CHAPTER

5

Classification of Medically Important Bacteria

CHAPTER CONTENTS

PRINCIPLES OF CLASSIFICATION

The current classification of bacteria is based primarily on morphologic and biochemical characteristics. A scheme that divides the medically important organisms by genus is shown in Table 5–1. For pedagogic purposes, this classification scheme deviates from those derived from strict taxonomic principles in two ways:

(1) Only organisms that are described in this book in the section on medically important bacteria are included.
(2) Because there are so many gram-negative rods, they are divided into three categories: respiratory organisms, zoonotic organisms, and enteric and related organisms.

The initial criterion used in the classification is the nature of the cell wall (i.e., is it rigid, flexible, or absent?). Bacteria with rigid, thick walls can be subdivided into free-living bacteria, which are capable of growing on laboratory medium in the absence of human or other animal cells, and non–free-living bacteria, which are obligate intracellular parasites and therefore can grow only within human or other animal cells. The free-living organisms are further subdivided according to shape and staining reaction into a variety of gram-positive and gram-negative cocci and rods with different oxygen requirements and spore-forming abilities. Bacteria with flexible, thin walls (the spirochetes) and those without cell walls (the mycoplasmas) form separate units.

Using these criteria, along with various biochemical reactions, many bacteria can be readily classified into separate genus and species. However, there have been several examples of these criteria placing bacteria into the same genus when DNA sequencing of their genome reveals they are significantly different and should be classified in a new or different genus. For example, an organism formerly known as *Pseudomonas cepacia* has been reclassified as *Burkholderia cepacia* because the base sequence of its DNA was found to be significantly different from the DNA of the members of the genus *Pseudomonas*.

PEARLS

- The classification of bacteria is based on various criteria, such as the nature of the cell wall, staining characteristics, ability to grow in the presence or absence of oxygen, and ability to form spores.
- The criterion currently used is the base sequence of the genome DNA. Several bacteria have been reclassified on the basis of this information.

PRACTICE QUESTIONS: USMLE & COURSE EXAMINATIONS

Questions on the topics discussed in this chapter can be found in the Basic Bacteriology section of Part XIII: USMLE (National Board) Practice Questions starting on page 709. Also see Part XIV: USMLE (National Board) Practice Examination starting on page 751.

TABLE 5–1 Classification of Medically Important Bacteria

Characteristics	Genus	Representative Diseases
I. Rigid, thick-walled cells		
A. Free-living (extracellular bacteria)		
1. Gram-positive		
a. Cocci	*Streptococcus*	Pneumonia, pharyngitis, cellulitis
	Staphylococcus	Abscess of skin and other organs
b. Spore-forming rods		
(1) Aerobic	*Bacillus*	Anthrax
(2) Anaerobic	*Clostridium*	Tetanus, gas gangrene, botulism
c. Non–spore-forming rods		
(1) Nonfilamentous	*Corynebacterium*	Diphtheria
	Listeria	Meningitis
(2) Filamentous	*Actinomyces*	Actinomycosis
	Nocardia	Nocardiosis
2. Gram-negative		
a. Cocci	*Neisseria*	Gonorrhea, meningitis
b. Rods		
(1) Facultative		
(a) Straight		
(i) Respiratory organisms	*Haemophilus*	Meningitis
	Bordetella	Whooping cough
	Legionella	Pneumonia
(ii) Zoonotic organisms	*Brucella*	Brucellosis
	Francisella	Tularemia
	Pasteurella	Cellulitis
	Yersinia	Plague
(iii) Enteric and related organisms	*Escherichia*	Urinary tract infection, diarrhea
	Enterobacter	Urinary tract infection
	Serratia	Pneumonia
	Klebsiella	Pneumonia, urinary tract infection
	Salmonella	Enterocolitis, typhoid fever
	Shigella	Enterocolitis
	Proteus	Urinary tract infection
(b) Curved	*Campylobacter*	Enterocolitis
	Helicobacter	Gastritis, peptic ulcer
	Vibrio	Cholera
(2) Aerobic	*Pseudomonas*	Pneumonia, urinary tract infection
(3) Anaerobic	*Bacteroides*	Peritonitis
3. Acid-fast	*Mycobacterium*	Tuberculosis, leprosy
B. Non–free-living (obligate intracellular parasites)	*Rickettsia*	Rocky Mountain spotted fever, typhus, Q fever
	Chlamydia	Urethritis, trachoma, psittacosis
II. Flexible, thin-walled cells (spirochetes)	*Treponema*	Syphilis
	Borrelia	Lyme disease
	Leptospira	Leptospirosis
III. Wall-less cells	*Mycoplasma*	Pneumonia

