservoirs
   (1) zoonoses = diseases that occur primarily in animals but can be transmitted to humans
   (2) rabies (mammals), Rocky Mountain spotted fever (ticks)

c. non-living reservoirs
   (1) soil and water
   (2) soil has low levels of pathogens (under normal conditions)
   (3) pathogens often introduced to water (or soil) by humans (e.g., fecal contamination)

6. disease transmission has three principle routes
   a. contact transmission = spread of agent by direct contact (person to person transmission)
      (1) respiratory diseases, measles, smallpox, STDs
      (2) can also be by indirect contact
         (a) indirect contact transmission relies on inanimate objects (handkerchiefs, eating utensils)
         (b) droplet transmission = discharged mucous droplets that only travel short distances (sneezing, coughing, laughing)
   b. vehicle transmission = agents like water, food, air
   c. vectors = animals that carry pathogens from host to host
      (1) insects (arthropods) most common
      (2) transmission can be passive (surface transfer) or active (transmitted through bites)

7. nosocomial infections = acquired as a result of a hospital stay
   a. MO in hospital environments and compromised hosts
      (1) gram positive cocci (Staphylococcus aureus) used to be most common
      (2) major causes today are gram negatives (E. coli and Pseudomonas aeruginosa), especially antibiotic-resistant strains
   b. compromised host = resistance to infection impaired by disease, therapy or burns
      (1) broken skin or mucous membranes remove protective barrier
      (2) suppressed immune system
   c. controlled by scrupulous cleaning and disinfection

8. pathogenicity = ability of a pathogen to produce a disease by overcoming host defenses

9. virulence = the degree of pathogenicity

E. Mechanisms of pathogenicity
   1. portal of entry = how pathogen gains access to the body
      a. mucous membranes
         (1) respiratory, gastrointestinal, genitourinary tracts; conjunctiva (membrane
around eyeball)

(2) gastrointestinal and respiratory tracts most common

b. skin

(1) usually a defense against disease
(2) microbes gain entrance through openings
  (a) sweat gland ducts
  (b) hair follicles

c. parenteral route = direct deposition into tissues beneath skin or mucous membranes

(1) wounds
(2) bites
d. use of preferred portal of entry often prerequisite to ability to cause disease

(1) ingested *Salmonella typhi* (preferred portal) produce typhoid fever, but no reaction when rubbed on skin
(2) inhaled streptococci can cause pneumonia, but if swallowed, usually no signs or symptoms

2. dose (number of invading cells) important, as defense mechanisms will eliminate many

3. **adherence** = attachment to host tissues

a. essential for most pathogens (to result in pathogenicity)
b. surface molecules (ligands or adhesins), often located on glycocalyx or fimbrae, bind to host receptors

(1) most adhesins are glycoproteins or lipoproteins
(2) most receptors are sugars (mannose)

4. Penetration of host defenses

a. capsules

(1) protects cells against phagocytosis
  (a) **phagocytosis** = process where certain body cells engulf and destroy microbes
  (b) in second line of defense, human body produces antibodies against the capsule which, when on the capsule surface, allow phagocytosis to occur

(2) common with pneumonia-causing organisms (*Streptococcus pneumoniae, Klebsiella pneumoniae, Hemophilus influenzae*)

b. cell wall components

(1) *Mycobacterium tuberculosis* has a waxy cell wall that resists digestion by phagocytes
(2) *Streptococcus pyogenes* has a heat- and acid-resistant M protein
  (a) aids attachment
  (b) resists phagocytosis

5. enzymes

a. extracellular enzymes that break cells open, dissolve materials between cells, form or dissolve blood clots, and other functions
b. leukocins destroy neutrophils (white blood cells) and macrophages
c. hemolysins lyse red blood cells
d. coagulases clot blood (may protect bacteria)

e. several enzymes that enhance spread from a focal infection
   (1) kinases dissolve blood clots formed to isolate infection
   (2) hyaluronidase breaks down connective tissue, allowing cells to spread
   (3) collagenase hydrolyzes connective tissue

