جامعة جنوب الوادي
South Valley University

MEDICAL MICROBIOLOGY

A Brief Introduction

FOR

POST-GRADUATE

FACULTY OF SCIENCE

EDITED BY

PROF. DR. WESAM SALEM

2023/2024

Medical Microbiology- A Brief Introduction

Medical Microbiology is the study of the causes and management of infectious diseases. These can be caused by viruses, bacteria, microfungi and protozoa. Medical Microbiology may overlap with parasitology, generally considered to be the study of diseases caused by multicellular parasites, where a parasite is defined as an organism that derives its nutrients from another living organism, often but not always to the detriment of its host. So, why do Medical students need to know about microbiology? We are only 10% human. It has been estimated that there are about 10^{14} cells in the human body. Of these only 10% are of human origin. The remainder is the microbes that comprise our **commensal flora**. These are the microbes that live in and on our various body surfaces. We provide these organisms with food and shelter. In return, the commensal flora can play an important role in preventing infection. The word "commensal" means to share a table. The vast majority of commensal microbes are **bacteria** but we also harbour **fungi** and **protozoa. Viruses** are also found causing latent infections. These are infections where no symptoms are apparent. Most of the microbes that live on or in humans do no harm. Indeed, they may be positively beneficial. The relationship is, however, finely balanced. Microbes are continually probing our defences and commensals that get into the "wrong" place can do untold damage. **Peritonitis,** for example is the life-threatening infection that results when gut microbes gain access to the peritoneal cavity, for example following a ruptured appendix. **Urinary tract infections** are most frequently caused when gut organisms or the skin flora gain access to the bladder. This is why, considering comparative anatomy, women are much more likely to suffer cystitis than men, for example. Some commensals can cause disease because of our life-styles. **Dental decay** in the Developed world is the result of metabolism of sugars in our diet by our oral flora to produce acid that subsequently etches our teeth. This initiates dental caries.

A very small minority of microbes are **primary pathogens**. These are capable

of infecting individuals and causing disease. Because infections are very common and may be life-threatening, it is easy to get the wrong perspective on microbes.

Microorganisms are friend and foe !

Humans are born into an environment laden with microorganisms, and colonization of the human body begins at the time of birth. Colonization simply implies the establishment of microorganisms on the body surface which, by extension, continues internally (oral cavity, gastrointestinal tract, ear canals, etc.). Throughout life, the skin and mucous membranes exposed to the outside world harbor a variety of indigenous bacteria, the **normal flora (**see, **H**ost **M**icrobe **I**nteraction for more details**)**.

A few hours after birth, the establishment of the normal flora on the surfaces of the body is well under way. Organisms acquired by an infant during passage through the birth canal are replaced by organisms derived from persons who attend the infant and from ingested foods. During the first day of life, many organisms find their niche for the life of the individual. Others take months or years to reach populations found in normal adults.

The number of different species of microorganisms living on the surfaces of the body is very large and even includes species that have not been fully characterized and classified. Some examples of the principal resident bacteria include *Staphylococcus*, *Micrococcus* and *Propionibacterium* found on the skin; *Lactobacillus*, *Streptococcus*, *Neisseria*, *Corynebacterium* and *Bacteroides* in the mouth; and *Bacteroides*, *Clostridium*, *Lactobacillus*, *Streptococcus* and many species of the Family Enterobacteriaceae in the intestinal tract. Usually the normal flora cause no disturbances in the health of their host. In fact, they often benefit the host by out competing pathogenic bacteria, yeasts and protozoa which are encountered occasionally.

In the healthy individual it is **not** normal for microorganisms to grow in areas that are inside the body; the lungs, heart, brain, spleen and muscles are typically free of

microbes. Penetration of these areas by the normal flora or pathogens will incur the wrath of the immune system and result in a diseased state. Your body has a vast array of defenses to keep microbes out of the internal regions.

In the diagnosis of bacterial diseases of the human body, a general knowledge of the normal flora is essential. Exogenous pathogens must be distinguished from the indigenous species for the correct interpretation of bacteriological findings. Also, an increasing number of clinically-diagnosed bacterial diseases involves bacteria that are indigenous yet potentially pathogenic. Given an opportunity to infect an individual whose resistance is depressed, **or**, more specifically, to colonize an uncommon site (such as *E. coli* in the eye or urinary tract); certain of the normal flora may produce an endogenous bacterial disease. Examples of factors which lower the resistance of a host include radiation damage, prolonged use of antibiotics or steroids, and the debilitating effects of AIDS or other diseases. As the lives of more patients with major illnesses are prolonged by improved methods of treatment, and as more infections caused by virulent exogenous bacteria are controlled by effective antimicrobial drugs, endogenous bacterial diseases have become more common.

Principles of Diagnosis

Some infectious diseases are distinctive enough to be identified clinically. Most pathogens, however, can cause a wide spectrum of clinical syndromes in humans. Conversely, a single clinical syndrome may result from infection with any one of many pathogens. Influenza virus infection, for example, causes a wide variety of respiratory syndromes that cannot be distinguished clinically from those caused by streptococci, mycoplasma, or more than 100 other viruses.

Most often, therefore, it is necessary to use **microbiologic laboratory methods** to identify a specific etiologic agent. Diagnostic medical microbiology is the discipline that identifies etiologic agents of disease. The job of the clinical microbiology

laboratory is to **test specimens from patients for microorganisms that are, or may be, a cause of the illness and to provide information (when appropriate) about the in vitro activity of antimicrobial drugs against the microorganisms identified** (Fig. 1).

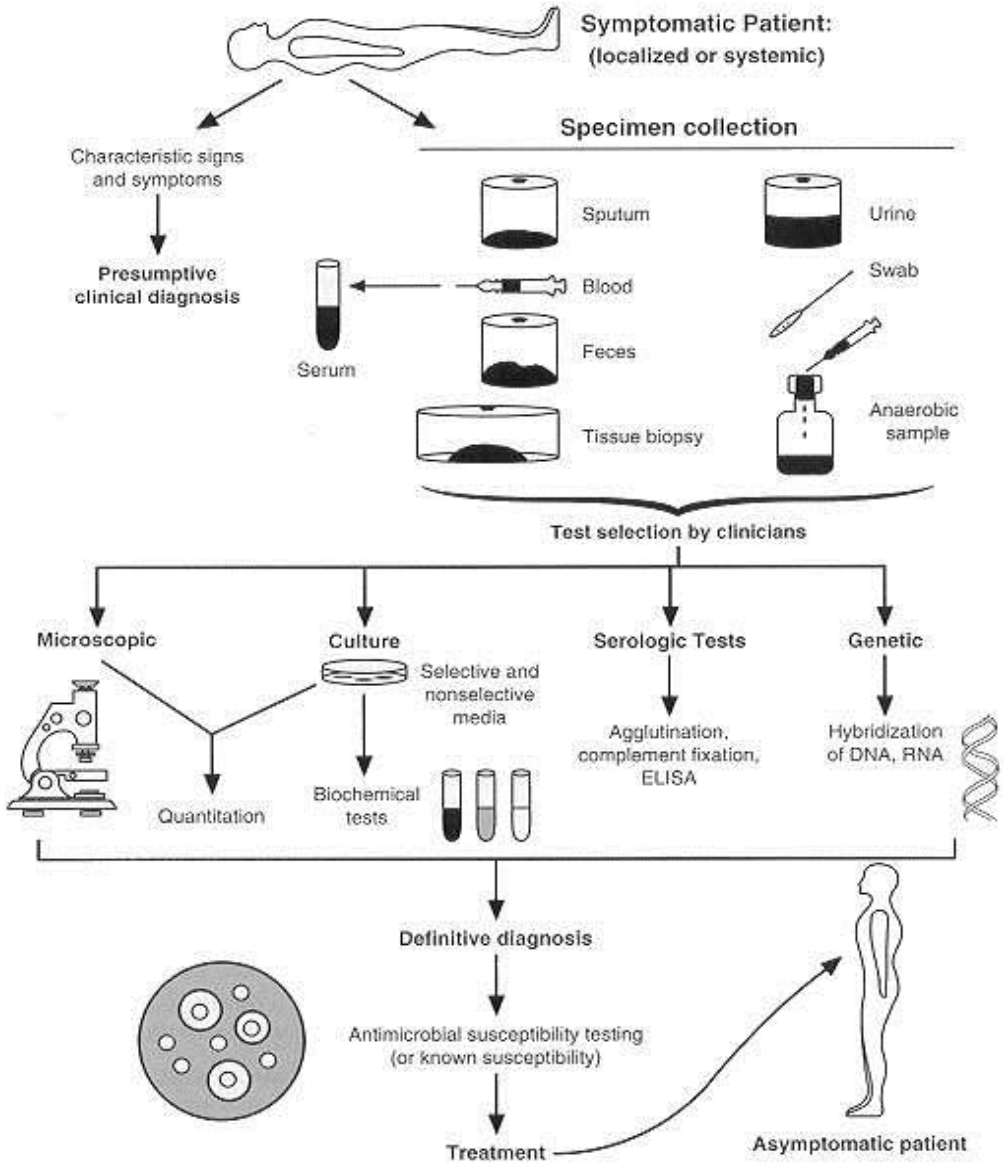


Fig. (1): Laboratory procedures used in confirming a clinical diagnosis of infectious disease with a bacterial etiology.

The staff of a clinical microbiology laboratory should be qualified to advise the physician as well as process specimens. The physician should supply salient information about the patient, such as age and sex, tentative diagnosis or details of the clinical syndrome, date of onset, significant exposures, prior antibiotic therapy, immunologic status, and underlying conditions. The clinical microbiologist participates in decisions regarding the microbiologic diagnostic studies to be performed, the type and timing of specimens to be collected, and the conditions for their transportation and storage. Above all, the clinical microbiology laboratory, whenever appropriate, should provide an interpretation of laboratory results.

Manifestations of Infection

It is the successful entry of an organism into the human body with subsequent growth, multiplication and invasion of body tissue.

--Microorganisms may be:

1- **Pathogenic**.

2- **Non pathogenic** including:-

a- Saprophytes from the cell.

b- Commensals from skin and mucous membranes of the host, which constitute the normal flora of the body which plays a role in the body defense against pathogenic organisms, examples of these are:-

-- Salivary *streptococci*, produce H_2O_2 which is lethal to diphtheria and meningococci.

-- *Lactobacillus acidophilus*, ferment glycogen derived from the vaginal epithelium and produce a highly acidic vaginal secretion inhibitory to many kinds of bacteria as streptococci and staphylococci.

-- Some Commensals microorganisms may cause infection if they leave their normal places so they called opportunistic, e.g. *E. coli* which is Commensal in the gut but it may cause urinary tract infection. Also, *Streptococcus viridans* Commensal in the

mouth but it may cause subacute bacterial endocarditis if they reach the blood stream.

Another example is *Clostridium welchii* which is Commensal in the intestine but they may cause gas gangrene in locally damaged tissue.

Pathogenic bacteria:

Those bacteria which can cause diseases by invasion of the tissue by itself or by its toxins.

Pathogens should have the following characteristics:

1- **Transmissibility**:

It is the ability to grow profusely or to be shed in large number in body fluids.

2- **Infectivity**:

It is the ability to initiate infection related to (or depend on) dosage of the pathogen & phase of growth, virulence.

This occurs by overcome of the first line of defense which is the skin & mucous membrane.

For example, the infectivity dosage of *Salmonella typhi* is very small where large number of food- poisoning Salmonella must be ingested to produce acute vomiting and diarrhea.

3- **Virulence**.

It is the ability of organism to invade tissue, multiply & produce toxic effect substances.

The degree of virulence is measured by inoculation of the organism into a laboratory animals and we record LD_{50} (Lethal dose fifty) = the dose which kill 50% of laboratory animals.

Virulence of bacteria depends on:

A) Production of toxins. B) Invasiveness or aggressiveness.

Source of infection

A) Exogenous. B) Endogenous.

Exogenous infection through:

1- Patients

This occurs by transmission of microorganisms from patient to healthy one e.g., T.B., syphilis, wheeping-cough, gonorrhoea Small-pox, Measles, & Influenza.

2- Healthy carriers.

Many pathogenic bacteria can affect certain persons and cause a limited sub- clinical infection, so it is unable to produce signs & symptoms of illness and they considered as source of infection to others.

3- Infected animals.

Here the disease is transmitted from animal to human by direct contamination. e.g., Anthrax, Rabies, Brucellosis & Bubonic- plug.

4- Soil.

Some saprophytic bacteria from the soil can infect human.

e.g., *Clostridium tetani* ---------- Tetanus.

Cl. Welchii ----------Gas gangrene.

Endogenous infection:

Here the persons carry the bacteria but they are harmless in their natural sites & pathogenic in other sites.

Examples of endogenous infection are:-

1- *E. coli*

They are harmless in the intestine but they case urinary tract infection in urinary tract.

2- *Staph. aureus*

They are harmless in throat but harmful in wounds.

3- Pneumenococci

They are harmless in the throat & harmful in lung causing bronchopneumonia.

Spread of infection

Infections may be spread from the following:

1- **Respiratory**: By direct contact, by sneezing, coughing, speaking or indirectly transmitted.
2- **Skin**: In wounds & burns by contaminated hands and clothes.
3- **Venereal infection**: It is transmitted directly by sexual intercourse.
4- **Alimentary infection**: by contaminated hands, water or food through faecal-oral route transmission.
5- **Laboratory infection**: By culture & laboratory animals as in Brucellosis and Rikettsiosis.
6- **Blood sucking arthropods**: such as

Mosquito ---------- Malaria.

Flea ---------- Plug.

Louse ---------- Epidemic typhus.

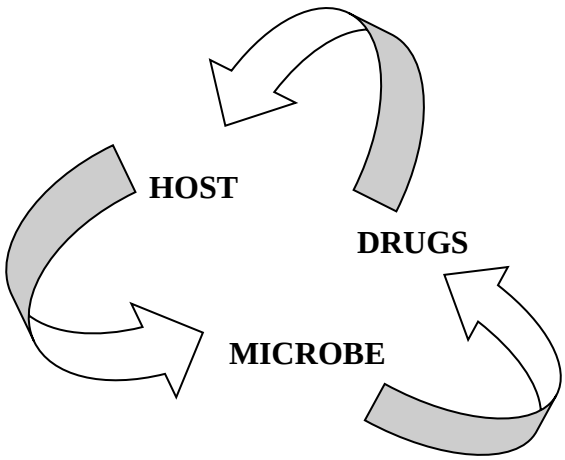
The manifestations of an infection depend on many factors, including the site acquisition or entry of the microorganism; organ or system tropisms of the microorganism; microbial virulence; the age, sex, and immunologic status of the patient; underlying diseases or conditions; and the presence of implanted prosthetic devices or materials. The signs and symptoms of infection may be localized, or they may be systemic, with fever, chills, and hypotension. In some instances the manifestations of an infection are sufficiently characteristic to suggest the diagnosis; however, they are often nonspecific.

The clinical presentation of an infectious disease reflects the interaction between the host and the microorganism. This interaction is affected by the host immune status and microbial virulence factors. Signs and symptoms vary according to the site and severity of infection. Diagnosis requires a composite of information, including history, physical examination, radiographic findings, and laboratory data.

Microbial Causes of Infection

Infections may be caused by bacteria, viruses, fungi, or parasites. Infection may be endogenous or exogenous. In endogenous infections, the microorganism (usually a bacterium) is a component of the patient's indigenous flora. Endogenous infections can occur when the microorganism is aspirated from the upper to the lower respiratory tract or when it penetrates the skin or mucosal barrier as a result of trauma or surgery. In contrast, in exogenous infections, the microorganism is acquired from the environment (e.g., from soil or water) or from another person or an animal. Although it is important to establish the cause of an infection, the differential diagnosis is based on a careful history, physical examination, appropriate radiographic and laboratory studies, including the selection of appropriate specimens for microbiologic examination. All of these, allow the physician to request tests for the microorganisms most likely to be the cause of the infection.

HOST-PARASITE RELATIONSHIPS



MECHANISMS OF PATHOGENESIS

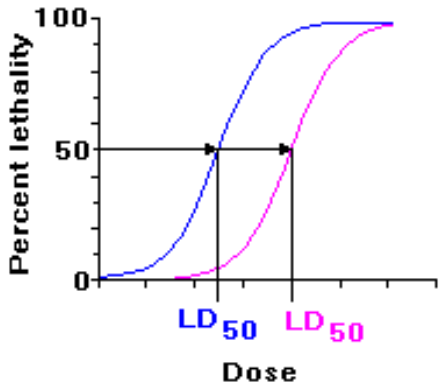
Pathogenic properties of bacteria: microorganisms cause disease by two basic mechanisms: 1) Invasion of tissue and 2) production of toxins.

Invasiveness: the ability to invade host tissues.

1. Intracellular pathogens vs extracellular pathogens: Intracellular pathogens generally produce chronic disease, extracellular pathogens generally produce acute disease (e.g. *Mycobacterium tuberculosis vs Streptococcus pyogenes*).
2. Capsules: Some bacteria produce hydrophilic gels that inhibit phagocytosis (e.g. smooth *vs* rough strains of *Streptococcus pneumoniae*).
3. Adaptation: microenvironments of the host body provide habitats for bacteria that are capable of selective tissue invasion (e.g. *Neisseria meningitidis vs Streptococcus pneumoniae*. Both inhabit the human nasopharynx, but only the latter invades the lower respiratory tract).
4. Extracellular enzymes: Some bacteria produce enzymes like hyaluronidase or collagenase that degrade host tissues.

Toxigenicity: the production of toxins.

1. Exotoxins: secreted proteins that are generally very toxic but heat labile. they are usually very good immunogens. Exotoxins are found mostly in Gram-positive organisms.
2. Endotoxins: complex polysaccharides (LPS) that are a part of the bacterial cell wall. they are usually poorly immunogenic. These toxins are released when cells lyse, are generally heat stable, and found mostly in Gram-negative bacteria.



Virulence: the combination of invasiveness and toxigenicity producing the ability to overcome host defenses.

1. Measurement of virulence: LD_{50} (% dead vs dose).
2. Variability in virulence potential may be genotypic (e.g. smooth *vs* rough strains of *Streptococcus pneumoniae*, lysogeny in *Corynebacterium diphtheriae*) or phenotypic (e.g. the production of capsular polysaccharides in the presence of rich carbohydrates).