CHAPTER

6

Normal Flora

CHAPTER CONTENTS

CONCEPT OF NORMAL FLORA

Normal flora is the term used to describe the various bacteria and fungi that are **permanent residents** of certain body sites, especially the skin, oropharynx, colon, and vagina **(Tables 6–1 and 6–2).** Viruses and parasites (protozoa and helminths), which are the other major groups of microorganisms, are usually not considered members of the normal flora, although they can be present in asymptomatic individuals. The normal flora organisms are often referred to as **commensals**. Commensals are organisms that derive benefit from another host but do not damage that host. The term **human microbiome** is often used to describe the normal flora (see later).

TABLE 6–1 Summary of the Members of Normal Flora and Their Anatomic Locations

Members of the Normal Flora[1]	Anatomic Location
Bacteroides species	Colon, throat, vagina
Candida albicans	Mouth, colon, vagina
Clostridium species	Colon
Corynebacterium species (diphtheroids)	Nasopharynx, skin, vagina
Enterococcus faecalis	Colon
Escherichia coli and other coliforms	Colon, vagina, outer urethra
Gardnerella vaginalis	Vagina
Haemophilus species	Nasopharynx
Lactobacillus species	Mouth, colon, vagina
Neisseria species	Mouth, nasopharynx
Propionibacterium acnes	Skin
Pseudomonas aeruginosa	Colon, skin
Staphylococcus aureus	Nose, skin
Staphylococcus epidermidis	Skin, nose, mouth, vagina, urethra
Viridans streptococci	Mouth, nasopharynx

[1]In alphabetical order.

The members of the normal flora vary in both number and kind from one site to another. Although the normal flora extensively populates many areas of the body, the internal organs usually are sterile. Areas such as the central nervous system, blood, lower bronchi and alveoli, liver, spleen, kidneys, and bladder are free of all but the occasional transient organism.

There is a distinction between the presence of these organisms and the **carrier state.** In a sense, we all are carriers of microorganisms, but that is not the normal use of the term in the medical context. The term *carrier* implies that an individual harbors a potential pathogen and therefore can be a source of infection of others. It is most frequently used in reference to a person with an asymptomatic infection or to someone who has recovered from a disease but continues to carry the organism and may shed it for a long period.

There is also a distinction to be made between members of the normal flora, which are the permanent residents, and the **colonization** of the individual with a new organism. In a sense, we are all colonized by the normal flora organisms, but the term *colonization* typically refers to the acquisition of a new organism. After the new organism colonizes (i.e., attaches and grows, usually on a mucosal membrane), it may cause an infectious disease, or it may be eliminated by our host defenses. Furthermore, the person colonized by a new organism can transmit that organism to others (i.e., act as a reservoir of infection for others).

TABLE 6–2 Medically Important Members of the Normal Flora

Location	Important Organisms[1]	Less Important Organisms[2]
Skin	*Staphylococcus epidermidis*	*Staphylococcus aureus, Corynebacterium* (diphtheroids), various streptococci, *Pseudomonas aeruginosa,* anaerobes (e.g., *Propionibacterium*), yeasts (e.g., *Candida albicans*)
Nose	*S. aureus*[3]	*S. epidermidis, Corynebacterium* (diphtheroids), various streptococci
Mouth	Viridans streptococci	Various streptococci, *Eikenella corrodens*
Dental plaque	*Streptococcus mutans*	*Prevotella intermedia, Porphyromonas gingivalis*
Gingival crevices	Various anaerobes (e.g., *Bacteroides, Fusobacterium,* streptococci, *Actinomyces*)	
Throat	Viridans streptococci	Various streptococci (including *Streptococcus pyogenes* and *Streptococcus pneumoniae), Neisseria* species, *Haemophilus influenzae, S. epidermidis*
Colon	*Bacteroides fragilis, Escherichia coli*	*Bifidobacterium, Eubacterium, Fusobacterium, Lactobacillus,* various aerobic gram-negative rods, *Enterococcus faecalis* and other streptococci, *Clostridium*
Vagina	*Lactobacillus, E. coli,*[3] group B streptococci[3]	Various streptococci, various gram-negative rods. *B. fragilis, Corynebacterium* (diphtheroids), *C. albicans*
Urethra		*S. epidermidis, Corynebacterium* (diphtheroids), various streptococci, various gram-negative rods (e.g., *E. coli*)[3]

[1]Organisms that are medically significant or present in large numbers.

