8 Control of Microorganisms in the Environment and
9 Antimicrobial Chemotherapy

For the exam be able to:
- compare and contrast the actions of disinfection, sanitization, antisepsis, chemotherapy and sterilization
- distinguish between cidal (killing) and static (inhibitory) agents
- understand the concept of decimal reduction time (D value)
- understand the factors that influence control (population, types of organisms, extraneous organic material, etc.)
- know the difference between the phenol coefficient and the use dilution method of evaluation
- explain the mechanism of action of: filtration, heat, radiation and chemicals: phenolics, alcohols, halogens (iodine and chlorine), heavy metals, quaternary ammonium chlorides, aldehydes and oxides
- explain the terms: chemotherapeutic agent, antibiotic, selective toxicity, therapeutic index, narrow and broad spectrum drugs,
- know the 4 groups of chemotherapeutic agents (antibiotics) by their mechanism/mode of action and know 2 examples of each

CHAPTER OUTLINE

I. Principles of Microbial Control
   A. Sterilization is the destruction or removal of all viable organisms (vegetative cells and endospores) from an object or from a particular environment
   B. Disinfection is the killing, inhibition, or removal of pathogenic microorganisms (usually on inanimate objects)
   C. Sanitization is the reduction of microbial populations to a safe level as determined by public health standards
   D. Antisepsis is the prevention of infection of living tissue by microorganisms
   E. Antimicrobial agents fall into one of two broad categories denoted by suffixes indicating effect
      1. -cide is a suffix indicating that the agent will kill the kind of organism in question (e.g., viricide, fungicide)
      2. -static is a suffix indicating that the agent will prevent the growth of the type of organism in question (e.g., bacteriostatic, fungistatic)

II. The Pattern of Microbial Death
   A. Microorganisms are not killed instantly when exposed to a lethal agent; rather, the population generally decreases by a constant fraction at constant intervals (exponential killing)
   B. Efficiency of killing agent
      a. The decimal reduction time (D, or D value) is the time required to kill 90% of the microorganisms or spores in a sample at a specific temperature
      b. The Z value is the increase in temperature required to reduce D to 1/10 of its previous value
   C. A microorganism is usually considered dead when it is unable to grow in conditions that would normally support its growth and reproduction; however, the issue is confounded by organisms that are in a viable but nonculturable (VBNC) state

III. Conditions Influencing the Effectiveness of Antimicrobial Agents
   A. Population size—larger populations take longer to kill than smaller populations
   B. Population composition—populations consisting of different species or of cells at different developmental stages (e.g., endospores versus vegetative cells or young cells versus old cells) differ markedly in their sensitivity to various agents
   C. Concentration or intensity of the antimicrobial agent—higher concentrations or intensities are generally more efficient, but the relationship is not linear
   D. Contact time—the longer the exposure, the greater the number of organisms killed
E. Temperature—a higher temperature will usually (but not always) increase the effectiveness of killing
F. Local environment—environmental factors (e.g., pH, viscosity, and concentration of organic matter) can
profoundly influence the effectiveness of a particular antimicrobial agent

IV. Mechanical Removal Methods
A. Filtration sterilizes heat-sensitive liquids and gases by removing microorganisms rather than destroying
   them
   1. Depth filters are thick fibrous or granular filters that remove microorganisms by physical screening,
      entrapment, and/or adsorption
   2. Membrane filters are thin filters with defined pore sizes that remove microorganisms, primarily by
      physical screening (we will be using in our water lab)
   3. Air can be sterilized by passage through surgical masks, cotton plugs in culture vessels, and high-
      efficiency particulate air (HEPA) filters; HEPA filters are used in laminar flow biological safety
      cabinets to stabilize the air circulating in the enclosure