f. necrotizing factors kill cells

g. hypothermic factors lower body temperature

h. proteases break down tissues

6. damage mechanisms
   a. three basic ways for successful invaders to damage host cells
      (1) direct damage in immediate vicinity of invasion
      (2) toxin production, transported by fluids to damage distant sites
      (3) induction of hypersensitivity reactions
   b. host cells destroyed when pathogens metabolize and multiply inside the host cells
   c. toxins = poisonous substances produced by specific MO
      (1) usually the primary mechanism of host damage (pathogenicity)
      (2) toxigenicity = ability to produce toxins
      (3) toxemia = symptoms caused by toxins in blood
   d. exotoxins = secreted proteins, usually enzymes
      (1) exotoxin, not bacterium, produces disease symptoms
         (a) usually produced by gram positives
         (b) genes often plasmid encoded
         (c) tend to be very toxic (1 mg botulinum toxin enough to kill 1 million guinea pigs)
      (2) antitoxins = antibodies produced against exotoxins
      (3) cytotoxins kill host cells or affect functions
         (a) diphtherotoxin (inhibits protein synthesis)
         (b) erythrogenic toxins (damage capillaries)
      (4) neurotoxins interfere with nerve impulses
         (a) botulinum toxin (prevents nerve transmission)
         (b) tetanus toxin (prevents inhibitory nerve transmission)
      (5) enterotoxins affect cells lining the GI tract, inducing fluid and electrolyte loss from host cells
         (a) *Vibrio* choleraigen
         (b) *staphylococcal enterotoxin*
   e. endotoxins = part of outer wall of cell wall of gram negative bacteria (lipid portion of lipopolysaccharide layer = lipid A)
      (1) endotoxins = lipopolysaccharides, exotoxins = proteins
      (2) endotoxin released when cells are lysed
         (a) cell death
         (b) antibiotics
         (c) antibodies
      (3) all produce fever (pyrogenic response), weakness, aches, sometimes shock
(4) do not promote formation of effective antitoxins
   (a) **antitoxin** = specific antibody produced in response to an exotoxin
       or its toxoid
   (b) antibodies are produced, but they do not counter the effect of the
       toxin and can often enhance its effect

7. **hypersensitivity** = allergy = exaggerated or heightened immune reaction that is
   injurious
   a. contact with heterophile antigens on bacteria can cause a host to produce
      antibodies against any A or B antigens they are lacking
   b. these antibodies can react with these antigens if the wrong blood type is
      transfused without regard to compatibility

F. Host defense mechanisms
1. **resistance** = ability to ward off diseases through body defenses
   a. **susceptibility** = lack of resistance
   b. **nonspecific resistance** = all defenses that protect host from any kind of
      pathogen
   c. **specific resistance** = antibodies against specific microorganisms

2. skin and mucous membranes protect through a combination of mechanical and
   chemical factors
   a. mechanical factors include physical barrier to microbial invasion
      (1) intact skin difficult to penetrate
      (2) keratin (skin protein) is waterproof
      (3) some pathogens in large numbers can penetrate mucous membranes
   b. bacteria can be washed from surfaces
      (1) lacrimal apparatus in eyes
      (2) saliva washes MO from teeth and gums
      (3) urine move MO out of urinary tract
      (4) vaginal secretions move MO out of vagina
      (5) perspiration washes MO off skin
   c. mucus traps many MO that enter respiratory or GI tracts
   d. sebum contains unsaturated fatty acids, which can inhibit pathogens
      (1) **sebum** = oily substance produced by sebaceous glands that coats some
         areas of skin and keeps hair from getting brittle
      (2) some skin bacteria metabolize sebum and cause the inflammatory response
         associated with acne
   e. lysozyme in tears, saliva, nasal secretions, and perspiration lyse bacteria
   f. high acidity (pH 1.2-3.0) of gastric juice prevents microbial growth in stomach
      and kills many ingested organisms
   g. normal flora prevent the growth of many pathogens

3. **phagocytosis** is the ingestion of MO or particulate matter by phagocytes
   a. **phagocytes** = white blood cells (or derivatives) = leukocytes
      (1) infection stimulates WBC synthesis (leukocytosis)
      (2) two types of phagocytic WBC
         (a) neutrophils (a type of granulocyte), commonly called
             polymorphonuclear leukocytes (PMNs)
(b) monocytes, which lack granules and mature into macrophages once they leave blood and enter tissues

(3) neutrophils are highly phagocytic and motile
   (a) able to leave the blood, enter tissues, and destroy microbes
   (b) active in initial stages of infection

(4) monocytes more active in later stages of infection

b. phagocytosis involves four major steps
   (1) phagocytes attracted to MO by chemotaxis
   (2) phagocyte adheres to the MO
   (3) phagocyte ingests MO
   (4) phagocyte digests MO

4. Inflammation is a response to bodily damage, characterized by redness, pain, heat, swelling, and sometimes loss of function
   a. vasodilation and increased permeability of blood vessels
      (1) vasodilation = increase in diameter of blood vessels
         (a) increases blood flow to damaged area
         (b) responsible for redness and heat
      (2) increased permeability allows defensive substances normally retained in blood to pass into injured area
         (a) responsible for swelling
      (3) vasodilation and increased permeability induced by histamines, kinins, prostaglandins, and leukotrienes
   b. phagocytes squeeze through blood vessels into infected tissue
   c. pus is the accumulation of damaged tissue and dead microbes, granulocytes, and macrophages
   d. repair = final stage of inflammation