Specimen Selection, Collection and Processing. Specimens selected for microbiologic examination should reflect the disease process and be collected in sufficient quantity to allow complete microbiologic examination. The number of microorganisms per milliliter of a body fluid or per gram of tissue is highly variable, ranging from less than 1 to 10^8 or 10^{10} colony-forming units (CFU). Swabs (Fig. 2), although popular for specimen collection, frequently yield too small a specimen for accurate microbiologic examination and should be used only to collect material from the skin and mucous membranes.

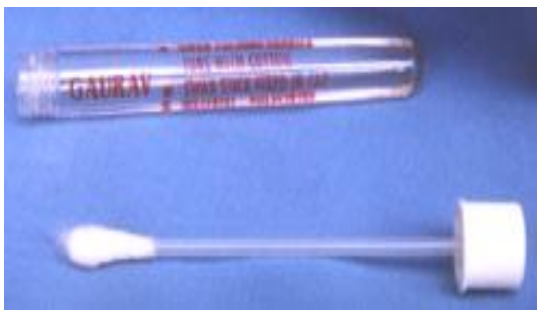


Figure 2: SWAB CULTURE TUBE With cotton screw cap (Disposable!)

Because skin and mucous membranes have a large and diverse indigenous flora, every effort must be made to minimize specimen contamination during collection. Contamination may be avoided by various means. The skin can be disinfected before aspirating or incising a lesion. Alternatively, the contaminated area may be bypassed altogether. Examples of such approaches are transtracheal puncture with aspiration of lower respiratory secretions or suprapubic bladder puncture with

aspiration of urine. It is often impossible to collect an uncontaminated specimen, and decontamination procedures, cultures on selective media, or quantitative cultures must be used. Specimens collected by invasive techniques, particularly those obtained intraoperatively, require special attention. Enough tissue must be obtained for both histopathologic and microbiologic examination. Histopathologic examination is used to distinguish neoplastic from inflammatory lesions and acute from chronic inflammations. The type of inflammation present can guide the type of microbiologic examination performed. If, for example, a caseous granuloma is observed histopathologically, microbiologic examination should include cultures for mycobacteria and fungi. The surgeon should obtain several samples for examination from a single large lesion or from each of several smaller lesions. If an abscess is found, the surgeon should collect several milliliters of pus, as well as a portion of the wall of the abscess, for microbiologic examination. Swabs should be kept out of the operating room. If possible, specimens should be collected before the administration of antibiotics. Above all, close communication between the clinician and the microbiologist is essential to ensure that appropriate specimens are selected and collected and that they are appropriately examined.

Parasitic and pathogenic interactions.

In these types of interactions the presence of the microbe is harmful to the host, and the microbe is considered a **parasite**. If the interaction causes a diseased state in the host, then the parasite is considered a **pathogen**. Many of the ideas in the ensuing discussion apply to both parasites and pathogens, but we will restrict our terminology to pathogens for simplicity and because these interactions are of greater concern. In addition to their ability to damage the host, pathogens have other traits that define them. Many have the ability to invade into areas where they are not wanted. This is in contrast to mutualistic and commensal organisms that avoid areas whose infection would cause a problem for the host. Pathogens also tend to have

more aggressive mechanisms for evading the host defenses than do mutualistic or commensal microbes, and these help them succeed in prohibited areas of the host.

A host being attacked by a pathogen is certainly an unwilling participant. The host actively avoids infection by trying to rid itself of the pathogens as quickly as possible. The defenses of hosts are focused on both preventing associations with pathogens and then getting rid of the microbes that succeed in associating. This process is literally a life-and-death struggle and generally, the host defenses are up to the task. *Streptococcus pneumoniae* is one example of a bacterial pathogen that colonizes the lungs if given the chance. It is a common inhabitant of the upper respiratory tract where it is commensal, but under the right circumstances, it will penetrate into the lungs. To prevent removal, the microbe has specific proteins that help it attach to lung tissue and once it is settled, it will grow in the lungs and cause disease. This growth and the production of certain toxic molecules harm the host and elicit a strong host immune reaction that is responsible for the high fever often associated with streptococcal pneumonia.

Challenges faced by a colonizing microbe

Here we will look at some general concepts associated with host-microbe interactions. We will examine the host response to pathogens at the end of this course, but now we will talk about the challenges faced by a microbe as it tries to colonize a host. We talk about these concepts here because they are faced by all microbes that interact with animal hosts, not just pathogens. These challenges can be broken into a series of steps that must be followed for a successful interaction: detecting a host, forming an interaction with a host, possibly invading a host, and dealing with hosts defenses. As we go through these different steps, we will describe specific examples of how microbes have overcome these challenges.

1.***Detecting a host.*** A microbe that has the ability to form an interaction with a host must first be able to find it in the environment. For the most part this involves

detecting some feature of the environment that is strongly correlated with the presence of the host. There are many different ways, by which different microbes can detect their respective host. *Pseudomonas aeruginosa*, an opportunistic pathogen of humans, shows somewhat less specificity in host detection by recognizing the level of iron present in its environment. The body sequesters most iron it comes in contact with and the concentration of free iron in the body is very low. When *P. aeruginosa* detects extremely low iron levels, it assumes it is in an appropriate host and turns on virulence genes. *Vibrio cholerae* uses temperature, pH, osmolarity, and certain amino acids as measures of the presence of the host. Outside the host, *V. cholerae* lives free in the ocean where the average temperature is 21°C or below and amino acids are at very low concentration. When ingested, the signals in the gastrointestinal tract turn on gene expression important in pathogenicity, including production of toxins and fimbriae. The fimbriae help the bacteria adhere to the intestine, while the toxin causes diarrhea.

Host specificity is often part of host detection. The microbe (and host in a mutualistic interaction) often send out signal molecules for reception by appropriate partners. Certain mutualistic interactions can be extremely selective as we described above. This type of specificity can also be found in pathogenic relationships. *Treponema pallidum* (the cause of syphilis) only infects humans, but other microbes are not so specific and infect a wide variety of hosts. Many commensal microbes such as some species of *Streptococcus* and *Staphylococcus* are found on a wide variety of mammals.

2. Attachment. Once near a host, a microbe has to associate with it in some manner to allow more than a transient interaction. In many cases this involves a specific molecular interaction between the microbe and the host (Table 1). There are numerous examples of this type of interaction. *E. coli* displays fimbriae on its surface that help it attach to the intestinal wall and uroepithelium. *Streptococcus mutans* uses an extracellular polysaccharide to bind tightly to teeth. *Neisseria*

meningitidis produces pili that help it hold onto the nasopharyngeal epithelium cells. In each of these cases a product produced by the microbe interacts with a specific molecule on the host that helps it to stick. Often this reaction is important in determining the microbes tissue specificity since it can only bind to cells producing the recognized receptor.

Table (1) Examples of attachment mechanisms.

Bacterium	**Adhesin**	**Receptor**	**Attachment site**	**Disease**
Streptococcus pyogenes	Protein F	Amino terminus of fibronectin	Pharyngeal epithelium	Sore throat
Streptococcus mutans	Glycosyl transferase	Salivary glycoprotein	Pellicle of tooth	Dental caries
Streptococcus salivarius	Lipoteichoic acid	Unknown	Buccal epithelium of tongue	None
Streptococcus pneumoniae	Cell-bound protein	N-acetylhexosamine-galactose disaccharide	Mucosal epithelium	pneumonia
Staphylococcus aureus	Cell-bound protein	Amino terminus of fibronectin	Mucosal epithelium	Various
Neisseria gonorrhoeae	N-methylphenyl- alanine pili	Glucosamine-galactose carbohydrate	Urethral/cervical epithelium	Gonorrhea
Enterotoxigenic E. coli	Type-1 fimbriae	Species-specific carbohydrate(s)	Intestinal epithelium	Diarrhea
Uropathogenic *E. coli*	Type 1 fimbriae	Complex carbohydrate	Urethral epithelium	Urethritis
Uropathogenic *E. coli*	P-pili (pap)	Globobiose linked to ceramide lipid	Upper urinary tract	Pyelonephritis
Bordetella pertussis	Fimbriae ("filamentous hemagglutinin")	Galactose on sulfated glycolipids	Respiratory epithelium	Whooping cough
Vibrio cholerae	N-methylphenylalanine pili	Fucose and mannose carbohydrate	Intestinal epithelium	Cholera
Treponema pallidum	Peptide in outer membrane	Surface protein(fibronectin)	Mucosal epithelium	Syphilis

3. *Invasion*

Many associations get no farther than attachment, where the microbe remains at its initial site of infection and has its effect, either positive or negative. The normal flora are a good example of this in a mutualistic/commensal interaction, but even some pathogenic microbes such as *Corynebacterium diphtheriae*, the causative agent of diphtheria, fails to penetrate beyond the initial site of attachment in the throat (Fig. 3).

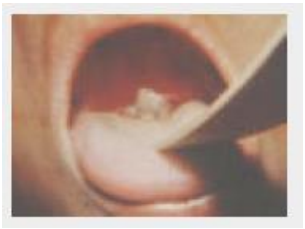


Fig. (3) Some stop here and go no further

In other cases, the microbe penetrates the host further. For microbes that *do* move beyond the initial interaction site, bacterial and sometimes host factors are necessary to allow further penetration.

In the case of mutualistic interactions host proteins help facilitate this movement, typically to a specific tissue in the host, and prevent further spread from there. In pathogenic interactions the host obviously tries to prevent penetration and as a result, pathogens have developed mechanisms to deal with the more aggressive host reaction.

Mammalian cells take up particles from the outside by a mechanism termed **endocytosis** and some pathogens have developed methods of exploiting this import mechanism to gain access to the inside of cells. Other defensive cells in the body, termed **phagocytes,** use engulfment (phagocytosis) to capture pathogens and kill them. Again, some pathogens are able to turn the tables on the phagocyte. They not only survive inside these cells but use them as a vehicle to spread throughout a host. In these cases, the microbe must have methods of surviving the harsh conditions faced when taken up by defensive host cells.

Mycobacterium tuberculosis is an example of a pathogen that can actually multiply inside a phagocyte and this immune cell then actually facilitates spread of the bacteria throughout the body. Table (2) lists some examples of microbes and factors involved in their invasion of a host.

Table (2) Invasion of a host by a microbe and some examples of factors that facilitate that invasion.

Microbe	**Host**	**Factors involved in invasion**	**Activity**
Rhizobia	Legumes	Nod factors	Induces nodulation reactions in plant root
S*treptococci, staphylococci and clostridia*	Humans	Hyaluronidase	Degrades hyaluronic of connective tissue
Staphylococci and streptococci	Mammals	Kinase	Converts plasminogen to plasmin, which digests fibrin
Staphylococcus aureus	Mammals	Leukocidin	Disrupts neutrophil membranes and causes discharge of lysosomal granules
Bacillus anthracis	Some mammals	Anthrax toxin	One component (EF or edema factor) is an adenylate cyclase, which causes increased levels of intracellular cyclic AMP. This in turn leads to edema.

Pathogens also have two other modes of entry besides penetration of healthy tissue that other symbionts do not seem to use. Many illnesses, including malaria, Lyme disease and the plague involve an insect vector that penetrates the initial outer defenses of the host. For example the bite of the deer tick allows *Borrelia*

burgdorferi--the microbe that causes Lyme disease--to penetrate the skin and directly enter the blood stream. There are examples of other organisms serving as vectors, but insects are the most common. The second entry mode involves tissues that have already been compromised by an injury or by the action of a previous pathogen. An example is infection by *Clostridium tetani*. The organism itself cannot penetrate the skin, but if allowed entry by a puncture wound, will grow deep within muscular tissue, causing tetanus. Interestingly, *C. tetani* is a strict anaerobe and requires deep wounds with an anoxic environment to grow. This is an example of a microbes basic physiology restricting its tissue tropism.

At this point, the results of a symbiosis diverge depending upon the outcome of the interaction. In light of this fact, we will treat these outcomes in separate sections beginning first with mutualistic interactions and then looking at pathogenic interactions. Note that commensal interactions fall somewhere between these two, with the host neither mounting a strong defense nor helping the commensal microorganism.

***Pathogenic outcomes*.**

After identifying and attaching to a host the pathogen typically spreads to various parts of the body and increases its population. This precipitates a vigorous host response and a pathogen has to be prepared to deal with it. This makes sense since the pathogen is invading places it ought not to be. There are many different **levels of defense**. Here are a few examples to give you an idea of the many different strategies microbes have evolved. *Staphylococcus aureus* produces leukocidin, a protein that is lethal to phagocytes. *S. aureus* also produces Protein A, which inhibits phagocytic engulfment. These bacteria then use the phagocyte as a method to spread throughout the body. *Pseudomonas aeruginosa* produces a capsule (an outer layer of polysaccharide) that helps it hide from the host immune system. Finally some microbes change their outer surface so that they are no longer recognized by previously generated immune responses. In all these cases the properties of the

microbe help it to evade some part of the immune system and then survive and grow.

Direct damage to host

By definition, pathogenic microorganisms damage their hosts. The type and the extent of this damage depends upon the **pathogen** and the **tissue**. For example, an infection that damages the muscle in the shoulder is a less devastating disease than an infection of the heart muscle. One pathogen may cause only a little damage while another causes severe damage and life-threatening disease. Some strains of *Bacillus cereus* can cause mild food poisoning that the body can deal with in a few days, whereas *Mycobacterium lepreae* (leprosy) causes many pathologies, including limb loss.

Damage may be directly caused by the pathogen or indirectly caused by the **hosts response to the pathogen**. Direct damage can be caused just by the mere growth of the microbe. *Streptococcus mutans* metabolizes sugars to lactic acid that then eats away at the enamel on the teeth, causing tooth decay. *Listeria monocytogenes* grows within host phagocytic cells and their high populations result in the death of the cell. However, pathogens very often make products that cause damage to the host and are important in the virulence of the microorganism. Many of these products are called toxins. Toxins come in two forms, **exotoxins** and **endotoxins**. Exotoxins are generally soluble proteins liberated from growing bacteria that then cause damage to the host. Endotoxins are lipopolysaccharides from the outer cell membrane of gram-negative bacteria that are normally released only after the death of the microbe.

Exotoxins

Each exotoxin-producing microorganism synthesizes a toxin that is typically unique to its species. For instance, *Bacillus anthracis* produces anthrax toxin and *Clostridium tetani* produces tetanus toxin. The exotoxin is often the major **virulence factor** and otherwise similar strains that do not produce the toxin often do not cause

illness. Because they are proteins, exotoxins are commonly heat-sensitive; however, many are resistant to inactivation by gastric pH.

Exotoxins attack very specific targets in the cell and the mechanisms of action of many of them are well established. Table (3) lists some exotoxins and their mechanisms of action. Some toxins work only on certain tissues or cells. For example, tetanus and botulism toxins only act at the synapses of neurons. Other toxins such as phospholipases act systemically, destroying many types of cells all over the body.

Table (3) Examples of various exotoxins and their activities.

Name of toxin	**Bacteria involved**	**Activity**
Anthrax toxin (EF, LF)	*Bacillus anthracis*	Edema factor (EF) is an adenylate cyclase that causes increased levels of intracellular cyclic AMP in host cells, leading to swelling. Lethal factor kills primarily macrophages by disrupting its signal transduction pathway.
Adenylate cyclase toxin	*Bordetella pertussis*	This toxin acts locally to increase levels of cyclic AMP in mucosal cells leading to increase in respiratory secretions and mucus production.
Cholera enterotoxin	*Vibrio cholerae*	ADP-ribosylation of G proteins stimulates adenylate cyclase and increases cAMP in cells of the GI tract, causing secretion of water and electrolytes.
E. coli LT toxin	*Escherichia coli*	Similar to cholera toxin.
E. coli ST toxin	*Escherichia coli*	Stimulates guanylate cyclase and promotes secretion of water and electrolytes from intestinal epithelium.
Shiga toxin	*Shigella dysenteriae*	Enzymatically cleaves rRNA resulting in inhibition of protein synthesis in large intestinal cells.
Clostridium perfringens enterotoxin	*Clostridium perfringens*	Stimulates adenylate cyclase leading to increased cAMP in small intestinal cells.
Botulinum toxin	*Clostridium botulinum*	Zn^{++}-dependent protease that inhibits neurotransmission at neuromuscular synapses resulting in flaccid paralysis.
Tetanus toxin	*Clostridium tetani*	Zn^{++}-dependent protease that inhibits neurotransmission at inhibitory synapses resulting in spastic paralysis.
Diphtheria toxin	*Corynebacterium*	ADP-ribosylation of translation elongation factor 2, which leads to

	diphtheriae	inhibition of protein synthesis in target cells.
Exotoxin A	*Pseudomonas aeruginosa*	Inhibits protein synthesis; similar to diphtheria toxin.
Pertussis toxin	*Bordeatella pertussis*	ADP-ribosylation of Gi proteins upregulates host cell adenylate cyclase, increasing concentration of cAMP in respiratory epithelium
Staphylococcus enterotoxins*	*Staphylococcus aureus*	Common cause of food poisoning. Certain serotypes have been associated with massive activation of the immune system resulting in toxic shock syndrome (TSS).
Toxic shock syndrome toxin (TSST-1)*	*Staphylococcus aureus*	Massive activation of the immune system resulting in TSS.
Exfoliative toxin*	*Staphylococcus aureus*	Cleavage of epidermis from dermis.
Pyrogenic toxin (formerly known as erythrogenic or scarlet fever toxin)*	*Streptococcus pyogenes*	Causes localized erythematous reactions.

*These toxins are called superantigens. They represent a family of molecules capable of massive activation of the immune system by interaction with T cells. The important feature is the production of lymphokines that appear to be the principal mediators of disease processes associated with these toxins.

Exotoxins are some of the most powerful human poisons known and are effective at very low concentrations, because most of the proteins are catalytic enzymes. These toxins can sometimes lead to death of the host, which in most cases is not a desirable outcome for the pathogen.