[2]Organisms that are less medically significant or present in smaller numbers.

[3]These organisms are not part of the normal flora in this location but are important colonizers.

The members of the normal flora play a role both in the maintenance of health and in the causation of disease in three significant ways:

(1) They can cause disease, especially in immunocompromised and debilitated individuals. Although these organisms are nonpathogens in their usual anatomic location, they can be pathogens in other parts of the body.

(2) They constitute a protective host defense mechanism. The nonpathogenic resident bacteria occupy attachment sites on the skin and mucosa that can interfere with colonization by pathogenic bacteria. The ability of members of the normal flora to limit the growth of pathogens is called **colonization resistance.** If the normal flora is suppressed, pathogens may grow and cause disease. For example, antibiotics can reduce the normal colonic flora that allows *Clostridium difficile,* which is resistant to the antibiotics, to overgrow and cause pseudomembranous colitis.

(3) They may serve a nutritional function. The intestinal bacteria produce several B vitamins and vitamin K. Poorly nourished people who are treated with oral antibiotics can have vitamin deficiencies as a result of the reduction in the normal flora. However, since germ-free animals are well-nourished, the normal flora is not essential for proper nutrition.

THE HUMAN MICROBIOME

The **human microbiome** is the term used to describe the thousands of microbes (“microbiota”) located on the skin, on mucosal surfaces, and within the lumen of the gastrointestinal tract. The majority of these microbes are bacteria, but yeasts and protozoa are also found in large numbers. Using sophisticated molecular techniques, many previously unknown bacteria have been identified. Routine cultures on bacteriological media typically reveal only a small subset of the existing resident organisms.

It is estimated that the adult human contains 10^{13} cells whereas the number of microbes in the human microbiome is approximately 10^{14}, that is, there are 10 times more microbial cells than human cells. The largest and most complex microbial population resides in the colon. Within the colon, the two largest phyla of bacteria are the *Firmicutes* (64%) and the *Bacteroidetes* (23%). The *Firmicutes* are gram-positive rods and members of the genera *Clostridium* and *Faecalibacterium* are prominent organisms. The *Bacteroidetes* are gram-negative rods and the genera, *Bacteroides* and *Prevotella* are important members. Species of *Proteobacteria* (gram-negative rods such as *Escherichia* and *Salmonella*) and *Actinobacteria* (gram-positive rods such as *Actinomyces*) make up a large percentage of the remainder.

There is mounting evidence that the organisms in the microbiome play an important role in several body functions and diseases, such as weight control (obesity), inflammatory bowel disease, the immune response in general, and resistance to infectious disease.

The effect on obesity is revealed by studies involving the transfer of fecal bacteria between strains of inbred mice. For example, fecal bacteria from obese mice transplanted into germ-free strains of nonobese mice resulted in the nonobese mice becoming obese. It appears that the fecal

CHAPTER

13

Sterilization & Disinfection

CHAPTER CONTENTS

STERILIZATION, DISINFECTION, AND STANDARD PRECAUTIONS

The purpose of sterilization and disinfection procedures is to prevent transmission of microbes to patients. In addition to sterilization and disinfection, other important measures to prevent transmission are included in the protocol of "**standard precautions**" (previously known as Universal Precautions).These standard precautions should be used in interaction with *all* patients because it is unknown whether any particular patient may be the reservoir of transmissible bacteria, viruses, or other microbes.

Standard precautions include (1) hand hygiene, (2) respiratory hygiene and cough etiquette, (3) safe injection practices, and (4) proper disposal of needles and scalpels. Further, if exposure to body fluids or aerosols is likely, personal protective equipment (PPE) such as masks or face shields, gloves, gowns, and protective eyewear should be used. The precautions taken should be specific for the task rather than for the particular patient.

In addition, there are **transmission-based precautions** that supplement the standard precautions and should be employed when the patient is infected (or suspected to be infected) with a highly transmissible organism. The three categories of transmission-based precautions are contact, droplet, and airborne. Table 13–1 describes some of the specific information referable to these categories. For additional information, please consult the CDC Web site <http://www.cdc.gov/hai/>, where health care associated infections (HAI) are discussed.