5. fever = abnormally high body temperature produced in response to bacterial or viral infection
   a. increased body temperature increased production of immune system components
   b. may inhibit growth of some bacteria
   c. may increase repair rate

6. the body produces certain antimicrobial substances in addition to chemical already mentioned
   a. interferons probably best known
   b. interferons are antiviral proteins
      (1) 3 types of human interferon
      (2) induce uninfected cells to produce antiviral proteins that prevent viral replication
         (a) good only on short-term basis
         (b) does not prevent viral multiplication in cells already infected
      (3) host-cell specific, but not virus specific

G. The immune system
   1. an inducible system that acts as the last line of defense
      a. activation indicates that general resistance mechanisms have failed
b. must have an extensive repertoire but focus on specific foreign substance
c. must be shut down upon removal of foreign material

2. types of immunity
   a. innate (genetic)
      (1) genetically determined
      (2) species immunity
   b. acquired immunity
      (1) natural (by having a disease)
      (2) artificial (vaccination)
   c. active vs. passive
      (1) active = own system produces antibodies
         (a) naturally acquired active immunity = from disease
         (b) artificially acquired active immunity = from vaccine
      (2) passive
         (a) naturally acquired passive immunity = antibodies from mother
            passed to offspring
         (b) artificially acquired passive immunity = antibodies obtained from
            other hosts

3. antigen = immunogen = substance the body identifies as foreign and mounts a
   defense against
   a. most are large proteins (MW > 10,000)
      (1) polysaccharides or protein complexes (glycoproteins, nucleoproteins)
         work well
      (2) lipid work poorly
   b. epitope = antigenic determinant

4. antibody = antiantigen protein produced by immune system against antigens

5. lymphocytes = cells that carry out specific immune responses
   a. develop from stem cells (like other wbc, rbc, and platelets)
   b. B cells or B lymphocytes mature in bursal cells of birds (or equivalent in
      humans)
      (1) most believe they develop in bone marrow or gut-associated lymphoid
          tissues
      (2) about one-fourth of lymphocytes circulating in blood
   c. T cells or T lymphocytes undergo differentiation in the thymus
      (1) as adults (thymus less active) differentiation occurs in blood marrow or
          tissues under the influence of hormones from the thymus
      (2) four different types
         (a) cytotoxic (killer) T cells
         (b) delayed-hypersensitivity T cells
         (c) helper T cells
         (d) suppressor T cells

6. humoral immunity is carried out by antibodies circulating in the blood
   a. B cells release antibodies
   b. most effective against bacterial toxins, bacteria, and viruses before they enter
      cells
c. IgG makes up about 80% of plasma antibodies
   (1) appears in all body fluids
   (2) major antibacterial and antiviral antibody
d. IgM is the first immunoglobulin produced during immune response
   (1) very large
   (2) usually found only in vascular system
e. IgA is found mainly in bodily secretions
   (1) saliva, sweat, tears, mucus, bile, and colostrum
   (2) defends against surface pathogens especially those that enter the respiratory and GI tracts
f. IgD is the predominant antibody on the surface of B cells and acts mainly as an antigen receptor
g. IgE is involved in hypersensitivity reactions
   (1) develops within minutes of exposure to antigen
   (2) stimulates the release of mast cell granules, which contain histamine and heparin
h. inflammatory response is part of humoral immunity
7. cell mediated immunity is carried out by T cells
   a. works against antigens in cell membranes or inside cells
   b. defends primarily against virus-infected cells, but can also work with eukaryotic parasites, cancers, and foreign tissues (transplants)
8. recognition of self
   a. major histocompatibility complex (MHC)
      (1) cell surface components
      (2) secondary interactions
   b. clonal selection theory
      (1) timing of exposure between lymphocytes and antigens determines if it is recognized as self or not
      (2) early exposure (embryonic development) leads to destruction of specific lymphocytes
9. specificity = different reactions of immune system to each foreign substance
10. heterogeneity = ability for immune system to respond to all the different antigens encountered
11. memory = recognition of antigens previously encountered
    a. memory cells produced
    b. memory cells have different lifetimes
12. immunoglobulins = antibodies
    a. Y-shaped molecules made up of two light chains and two heavy chains
    b. constant and variable regions responsible for different combinations
H. Immune Disorders
1. hypersensitivity (allergies)
   a. immune system over-reacts
   b. can harm the host
   c. usually treated with antihistamines
   d. several different manifestations
1. allergic rhinitis (hay fever)
2. urticaria (hives)
3. asthma