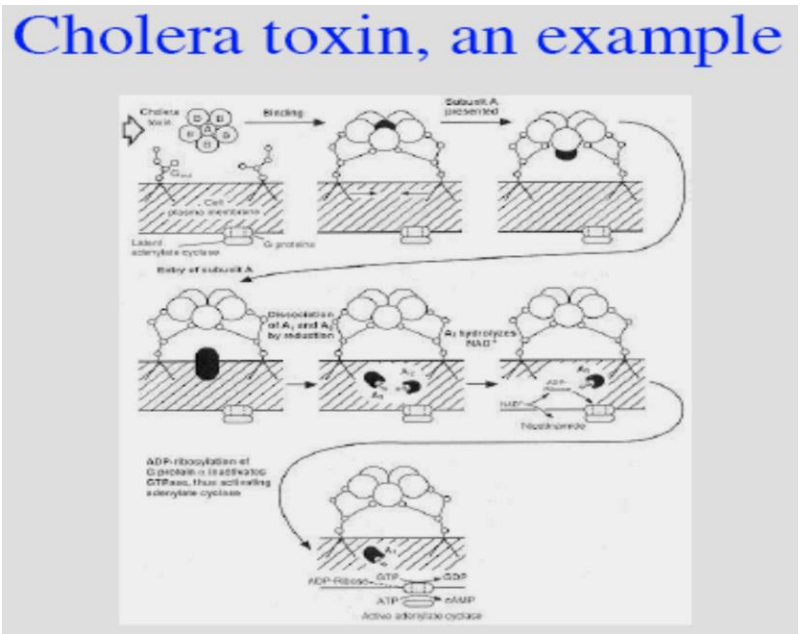
Structure and entry of exotoxins

Many of the protein toxins consist of two components. One component **(subunit A)** is responsible for the toxic activity, while the second component **(subunit B)** is necessary for attachment and transport of subunit A across the membrane of the target cells. Subunit A is not active until it is released from subunit B. There are two mechanisms of entry of toxins into target cells (Fig. 4). In direct entry, subunit B binds to a specific receptor on the membrane of the target cell, creates a pore and transports subunit A across the membrane. Subunit A then has its toxic effect. In the second method the A/B toxin binds to the target cell and is taken into the cell by receptor-mediated endocytosis. Inside the endosome the influx of protons triggers an

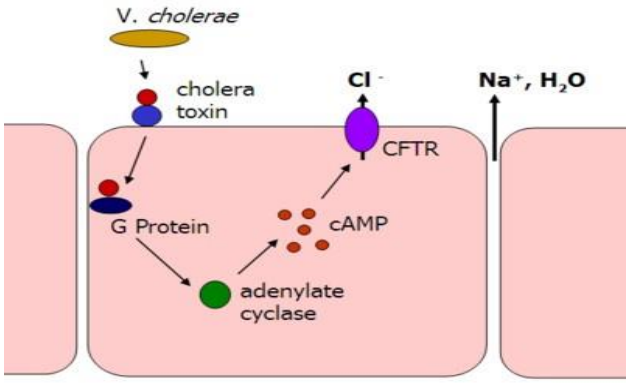
unknown mechanism that results in the release of the A subunit. The B subunit remains in the endosome, eventually exiting the cell by exocytosis. By either method, a toxic effect reflects the transfer of the A subunit into the cell cytoplasm.

Curiously, the genes for many toxins (e.g., diphtheria, botulism and some streptoccocal toxins) are located on temperate phages, while the genes for others (*E. coli* and *Staphylococcus aureus*) are on plasmids. This association with phage and plasmids is not important for the expression of the genes or for the function of the gene products. We therefore assume that these locations reflect the fact that these genes have been moving among different hosts, since phage and plasmids share this property. Genetic exchange by transduction and conjugation can therefore result in the transfer of toxin production from strain to strain, which must play a role in the virulence of particular bacteria in nature. It is still somewhat odd that such critical players in pathogenesis would be located on such potentially transient DNA as plasmids and temperate phages.

Fig. (4) Entry of Toxins.



The cholera toxin forces intestinal endothelia cells (cells that line the intestine) to purge Cl^- into the lumen of the intestine (hollow tube). Water follows. The end result, diarrhea as in the following figure.



Curiously, the genes for many toxins (e.g., diphtheria, botulism and some streptoccocal toxins) are located on temperate phages, while the genes for others (*E. coli* and *Staphylococcus aureus*) are on plasmids. This association with phage and plasmids is not important for the expression of the genes or for the function of the gene products. We therefore assume that these locations reflect the fact that these genes have been moving among different hosts, since phage and plasmids share this property. Genetic exchange by transduction and conjugation can therefore result in the transfer of toxin production from strain to strain, which must play a role in the virulence of particular bacteria in nature. It is still somewhat odd that such critical players in pathogenesis would be located on such potentially transient DNA as plasmids and temperate phages.

Endotoxins

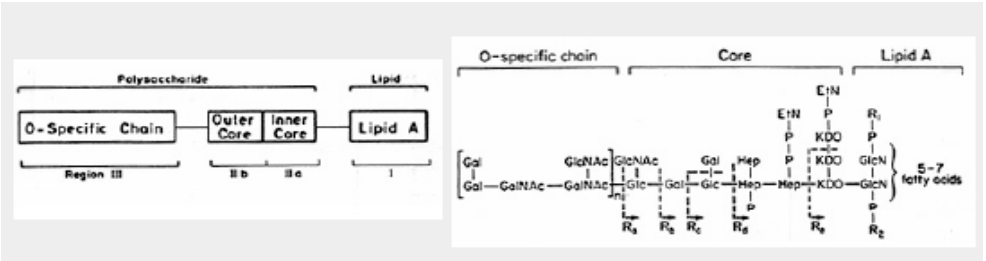
A second type of toxin is produced by Gram-negative pathogens. The outer layer of all gram-negative bacteria consists of an outer membrane that contains lipids, proteins and lipopolysaccharide (LPS). The LPS in the outer membrane has toxic systemic effects in mammals and has been given the name endotoxin. Endotoxins

are less potent than exotoxins, since they do not act enzymatically; they are also less specific. They are not destroyed by heat, i.e., boiling for 30 minutes has no effect on LPS. But powerful oxidizing agents such as hypochlorite can destroy them. Since LPS is part of the living microbial cell, it is not released in large quantities until the microbe dies, which is in contrast to exotoxins, which are released from growing cells. The levels of toxicity of endotoxins vary depending upon the producing microbe. Endotoxin from *E. coli* is more toxic than that from *Pseudomonas aeruginosa*, which is in turn more toxic than that from *Brucella sp.*

Structure

The LPS molecules from all known sources have roughly similar structures and are composed of three regions (Fig. 5).

Fig. (5) The stucture of LPS



Lipid A is a highly conserved region consisting of a phosphorylated N-acetylglucosamine (NAG) dimer with six or seven fatty acids attached. The toxicity of LPS is associated with Lipid A. Lipid A is absolutely required by the microbial cell, as it has been impossible to isolate mutants unable to synthesize lipid A. Lipid A is likely essential for the assembly of the outer membrane.

Core polysaccharide or **R antigen** consists of a short chain of sugars. For example in *E. coli* the sugars are 2-keto-3-deoxyoctonoic acid (KDO), heptose, glucose, galactose and N-acetyl glucosamine. KDO is unique to LPS and invariably present. This core polysaccharide is conserved within all strains in a species but will vary somewhat between different species. For example, the core polysaccharide of

Salmonella enterica subsp. Typhimurium contains ethanolamine, while that from *E. coli* does not.

The core polysaccharide seems to be important in general cell function and specifically for outer membrane integrity. Loss of the core polysaccharide by mutation causes the microbe to be more sensitive to a range of hydrophobic compounds, including antibiotics, detergents, bile salts and mutagens. The sugars of the core polysaccharide contain a number of charged groups, and these are thought to be important in maintaining the permeability of the outer membrane. Loss of the core probably weakens the outer membrane, allowing many normally excluded molecules to come into contact with the cytoplasmic membrane.

O antigen is attached to the core antigen and consists of repeating units of three to five sugars. It varies in length and can contain up to 40 repeating units. At least 20 different sugars are known to occur in O antigens and many of these sugars are only found in Gram-negative cell walls. The sugars present in the O antigen vary greatly between species and even within them. Sugars in the structure, especially the terminal ones, are what interact with the microbe's environment.

LPS is a major determinant of virulence for Gram-negative pathogens. Small changes in the O-polysaccharide can make large changes in the virulence of a strain. Strains that lose the ability to make O-polysaccharide are called rough strains due to their colony appearance, and these are much less pathogenic than regular strains (termed smooth). O antigen is important for interacting with the host structures, and mutants lacking the O antigen are also more easily taken up and destroyed by phagocytes.

Pathologies of endotoxin

Endotoxin from all pathogenic species causes the same range of biological activities affecting almost every organ in the body. When LPS is released into the blood stream it is bound by LPS-binding serum proteins. This is then presented to immune system cells, causing several important effects.

- The release of **cytokines**, a group of small peptides that are powerful mediators of inflammation, is stimulated. One important effect of this is the induction of vasodilation causing drop in blood pressure and setting the stage for septic shock.
- Endotoxins are pyrogenic (fever-inducing), because they cause the release of interleukin 1 (a type of cytokine produced by macrophages), which acts on the hypothalamus, inducing fever.
- Clotting cascade is activated, causing intravascular coagulation.
- Endotoxins induce various parts of the immune system, causing the production of antibodies and activation of immune cells. Table 4 summarizes the properties of endotoxins and exotoxins.

Table 4. A comparison of the properties of endotoxins and exotoxins.

Property	**Endotoxin**	**Exotoxin**
Chemical nature	Lipopolysaccharide (mw = 10kDa)	Protein (mw = 50-1000 kDa)
Relationship to cell	Part of outer membrane and has major non-toxin use for the molecule	Specifically synthesized protein that causes a toxic effect; extracellular and diffusible.
Denatured by boiling	No	Usually
Antigenic	Yes	Yes
Relative potency	Low (>100 g)	High (1 g)
Specificity	Low degree	High degree
Enzymatic action	No	Usually
Pyrogenicity	Yes	Occasionally

Indirect damage to host

The host causes indirect damage to itself by its very reaction to the presence of the microbe. Generally, this involves the inflammatory response to products from the pathogen. For example, lung infection by *Mycobacterium tuberculosis* causes very little direct damage. However, the host mounts a vigorous response to the presence of the organisms and forms large granulomas, which are grainy growths of host mononuclear cells. These wall off the bacteria, but also damage the lung tissue, impairing its function. In weakened hosts, *M. tuberculosis* can escape these granulomas, causing lysis of the cells. Cell contents, including lysosomal enzymes, spill out and damage lung tissue. Over many months, the infection destroys the lung tissue with most of the damage being caused by the immune system.

Indirect damage can also be caused by a variety of other host responses to infection. Infection by a microorganism precipitates the stress response, which involves the release of the hormones epinephrine and cortisol. This causes high blood pressure and high blood sugar, which is detrimental to the body. Inflammation causes blood vessels to become leaky, which then allows the immune system to reach the tissue. During this process, the blood vessels and the tissue, into which the blood leaks, are damaged, and platelets are depleted. Finally, infection with one microorganism can weaken the local defenses enough to allow invasion by a second microorganism that causes the actual damage. The classic example of this is an ear infection, where a viral infection causes buildup of fluid in the ears, creating an environment for growth of bacteria such as *Streptococcus pneumoniae* or *Haemophilus influenzae*. Another example is infection with HIV (human immunodeficiency virus). Growth of the viruses causes the destruction of the immune system cells, thus allowing an uncontrolled proliferation of organisms such as *Mycobacterium tuberculosis* that can kill the host.

THE COMPOSITION OF THE NORMAL FLORA

The normal flora of corresponding anatomical sites in different animal species varies widely. Within a single species (e.g. humans) there is additional variation in the normal flora that is related to factors such as age, sex, diet and nutrition. Some bacteria are found regularly at particular anatomical locales; others are present only occasionally, or at certain times during life. Developmental changes in humans such as weaning, the eruption of the teeth, and the onset and cessation of ovarian functions, invariably affect the composition of the normal flora in the intestinal tract, the oral cavity, and the vagina, respectively. However, within the limits of these fluctuations, the bacterial flora of humans is sufficiently constant to a give general description of the situation.

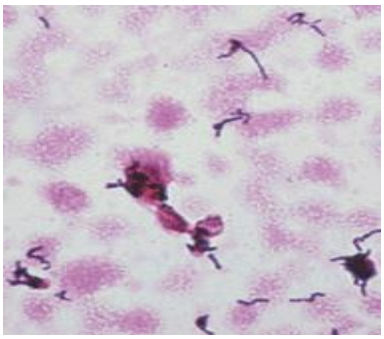
It has been calculated that the normal human houses about 10^{12} bacteria on the skin, 10^{10} in the mouth, and 10^{14} in the gastrointestinal tract. The latter number is far in excess of the number of eukaryotic cells in all organs which comprise the human host.

Normal Flora of the Skin. The adult human is covered with approximately 2 square meters of skin. The density and composition of the normal flora of the skin vary with anatomical locale. The high moisture content of the axilla, and areas between the toes supports the activity and growth of relatively high densities of bacterial cells, but the density of bacterial populations at most other sites is fairly low, generally in 100s or 1000s per square cm. Qualitatively, the bacteria on the skin near any body orifice (e.g. mouth, nose and anus) may be similar to those in the orifice.

The majority of skin microorganisms are found in the most superficial layers of the epidermis and the upper parts of the hair follicles. They consist largely of micrococci (*Staphylococcus epidermidis* and *Micrococcus* sp.) and corynebacteria. These are generally non-pathogenic and considered to be commensal, although

mutualistic and parasitic roles have been assigned to them. Sometimes potentially pathogenic *Staphylococcus aureus* is found on the face and hands.

Normal Flora of the Cunjunctiva. A variety of bacteria may be cultivated from the normal conjunctiva but the number of organisms is usually small. *Staphylococcus epidermidis* and certain coryneforms (Propoinibacterium acnes) are dominant. *Staphylococcus aureus, some* streptococci, *Haemophilus* sp. and *Neisseria* sp. are occasionally found. The conjunctiva is kept moist and healthy by the continuous secretions from the lachrymal glands. Blinking wipes the conjunctiva every few seconds mechanically washing away foreign objects including bacteria. Lachrymal secretions (tears) also contain bactericidal substances including lysozyme. There is little or no opportunity for microorganisms to colonize the conjunctiva without special mechanisms to attach to the epithelial surfaces and some ability to withstand attack by lysozyme. Pathogens which do infect the conjunctiva (e.g. *Neisseria gonorrhoeae* and *Chlamydia trachomatis*) are thought to be able to specifically attach to the conjunctival epithelium by means of sialic acid receptors on epithelial cells, but this is not certain.



Propionibacterium acnes

Normal Flora of the Respiratory Tract. The nares (nostrils) are always heavily colonized, predominantly with *Staphylococcus epidermidis* and corynebacteria, and often (about 20% of the general population) with *Staphylococcus aureus,* this being the main carrier site of this important pathogen. The healthy sinuses, in contrast are sterile. A large number of bacterial species colonize the upper respiratory tract

(nasopharynx). The predominant species are non-hemolytic and alpha-hemolytic streptococci and *Neisseria,* but sometimes pathogens such as *Streptococcus pneumoniae, Streptococcus pyogenes, Haemophilus influenzae* and *Neisseria meningitidis* colonize the pharynx.

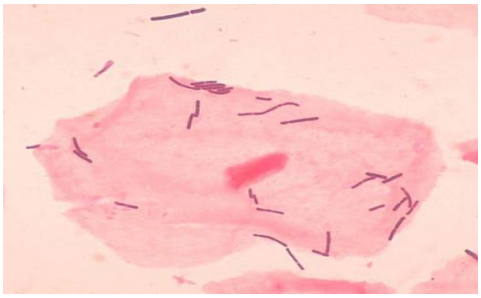
The lower respiratory tract (trachea, bronchi, and pulmonary tissues) are virtually free of microorganisms, mainly because of the efficient cleansing action of the ciliated epithelium which lines the tract. Any bacteria reaching the lower respiratory tract are swept upward by the action of the mucociliary blanket that lines the bronchi, to be removed subsequently by coughing, sneezing, swallowing, etc. If the respiratory tract epithelium becomes damaged, as in bronchitis or viral pneumonia, the individual may become susceptible to infection by pathogens descending from the nasopharynx (e.g. *H. influenzae* or *S. pneumoniae*). The pathogen *Bordetella pertussis* is specifically able to colonize the tracheal epithelium of humans, allowing it to produce the disease, pertussis (whooping cough)

Normal flora of the Urogenital Tract. The urogenital tract consists of the **bladder**, the **ureter**, the **urethra** and the **genitalia**. The bladder and internal regions of the ureter and urethra are typically devoid of microorganisms. The few found in urine cultures are probably contaminants from end of the urethra and the genitals, which have bacteria that are common on the skin and the colon. These include *S. epidermidis, Enterococcus faecalis, E. coli, Proteus spp.*, corynebacteria and streptococci.

The vagina of the healthy female is colonized soon after birth by typical microbes found on the skin and rectum, including corynebacteria, staphylococci, non-pyogenic streptococci, *E. coli*, and a lactic acid bacterium named Dderleins bacilli (most likely, *Lactobacillus acidophilus*). After the onset of puberty and throughout reproductive life, circulating estrogen causes the secretion of glycogen in the vagina. Metabolism of glycogen to lactic acid by Dderleins bacilli drops the pH and inhibits

colonization by all except this microbe and a few others. Significantly, this helps prevent colonization by *Candida albicans*, the cause of yeast infections.

Urine is normally sterile, and since the urinary tract is flushed with urine every few hours, microorganisms have problems gaining access and becoming established. The flora of the anterior urethra, as indicated principally by urine cultures, suggests that the area may be inhabited by a relatively consistent normal flora consisting of S*taphylococcus epidermidis, Enterococcus faecalis* and some alpha-hemolytic streptococci. Their numbers are not plentiful, however. In addition, some enteric bacteria (e.g. *E. coli, Proteus*) and corynebacteria, which are probably contaminants from the skin, vulva or rectum, may occasionally be found at the anterior urethra.



Lactic acid bacteria, possibly Doderlein's bacillus, in ascociation with a vaginal epithelial cell.

Normal Flora of the Human Oral Cavity.