PRINCIPLES OF STERILIZATION & DISINFECTION

Sterilization is the killing or removal of *all* microorganisms, including bacterial spores, which are highly resistant. Sterilization is usually carried out by autoclaving, which consists of exposure to steam at 121°C under a pressure of 15 lb/in^2 for 15 minutes. Surgical instruments that can be damaged by moist heat are usually sterilized by exposure to ethylene oxide gas, and most intravenous solutions are sterilized by filtration.

Disinfection is the killing of many, but not all, microorganisms. For adequate disinfection, pathogens must be killed, but some organisms and bacterial spores may survive. Disinfectants vary in their tissue-damaging properties from the corrosive phenol-containing compounds, which should be used only on inanimate objects, to less toxic materials such as ethanol and iodine, which can be used on skin surfaces. Chemicals used to kill

TABLE 13–1 Infection Control Precautions and Practices

Type of Precaution	Example of Type of Patient or Type of Infection	Important Precaution Practice Employed
Standard	All patients	1. Hand hygiene 2. Respiratory hygiene and cough etiquette 3. Safe injection practices 4. Proper disposal of needles and scalpels
Standard	If exposure to blood, secretions, or body fluids is likely to occur	Personal protective equipment (PPE) such as mask, face shield, goggles, gloves, or gown
Contact	1. Stool incontinence, e.g., *Clostridium difficile*, norovirus 2. Generalized rash, e.g., varicella (chickenpox) 3. Draining wounds	1. Wear gloves and gown 2. Disinfect room
Droplet	1. Respiratory viruses, e.g., influenza 2. *Bordetella pertussis* 3. Early infection with *Neisseria meningitidis*	1. Face mask or face shield for patient and provider 2. Disinfect room
Airborne	1. Tuberculosis 2. Measles 3. Varicella (chickenpox) when patient is coughing	1. Isolation room; negative pressure room 2. Face mask or face shield for patient and provider. N-95 respirator, if available. 3. Disinfect room

TABLE 13–2 Clinical Use of Disinfection and Sterilization

Clinical Use	Commonly Used Disinfectant or Method of Sterilization
Disinfect surgeon's hands prior to surgery	Chlorhexidine
Disinfect surgical site prior to surgery	Iodophor
Disinfect skin prior to venipuncture or immunization	70% ethanol
Disinfect skin prior to blood culture or inserting vascular catheter	Tincture of iodine followed by 70% ethanol, or iodophor, or chlorhexidine
Cleanse wounds	Thimerosal, chlorhexidine, hydrogen peroxide
Cleanse burn wounds	Silver sulfadiazine
Cleanup of blood spill from a patient with hepatitis B or C (disinfect area)	Hypochlorite (bleach, Clorox)
Sterilize surgical instruments and heat-sensitive materials (e.g., endoscopes, respiratory therapy equipment)	Ethylene oxide or glutaraldehyde
Sterilize non–heat-sensitive materials (e.g., surgical gowns, drapes)	Autoclave
Sterilize intravenous solutions	Filtration
Disinfect air in operating room (when not in use)	Ultraviolet light
Disinfect floor of operating room	Benzalkonium chloride (Lysol)
Disinfect stethoscope	70% ethanol
Preservative in vaccines	Thimerosal

microorganisms on the surface of skin and mucous membranes are called **antiseptics**.

Table 13–2 describes the clinical uses of common disinfectants and modes of sterilization.

RATE OF KILLING OF MICROORGANISMS

Death of microorganisms occurs at a certain rate dependent primarily on two variables: the concentration of the killing agent and the length of time the agent is applied. The rate of killing is defined by the relationship

$$N \propto 1/CT$$

which shows that the number of survivors, N, is inversely proportionate to the concentration of the agent, C, and to the time of application of the agent, T. Collectively, CT is often referred to as the dose. Stated alternatively, the number of microorganisms killed is directly proportionate to CT. The relationship is usually stated in terms of survivors because they are easily measured by colony formation. Death is defined as the inability to reproduce. In certain circumstances, the physical remains of dead bacteria can still cause problems (see page 46).

CHEMICAL AGENTS

Chemicals vary greatly in their ability to kill microorganisms. A quantitative measure of this variation is expressed as the **phenol coefficient,** which is the ratio of the concentration of phenol to the concentration of the agent required to cause the same amount of killing under the standard conditions of the test.