2. autoimmunity
   a. immune system attacks host
   b. several manifestations
      1. rheumatoid arthritis
      2. lupus erythematosus

IV. Control of Growth (inanimate objects)

A. Terminology
   1. sterilization = destruction of living cells, viable spores, viruses, viroids
   2. disinfection = killing, inhibition, or removal of organisms
   3. sanitization = reduction of microorganisms to safe health levels
   4. antiseptic = chemical agents applied to tissues to kill or inhibit pathogens
   5. -cide = kills organisms
   6. -lytic = lyses organisms
   7. -static = inhibits growth of organisms

B. Effectiveness of antimicrobials
   1. population death is generally exponential or logarithmic
   2. efficiency of antimicrobials influenced by at least 6 factors
      a. population size - larger population requires more time to die
      b. population composition - different degrees of resistance between different organisms or structures
         1. spores more resistant than vegetative cells
         2. *Mycobacterium tuberculosis* (acid-fast) more resistant than most bacteria
         3. young cells more readily destroyed than older cells
      c. concentration of antimicrobial
         1. usually greater concentration = greater effectiveness
         2. 70% ethanol more effective than 95% ethanol
      d. exposure duration
         1. longer exposure = greater death
         2. sterilization = reduction of survival probability to $10^{-6}$
      e. temperature; increase usually increases effectiveness of chemical
      f. local environment
         1. heat kills better at acid pH
         2. efficiency higher with lower organic matter

C. Heat
   1. moist heat
      a. boiling kills viruses, bacteria, fungi
      b. 10 minutes boiling kills vegetative cells, not endospores
      c. pressurized steam (autoclave)
         1. autoclave = fancy pressure cooker
         2. combines wet heat with pressure; allows temperatures above 100° C
         3. temperatures above 100° C required to destroy endospores
         4. chamber filled with saturated steam for 121° C, 15 psi
(5) materials exposed for 15 minutes
d. thermal death time (TDT) = shortest period of time to kill all organisms at a specific temperature under defined conditions
e. decimal reduction time (D) or D value = time to kill 90%
   (1) important to food industry
   (2) usually assumed population of $10^{12}$ cells, reduced to $10^6$
   (3) if D for *C. botulinum* spores is 0.204 minutes at 121° C, 12D = 2.5 minutes
   (4) z value = increase in temperature required to reduce D to 1/10 its value
      (a) for *C. botulinum*, z = 10° C
      (b) at 111° C, D = 2.04 minutes, 12D = 24.5 minutes
f. pasteurization
   (1) reduces microorganism numbers but retains flavor of foods, especially dairy, beer, and other beverages
   (2) brief heating, followed by rapid cooling
   (3) 63° - 66° C for 30 minutes (batch or older method)
   (4) flash pasteurization = 72° C for 15 seconds
   (5) UHT = 134° C for 1-2 seconds
g. tyndallization = discontinuous boiling or fractional steam sterilization
   (1) heat material to 90°-100° C for 30 minutes on 3 consecutive days, incubated at 37° C
   (2) 1st heating destroys cells but leaves endospores
   (3) 2nd and 3rd heating destroys germinating endospores

2. Dry heat sterilization
   a. 160°-170° C for 2-3 hours
   b. cell constituents oxidize
   c. less effective than moist heat

3. Filtration
   a. physical removal of microorganisms
      (1) excellent for heat sensitive materials
      (2) can be used with gases
   b. depth filters = thick layers of fibrous or granular material
      (1) twisting channels of small diameter
      (2) microbes removed by physical entrapment and adsorption to filter material
   c. membrane filters = thin (0.1 mm) membranes
      (1) made of cellulose acetate, cellulose nitrate, polycarbonate, polyvinylidene chloride, or other synthetic materials
      (2) vegetative cells removed with 0.2 μm pore size