The presence of nutrients, epithelial debris, and secretions makes the mouth a favorable habitat for a great variety of bacteria. Oral bacteria include streptococci, lactobacilli, staphylococci and corynebacteria, with a great number of anaerobes, especially bacteroides.

The mouth presents a succession of different ecological situations with age, and this corresponds with changes in the composition of the normal flora. At birth the oral cavity is composed solely of the soft tissues of the lips, cheeks, tongue and palate, which are kept moist by the secretions of the salivary glands. At birth the oral cavity is sterile but rapidly becomes colonized from the environment, particularly from the

mother in the first feeding. *Streptococcus salivarius* is dominant and may make up 98% of the total oral flora until the appearance of the teeth (6 - 9 months in humans). The eruption of the teeth during the first year leads to colonization by *S. mutans* and *S. sanguis*. These bacteria require a nondesquamating (nonepithelial) surface in order to colonize. They will persist as long as teeth remain. Other strains of streptococci adhere strongly to the gums and cheeks but not to the teeth. The creation of the gingival crevice area (supporting structures of the teeth) increases the habitat for the variety of anaerobic species found. The complexity of the oral flora continues to increase with time, and bacteroides and spirochetes colonize around puberty. Several oral bacteria have been implicated in diseases of the oral cavity, including dental caries and gum diseases such as gingivitis and periodontitis. The oral bacteria can invade compromised tissues in their hosts and produce disease outside the oral cavity. Oral bacteria invade deeper tissues they may cause abscesses of alveolar bone, lung, brain, or the extremities. Such infections usually contain mixtures of bacteria with *Bacteroides melaninogenicus* often playing a dominant role. If oral streptococci are introduced into wounds created by dental manipulation or treatment, they may adhere to heart valves and initiate subacute bacterial endocarditis.

Dental Plaque, Caries, and Gingivitis

Dental plaque, which is material adhering to the teeth, consists of bacterial cells (60-70% the volume of the plaque), salivary polymers, and bacterial extracellular products. Plaque is a naturally-constructed biofilm, in which the consortia of bacteria may reach a thickness of 300-500 cells on the surfaces of the teeth. These accumulations subject the teeth and gingival tissues to high concentrations of bacterial metabolites, which result in dental disease.

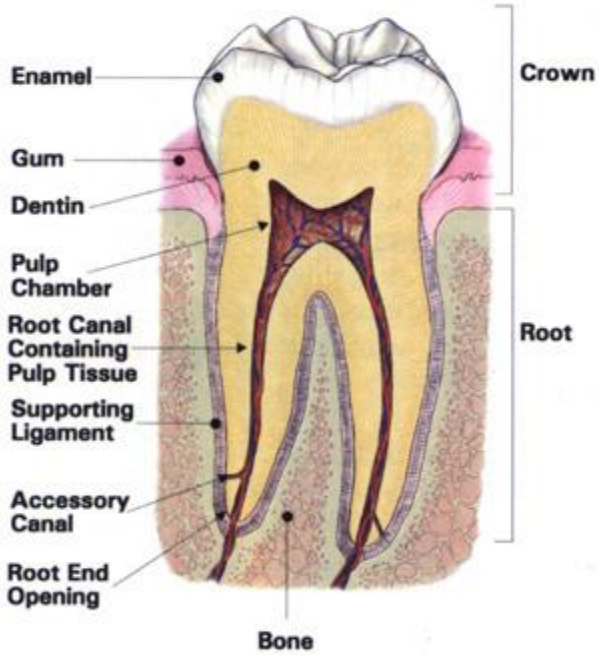
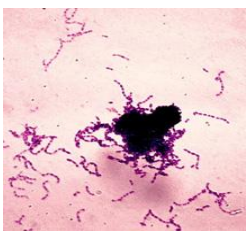


Fig. Cross section of a tooth illustrating the various structural regions susceptible to colonization or attack by microbes.

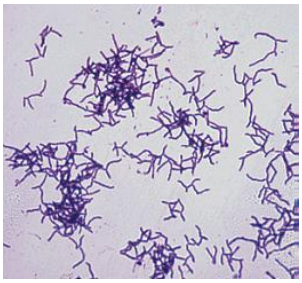
In relationship dental caries and periodontitis oral bacteria produce three forms of toxins: soluble protein toxins (exotoxins) which work as extracellular enzymes; endotoxins which are lippolysaccharides in the Gran-negative cell wall; products of their metabolism (metabolites) which may be toxic. The latter include volatile sulfur compounds such as hydroghen sulfide and methylmercaptan; polyamines with names like putrescine, cadaverine and spermidine; and fatty acids such as propionic acid and butyric acid. By far the dominant bacterial species in dental plaque are *Streptococcus sanguis* and *Streptococcus mutans,* both of which are considered responsible for plaque.



Streptococcus mutans. **Gram stain.**

Plaque formation is initiated by a weak attachment of the streptococcal cells to salivary glycoproteins forming a pellicle on the surface of the teeth. This is followed by a stronger attachment by means of extracellular sticky polymers of glucose (glucans) which are synthesized by the bacteria from dietary sugars (principally sucrose). An enzyme on the cell surface of *Streptococcus mutans*, glycosyl transferase, is involved in initial attachment of the bacterial cells to the tooth surface and in the conversion of sucrose to dextran polymers (glucans) which form plaque.

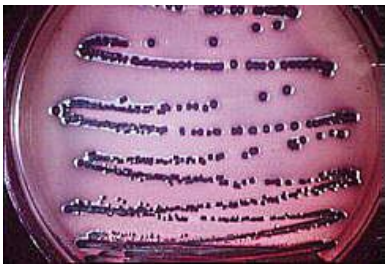
Dental Caries is the destruction of the enamel, dentin or cementum of teeth due to bacterial activities. Caries are initiated by direct demineralization of the enamel of teeth due to lactic acid and other organic acids which accumulate in dental plaque. Lactic acid bacteria in the plaque produce lactic acid from the fermentation of sugars and other carbohydrates in the diet of the host. Streptococcus mutans has most consistently been associated with the initiation of dental caries, but other lactic acid bacteria are probably involved as well. These organisms normally colonize the occlusal fissures and contact points between the teeth, and this correlates with the incidence of decay on these surfaces.



Actinomyces israelii

Lactobacilli, *Actinomyces,* and various proteolytic bacteria are commonly found in human carious dentin and cementum, which suggests that they are secondary invaders that contribute to the progression of the lesions.

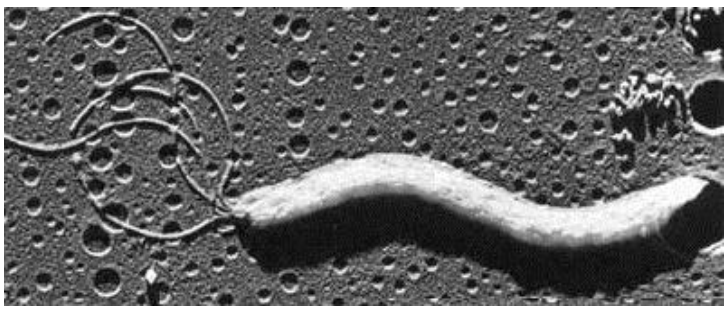
Normal Flora of the Gastrointestinal Tract.



Colonies of *E. coli* growing on EMB agar.

The bacterial flora of the GI tract of animals has been studied more extensively than that of any other site. The composition differs between various animal species. In humans, there are differences in the composition of the flora which are influenced by age, diet, cultural conditions, and the use of antibiotics. The latter greatly perturbs the composition of the intestinal flora.

Because of the high acidity of the gastric juice very few bacteria (mainly acid-tolerant lactobacilli) can be cultured from the normal stomach. However, at least half the population in our country is colonized by a pathogenic bacterium, *Helicobacter pylori.* Since the 1980s, this bacterium has been known to be the cause of gastric ulcers, and it is probably a cause of gastric and duodenal cancer as well.



Helicobacter pylori

The proximal small intestine has a relatively sparse Gram-positive flora, consisting mainly of lactobacilli and *Enterococcus faecalis.* This region has about 10^5 - 10^7 bacteria per ml of fluid. The distal part of the small intestine contains greater numbers of bacteria (10^8/ml) and additional species including coliforms and

Bacteroides, in addition to lactobacilli and enterococci. The flora of the large intestine (colon) is qualitatively similar to that found in feces. Populations of bacteria in the colon reach levels of 10^{11}/ml feces. Coliforms become more prominent, and enterococci, clostridia and lactobacilli can be regularly found, but the predominant species are anaerobic *Bacteroides* and anaerobic lactic acid bacteria in the genus *Bifidobacterium* (*Bifidobacterium bifidum*). These organisms may outnumber *E. coli* by 1,000:1 to 10,000:1. It is now known that significant numbers of anaerobic methanogenic bacteria (up to 10^{10}/gm) also reside in the colon of humans. The range of incidence of certain bacteria in the large intestine of humans is shown below.

At birth the entire intestinal tract is sterile, but bacteria enter with the first feed. The initial colonizing bacteria vary with the food source of the infant. In breast-fed infants bifidobacteria account for more than 90% of the total intestinal bacteria. *Enterobacteriaceae* and enterococci are regularly present, but in low proportions, while bacteroides, staphylococci, lactobacilli and clostridia are practically absent. In bottle-fed infants, bifidobacteria are not predominant. When breast-fed infants are switched to a diet of cow's milk or solid food, bifidobacteria are progressively joined by enterics, bacteroides, enterococci lactobacilli and clostridia. Apparently, human milk contains a growth factor that enriches for growth of bifidobacteria, and these bacteria play an important role in preventing colonization of the infant intestinal tract by non indigenous or pathogenic species.

Clostridium difficile. Gram stain.

THE BENEFITS OF THE NORMAL FLORA

Many microorganisms enjoy the lush environment our bodies provide for them, but what are the benefits for the host?

1. The normal flora synthesize and excrete vitamins in excess of their own needs, which can be absorbed as nutrients by the host. For example, enteric bacteria secrete Vitamin K and Vitamin B12, and lactic acid bacteria produce certain B-vitamins. Germ-free animals may be deficient in Vitamin K to the extent that it is necessary to supplement their diets.

2. The normal flora prevent colonization by pathogens by competing for attachment sites or for essential nutrients. This is thought to be their most important beneficial effect, which has been demonstrated in the oral cavity, the intestine, the skin, and the vaginal epithelium. In some experiments, germ-free animals can be infected by 10 *Salmonella* bacteria, while the infectious dose for conventional animals is near 10^6 cells.

3. The normal flora may antagonize other bacteria through the production of substances which inhibit or kill nonindigenous species. The intestinal bacteria produce a variety of substances ranging from relatively nonspecific fatty acids and peroxides to highly specific bacteriocins, which inhibit or kill other bacteria.

4. The normal flora stimulate the development of certain tissues, i.e., the caecum and certain lymphatic tissues (Peyer's patches) in the GI tract. The caecum of germ-free animals is enlarged, thin-walled, and fluid-filled, compared to that organ in conventional animals.

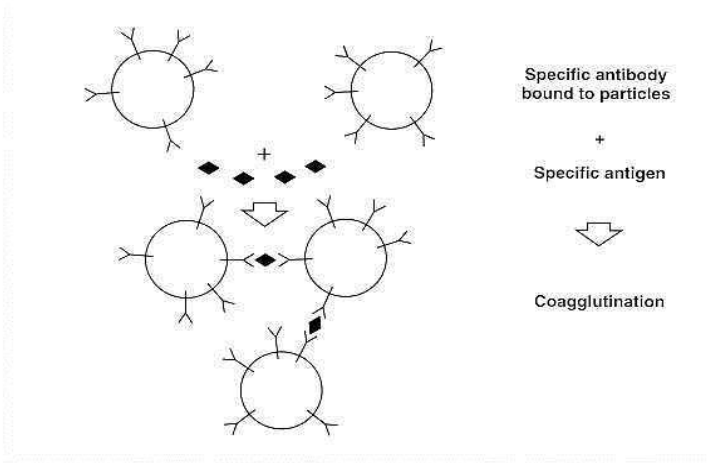
5. The normal flora stimulate the production of cross-reactive antibodies. Since the normal flora behave as antigens in an animal, they induce an immunological response, in particular, an antibody-mediated immune (AMI) response. Low levels of antibodies produced against components of the normal flora are known to cross react with certain related pathogens, and thereby prevent infection or

invasion. Antibodies produced against antigenic components of the normal flora are sometimes referred to as "natural" antibodies, and such antibodies are lacking in germ-free animals.

Most Common Lab. Techniques Used

For microscopic examination it is sufficient to have a compound binocular microscope equipped with low-power (1OX), high-power (40X), and oil immersion (1OOX) achromatic objectives, 10X wide-field oculars, a mechanical stage, a substage condenser, and a good light source. For examination of wet-mount preparations, a darkfield condenser or condenser and objectives for phase contrast increases image contrast. An exciter barrier filter, darkfield condenser, and ultraviolet light source are required for fluorescence microscopy.

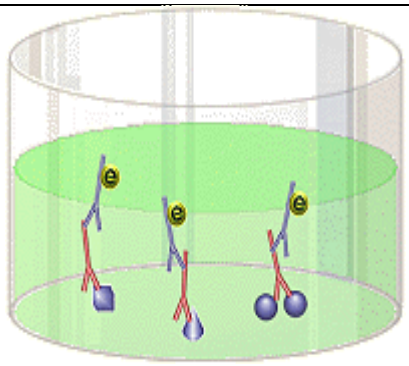
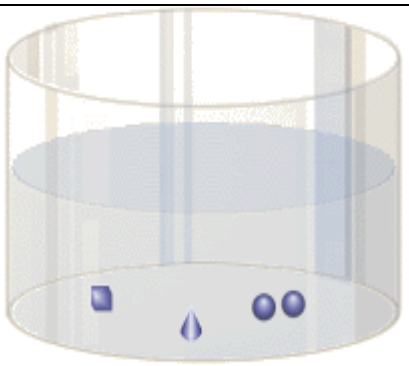
For immunologic detection of microbial antigens, latex particle agglutination, coagglutination, and enzyme-linked immuno sorbent assay (ELISA) are the most frequently used techniques in the clinical laboratory. Antibody to a specific antigen is bound to latex particles or to a heat-killed and treated protein A-rich strain of *Staphylococcus aureus* to produce agglutination. There are several approaches to ELISA; the one most frequently used for the detection of microbial antigens uses an antigen-specific antibody that is fixed to a solid phase, which may be a latex or metal bead or the inside surface of a well in a plastic tray. Antigen present in the specimen binds to the antibody as in. The test is then completed by adding a second antigen-specific antibody bound to an enzyme that can react with a substrate to produce a colored product. The initial antigen antibody complex forms in a manner similar to that shown in. When the enzyme-conjugated antibody is added, it binds to previously unbound antigenic sites, and the antigen is, in effect, sandwiched between the solid phase and the enzyme-conjugated antibody. The reaction is completed by adding the enzyme substrate.



Agglutination test in which inert particles (latex beads or heat-killed *S. aureus* Cowan 1 strain with protein A) are coated with antibody to any of a variety of antigens and then used to detect the antigen in specimens or in isolated bacteria.

The ELISA Method

	Partially purified, inactivated HIV antigens pre-coated onto an ELISA plate
	Patient serum which contains antibodies. If the patient is HIV+, then this serum will contain antibodies to HIV, and those antibodies will bind to the HIV antigens on the plate.
	Anti-human immunoglobulin coupled to an enzyme. This is the second antibody, and it binds to human antibodies.
	Chromogen or substrate which changes color when cleaved by the enzyme attached to the second antibody.

	
Positive ELISA Test	**Negative ELISA Test**

Genetic probes are based on the detection of unique nucleotide sequences with the DNA or RNA of a microorganism. Once such a unique nucleotide sequence, which may represent a portion of a virulence gene or of chromosomal DNA, is found, it is isolated and inserted into a cloning vector (plasmid), which is then transformed into Escherichia coli to produce multiple copies of the probe. The sequence is then reisolated from plasmids and labeled with an isotope or substrate for diagnostic use. Hybridization of the sequence with a complementary sequence of DNA or RNA follows cleavage of the double-stranded DNA of the microorganism in the specimen.

The use of molecular technology in the diagnoses of infectious diseases has been further enhanced by the introduction of gene amplication techniques, such as the polymerase chain reaction (PCR) in which DNA polymerase is able to copy a strand of DNA by elongating complementary strands of DNA that have been initiated from a pair of closely spaced oligonucleotide primers. This approach has had major applications in the detection of infections due to microorganisms that are difficult to culture (e.g. the human immunodeficiency virus) or that have not as yet been successfully cultured (e.g. the Whipple's disease bacillus).

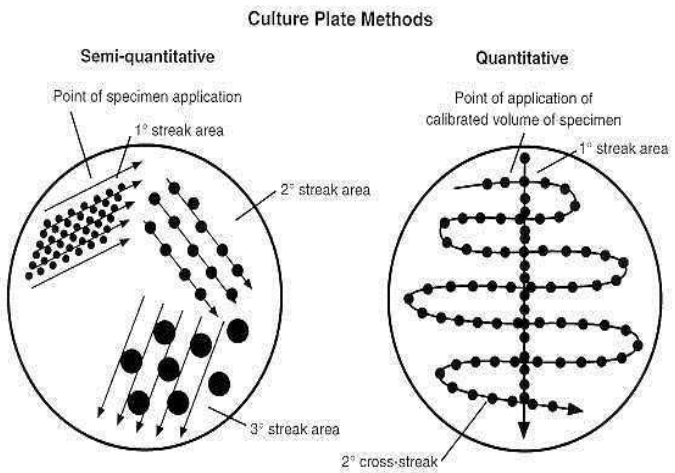
Culture

In many instances, the cause of an infection is confirmed by isolating and culturing microorganism either in artificial media or in a living host. Bacteria (including mycobacteria and mycoplasmas) and fungi are cultured in either liquid (broth) or on solid (agar) artificial media. Liquid media provide greater sensitivity for the isolation of small numbers of microorganisms; however, identification of mixed cultures growing in liquid media requires subculture onto solid media so that isolated colonies can be processed separately for identification. Growth in liquid media also cannot ordinarily be quantitated. Solid media, although somewhat less sensitive than liquid media, provide isolated colonies that can be quantified if necessary and identified. Some genera and species can be recognized on the basis of their colony morphologies.