Chemical agents act primarily by one of the three mechanisms: (1) disruption of the lipid-containing cell membrane, (2) modification of proteins, or (3) modification of DNA. Each of the following chemical agents has been classified into one of the three categories, but some of the chemicals act by more than one mechanism.

DISRUPTION OF CELL MEMBRANES

Alcohol

Ethanol is widely used to clean the skin before immunization or venipuncture. It acts mainly by disorganizing the lipid structure in membranes, but it denatures proteins as well. Ethanol requires the presence of water for maximal activity (i.e., it is far more effective at 70% than at 100%). **Seventy percent ethanol** is often used as an antiseptic to clean the skin prior to venipuncture. However, because it is not as effective as iodine-containing compounds, the latter should be used prior to obtaining a blood culture and installing intravenous catheters. Ethanol will not kill bacterial spores and therefore cannot be used for sterilization.

Detergents

Detergents are "surface-active" agents composed of a long-chain, lipid-soluble, hydrophobic portion and a polar hydrophilic group, which can be a cation, an anion, or a nonionic group. These surfactants interact with the lipid in the cell membrane through their hydrophobic chain and with the surrounding water through their polar group and thus disrupt the membrane. **Quaternary ammonium compounds (e.g., benzalkonium chloride)** are cationic detergents widely used for skin antisepsis. Benzalkonium chloride is the active ingredient in Lysol, a commonly used disinfectant for floors and other surfaces.

Phenols

Phenol was the first disinfectant used in the operating room (by Lister in the 1860s), but it is rarely used as a disinfectant today because it is too caustic. **Chlorhexidine** is a chlorinated phenol that is widely used as a hand disinfectant prior to surgery ("surgical scrub") and in the cleansing of wounds. **Hexachlorophene**, which is a biphenol with six chlorine atoms, is used in germicidal soaps, but concern over possible neurotoxicity has limited its use. Phenols not only damage membranes, but also denature proteins.

MODIFICATION OF PROTEINS

Chlorine

Chlorine is used as a disinfectant to purify the water supply and to treat swimming pools. It is also the active component of **hypochlorite (bleach, Clorox),** which is used as a disinfectant in the home and in hospitals. Chlorine is a powerful oxidizing agent that kills by cross-linking essential sulfhydryl groups in enzymes to form the inactive disulfide.

Iodine

Iodine is the most effective skin antiseptic used in medical practice and should be used prior to obtaining a blood culture and installing intravenous catheters because contamination with skin flora such as *Staphylococcus epidermidis* can be a problem. Iodine, like chlorine, is an oxidant that inactivates sulfhydryl-containing enzymes. It also binds specifically to tyrosine residues in proteins.

Iodine is supplied in two forms:

(1) **Tincture of iodine** (2% solution of iodine and potassium iodide in ethanol) is used to prepare the skin prior to blood culture. Because tincture of iodine can be irritating to the skin, it should be removed with alcohol.

(2) Iodophors are complexes of iodine with detergents that are frequently used to prepare the skin prior to surgery because they are less irritating than tincture of iodine. **Povidone-iodine** is an iodophor commonly used as an antiseptic.

Heavy Metals

Mercury and silver have the greatest antibacterial activity of the heavy metals and are the most widely used in medicine. They act by binding to sulfhydryl groups, thereby blocking enzymatic activity. **Thimerosal** (Merthiolate) and merbromin (Mercurochrome), which contain mercury, are used as skin antiseptics. **Silver nitrate** drops are effective in preventing gonococcal neonatal conjunctivitis (ophthalmia neonatorum). Silver sulfadiazine is used to prevent infection of burn wounds.

Hydrogen Peroxide

Hydrogen peroxide is used as an antiseptic to clean wounds and to disinfect contact lenses. Its effectiveness is limited by the organism's ability to produce catalase, an enzyme that degrades H_2O_2. (The bubbles produced when peroxide is used on wounds which are formed by oxygen arising from the breakdown of H_2O_2 by tissue catalase.) Hydrogen peroxide is an oxidizing agent that attacks sulfhydryl groups, thereby inhibiting enzymatic activity.