V. Physical Control Methods
A. Heat
   1. Killing with moist heat; *acts by degrading nucleic acids, denaturing proteins and disrupting membranes*
      a. Boiling water is effective against vegetative cells and eukaryotic spores, but not bacterial
         spores
      b. Autoclaving (steam under pressure) at 121°C is effective against most vegetative cells and
         bacterial endospores
      c. Pasteurization, a process involving brief exposure to temperatures below the boiling point of
         water, reduces the total microbial population and thereby increases the shelf life of the treated
         material; it is often used for heat-sensitive materials that cannot withstand prolonged exposure
         to high temperatures
      d. Tyndallization uses exposures to steam to kill vegetative cells; spores are allowed to grow and
         then the steam treatment is repeated; after several cycles the material is sterilized, not hot
         enough to kill spores or cause damage to materials
   2. Dry heat can be used to sterilize moisture-sensitive materials such as powders, oils, and similar
      items; it is less efficient than moist heat and usually requires higher temperatures (160 to 170°C) and
      longer exposure times (2 to 3 hrs); *acts by oxidizing cell components and denatures proteins*
B. Radiation
   1. Ultraviolet (UV) radiation is effective, but its use is limited to surface sterilization because UV
      radiation does not penetrate glass, dirt films, water, and other substances; *acts by causing thymine
      dimers preventing replication and transcription*
   2. Ionizing radiation (gamma radiation) is effective and penetrates the material; *acts by forming
      reactive high energy radicals which are destructive to the DNA and proteins*

VI. Chemical Control Agents
A. Phenolics—laboratory and hospital disinfectants; *act by denaturing proteins and disrupting cell
   membranes*; widely used agents include Lysol and triclosan
B. Alcohols—widely used disinfectants and antiseptics; will not kill endospores; ethanol and isopropanol are
   most widely used; *act by denaturing proteins and possibly dissolving membrane lipids*
C. Halogens—widely used antiseptics and disinfectants; *iodine acts by oxidizing cell constituents and
   iodinating cell proteins*; chlorine acts primarily by oxidizing cell constituents
D. Heavy metals—effective but usually toxic; *act by combining with proteins and inactivating them*
E. Quaternary ammonium compounds—cationic detergents used as disinfectants for food utensils and small
   instruments, and because of low toxicity, as antiseptics for skin; *act by disrupting biological membranes
   and possibly by denaturing proteins*; benzalkonium chloride and cetylpyridinium chloride are widely used
F. Aldehydes—reactive molecules that can be used as chemical sterilants; may irritate the skin; *act by
   combining with nucleic acids and proteins and inactivating them*; formaldehyde and glutaraldehyde are
   widely used
G. Sterilizing gases (e.g., ethylene oxide, betapropiolactone)—can be used to sterilize heat-sensitive
   materials such as plastic culture dishes and disposable syringes; *act by combining with proteins and
   inactivating them*; vaporized hydrogen peroxide has been used to decontaminate large facilities
VII. Evaluation of Antimicrobial Agent Effectiveness
A. The phenol coefficient test is a useful initial screening test in which the potency of a disinfectant is compared to that of phenol
B. A more realistic test is the use dilution test; in this test, stainless steel cylinders (we used beads) are contaminated with specific bacterial species under carefully controlled conditions and then exposed to the disinfectant

VIII. Biological Control of Microorganisms
An emerging group of control agents rely on one microbe to kill another—*Bdellovibrio*; processes include predation, viral-mediated lysis by bacteriophage, and bacterial toxin-mediated killing

IX. General Characteristics of Antimicrobial Drugs
A. Selective toxicity—ability to kill or inhibit microbial pathogen with minimal side effects in the host
   1. Therapeutic dose—the drug level required for clinical treatment of a particular infection
   2. Toxic dose—the drug level at which the agent becomes too toxic for the host (produces undesirable side effects)
   3. Therapeutic index—the ratio of the minimum toxic dose to the host/the minimum effective microbial lethal dose; the larger the better
B. Chemotherapeutic agents can occur naturally, be synthetic, or semisynthetic (chemical modifications of naturally occurring antibiotics)
C. Drugs with narrow-spectrum activity are effective against a limited variety of pathogens; drugs with broad-spectrum activity are effective against a wide variety of pathogens
D. Drug can be cidal (able to kill) or static (able to reversibly inhibit growth)
E. Minimal inhibitory concentration (MIC) is the lowest concentration of the drug that prevents growth of a pathogen; minimal lethal concentration (MLC) is the lowest drug concentration that kills the pathogen