In some instances one can take advantage of differential carbohydrate fermentation capabilities of microorganisms by incorporating one or more carbohydrates in the medium along with a suitable pH indicator. Such media are called differential media (e.g., eosin methylene blue or MacConkey agar) and are commonly used to isolate enteric bacilli. Different genera of the Enterobacteriaceae can then be presumptively identified by the color as well as the morphology of colonies.

Culture media can also be made selective by incorporating compounds such as antimicrobial agents that inhibit the indigenous flora while permitting growth of specific microorganisms resistant to these inhibitors. One such example is Thayer-Martin medium, which is used to isolate *Neisseria gonorrhoeae*. This medium contains vancomycin to inhibit Gram-positive bacteria, colistin to inhibit most Gram-negative bacilli, trimethoprim-sulfamethoxazole to inhibit *Proteus* species and other species that are not inhibited by colistin and anisomycin to inhibit fungi. The pathogenic *Neisseria* species, *N gonorrhoeae* and *N meningitidis,* are ordinarily resistant to the concentrations of these antimicrobial agents in the medium.

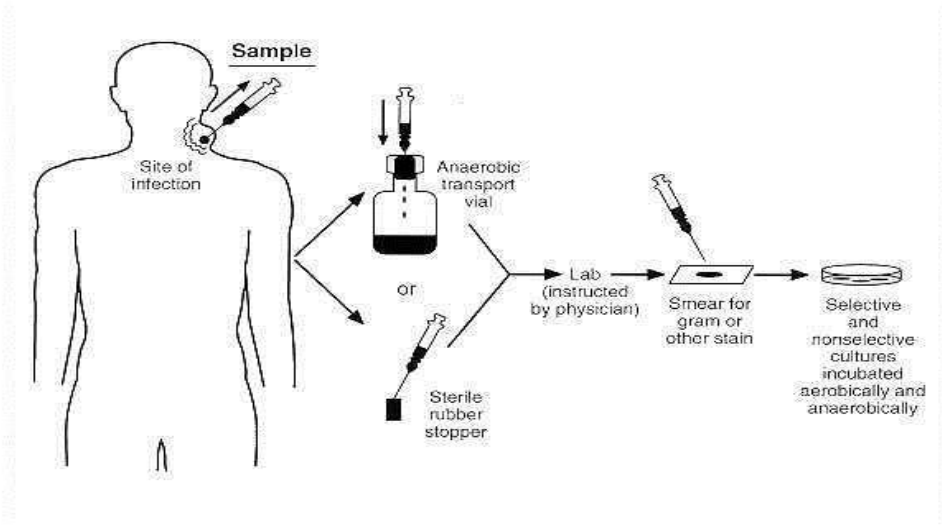
The number of bacteria in specimens may be used to define the presence of infection. For example, there may be small numbers (< 10^3 CFU/ml) of bacteria in clean-catch, midstream urine specimens from normal, healthy women; with a few exceptions, these represent bacteria that are indigenous to the urethra and periurethral region. Infection of the bladder (cystitis) or kidney (pyelone-phritis) is usually accompanied by bacteriuria of about > 10^4 CFU/ml. For this reason, quantitative cultures **(Fig. 4)** of urine must always be performed. For most other specimens a semiquantitative streak method **(Fig. 4)** over the agar surface is sufficient. For quantitative cultures, a specific volume of specimen is spread over the agar surface and the number of colonies per milliliter is estimated. For semiquantitative cultures, an unquantitated amount of specimen is applied to the agar and diluted by being streaked out from the inoculation site with a sterile bacteriologic loop **(Fig. 4)**. The amount of growth on the agar is then reported semiquantitatively as many, moderate, or few (or 3+, 2+, or 1+), depending on how far out from the inoculum site colonies appear. An organism that grows in all streaked areas would be reported as 3+.



Quantitative versus semi quantitative culture, revealing the number of bacteria in specimens.

Chlamydiae and viruses are cultured in cell culture systems, but virus isolation occasionally requires inoculation into animals, such as suckling mice, rabbits, guinea pigs, hamsters, or primates. Rickettsiae may be isolated with some difficulty and at some hazard to laboratory workers in animals or embryonated eggs. For this reason, rickettsial infection is usually diagnosed serologically. Some viruses, such as the hepatitis viruses, cannot be isolated in cell culture systems, so that diagnosis of hepatitis virus infection is based on the detection of hepatitis virus antigens or antibodies.

Cultures are generally incubated at 35 to 37°C in an atmosphere consisting of air, air supplemented with carbon dioxide (3 to 10 percent), reduced oxygen (microaerophilic conditions), or no oxygen (anaerobic conditions), depending upon requirements of the microorganism. Since clinical specimens from bacterial infections often contain aerobic, facultative anaerobic, and anaerobic bacteria, such specimens are usually inoculated into a variety of general purpose, differential, and selective media, which are then incubated under aerobic and anaerobic conditions.



General procedure for collecting and processing specimens for aerobic and/or anaerobic bacterial culture.

The duration of incubation of cultures also varies with the growth characteristics of the microorganism. Most aerobic and anaerobic bacteria will grow overnight, whereas some mycobacteria require as many as 6 to 8 weeks.

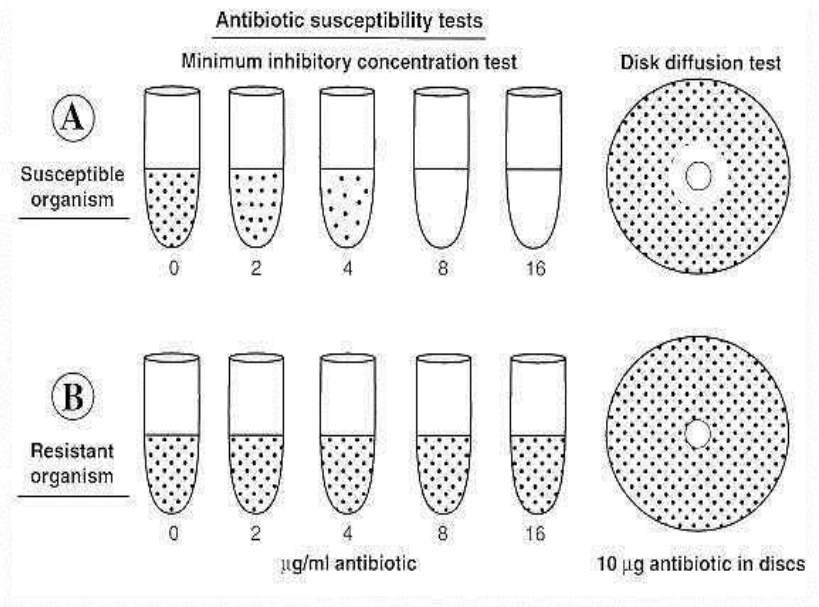
Antimicrobial Susceptibility

The responsibility of the microbiology laboratory includes not only microbial detection and isolation but also the determination of microbial susceptibility to antimicrobial agents. Many bacteria, in particular, have unpredictable susceptibilities to antimicrobial agents, and their susceptibilities can be measured in vitro to help guide the selection of the most appropriate antimicrobial agent.

Antimicrobial susceptibility tests are performed by either disk diffusion or a dilution method. In the former, a standardized suspension of a particular microorganism is inoculated onto an agar surface to which paper disks containing various antimicrobial agents are applied. Following overnight incubation, any zone diameters of inhibition about the disks are measured and the results are reported as indicating susceptibility or resistance of the microorganism to each antimicrobial agent tested. An alternative method is to dilute on a log2 scale each antimicrobial agent in broth to provide a range of concentrations and to inoculate each tube or, if a microplate is used, each well containing the antimicrobial agent in broth with a standardized suspension of the microorganism to be tested. **The lowest concentration of antimicrobial agent that inhibits the growth of the microorganism is the minimal inhibitory concentration** (**MIC**). The MIC and the zone diameter of inhibition are inversely correlated. In other words, the more susceptible the microorganism is to the antimicrobial agent, the lower the MIC and the larger the zone of inhibition. Conversely, the more resistant the microorganism, the higher the MIC and the smaller the zone of inhibition.

The term **susceptible** means that the microorganism is inhibited by a concentration of antimicrobial agent that can be attained in blood with the normally recommended

dose of the antimicrobial agent and implies that an infection caused by this microorganism may be appropriately treated with the antimicrobial agent. The term **resistant** indicates that the microorganism is resistant to concentrations of the antimicrobial agent that can be attained with normal doses and implies that an infection caused by this microorganism could not be successfully treated with this antimicrobial agent.



Two methods for performing antibiotic susceptibility tests.

(A) Disk diffusion method. (B) Minimum inhibitory concentration (MIC) method. In the example shown, two different microorganisms are tested by both methods against the same antibiotic. The MIC of the antibiotic for the susceptible microorganism is 8 μg/ml. The corresponding disk diffusion test shows a zone of inhibition surrounding the disk. In the second sample, a resistant microorganism is not inhibited by the highest antibiotic concentration tested (MIC > 16 μg/ml) and there is no zone of inhibition surrounding the disk. The diameter of the zone of inhibition is inversely related to the MIC.

Introduction to Infectious Diseases

The record of human suffering and death caused by smallpox, cholera, typhus, dysentery, malaria, etc. establishes the eminence of the infectious diseases. Despite the outstanding successes in control afforded by improved sanitation, immunization, and antimicrobial therapy, the infectious diseases continue to be a common and significant problem of modern medicine. The most common disease of mankind, the common cold, is an infectious disease, as is the feared modern disease AIDS. Some chronic neurological diseases that were thought formerly to be degenerative diseases have proven to be infectious. There is little doubt that the future will continue to reveal the infectious diseases as major medical problems.

In the study and care of patients with infectious disease, physicians use some terms that are not easy to define precisely. A definition of infection as growth of a microorganism in an animal with any resulting host response will include essentially all of the infectious diseases of humans. Many of the body surfaces of humans that communicate with the external environment (e.g., the skin and the gastrointestinal and respiratory tracts) support a normal flora, but these microorganisms usually do not invade and cause disease. Under the right circumstances, however, elements of the flora can invade and produce an infection.

A number of other terms are commonly used in describing the infectious diseases. Pathology refers to the abnormality induced by an infection, and pathogenesis to the events producing the pathology. A pathogenic microorganism is a microbe that can cause pathology. Disease refers to the existence of pathology and an infectious disease is a disease caused by a microorganism. Virulence is a term referring to the power of a microbe to produce disease in a particular host. For example, a microorganism may be avirulent for a normal host and highly virulent for an immunosuppressed host. Immunity refers to the degree of resistance of the host for a particular microbe. Finally, it must be appreciated that the occurrence of an

infectious disease in a human is a dynamic process that represents a host-parasite interaction. The parasite attempts to multiply and the host defenses seek to control this effort. The task of the physician is to recognize that such a process accounts for the patient's problem and to intervene for the benefit of the patient.

The infectious diseases are usually characterized by the dominant organ system involved. This classification is useful as a guide in approaching patients. For example, patients do not present complaining of pneumococcal pneumonia; patients present complaining of fever, cough, and chest pain. The physician localizes the disease to the chest (respiratory infection) and then proceeds to develop data proving the presence of a pneumonia caused by the pneumococcus. Thus, we classify infections as respiratory infections, gastrointestinal infections, genitourinary infections, nervous system infections, skin and soft tissue infections, bone and joint infections, cardiovascular infections, and generalized (disseminated) infections. The chapters in this section are organized according to this scheme. The section is intended primarily to help the student begin to integrate the knowledge of microbiology and immunology into a framework useful for the practice of medicine. The diagnosis, prevention, and treatment of the infectious diseases is a stimulating and gratifying process.

General Concepts

1. Upper Respiratory Infections: Common Cold, Sinusitis, Pharyngitis, Epiglottitis and Laryngotracheitis

Etiology: Most upper respiratory infections are of viral etiology. Epiglottitis and laryngotracheitis are exceptions with severe cases likely caused by *Haemophilus influenzae* type b. Bacterial pharyngitis is often caused by *Streptococcus pyogenes*.

Pathogenesis: Organisms gain entry to the respiratory tract by inhalation of droplets and invade the mucosa. Epithelial destruction may ensue, along with redness, edema, hemorrhage and sometimes an exudate.

Clinical Manifestations: Initial symptoms of a cold are runny, stuffy nose and sneezing, usually without fever. Other upper respiratory infections may have fever. Children with epiglottitis may have difficulty in breathing, muffled speech, drooling and stridor. Children with serious laryngotracheitis (croup) may also have tachypnea, stridor and cyanosis.

Microbiologic Diagnosis: Common colds can usually be recognized clinically. Bacterial and viral cultures of throat swab specimens are used for pharyngitis, epiglottitis and laryngotracheitis. Blood cultures are also obtained in cases of epiglottitis.

Prevention and Treatment: Viral infections are treated symptomatically. Streptococcal pharyngitis and epiglottitis caused by *H influenzae* are treated with antibacterials. *Haemophilus influenzae* type b vaccine is commercially available and is now a basic component of childhood immunization program.

2. Lower Respiratory Infections: Bronchitis, Bronchiolitis and Pneumonia

Etiology: Causative agents of lower respiratory infections are viral or bacterial. Viruses cause most cases of bronchitis and bronchiolitis. In community-acquired pneumonias, the most common bacterial agent is *Streptococcus pneumoniae*. Atypical pneumonias are cause by such agents as *Mycoplasma pneumoniae, Chlamydia spp, Legionella, Coxiella burnetti* and viruses. Nosocomial pneumonias and pneumonias in immunosuppressed patients have protean etiology with gram-negative organisms and staphylococci as predominant organisms.

Pathogenesis: Organisms enter the distal airway by inhalation, aspiration or by hematogenous seeding. The pathogen multiplies in or on the epithelium, causing inflammation, increased mucus secretion, and impaired mucociliary function; other lung functions may also be affected. In severe bronchiolitis, inflammation and necrosis of the epithelium may block small airways leading to airway obstruction.

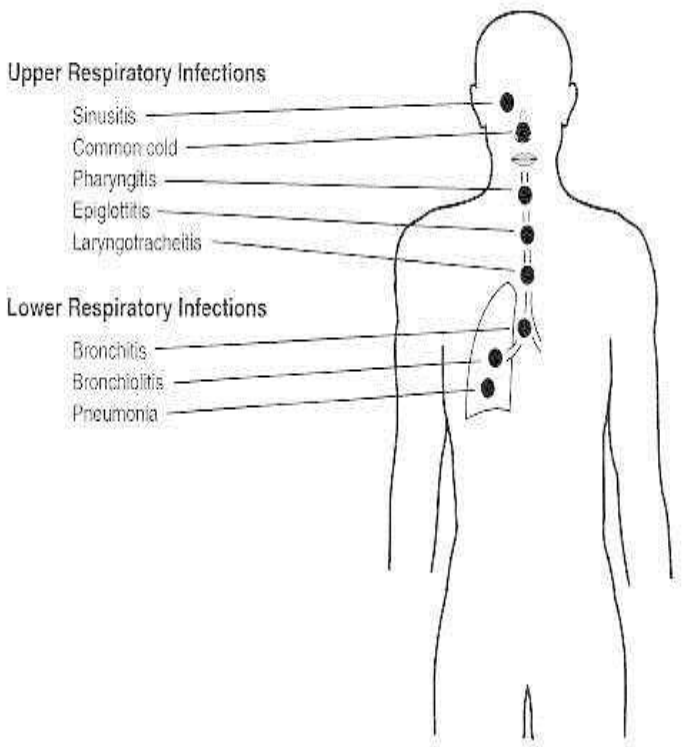
Clinical Manifestations: Symptoms include cough, fever, chest pain, tachypnea and sputum production. Patients with pneumonia may also exhibit non-respiratory symptoms such as confusion, headache, myalgia, abdominal pain, nausea, vomiting and diarrhea.

Microbiologic Diagnosis: Sputum specimens are cultured for bacteria, fungi and viruses. Culture of nasal washings is usually sufficient in infants with bronchiolitis. Fluorescent staining technic can be used for legionellosis. Blood cultures and/or serologic methods are used for viruses, rickettsiae, fungi and many bacteria. Enzyme-linked immunoassay methods can be used for detections of microbial antigens as well as antibodies. Detection of nucleotide fragments specific for the microbial antigen in question by DNA probe or polymerase chain reaction can offer a rapid diagnosis.

Prevention and Treatment: Symptomatic treatment is used for most viral infections. Bacterial pneumonias are treated with antibacterials. A polysaccharide vaccine against 23 serotypes of *Streptococcus pneumoniae* is recommended for individuals at high risk.

Upper Respiratory Infections. Infections of the respiratory tract are grouped according to their symptomatology and anatomic involvement. Acute upper respiratory infections (URI) include the common cold, pharyngitis, epiglottitis, and laryngotracheitis. These infections are usually benign, transitory and self-limited,

altho ugh epiglottitis and laryngotracheitis can be serious diseases in children and young infants. Etiologic agents associated with URI include viruses, bacteria, mycoplasma and fungi. Respiratory infections are more common in the fall and winter when school starts and indoor crowding facilitates transmission.



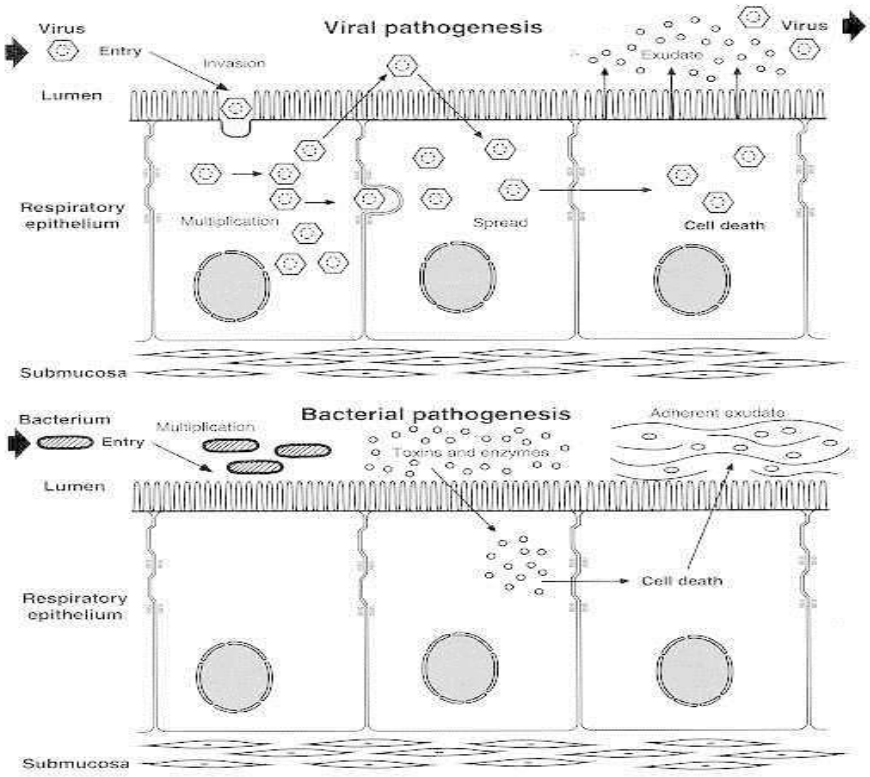
Upper and lower respiratory tract infections.