4. Radiation
   a. alters DNA, causing lethal mutations
   b. UV= ultraviolet light (260 nm)
      (1) doesn't penetrate glass, dirt films, water, many plastics
      (2) often used to sterilize cabinets or entire rooms
   c. ionizing (e.g., gamma)
      (1) penetrates objects
      (2) also called cold sterilization
D. Chemical methods
1. chemicals react with and destroy function of cell components
2. phenolics
   a. early use by Lister
   b. Lysol contains a mixture of phenolics
   c. denature proteins and disrupt cell membranes
   d. excellent for surfaces, but can cause skin irritation
3. alcohols
   a. bactericidal and fungicidal, but not sporicidal
   b. denature proteins and dissolve membrane lipids
4. halogens
   a. iodine most common, followed by chlorine
   b. **tincture of iodine** = 2% iodine in water/ethanol solution of potassium iodide
      (1) effective antiseptic
      (2) stains and may damage skin
   c. chlorine disinfectant of choice for municipal water supplies and swimming pools
      (1) added in many forms; forms hypochlorous acid
      (2) oxidizes several materials, destroying cells but not endospores
      (3) Halzone tablets used for personal drinking water
      (4) excellent household disinfectant
         (a) 1:100 dilution of household bleach (1.3 oz/gal) + 0.7% nonionic detergent
            (1 oz/gal)
         (b) cleans and kills bacteria
5. heavy metals
   a. Hg, Ag, As, Zn, Cu used to be common germicides
      (1) most heavy metals are bacteriostatic, not bactericidal
      (2) currently using less toxic, more effective germicides
      (3) 1% silver nitrate added to eyes of infants to prevent ophthalmic gonorrhea;
         being replaced by erythromycin, which is also effective against *Chlamydia* and *Neisseria*
      (4) silver sulfadiazine used on burns
      (5) copper sulfate used as algicide in lakes and swimming pools
   b. combine with proteins (sulphydryl groups), inactivating them
6. quaternary ammonium compounds
   a. **detergents** = organic molecules (non-soaps) that serve as wetting agents and
      emulsifiers
      (1) amphipathic molecules
      (2) effective cleansing agents
   b. cationic detergents more antimicrobial than anionic detergents
      (1) quaternary ammonium compounds most popular
      (2) positively charged quaternary nitrogen with long hydrophobic aliphatic chain
c. disrupt membranes and may denature proteins
d. kill most cells, but not endospores or *M. tuberculosis*
e. often used as disinfectants for food utensils, small instruments, and skin antiseptics
7. aldehydes
   a. formaldehyde and glutaraldehyde most common
   b. combine with and deactivate proteins
   c. 2% glutaraldehyde commonly used to disinfect hospital equipment
8. gases
   a. ethylene oxide is both microbicidal and sporicidal
      (1) combines with cell proteins
      (2) penetrates packing materials, even plastic wraps
   b. explosive, usually done in special sterilizer
V. Antimicrobial chemotherapy (living systems)
A. History
   1. chemotherapeutic agents = chemical agents used to treat disease
      a. destroy or inhibit growth of pathogens
      b. concentrations low enough not to damage host
      c. include antibiotics = microbial products or their derivatives that can kill or inhibit growth of microorganisms
   2. Paul Erlich began the modern age of chemotherapy
      a. magic bullet = chemical (toxic dye) that would specifically bind to and destroy pathogens
         (1) particularly interested in syphilis and African sleeping sickness
         (2) normal syphilis treatment was ingestion of toxic mercury compounds (often patients died)
         (3) trypanosomiasis treated similarly with arsenic compounds
         (4) deliberately tried preparing organic material containing arsenic which would be less lethal to humans
      b. arsphenamine (Erlich 606) was effective against syphilis (trade name Salversan)
      c. noted that dyes were strongly taken up by bacteria, so studied those for specific toxicity
         (1) trypan red was effective against the trypanosome that causes African sleeping sickness
         (2) acriflavine (yellow dye still in use)
            (a) good for superficial wounds and skin infections
            (b) too toxic for internal use
   d. established the concept of selective toxicity; led to testing of hundreds of compounds
      (1) Gerhard Domagk found that Prontosil Red killed pathogenic staphylococci and
streptococci, but didn't harm the animal

2. Jacques and Therese Trefouel discovered that Prontosil Red was converted to sulfanilamide, the true active factor

\[
\text{Sulfa Drugs (Sulphonamides)}
\]

3. led to development of sulfa drugs
   a. specifically designed to stay in gut or be absorbed in bloodstream
   b. often more active against microbes and less toxic to humans than sulfanilamide
   c. tremendously effective against pneumonia, puerperal fever (systemic infection due to *Streptococcus pyogenes*)
   d. general structure similar to PABA
   e. some microbes require PABA for growth
      f. sulfa drugs are competitive inhibitor; prevent growth and allow body's defense mechanisms to deal with microbes
      g. during 40's and 50's, many vitamins discovered and inhibitory analogs made
         (1) worked in test tubes
         (2) too toxic in humans, eliminated too well by kidneys, vitamin concentration in tissues too high, or infecting bacteria did not require vitamin
         (3) not one drug was of practical therapeutic importance