Formaldehyde & Glutaraldehyde

Formaldehyde, which is available as a 37% solution in water (formalin), denatures proteins and nucleic acids. Both proteins and nucleic acids contain essential $-NH_2$ and $-OH$ groups, which are the main sites of alkylation by the hydroxymethyl group of formaldehyde. **Glutaraldehyde**, which has two reactive aldehyde groups, is 10 times more effective than formaldehyde and is less toxic. In hospitals, it is used to sterilize respiratory therapy equipment, endoscopes, and hemodialysis equipment.

Ethylene Oxide

Ethylene oxide gas is used extensively in hospitals for the sterilization of heat-sensitive materials such as surgical instruments and plastics. It kills by alkylating both proteins and nucleic acids (i.e., the hydroxyethyl group attacks the reactive hydrogen atoms on essential amino and hydroxyl groups). It is classified as a mutagen and a carcinogen.

Acids & Alkalis

Strong acids and alkalis kill by denaturing proteins. Although most bacteria are susceptible, it is important to note that *Mycobacterium tuberculosis* and other mycobacteria are relatively resistant to 2% NaOH, which is used in the clinical laboratory to liquefy sputum prior to culturing the organism. Weak acids, such as **benzoic, propionic, and citric acids**, are frequently used as food preservatives because they are bacteriostatic. The action of these acids is partially a function of the organic moiety (e.g., benzoate), as well as the low pH.

MODIFICATION OF NUCLEIC ACIDS

A variety of dyes not only stain microorganisms, but also inhibit their growth. One of these is crystal violet (gentian violet), which is used as a skin antiseptic. Its action is based on binding of the positively charged dye molecule to the negatively charged phosphate groups of the nucleic acids. Malachite green, a triphenylamine dyelike crystal violet, is a component of Löwenstein-Jensen's medium, which is used to grow *M. tuberculosis*. The dye inhibits the growth of unwanted organisms in the sputum during the 6-week incubation period.

PHYSICAL AGENTS

The physical agents act either by imparting energy in the form of heat or radiation or by removing organisms through filtration.

HEAT

Heat energy can be applied in three ways: in the form of moist heat (either boiling or autoclaving) or dry heat or by pasteurization. In general, heat kills by denaturing proteins, but membrane damage and enzymatic cleavage of DNA may also be involved. Moist heat sterilizes at a lower temperature than dry heat, because water aids in the disruption of noncovalent bonds (e.g., hydrogen bonds), which hold protein chains together in their secondary and tertiary structures.

Moist heat sterilization, usually **autoclaving,** is the most frequently used method of sterilization. Because bacterial **spores are resistant to boiling** (100°C at sea level), they must be exposed to a higher temperature; this cannot be achieved unless the pressure is increased. For this purpose, an autoclave chamber is used in which steam, at a pressure of 15 lb/in^2, reaches a temperature of 121°C and is held at that temperature for 15 to 20 minutes. This kills even the highly heat-resistant spores of *Clostridium botulinum*, the cause of botulism, with a margin of safety. To test the effectiveness of the autoclaving process, spore-forming organisms, such as members of the genus *Clostridium,* are used.

Sterilization by dry heat, on the other hand, requires temperatures in the range of 180°C for 2 hours. This process is used primarily for glassware and is used less frequently than autoclaving.

Pasteurization, which is used primarily for milk, consists of heating the milk to 62°C for 30 minutes followed by rapid cooling. ("Flash" pasteurization at 72°C for 15 seconds is often used.) This is sufficient to kill the vegetative cells of the milk-borne pathogens (e.g., *Mycobacterium bovis, Salmonella, Streptococcus, Listeria*, and *Brucella*), but not to sterilize the milk.

RADIATION

The two types of radiation used to kill microorganisms are **ultraviolet (UV) light and X-rays.** The greatest antimicrobial activity of UV light occurs at 250 to 260 nm, which is the wavelength region of maximum absorption by the purine and pyrimidine bases of DNA. The most significant lesion caused by UV irradiation is the formation of thymine dimers, but addition of hydroxyl groups to the bases also occurs. As a result, DNA replication is inhibited and the organism cannot grow. Cells have repair mechanisms against UV-induced damage that involve either cleavage of dimers in the presence of visible light (photoreactivation) or excision of damaged bases, which is not dependent on visible light (dark repair). Because UV radiation can damage the cornea and skin, the use of UV irradiation in medicine is limited. However, it is used in hospitals to kill airborne organisms, especially in operating rooms when they are not in use. Bacterial spores are quite resistant and require a dose up to 10 times greater than do the vegetative bacteria.