Common Cold

Etiology. Common colds are the most prevalent entity of all respiratory infections and are the leading cause of patient visits to the physician, as well as work and school absenteeism. Most colds are caused by viruses. Rhinoviruses with more than 100 serotypes are the most common pathogens, causing at least 25% of colds in adults. Coronaviruses may be responsible for more than 10% of cases. Parainfluenza

viruses, respiratory syncytial virus, adenoviruses and influenza viruses have all been linked to the common cold syndrome. All of these organisms show seasonal variations in incidence. The cause of 30% to 40% of cold syndromes has not been determined.

Pathogenesis. The viruses appear to act through direct invasion of epithelial cells of the respiratory mucosa, but whether there is actual destruction and sloughing of these cells or loss of ciliary activity depends on the specific organism involved. There is an increase in both leukocyte infiltration and nasal secretions, including large amounts of protein and immunoglobulin, suggesting that cytokines and immune mechanisms may be responsible for some of the manifestations of the common cold.



Pathogenesis of viral and bacterial mucosal respiratory infections.

Clinical Manifestations. After an incubation period of 48–72 hours, classic symptoms of nasal discharge and obstruction, sneezing, sore throat and cough occur in both adults and children. Myalgia and headache may also be present. Fever is rare. The duration of symptoms and of viral shedding varies with the pathogen and the age of the patient. Complications are usually rare, but sinusitis and otitis media may follow.

Microbiologic Diagnosis. The diagnosis of a common cold is usually based on the symptoms (lack of fever combined with symptoms of localization to the nasopharynx). Unlike allergic rhinitis, eosinophils are absent in nasal secretions. Although it is possible to isolate the viruses for definitive diagnosis, that is rarely warranted.

Prevention and Treatment. Treatment of the uncomplicated common cold is generally symptomatic. Decongestants, antipyretics, fluids and bed rest usually suffice. Restriction of activities to avoid infecting others, along with good hand washing, are the best measures to prevent spread of the disease. No vaccine is commercially available for cold prophylaxis.

Sinusitis. Sinusitis is an acute inflammatory condition of one or more of the paranasal sinuses. Infection plays an important role in this affliction. Sinusitis often results from infections of other sites of the respiratory tract since the paranasal sinuses are contiguous to, and communicate with, the upper respiratory tract.

Etiology. Acute sinusitis most often follows a common cold which is usually of viral etiology. Vasomotor and allergic rhinitis may also be antecedent to the development of sinusitis. Obstruction of the sinusal ostia due to deviation of the nasal septum, presence of foreign bodies, polyps or tumors can predispose to sinusitis. Infection of the maxillary sinuses may follow dental extractions or an

extension of infection from the roots of the upper teeth. The most common bacterial agents responsible for acute sinusitis are *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis*. Other organisms including *Staphylococcus aureus, Streptococcus pyogenes*, gram-negative organisms and anaerobes have also been recovered. Chronic sinusitis is commonly a mixed infection of aerobic and anaerobic organisms.

Pathogenesis. Infections caused by viruses or bacteria impair the ciliary activity of the epithelial lining of the sinuses and increased mucous secretions. This leads to obstruction of the paranasal sinusal ostia which impedes drainage. With bacterial multiplication in the sinus cavities, the mucus is converted to mucopurulent exudates. The pus further irritates the mucosal lining causing more edema, epithelial destruction and ostial obstruction. When acute sinusitis is not resolved and becomes chronic, mucosal thickening results and the development of mucoceles and polyps may ensue.

Clinical Manifestations. The maxillary and ethmoid sinuses are most commonly involved in sinusitis. The frontal sinuses are less often involved and the sphenoid sinuses are rarely affected. Pain, sensation of pressure and tenderness over the affected sinus are present. Malaise and low grade fever may also occur. Physical examination usually is not remarkable with no more than an edematous and hyperemic nasal mucosa.

In uncomplicated chronic sinusitis, a purulent nasal discharge is the most constant finding. There may not be pain nor tenderness over the sinus areas. Thickening of the sinus mucosa and a fluid level are usually seen in x-ray films or magnetic resonance imaging.

Microbiologic Diagnosis. For acute sinusitis, the diagnosis is made from clinical findings. A bacterial culture of the nasal discharge can be taken but is not very helpful as the recovered organisms are generally contaminated by the resident flora from the nasal passage. In chronic sinusitis, a careful dental examination, with sinus x-rays may be required. An antral puncture to obtain sinusal specimens for bacterial culture is needed to establish a specific microbiologic diagnosis.

Prevention and Treatment. Symptomatic treatment with analgesics and moist heat over the affected sinus pain and a decongestant to promote sinus drainage may suffice. For antimicrobial therapy, a beta-lactamase resistant antibiotic such as amoxicillin-clavulanate or a cephalosporin may be used. For chronic sinusitis, when conservative treatment does not lead to a cure, irrigation of the affected sinus may be necessary. Culture from an antral puncture of the maxillary sinus can be performed to identify the causative organism for selecting antimicrobial therapy. Specific preventive procedures are not available. Proper care of infectious and/or allergic rhinitis, surgical correction to relieve or avoid obstruction of the sinusal ostia are important. Root abscesses of the upper teeth should receive proper dental care to avoid secondary infection of the maxillary sinuses.

Otitis. Infections of the ears are common events encountered in medical practice, particularly in young children. Otitis externa is an infection involving the external auditory canal while otitis media denotes inflammation of the middle ear.

Etiology. For otitis externa, the skin flora such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, diphtheroids and occasionally an anaerobic organism, *Propionibacterium acnes* are major etiologic agents. In a moist and warm environment, a diffuse acute otitis externa (Swimmer's ear) may be caused by *Pseudomonas aeruginosa*, along with other skin flora. Malignant otitis externa is a severe necrotizing infection usually caused by *Pseudomonas aeruginosa*.

For otitis media, the commonest causative bacteria are *Streptococcus pneumoniae*, *Hemophilus influenzae* and beta-lactamase producing *Moraxella catarrhalis*. Respiratory viruses may play a role in otitis media but this remains uncertain. *Mycoplasma pneumoniae* has been reported to cause hemorrhagic bullous myringitis in an experimental study among nonimmune human volunteers inoculated with *M pneumoniae*. However, in natural cases of *M pneumoniae* infection, clinical bullous myringitis or otitis media is uncommon.

Pathogenesis. The narrow and tortuous auditory canal is lined by a protective surface epithelium. Factors that may disrupt the natural protective mechanisms, such as high temperature and humidity, trauma, allergy, tissue maceration, removal of cerumen and an alkaline pH environment, favor the development of otitis externa. Prolonged immersion in a swimming pool coupled with frequent ear cleansing increases the risk of otitis externa. Acute otitis media commonly follows an upper respiratory infection extending from the nasopharynx via the eustachian tube to the middle ear. Vigorous nose blowing during a common cold, sudden changes of air pressure, and perforation of the tympanic membrane also favor the development of otitis media. The presence of purulent exudate in the middle ear may lead to a spread of infection to the inner ear and mastoids or even meninges

Clinical Manifestations

Otitis externa. Furuncles of the external ear, similar to those in skin infection, can cause severe pain and a sense of fullness in the ear canal. When the furuncle drains, purulent otorrhea may be present. In generalized otitis externa, itching, pain and tenderness of the ear lobe on traction are present. Loss of hearing may be due to obstruction of the ear canal by swelling and the presence of purulent debris.

Malignant otitis externa tends to occur in elderly diabetic patients. It is characterized by severe persistent earache, foul smelling purulent discharge and the presence of granulation tissue in the auditory canal. The infection may spread and lead to osteomyelitis of the temporal bone or externally to involve the pinna with osteochondritis.

Otitis media. Acute otitis media occurs most commonly in young children. The initial complaint usually is persistent severe earache (crying in the infant) accompanied by fever, and, and vomiting. Otologic examination reveals a bulging, erythematous tympanic membrane with loss of light reflex and landmarks. If perforation of the tympanic membrane occurs, serosanguinous or purulent discharge may be present. In the event of an obstruction of the eustachian tube, accumulation of a usually sterile effusion in the middle ear results in serous otitis media. Chronic otitis media frequently presents a permanent perforation of the tympanic membrane. A central perforation of the pars tensa is more benign. On the other hand, an attic perforation of the pars placcida and marginal perforation of the pars tensa are more dangerous and often associated with a cholesteatoma.

Diagnosis. The diagnosis of both otitis externa and otitis media can be made from history, clinical symptomatology and physical examinations. Inspection of the tympanic membrane is an indispensable skill for physicians and health care workers. All discharge, ear wax and debris must be removed and to perform an adequate otoscopy. In the majority of patients, routine cultures are not necessary, as a number of good bacteriologic studies have shown consistently the same microbial pathogens mentioned in the section of etiology. If the patient is immunocompromised or is toxic and not responding to initial antimicrobial therapy tympanocentesis (needle aspiration) to obtain middle ear effusion for microbiologic culture is indicated.

Prevention and Treatment

Otitis externa. Topical therapy is usually sufficient and systemic antimicrobials are seldom needed unless there are signs of spreading cellulitis and the patient appears toxic. A combination of topical antibiotics such as neomycin sulfate, polymyxin B sulfate and corticosteroids used as eardrops, is a preferred therapy. In some cases, acidification of the ear canal by applying a 2% solution of acetic acid topically may also be effective. If a furuncle is present in the external canal, the physician should allow it to drain spontaneously.

Otitis media. Amoxicillin is an effective and preferred antibiotic for treatment of acute otitis media. Since beta-lactamase producing *H influenzae* and *M catarrhalis* can be a problem in some communities, amoxicillin-clavulanate is used by many physicians. Oral preparations of trimethoprim/sulfamethoxazole, second and third generation cephalosporins, tetracyclines and macrolides can also be used. When there is a large effusion, tympanocentesis may hasten the resolution process by decreasing the sterile effusion. Use of polyvalent pneumococcal vaccines has been evaluated for the prevention of otitis media in children.

Pharyngitis

Etiology. Pharyngitis is an inflammation of the pharynx involving lymphoid tissues of the posterior pharynx and lateral pharyngeal bands. The etiology can be bacterial, viral and fungal infections as well as noninfectious etiologies such as smoking. Most cases are due to viral infections and accompany a common cold or influenza. Type A coxsackieviruses can cause a severe ulcerative pharyngitis in children (herpangina), and adenovirus and herpes simplex virus, although less common, also can cause severe pharyngitis. Pharyngitis is a common symptom of Epstein-Barr virus and cytomegalovirus infections. Group A beta-hemolytic streptococcus or *Streptococcus pyogenes* is the most important bacterial agent associated with acute pharyngitis and tonsillitis. *Corynebacterium diphtheriae* causes occasional cases of

acute pharyngitis, as do mixed anaerobic infections (Vincent's angina), *Corynebacterium haemolyticum*, *Neisseria gonorrhoeae*, and *Chlamydia trachomatis*. Outbreaks of *Chlamydia pneumoniae* (TWAR agent) causing pharyngitis or pneumonitis have occurred in military recruits. *Mycoplasma pneumoniae* and *Mycoplasma hominis* have been associated with acute pharyngitis. *Candida albicans*, which causes oral candidiasis or thrush, can involve the pharynx, leading to inflammation and pain.

Pathogenesis. As with common cold, viral pathogens in pharyngitis appear to invade the mucosal cells of the nasopharynx and oral cavity, resulting in edema and hyperemia of the mucous membranes and tonsils. Bacteria attach to and, in the case of group A beta-hemolytic streptococci, invade the mucosa of the upper respiratory tract. Many clinical manifestations of infection appear to be due to the immune reaction to products of the bacterial cell. In diphtheria, a potent bacterial exotoxin causes local inflammation and cell necrosis.

Clinical Manifestations. Pharyngitis usually presents with a red, sore, or "scratchy" throat. An inflammatory exudate or membranes may cover the tonsils and tonsillar pillars. Vesicles or ulcers may also be seen on the pharyngeal walls. Depending on the pathogen, fever and systemic manifestations such as malaise, myalgia, or headache may be present. Anterior cervical lymphadenopathy is common in bacterial pharyngitis and difficulty in swallowing may be present.

Microbiologic Diagnosis. The goal in the diagnosis of pharyngitis is to identify cases that are due to group A beta-hemolytic streptococci, as well as the more unusual and potentially serious infections. The various forms of pharyngitis cannot be distinguished on clinical grounds. Routine throat cultures for bacteria are inoculated onto sheep blood and chocolate agar plates. Thayer-Martin medium is used if *N gonorrhoeae* is suspected. Viral cultures are not routinely obtained for

most cases of pharyngitis. Serologic studies may be used to confirm the diagnosis of pharyngitis due to viral, mycoplasmal or chlamydial pathogens. Rapid diagnostic tests with fluorescent antibody or latex agglutination to identify group A streptococci from pharyngeal swabs are available. Gene probe and polymerase chain reaction can be used to detect unusual organisms such as *M pneumoniae*, chlamydia or viruses but these procedures are not routine diagnostic methods.

Prevention and Treatment. Symptomatic treatment is recommended for viral pharyngitis. The exception is herpes simplex virus infection, which can be treated with acyclovir if clinically warranted or if diagnosed in immunocompromised patients. The specific antibacterial agents will depend on the causative organism, but penicillin G is the therapy of choice for streptococcal pharyngitis. Mycoplasma and chlamydial infections respond to erythromycin, tetracyclines and the new macrolides.

Epiglottitis and Laryngotracheitis

Etiology. Inflammation of the upper airway is classified as epiglottitis or laryngotracheitis (croup) on the basis of the location, clinical manifestations, and pathogens of the infection. *Haemophilus influenzae* type b is the most common cause of epiglottitis, particularly in children age 2 to 5 years. Epiglottitis is less common in adults. Some cases of epiglottitis in adults may be of viral origin. Most cases of laryngotracheitis are due to viruses. More serious bacterial infections have been associated with *H influenzae* type b, group A beta-hemolytic streptococcus and *C diphtheriae*. Parainfluenza viruses are most common but respiratory syncytial virus, adenoviruses, influenza viruses, enteroviruses and *Mycoplasma pneumoniae* have been implicated.

Pathogenesis. A viral upper respiratory infection may precede infection with *H influenzae* in episodes of epiglottitis. However, once *H influenzae* type b infection starts, rapidly progressive erythema and swelling of the epiglottis ensue, and bacteremia is usually present. Viral infection of laryngotracheitis commonly begins in the nasopharynx and eventually moves into the larynx and trachea. Inflammation and edema involve the epithelium, mucosa and submucosa of the subglottis which can lead to airway obstruction.

Clinical Manifestations. The syndrome of epiglottitis begins with the acute onset of fever, sore throat, hoarseness, drooling, dysphagia and progresses within a few hours to severe respiratory distress and prostration. The clinical course can be fulminant and fatal. The pharynx may be inflamed, but the diagnostic finding is a "cherry-red" epiglottis. A history of preceding cold-like symptoms is typical of laryngotracheitis, with rhinorrhea, fever, sore throat and a mild cough. Tachypnea, a deep barking cough and inspiratory stridor eventually develop. Children with bacterial tracheitis appear more ill than adults and are at greater risk of developing airway obstruction.

Haemophilus influenzae type b is isolated from the blood or epiglottis in the majority of patients with epiglottis; therefore a blood culture should always be performed. Sputum cultures or cultures from pharyngeal swabs may be used to isolate pathogens in patients with laryngotracheitis. Serologic studies to detect a rise in antibody titers to various viruses are helpful for retrospective diagnosis. Newer, rapid diagnostic techniques, using immunofluorescent-antibody staining to detect virus in sputum, pharyngeal swabs, or nasal washings, have been successfully used. Enzyme-linked immunosorbent assay (ELISA), DNA probe and polymerase chain reaction procedures for detection of viral antibody or antigens are now available for rapid diagnosis.

Prevention and Treatment. Epiglottitis is a medical emergency, especially in children. All children with this diagnosis should be observed carefully and be intubated to maintain an open airway as soon as the first sign of respiratory distress is detected. Antibacterial therapy should be directed at *H influenzae*. Patients with croup are usually successfully managed with close observation and supportive care, such as fluid, humidified air, and racemic epinephrine. For prevention, *Haemophilus influenzae* type b conjugated vaccine is recommended for all pediatric patients, as is immunization against diphtheria.

Lower Respiratory Infections

Infections of the lower respiratory tract include bronchitis, bronchiolitis and pneumonia. These syndromes, especially pneumonia, can be severe or fatal. Although viruses, mycoplasma, rickettsiae and fungi can all cause lower respiratory tract infections, bacteria are the dominant pathogens; accounting for a much higher percentage of lower than of upper respiratory tract infections.

Bronchitis and Bronchiolitis

Etiology. Bronchitis and bronchiolitis involve inflammation of the bronchial tree. Bronchitis is usually preceded by an upper respiratory tract infection or forms part of a clinical syndrome in diseases such as influenza, rubeola, rubella, pertussis, scarlet fever and typhoid fever. Chronic bronchitis with a persistent cough and sputum production appears to be caused by a combination of environmental factors, such as smoking, and bacterial infection with pathogens such as *H influenzae* and *S pneumoniae*. Bronchiolitis is a viral respiratory disease of infants and is caused primarily by respiratory syncytial virus. Other viruses, including parainfluenza viruses, influenza viruses and adenoviruses (as well as occasionally *M pneumoniae*) are also known to cause bronchiolitis.