4. penicillin = first antibiotic to be used therapeutically
   a. originally observed by 21-year old French medical student Ernest Duchesne, rediscovered by Alexander Fleming
   b. Fleming observed a contaminant mold which seemed to be dissolving *Staphylococcus* colonies
      (1) found that broth from a *Penicillium* culture could destroy a number of pathogens
      (2) unable to purify the active compound very well
      (3) published some papers and abandoned the area
   c. Howard Florey and Ernst Chain (1939) obtained *Penicillium notatum* from Fleming
      (1) purified penicillin
      (2) destroyed staph and strep infections in mice
      (3) Fleming, Florey, and Chain shared 1945 Nobel

5. Selman Waksman (1944) discovered *streptomycin*, an antibiotic produced by the actinomycete *Streptomyces griseus* (Nobel 1952)
a. led to increased search for other antibiotic producers
b. by 1953, microbes producing chloramphenicol, neomycin, terramycin, and tetracycline were isolated

B. Characteristics of antimicrobials
1. **selective toxicity** = kill or inhibit pathogen without damaging host
   a. **therapeutic dose** = drug level required for clinical treatment of a particular infection
   b. **toxic dose** = drug level at which the agent becomes too toxic for host
   c. **therapeutic index** = ratio of toxic dose to therapeutic dose (larger number = better)
   d. drugs specific for microbial function that doesn't occur in host(e.g., cell wall synthesis) have highest therapeutic index

2. range of effectiveness varies
   a. **narrow-spectrum** = effective against a limited number
   b. **broad-spectrum** = effective against many types of pathogens
   c. can be classified based on targeted group
      (1) antibacterial
      (2) antifungal
      (3) antiprotozoan
      (4) antiviral
   d. some agents are effective against more than one group (e.g., sulfa drugs work against bacteria and some protozoa)

3. chemotherapeutic agents can be **natural**, **synthetic**, or **semi-synthetic**
   a. synthetics are
      (1) sulfa drugs
      (2) trimethoprim
      (3) chloramphenicol
      (4) isoniazid
      (5) dapson
      (6) many antiviral and antiprotozoan drugs
   b. semisynthetic are natural antibiotics that have been chemically modified to make them less susceptible to inactivation
      (1) ampicillin
      (2) carbenicillin
      (3) methicillin

4. chemotherapeutic agents can be cidal or static
   a. can be concentration dependent
   b. effect varies with species
   c. static effect relies on host's defense mechanism for elimination of infection

C. Determining activity levels
1. **dilution susceptibility tests**
   a. a series of broth tubes containing a range of antibiotic concentrations inoculated with test organism
   b. **minimal inhibitory concentration (MIC)** = lowest concentration that prevents growth (no growth after 16-20 hr)
c. **minimal lethal concentration (MLC)** = lowest concentration that kills the organism (no growth in subculture)

d. **cidal drugs** usually kill at 2-4x MIC, static drugs kill at much higher concentrations (if at all)

### 2. **disk diffusion tests**

a. antibiotic impregnated disks are placed on agar previously inoculated with the test bacterium
   (1) antibiotic diffuses, forming a gradient
   (2) resistant organisms grow up to the disk
   (3) susceptible organisms grow some distance from the disk, displaying a clear zone around the disk
      (a) wider the clear zone = more susceptible
      (b) zone width is a function of initial concentration, solubility, diffusion rate, susceptibility of organism
      (c) zone width cannot be used to compare 2 antibiotics

b. **Kirby-Bauer** most used disk diffusion test
   (1) Mueller-Hinton agar inoculated with lawn of bacteria
   (2) disks placed on surface
   (3) incubation at 35° C for 16-20 hr
   (4) diameters of zones measured and compared to tabulated values to determine degree of microbial resistance
      (a) plot MIC vs zone diameters for different strains
      (b) determine from plot if treatment dosage would result in MIC

### D. General mechanisms of activity

1. Pathogen damage can occur through several mechanisms
   a. most selective antibiotics interfere with cell wall synthesis
   b. high therapeutic index since cell walls not sound in eucaryotes

2. **Cell wall synthesis inhibition**
   a. penicillin, ampicillin, carbenicillin, methicillin, cephalosporins
   b. inhibit enzymes for peptidoglycan cross-linking; activate cell wall lytic enzymes
   c. bacitracin inhibits CW synthesis by interfering with lipid carrier that transports precursors across the plasma membrane

3. **protein synthesis inhibition**
   a. streptomycin, gentamicin; bind to 30S ribosome subunit and causes misreading of mRNA
   b. chloramphenicol binds to 50S ribosomal subunit, inhibits peptidyl transferase, blocking peptide bond formation
   c. tetracyclines bind to 30S, interfere with aminoacyl-tRNA binding
   d. erythromycin binds to 50S, inhibits peptide chain elongation
   e. high therapeutic index because drugs differentiate between procaryotic and eucaaryotic ribosomes