X-rays have higher energy and penetrating power than UV radiation and kill mainly by the production of free radicals (e.g., production of hydroxyl radicals by the hydrolysis of water). These highly reactive radicals can break covalent bonds in DNA, thereby killing the organism. Sulfhydryl-containing compounds, such as the amino acid cysteine, can protect DNA from free-radical attack. Another mechanism is a direct hit on a covalent bond in DNA, resulting in chain breakage, but this is probably less important than the mechanism involving free radicals.

X-rays kill vegetative cells readily, but spores are remarkably resistant, probably because of their lower water content. X-rays are used in medicine for sterilization of heat-sensitive items, such as sutures and surgical gloves, and plastic items, such as syringes.

FILTRATION

Filtration is the preferred method of **sterilizing certain solutions** (e.g., those with heat-sensitive components). In the past, solutions for intravenous use were autoclaved, but heat-resistant endotoxin in the cell walls of the dead gram-negative bacteria caused fever in recipients of the solutions. Therefore, solutions are now filtered to make them **pyrogen-free** prior to autoclaving.

The most commonly used filter is composed of nitrocellulose and has a pore size of 0.22 µm. This size will retain all bacteria and spores. Filters work by physically trapping particles larger than the pore size and by retaining somewhat smaller particles via electrostatic attraction of the particles to the filters.

PEARLS

- Sterilization is the **killing of all** forms of microbial life, including bacterial spores. **Spores** are **resistant to boiling,** so sterilization of medical equipment is typically achieved at 121°C for 15 minutes in an autoclave. Sterilization of heat-sensitive materials is achieved by exposure to ethylene oxide, and liquids can be sterilized by filtration.
- **Disinfection** is **reducing the number of bacteria** to a level low enough that disease is unlikely to occur. Spores and some bacteria will survive. For example, disinfection of the water supply is achieved by treatment with chlorine. Disinfection of the skin prior to venipuncture is achieved by treatment with 70% ethanol. Disinfectants that are mild enough to use on skin and other tissues, such as 70% ethanol, are called **antiseptics.**
- The killing of microbes by either chemicals or radiation is proportional to the **dose,** which is defined as the product of the concentration multiplied by the time of exposure.
- Chemical agents kill bacteria by one of three actions: disruption of lipid in cell membranes, modification of proteins, or modification of DNA.
- Physical agents kill (or remove) bacteria by one of three processes: heat, radiation, or filtration.
- Heat is usually applied at temperatures above boiling (121°C) to kill spores, but heat-sensitive materials such as milk are exposed to temperatures below boiling (**pasteurization**) that kill the pathogens in milk but do not sterilize it.
- Radiation, such as **ultraviolet light** and X-radiation, is often used to sterilize heat-sensitive items. Ultraviolet light and X-radiation **kill by damaging DNA.**
- Filtration can sterilize liquids if the pore size of the filter is small enough to retain all bacteria and spores. Heat-sensitive liquids (e.g., intravenous fluids) are often sterilized by filtration.

SELF-ASSESSMENT QUESTIONS

1. Regarding sterilization and disinfection, which one of the following is the most accurate statement?
 (A) Seventy percent alcohol is a better antiseptic than iodine, so 70% alcohol should be used to disinfect the skin prior to drawing a blood culture rather than iodine.
 (B) Disinfectants kill both bacterial cells and bacterial spores.
 (C) During sterilization by autoclaving, the temperature must be raised above boiling in order to kill bacterial spores.
 (D) Transmission of milk-borne diseases can be prevented by pasteurization, which kills both bacterial cells and spores.
 (E) Ultraviolet light used in the operating room to disinfect the room kills bacteria primarily by causing oxidation of lipids in the cell membrane.

2. Which one of the following chemicals is used to sterilize heat-sensitive materials, such as surgical instruments, in the hospital?
 (A) Benzalkonium chloride
 (B) Cresol (Lysol)
 (C) Ethylene oxide
 (D) Thimerosal
 (E) Tincture of iodine

ANSWERS

1. **(C)**
2. **(C)**

PRACTICE QUESTIONS: USMLE & COURSE EXAMINATIONS

Questions on the topics discussed in this chapter can be found in the Basic Bacteriology section of Part XIII: USMLE (National Board) Practice Questions starting on page 709. Also see Part XIV: USMLE (National Board) Practice Examination starting on page 751.