Pathogenesis. When the bronchial tree is infected, the mucosa becomes hyperemic and edematous and produces copious bronchial secretions. The damage to the mucosa can range from simple loss of mucociliary function to actual destruction of the respiratory epithelium, depending on the organisms(s) involved. Patients with chronic bronchitis have an increase in the number of mucus-producing cells in their airways, as well as inflammation and loss of bronchial epithelium, Infants with bronchiolitis initially have inflammation and sometimes necrosis of the respiratory epithelium, with eventual sloughing. Bronchial and bronchiolar walls are thickened. Exudate made up of necrotic material and respiratory secretions and the narrowing of the bronchial lumen lead to airway obstruction. Areas of air trapping and atelectasis develop and may eventually contribute to respiratory failure.

Clinical Manifestations. Symptoms of an upper respiratory tract infection with a cough is the typical initial presentation in acute bronchitis. Mucopurulent sputum may be present, and moderate temperature elevations occur. Typical findings in chronic bronchitis are an incessant cough and production of large amounts of sputum, particularly in the morning. Development of respiratory infections can lead to acute exacerbations of symptoms with possibly severe respiratory distress.

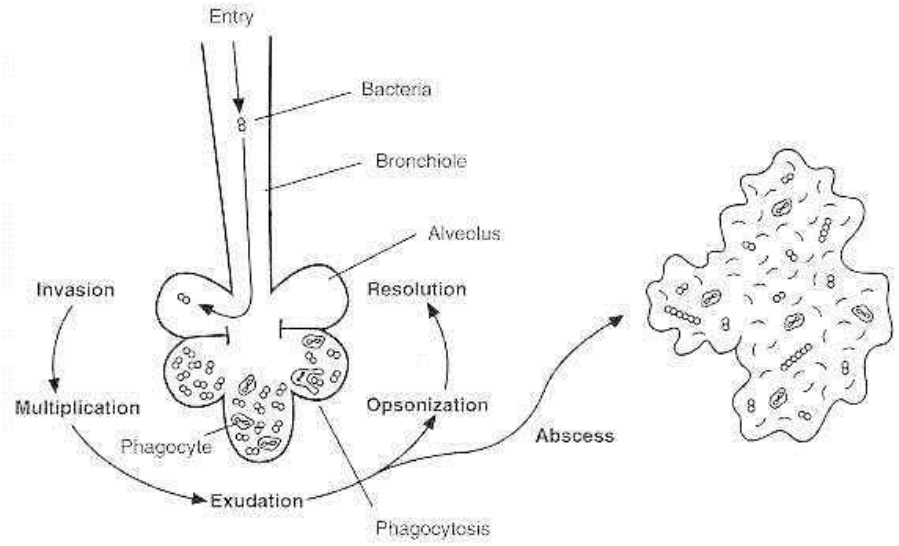
Coryza and cough usually precede the onset of bronchiolitis. Fever is common. A deepening cough, increased respiratory rate, and restlessness follow. Retractions of the chest wall, nasal flaring, and grunting are prominent findings. Wheezing or an actual lack of breath sounds may be noted. Respiratory failure and death may result.

Microbiologic Diagnosis. Bacteriologic examination and culture of purulent respiratory secretions should always be performed for cases of acute bronchitis not associated with a common cold. Patients with chronic bronchitis should have their sputum cultured for bacteria initially and during exacerbations. Aspirations of nasopharyngeal secretions or swabs are sufficient to obtain specimens for viral

culture in infants with bronchiolitis. Serologic tests demonstrating a rise in antibody titer to specific viruses can also be performed. Rapid diagnostic tests for antibody or viral antigens may be performed on nasopharyngeal secretions by using fluorescent-antibody staining, ELISA or DNA probe procedures.

Prevention and Treatment. With only a few exceptions, viral infections are treated with supportive measures. Respiratory syncytial virus infections in infants may be treated with ribavirin. Amantadine and rimantadine are available for chemoprophylaxis or treatment of influenza type A viruses. Selected groups of patients with chronic bronchitis may receive benefit from use of corticosteroids, bronchodilators, or prophylactic antibiotics.

Pneumonia. Pneumonia is an inflammation of the lung parenchyma. Consolidation of the lung tissue may be identified by physical examination and chest x-ray. From an anatomical point of view, lobar pneumonia denotes an alveolar process involving an entire lobe of the lung while bronchopneumonia describes an alveolar process occurring in a distribution that is patchy without filling an entire lobe. Numerous factors, including environmental contaminants and autoimmune diseases, as well as infection, may cause pneumonia. The various infectious agents that cause pneumonia are categorized in many ways for purposes of laboratory testing, epidemiologic study and choice of therapy. Pneumonias occurring in usually healthy persons not confined to an institution are classified as community-acquired pneumonias. Infections arise while a patient is hospitalized or living in an institution such as a nursing home are called hospital-acquired or nosocomial pneumonias. Etiologic pathogens associated with community-acquired and hospital-acquired pneumonias are somewhat different. However, many organisms can cause both types of infections.



Pathogenesis of bacterial pneumonias.

Etiology

Bacterial pneumonias. *Streptococcus pneumoniae* is the most common agent of community-acquired acute bacterial pneumonia. More than 80 serotypes, as determined by capsular polysaccharides, are known, but 23 serotypes account for over 90% of all pneumococcal pneumonias in the United States. Pneumonias caused by other streptococci are uncommon. *Streptococcus pyogenes* pneumonia is often associated with a hemorrhagic pneumonitis and empyema. Community-acquired pneumonias caused by *Staphylococcus aureus* are also uncommon and usually occur after influenza or from staphylococcal bacteremia. Infections due to *Haemophilus influenzae* (usually nontypable) and *Klebsiella pneumoniae* are more common among patients over 50 years old who have chronic obstructive lung disease or alcoholism. The most common agents of nosocomial pneumonias are aerobic gram-negative bacilli that rarely cause pneumonia in healthy individuals. *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter*, *Proteus*, and *Klebsiella* species are

often identified. Less common agents causing pneumonias include *Francisella tularensis*, the agent of tularemia; *Yersinia pestis*, the agent of plague; and *Neisseria meningitidis*, which usually causes meningitis but can be associated with pneumonia, especially among military recruits. *Xanthomonas pseudomallei* causes melioidosis, a chronic pneumonia in Southeast Asia.

Mycobacterium tuberculosis can cause pneumonia. Although the incidence of tuberculosis is low in industrialized countries, *M tuberculosis* infections still continue to be a significant public health problem in the United States, particularly among immigrants from developing countries, intravenous drug abusers, patients infected with human immunodeficiency virus (HIV), and the institutionalized elderly. Atypical *Mycobacterium* species can cause lung disease indistinguishable from tuberculosis.

Aspiration pneumonias. Aspiration pneumonia from anaerobic organisms usually occurs in patients with periodontal disease or depressed consciousness. The bacteria involved are usually part the oral flora and cultures generally show a mixed bacterial growth. *Actinomyces, Bacteroides, Peptostreptococcus, Veilonella, Propionibacterium, Eubacterium,* and *Fusobacterium* spp are often isolated.

Atypical pneumonias. Atypical pneumonias are those that are not typical bacterial lobar pneumonias. *Mycoplasma pneumoniae* produces pneumonia most commonly in young people between 5 and 19 years of age. Outbreaks have been reported among military recruits and college students.

Pathogenesis and Clinical Manifestations. Infectious agents gain access to the lower respiratory tract by the inhalation of aerosolized material, by aspiration of upper airway flora, or by hematogenous seeding. Pneumonia occurs when lung defense mechanisms are diminished or overwhelmed. The major symptoms or

pneumonia are cough, chest pain, fever, shortness of breath and sputum production. Patients are tachycardic. Headache, confusion, abdominal pain, nausea, vomiting and diarrhea may be present, depending on the age of the patient and the organisms involved.

Microbiologic Diagnosis. Etiologic diagnosis of pneumonia on clinical grounds alone is almost impossible. Sputum should be examined for a predominant organism in any patient suspected to have a bacterial pneumonia; blood and pleural fluid (if present) should be cultured. A sputum specimen with fewer than 10 while cells per high-power field under a microscope is considered to be contaminated with oral secretions and is unsatisfactory for diagnosis. Acid-fast stains and cultures are used to identify *Mycobacterium* and *Nocardia* spp. Most fungal pneumonias are diagnosed on the basis of culture of sputum or lung tissue. Viral infection may be diagnosed by demonstration of antigen in secretions or cultures or by an antibody response. Serologic studies can be used to identify viruses, *M pneumoniae, C. burnetii, Chlamydia species, Legionella, Francisella,* and *Yersinia*. A rise in serum cold agglutinins may be associated with *M pneumoniae* infection, but the test is positive in only about 60% of patients with this pathogen.

Rapid diagnostic tests, as described in previous sections, are available to identify respiratory viruses: the fluorescent-antibody test is used for *Legionella*. A sputum quellung test can specify *S pneumoniae* by serotype. Enzyme-linked immunoassay, DNA probe and polymerase chain reaction methods are available for many agents causing respiratory infections.

Some organisms that may colonize the respiratory tract are considered to be pathogens only when they are shown to be invading the parenchyma. Diagnosis of pneumonia due to cytomegalovirus, herpes simplex virus, *Aspergillus* spp. or *Candida* spp require specimens obtained by transbronchial or open-lung biopsy.

Pneumocystis carinii can be found by silver stain of expectorated sputum. However, if the sputum is negative, deeper specimens from the lower respiratory tract obtained by bronchoscopy or by lung biopsy are needed for confirmatory diagnosis.

Prevention and Treatment. Until the organism causing the infection is identified, decisions on therapy are based upon clinical history, including history of exposure, age, underlying disease and previous therapies, past pneumonias, geographic location, severity of illness, clinical symptoms, and sputum examination. Once a diagnosis is made, therapy is directed at the specific organism responsible.

The pneumococcal vaccine should be given to patients at high risk for developing pneumococcal infections, including asplenic patients, the elderly and any patients immunocompromised through disease or medical therapy. Yearly influenza vaccinations should also be provided for these particular groups. An enteric-coated vaccine prepared from certain serotypes of adenoviruses is available, but is only used in military recruits. In AIDS patients, trimethoprim/sulfamethoxazole, aerosolized pentamidine or other antimicrobials can be given for prophylaxis of *Pneumocystis carinii* infections.

Microbiology of the Circulatory System

General Concepts

Microbemia

Etiology. Gram-negative enteric bacilli, *Staphylococcus aureus*, and *Streptococcus pneumoniae* are the most common pathogens in the United States. Of these, the most likely agent of a given case of microbemia depends on host characteristics (age, granulocyte count, associated conditions, prior antimicrobial therapy) and

epidemiologic setting (community vs. hospital-acquired, travel, animal exposure, etc.).

Pathogenesis. Microbes generally enter the circulatory system via the lymphatics from areas of localized infection or from diseased skin and mucous membranes colonized by members of the normal bacterial flora.

Clinical Manifestations. Microbemias may be asymptomatic, symptomatic, transient, continuous, or intermittent. Microbemias due to small numbers of relatively nonpathogenic microorganisms are usually asymptomatic. Larger inocula or more pathogenic organisms may produce systemic signs and symptoms: fever, chills, rigors, sweating, malaise, sleepiness, and fatigue.

Microbiologic Diagnosis. Techniques used in diagnosis include cultures of localized sites of infection, multiple blood cultures, and (rarely) blood serology.

Prevention and Treatment. Prevention in hospitals consists of hand-washing by personnel in contact with patients and avoidance of unnecessary urinary and intravenous catheterization. After samples are taken for culturing, treatment with intravenous broad-spectrum antimicrobial agents is usually begun, based on an estimate of the most likely organisms and their usual antimicrobial susceptibility patterns. This empirical therapy is modified if necessary when the pathogen and its susceptibility pattern are identified.

Septic Shock

Etiology. Gram-negative enteric bacilli are the most common causes of septic shock, but the syndrome may be produced by a wide range of microorganisms.

Pathogenesis. Vascular injury from the microbes and release of inflammatory mediators cause local circulatory failure and multiorgan failure.

Clinical Manifestations. Manifestations of septic shock are widespread; they include hypotension, hypoxia, respiratory failure, lactic acidosis, renal failure, disseminated intravascular coagulation, and bleeding.

Microbiologic Diagnosis. Diagnosis is made by culturing local infections thought to be the source of microbemia and by culturing the blood.

Prevention and Treatment. Preventive measures are the same as for microbemia. Treatment consists of high-dose intravenous broad-spectrum antimicrobial agents, intravenous fluids, supplemental oxygen therapy, mechanical ventilation, hemodialysis, and transfusions of blood products and clotting factors, as needed.

Infective Endocarditis

Etiology. *Staphylococcus aureus,* viridans streptococci, and enterococci are the most common causes of endocarditis.

Pathogenesis. Microbes that enter the blood lodge on heart valves. Previously damaged heart valves are more susceptible. Bacterial colonies become covered with fibrin and platelets, which protect the organisms from phagocytes and complement. Clots may dislodge as infected emboli.

Clinical Manifestations. Infective endocarditis may affect native or abnormal cardiac valves, prosthetic valves, and, secondarily, other intravascular sites. Manifestations include fever, malaise, fatigue, weight loss, skin petechiae, embolic infarction of vital organs, and valve dysfunction with congestive failure. Metastatic infection in acute endocarditis is caused by virulent organisms.

Microbiologic Diagnosis. Infective endocarditis is diagnosed through blood cultures.

Prevention and Treatment. Antimicrobial prophylaxis is administered to patients with defective heart valves who are undergoing dental and other procedures known to produce bacteremia. Therapy consists of prolonged intravenous treatment with bactericidal antibiotics to eradicate bacteria within the protective clot. Surgical replacement of infected valves may be required to cure prosthetic valve infections.

Introduction

The circulatory system, consisting of the blood, blood vessels, and the heart, is normally free of microbial organisms. Isolation of bacteria or fungi from the blood of ill patients usually signifies serious and uncontrolled infection that may result in death. The presence of bacteria (bacteremia) and fungi (fungemia) in the blood occurs in more than 250,000 individuals per year in the United States and causes at least 50,000 deaths annually. Because rapid isolation, identification, and performance of antimicrobial susceptibility tests may lead to initiation of lifesaving measures, the culturing of blood to detect microbemia is one of the most important clinical microbiology laboratory procedures. Bacteremia may be prevented in some instances by the early recognition of localized infection and initiation of appropriate treatment with antimicrobial agents and surgical drainage of abscesses.

Clinical Syndromes.

Microbemia

Asymptomatic Microbemia. Microbes enter the circulatory system via lymphatic drainage from localized sites of infection or mucosal surfaces that are subject to trauma and are colonized with members of the normal bacterial flora. Organisms

may also be introduced directly into the bloodstream by infected intravenous needles or catheters or contaminated intravenous infusions. A number of disseminated viral infections are also spread through the body via the bloodstream. Small numbers of organisms or nonvirulent microbes are removed from the circulation by fixed macrophages in the liver, spleen, and lymph nodes. The phagocytes are assisted by circulating antibodies and complement factors present in serum. Under certain conditions, antibodies and complement factors may kill Gram-negative bacteria by lysis of the cell wall. Also, they may promote phagocytosis by coating bacteria (opsonization) with antibody and complement factors that have receptor sites for neutrophils and macrophages.

When defense mechanisms effectively remove small numbers of organisms, clinical signs or symptoms of microbemia may not occur (asymptomatic microbemia). Asymptomatic bacteremias caused by members of the endogenous bacterial flora have been observed in normal individuals after vigorous chewing, dental cleaning or tooth extraction, insertion of urinary bladder catheters, colon surgery, and other manipulative procedures. Asymptomatic bacteremias may occur if localized infections are subjected to trauma or surgery.

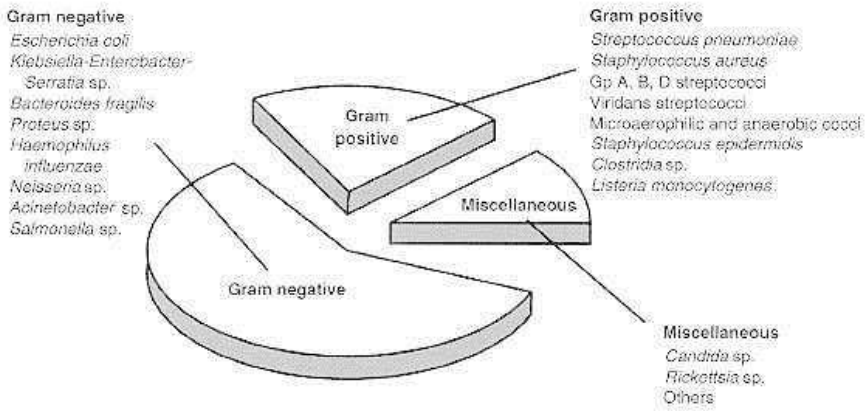
Most asymptomatic bacteremias are of no consequence; however, occasionally, virulent organisms that cause a localized infection (such as a *Staphylococcus aureus* skin boil) may produce infection at a distant site (e.g., bone infection) by means of asymptomatic bacteremia. Similarly, artificial or damaged heart valves may be colonized by viridans streptococci during asymptomatic bacteremia induced by dental manipulation. Infection of the heart valve (infective endocarditis) is fatal if not treated. Therefore, individuals with known valvular heart disease who undergo dental work or other procedures that produce asymptomatic bacteremias are given antibiotics to prevent colonization of the heart.

Symptomatic Microbemia

When a sufficient number of organisms are introduced into the bloodstream, an individual will develop fever, chills, shivering (rigors), and sweating (diaphoresis). Patients with symptomatic microbemias usually look and feel ill. As macrophages and polymorphonuclear leukocytes phagocytose microbes, they synthesize and release interleukin-1 into the circulation. This small protein acts on the temperature-regulatory center in the brain and sets the body thermostat at a higher level. The thermoregulatory center acts to decrease heat loss by reducing peripheral blood flow to the skin (pale appearance) and increases heat production by muscular activity (shivering), resulting in a rise in body temperature. When either a high body temperature level is attained or the microbemia terminates, the central nervous system thermostat becomes reset at a lower level and acts to reduce body temperature by increased peripheral blood flow to the skin (flushed appearance) and by sweating.