4. **nucleic acid synthesis inhibition**
   a. rifampicin
   b. inhibits DNA-dependent RNA polymerase, blocking RNA synthesis
   c. often toxic to eucaaryotic systems also
5. Cell membrane disruption
   a. polymyxin B
   b. binds to cell membrane, disrupts structure and permeability

6. metabolic antagonism (antimetabolites)
   a. sulfa drugs compete with PABA, inhibits folic acid synthesis
   b. trimethoprim inhibits dihydrofolate reductase, blocking tetrahydrofolate synthesis
   c. dapsone interferes with folic acid synthesis
   d. isoniazid may disrupt pyridoxal or NAD metabolism and functioning; inhibits synthesis of mycolic acid "cord factor"

7. Several factors determine effectiveness of antimicrobial drugs
   a. drug must reach site of infection, so delivery system important
      (1) penicillin G unstable in stomach acid
      (2) gentamicin (aminoglycosides) not well absorbed through gut and must be injected intramuscularly
      (3) parenteral routes = non-oral administration
   b. concentration must exceed MIC
      (1) dependent on amount administered,
      (2) speed of uptake,
      (3) rate of elimination from body
      (4) best if drug is absorbed slowly over a long period and excreted slowly
   c. infecting organism
      (1) dormant bugs less susceptible
      (2) pathogen must have proper target site
   d. many agents less effective due to resistance mechanisms, spread quickly via plasmids

E. Classes of antibiotics
1. sulfa drugs
   a. structural analog (similar to metabolic intermediate)
   b. similar to PABA, necessary for synthesis of folic acid

2. quinolones
   a. synthetic drug, broad spectrum, bactericidal
   b. inhibits DNA replication and repair, transcription
   c. nalidixic acid, fluoroquinolones (ciprofloxacin, norfoxacin, ofloxacin)

3. penicillins
   a. -lactam ring is common feature (side chains vary)
   b. penicillinase destroys ring
   c. block peptidoglycan cross-linking, leading to lysis
   d. many people are allergic

4. cephalosporins
   a. originally isolated from Cephalosporium (fungus)
   b. -lactam ring, like penicillins
   c. useful for people allergic to penicillin
   d. broad spectrum

5. tetracyclines
   a. naturally produced by Streptomyces or semi-synthetic
6. aminoglycoside antibiotics
   a. *Streptomyces* make streptomycin, kanamycin, tobramycin
   b. *Micromonospora purpurea* synthesizes gentamicin
   c. bind to small ribosomal subunit, inhibiting protein synthesis
   d. bactericidal, most effective against gram negatives
   e. quite toxic to humans
   f. widespread resistance

7. erythromycin
   a. macrolide, synthesized by *Streptomyces erythraeus*
   b. broad spectrum, bacteriostatic, most effective against G+
   c. bind to 23S rRNA of 50S ribosomal subunit, inhibiting protein elongation
   d. macrolides have 12- to 22-carbon lactone rings

8. chloramphenicol
   a. synthetic, but originally from *Streptomyces venezuelae*
   b. acts like erythromycin
   c. broad spectrum, bacteriostatic
   d. quite toxic to humans

F. Mechanisms of drug resistance
1. drug cannot enter cell
   a. G- unaffected by penicillin G because it can't penetrate the outer membrane
   b. changes in binding proteins render cells resistant

2. chemical modification
   a. penicillinase hydrolyzes the \( \beta \)-lactam ring
   b. groups can be added which inactivate drugs

3. modification of target
   a. changes in 23S rRNA protects against chloramphenicol or erythromycin
   b. change binding site for sulfanilamide

4. genes for drug resistance can be chromosomal or on plasmids
   a. spontaneous mutations in chromosome are rare
   b. chromosomal changes usually result in changes in drug receptors, preventing binding
   c. R plasmids (resistance plasmids) often code for enzymes that destroy or modify drugs
      (1) implicated in resistance to aminoglycosides, penicillins, cephalosporans, erythromycin, tetracyclines, sulfonamides, chloramphenicol, and others
      (2) plasmids transferred rapidly through populations
      (3) single plasmid can carry resistance to many drugs

5. Overuse of antibiotics has led to many resistant strains
   a. increase drug concentrations to destroy susceptible and spontaneous mutants
   b. use two drugs together
   c. limit use, especially broad-spectrum antibiotics

G. Antifungal drugs
1. Eukaryotic, so drugs often toxic to humans
2. Most fungi have efficient detoxification mechanisms
3. Often target membrane sterols or cell walls