X. Antibacterial Drugs
A. Inhibitors of cell wall synthesis are effective and selective because bacterial cell walls have unique structures not found in eukaryotic cells
   1. Penicillins—inhibit cell wall synthesis; many types have been identified or synthesized including ampicillin, carbenicillin, and methicillin; they differ in spectrum of activity and administration route but all have a β-lactam ring that is crucial for activity; resistance is an increasing problem, often due to penicillinase; some patients are allergic to these antibiotics
   2. Cephalosporins—inhibit cell wall synthesis; broad spectrum of activity; they contain a β-lactam ring, but are not subject to degradation by penicillinase; they can be given to some patients with penicillin allergies; they include cephalexin, cefoxitin, and ceftriaxone
   3. Vancomycin and teicoplanin—glycopeptide antibiotics that block peptidoglycan synthesis; vancomycin is particularly important as the last line of defense against antibiotic-resistant staphylococcal and enterococcal infections
B. Protein synthesis inhibitors exploit the differences between prokaryotic and eukaryotic ribosomes
   1. Aminoglycosides—contain cyclohexane ring and amino sugars; includes kanamycin, streptomycin, neomycin, and gentamicin; can be quite toxic to patients; act through interference with 30S ribosomal subunit, causing mistranslation with damaged proteins activating the envelope stress response and oxidative damage
   2. Tetracyclines—contain a four-ring structure with side chains; very broad spectrum that includes intracellular parasites and mycoplasmas; bacteriostatic
   3. Macrolides—12- to 22-carbon lactone rings linked to sugars; broad spectrum similar to that of penicillin; includes erythromycin and clindamycin; inhibit peptidyl chain elongation by binding rRNA
   4. Chloramphenicol—has a broad spectrum but is quite toxic; inhibits peptidyl transfer reaction
C. Metabolic antagonists are structural analogs of metabolic intermediates that act as antimetabolites, inhibiting metabolic pathways; bacteriostatic
   1. Sulfonamides or sulfa drugs—inhibit folic acid synthesis in bacteria (humans don’t synthesize folic acid, so are not affected); resistance is increasing and many patients are allergic to these drugs; includes p-aminobenzoic acid (PABA)
   2. Trimethoprim—synthetic antibiotic that blocks folic acid production; broad spectrum often combined with sulfa drugs
D. Nucleic acid synthesis inhibitors block enzymes of transcription and translation; generally not as selectively toxic
   1. Quinolones—synthetic drugs that inhibit bacterial DNA gyrase or topoisomerase II, thereby disrupting replication, repair, and other processes involving DNA; broad spectrum; includes nalidixic acid and ciprofloxacin (Cipro)

XI. Factors Influencing the Antimicrobial Drug Effectiveness
   A. Drug effectiveness—this is greatly influenced by the mode of administration (e.g., oral, topical, parenteral), but also can be influenced by exclusion from the site of infections (e.g., blood clots or necrotic tissue protects bacterium)
   B. Susceptibility of pathogen—affected by growth rate and by inherent properties (e.g., whether or not pathogen has target of the drug)
   C. Factors influencing drug concentration in the body—must exceed the pathogen’s MIC for the drug to be effective; this will depend on the amount of drug administered, the route of administration, the speed of uptake, and the rate of clearance (elimination) from the body
   D. Overcoming drug resistance
      1. Several strategies can be used to discourage emergence of drug resistance (e.g., administration of high doses, simultaneous treatment with more than one drug, limited use of broad-spectrum antibiotics)
      2. Drug resistance has become an increasing problem; new drugs are constantly being developed and new treatment methods (e.g., phage treatment of bacterial infections) are being explored