H. Antiviral drugs
   1. Most drugs disrupt critical stages in virus life cycle or synthesis of viral-specific nucleic acids
   2. Difficult to use drug therapy, since viruses use host's cell machinery
# Terminology for Control of Microbial Growth

## Chemical Methods

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>antimicrobial agent</td>
<td>chemical that kills or inhibits growth of microorganisms</td>
</tr>
<tr>
<td>cidal agents</td>
<td>kill organisms (e.g., bactericidal, fungicidal, algicidal)</td>
</tr>
<tr>
<td>static agents</td>
<td>inhibit growth (e.g., bacteriostatic, fungistatic, algistatic)</td>
</tr>
<tr>
<td>lytic agents</td>
<td>induce cell lysis (e.g., bacteriolytic)</td>
</tr>
<tr>
<td>minimum inhibitory concentration (MIC)</td>
<td>smallest amount of an agent needed to inhibit growth of a test organism</td>
</tr>
<tr>
<td>agar diffusion method</td>
<td>antimicrobial action determined by zones of inhibition (concentration at zone edge equivalent to MIC)</td>
</tr>
<tr>
<td>disinfectant</td>
<td>chemical used to kill microorganisms on inanimate objects</td>
</tr>
<tr>
<td>sepsis</td>
<td>presence of microorganisms in tissues or blood</td>
</tr>
<tr>
<td>antiseptic</td>
<td>chemical agent that kills or inhibits microorganisms on living tissues</td>
</tr>
<tr>
<td>germicide</td>
<td>chemical agent that kills pathogenic microbes on inanimate objects</td>
</tr>
</tbody>
</table>

## Physical Methods

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>thermal death time (TDT)</td>
<td>shortest time required to kill all microbes at a specified temperature</td>
</tr>
<tr>
<td>tyndallization</td>
<td>discontinuous boiling</td>
</tr>
<tr>
<td>pasteurization</td>
<td>heated at 63°-66° C for 30 minutes (batch method) or 71.6° C for 15 seconds (flash method) to reduce microbe numbers and retain flavor</td>
</tr>
<tr>
<td>ultra high temperature (UHT) processing</td>
<td>heat at 134° C for 1-2 seconds; can sterilize some foods (i.e., milk), allowing unrefrigerated shelf lives of several months</td>
</tr>
<tr>
<td>incineration</td>
<td>most rigorous heat treatment</td>
</tr>
<tr>
<td>irradiation</td>
<td>bombardment with radiation; causes mutations</td>
</tr>
<tr>
<td>filtration</td>
<td>microbes removed by passage through small pore (e.g., 22 μm) membranes</td>
</tr>
</tbody>
</table>
"..."... in fact, the body has at least four lines of defence. The first is an enzyme called lysozyme, which is found in saliva, tears and nose mucus and has the property of dissolving many bacteria. The second is a group of substances collectively called interferon, proteins produced by virus-infected cells which interfere with the further growth of viruses. The body's third line of defence is based on the fact that the blood contains certain white corpuscles (leucocytes) which are rather like domesticated protozoa and live in the blood stream. Some of them, known as phagocytes, actually eat up and digest any extraneous microbes that get in. If a slight wound occurs, the damaged tissue causes these phagocytes to congregate near the site of damage and thus be ready to forestall infection."

How does the body cope with a well-established infection involving billions of cells?

"Massive microbial growth" only occurs if the body's initial defences have been broken down, and then one is very ill and, if the bacteria produce particularly nasty toxins, one may die. If one recovers, the nasty toxins, one may die. If one recovers, the reason is that the fourth defence mechanism has been successful: the body has made certain proteins called antibodies which, dissolved in the blood stream, react with the invading microbes and cause them to coagulate in lumps. In this condition they do less harm and are more easily ingested by the phagocytes. The serum of the blood is now immune to the particular microbe and this immunity can be retained, sometimes only for a few months, sometimes for many years, even a whole lifetime."
<table>
<thead>
<tr>
<th>Causes of Death</th>
<th>Lifestyle</th>
<th>Environment</th>
<th>Genetics</th>
<th>Health Care Services</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Disease</td>
<td>54</td>
<td>9</td>
<td>25</td>
<td>12</td>
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<tr>
<td>Cancer</td>
<td>37</td>
<td>24</td>
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<td>Stroke</td>
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<tr>
<td>COPD</td>
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<td>Unintentional Injuries</td>
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<td>Diabetes</td>
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<td>Suicide</td>
<td>60</td>
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<td>3</td>
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<tr>
<td>Liver Disease</td>
<td>70</td>
<td>9</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>All Ten Causes</td>
<td>56</td>
<td>15</td>
<td>21</td>
<td>8</td>
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</table>