Symptomatic microbemias are most commonly caused by the organisms listed in. In recent years, the incidence of Gram-positive coccal bacteremias resulting from intravascular access infections in debilitated patients with serious underlying conditions has increased steadily, but Gram-negative bacillary infection still predominates. Hospitalized patients frequently have had surgery, severe trauma, or neoplasms that predispose to complicated local infections; also, these individuals' host defenses have been compromised by malnutrition, age, or corticosteroid or cancer chemotherapy. Granulocytopenia due to leukemia, cancer, or cancer chemotherapy is a frequent predisposing cause of microbemia and a reason for poor response to antimicrobial therapy. Gram-negative bacteremia is frequently due to pulmonary infections in intubated patients receiving ventilator therapy or to urinary tract infections caused by indwelling urinary catheters. Organisms other than those listed in Table 94-1 may produce microbemia in severely compromised hosts. Skin

contaminants, such as *Staphylococcus epidermidis* and diphtheroid species, may cause significant microbemias (indicated by isolation from multiple blood cultures). Bacteremias of this type are associated with intravenous catheters or prosthetic heart valves.



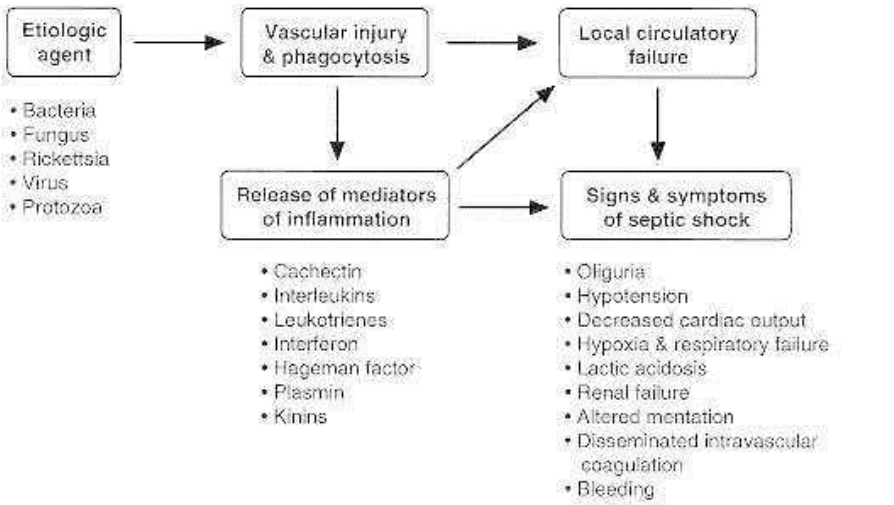
Common causes of symptomatic microbemia.

Transient microbemias are self:limited and often due to manipulation of infected tissues, such as incision and drainage of an abscess; early phases of localized infection, such as pneumococcal bacteremia in pneumococcal pneumonias; or bacteremias associated with trauma to mucosal surfaces colonized by the normal host flora. When multiple blood cultures are positive over a period of 12 hours or more, a continuous microbemia is present. The presence of continuous microbemia suggests a severe spreading infection that has overwhelmed host defenses. A continuous microbemia may originate from an intravascular site of infection in which organisms are shed directly into the bloodstream (e.g., infective endocarditis or an infected intravascular catheter), or from an early phase of a specific infection characterized by a continuous microbemia (e.g., typhoid fever).

Microbemias may persist despite treatment with antimicrobial agents to which the organisms are susceptible. Therefore, repeated blood cultures should be performed in patients who do not appear to respond to sustained antimicrobial treatment. During the first 3 days of treatment, positive blood cultures often are associated with inadequate antimicrobial dosage. Microbemias that persist longer than 3 days may be caused by organisms resistant to multiple antimicrobial agents, by undrained abscesses, or by intravascular foci of infection. When positive blood cultures with the same organism are separated by negative cultures, an intermittent microbemia is present.

Septic Shock. Septic shock occurs in approximately 40 percent of patients with Gram-negative bacillary bacteremia and 5 percent of patients with Gram-positive bacteremia. The septic shock syndrome consists of a fall in systemic arterial blood pressure with resultant decreased effective blood flow to vital organs. Septic shock patients frequently develop renal and pulmonary insufficiency and coma as part of a generalized metabolic failure caused by inadequate blood flow. Survival depends on rapid institution of broad-spectrum antimicrobial therapy, intravenous fluids, and other supportive measures. Elderly patients and those with severe underlying surgical or medical diseases are less likely to survive. Mortality from Gram-negative septic shock ranges from 40 to 70 percent. Septic shock may also occur with rickettsial, viral, and fungal infections. Septic shock due to Gram-negative bacillary bacteremias constitutes the most common serious infectious disease problem in hospitalized patients. The high frequency of septic shock in Gram-negative bacillary infeHtion is attributed to the toxic effect on the circulatory system of lipopolysaccharides (endotoxin) found in the cell wall of Gram-negative organisms. Endotoxin within the circulatory system has multiple and complex effects on neutrophils, platelets, complement, clotting factors, and inflammatory mediators in

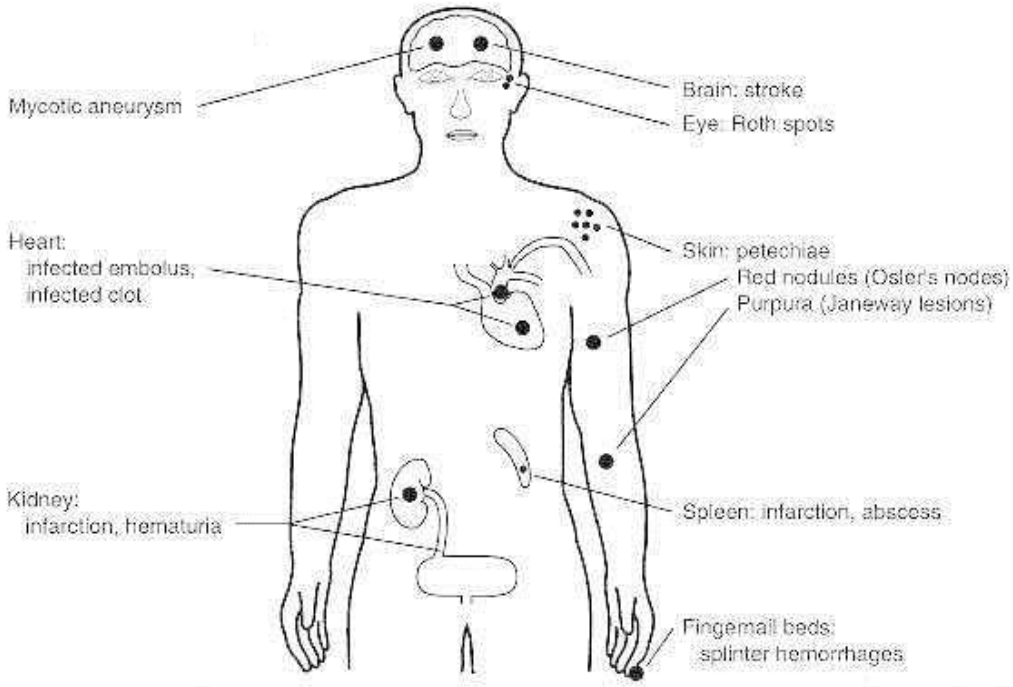
the blood. The symptoms of bacteremia and septic shock are reproduced when purified cell wall endotoxin is injected into the circulation.



Pathogenesis of septic shock.

Infective Endocarditis. Heart valve infections generally are classified as acute endocarditis, subacute endocarditis, and prosthetic valve endocarditis. If they are untreated, these infections are fatal. With treatment, mortality averages 30 percent; it is higher in acute and prosthetic valve infections. Acute endocarditis usually occurs when heart valves are colonized by virulent bacteria in the course of microbemia. The most common cause of acute endocarditis is *Staphylococcus aureus*; other less common causes are *Streptococcus pneumoniae, Neisseria gonorrhoeae, Streptococcus pyogenes*, and Enterococcus *faecalis*. Patients with acute endocarditis usually have fever, marked prostration, and signs of infection at other sites. Infected heart valves may be destroyed rapidly, leading to heart failure from valve leaflet perforation and acute valvular insufficiency. Infected pieces of fibrin and platelet vegetations on the valves may break loose into the circulation and lodge at distant

sites, producing damage to target organs. Metastatic infection due to emboli may involve arterial walls (mycotic aneurysm) or produce abscesses.



Infective endocarditis: metastatic infections due to emboli.

Patients with subacute endocarditis usually have underlying valvular heart disease and are infected by less virulent organisms such as viridans streptococci, enterococci, nonenterococcal group D streptococci, microaerophilic streptococci, and *Haemophilus* species. Frequently, the source and onset of infection are not clear, and patients consult physicians with complaints of fever, weight loss, or symptoms related to embolic phenomenon and congestive heart failure.

Prosthetic valvular endocarditis may present either acute or subacute in onset, and the infecting organisms differ, depending on whether endocarditis develops within 2 months of surgery or later. Whereas infections on nonprosthetic valves usually are eradicated by antimicrobial therapy alone, prosthetic valve infections frequently

require surgical removal of the infected valve before the infection is eliminated. Antimicrobial therapy of endocarditis is prolonged and should be guided by susceptibility studies. Fungal endocarditis is rare, but *Candida* infections occur in those with prosthetic valves and in drug addicts. *Aspergillus* endocarditis may occur after cardiac valve surgery.

Blood Cultures

Because several commercial blood culture systems are used by clinical microbiology laboratories, blood culture specimens may be processed differently by different laboratories. Most clinical laboratories will give a preliminary report of a negative culture if no growth is detected after 4 days of incubation. A final negative report is made if there is no growth after 7 days of incubation.

Clinicians should know when it is necessary for the laboratory to use special or nonroutine blood culture techniques to detect microorganisms. Failure to tell the clinical laboratory about the need for special culture conditions may result in false-negative blood culture reports.

If the patient has received antimicrobial agents before the blood specimen was obtained, the clinical laboratory can add penicillinase to remove β-lactam antibiotics, use an antimicrobial removal device or special resin bottle to remove or inactivate the antimicrobial agent, or prolong blood incubation for 2 weeks to improve the chances of obtaining a positive culture. If infective endocarditis is suspected, the blood culture bottles should be incubated for 2 weeks to allow growth of slow-growing or fastidious microorganisms. When fungemia is suspected, special media and techniques are used to grow fungi. When *Mycobacterium avium-intracellulare* bacteremia is suspected in patients with human immunodeficiency virus (HIV) infection, the laboratory must be alerted to use special mycobacterium culture bottles and media. Special culture techniques or media are required for the

isolation of brucellae, *Listeria monocytogenes*, leptospires, *Francisella tularensis*, and *Mycoplasma hominis*.

If a central venous catheter infection is suspected, blood should be drawn both from the line and from a peripheral vein, and the results of quantitative cultures compared. If the catheter blood culture has a 10-fold greater count than the peripheral blood culture or has more than 100 CFU/ml, the catheter is probably infected. Semi-quantitative culture of peripheral intravenous catheters may also help establish whether they are the portal of entry for bacteremia. When the results of blood cultures do not fit with the clinical condition of an infected patient, the clinician should review the situation with the clinical microbiology laboratory director or an infectious diseases specialist.

Microbiology of the Gastrointestinal Tract

General Concepts

Composition and Distribution of the Intestinal Microflora

The intestinal microflora is a complex ecosystem containing over 400 bacterial species. Anaerobes outnumber facultative anaerobes. The flora is sparse in the stomach and upper intestine, but luxuriant in the lower bowel. Bacteria occur both in the lumen and attached to the mucosa, but do not normally penetrate the bowel wall

Metabolic Activities. Intestinal bacteria are a crucial component of the enterohepatic circulation in which metabolites that are conjugated in the liver and excreted in the bile are deconjugated in the intestine by bacterial enzymes, then absorbed across the mucosa and returned to the liver in the portal circulation. Many drugs and endogenous compounds undergo enterohepatic circulation. Antibiotics that suppress the flora can alter the fecal excretion and hence the blood levels of

these compounds. The flora also plays a role in fiber digestion and synthesizes certain vitamins.

The Intestinal Microflora. The intestinal microflora may prevent infection by interfering with pathogens. The flora includes low populations of potentially pathogenic organisms such as *Clostridium difficile*. Antibiotics that upset the balance of the normal flora can favor both infection by exogenous pathogens and overgrowth by endogenous pathogens. If the bowel wall is breached, enteric bacteria can escape into the peritoneum and cause peritonitis and abscesses.

Bacterial Diarrheas. *Enterotoxin-Mediated Diarrheas*: Enterotoxigenic bacteria, such as *Vibrio cholerae* and enterotoxigenic *Escherichia coli* strains, colonize the upper bowel and cause watery diarrhea by producing an enterotoxin that stimulates mucosal cells to secrete fluid via an increase in intracellular AMP.

Invasive Diarrheas: Invasive bacteria, such as *Shigella* and *Campylobacter*, penetrate the intestinal mucosa. A bloody, mucoid diarrheal stool with inflammatory exudate is produced.

Viral Diarrheas. Rotavirus and Calicivirus (formerly Norwalk virus) are major causes of diarrheal disease. Rotavirus diarrhea affects mostly young children; Calicivirus causes disease in all age groups

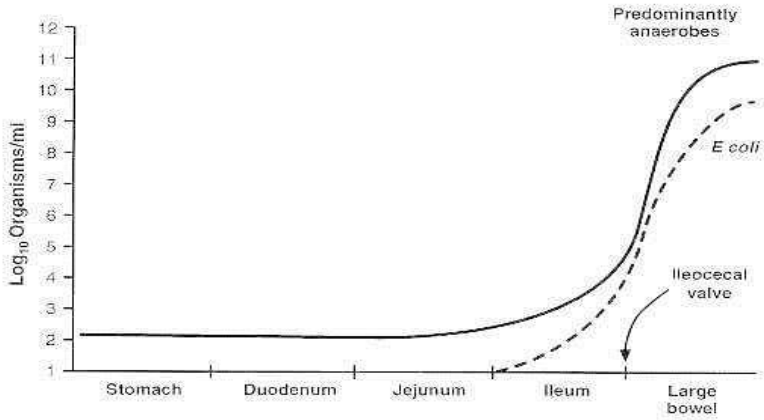
Parasitic Diarrheas. Some protozoa (especially *Entamoeba histolytica* and *Giardia lamblia*) as well as some intestinal helminths can cause diarrheal disease.

Clinical Diagnosis. In general, enterotoxigenic bacteria and viruses affect the upper bowel, causing watery diarrhea and periumbilical pain. The invasive bacteria act primarily in the colon (*Shigella* and *Campylobacter*) or lower ileum (*Salmonella*).

The stool in these diseases may contain blood. Colitis is marked by painful straining at stool (tenesmus).

Composition and Distribution of the Microflora

The bacterial inhabitants of the human gastrointestinal tract constitute a complex ecosystem. More than 400 bacterial species have been identified in the feces of a single person. Anaerobic bacteria predominate. The upper gastrointestinal tract (the stomach, duodenum, jejunum, and upper ileum) normally contains a sparse microflora; the bacterial concentrations is less than 10^4 organisms/ml of intestinal secretions. Most of these organisms are derived from the oropharynx and pass through the gut with each meal. Colonization of the upper intestine by coliform organisms is an abnormal event and is characteristic of certain infectious pathogens such as *Vibrio cholerae* and enterotoxigenic *Escherichia coli*. In contrast, the large intestine normally contains a luxuriant microflora with total concentrations of 10^{11} bacteria/g of stool. Anaerobes such as *Bacteroides*, anaerobic streptococci, and clostridia outnumber facultative anaerobes such as *E coli* by a factor of 1,000.



Concentration of the bacterial flora in regions of the gastrointestinal tract.

The character of the bacterial flora changes not only along the length of the gastrointestinal tract but also cross-sectionally with regard to the mucosal surface. Bacteria occupy the lumen, overlie the epithelial cells, and adhere to the mucosa. Penetration of bacteria through the mucosal surface is an abnormal event; pathogens such as *Shigella*, *Salmonella*, and *Campylobacter* invade in this way.

The same mechanisms that control the normal flora also protect the bowel from invasion by pathogens. Gastric acid in the stomach kills most organisms that are swallowed. Individuals with reduced or absent gastric acid have a high incidence of bacterial colonization in the upper small bowel and are more susceptible to bacterial diarrheal disease. Bile has antibacterial properties and thus may be another factor in controlling the flora. Forward propulsive motility (peristalsis) is a key element in suppressing the flora of the upper bowel. Finally, the microflora itself, by producing its own antibacterial substances (e.g., bacteriocins and fatty acids), stabilizes the normal populations and prevents implantation of pathogens.

Metabolic Activites of the Microflora

The metabolic capacities of the intestinal bacteria are extremely diverse. Bacterial enzymes can use as substrate virtually any compound in the intestinal lumen, whether taken orally or entering the intestine by secretion through the biliary tract or directly across the mucosa.

The Enterohepatic Circulation. Enzymes produced by intestinal bacteria play a central role in the enterohepatic circulation. Substances that undergo enterohepatic circulation are metabolized in the liver, excreted in the bile, and passed into the intestinal lumen, where they are reabsorbed across the intestinal mucosa and returned to the liver via the portal circulation. The enterohepatic circulation generally involves compounds that are conjugated in the liver to a polar group such as glucuronic acid, sulfate, taurine, glycine, or glutathione. Conjugation increases

the solubility of the metabolite in bile, but the conjugated compounds are poorly absorbed by the intestinal mucosa. Enzymes produced by intestinal bacteria—such as ß-glucuronidase, sulfatase, and various glycosidases—deconjugate these compounds, releasing the parent compounds which are readily absorbed across the intestinal wall. Many endogenous compounds undergo enterohepatic circulation, including bilirubin, bile acids, cholesterol, estrogens, and metabolites of vitamin D. In addition, many drugs that are excreted by the liver, including digitalis, diethylstilbestrol, morphine, colchicine, rifampin, and chloramphenicol, enter this pathway.

Antibiotics block the enterohepatic circulation by suppressing the intestinal flora and thereby reducing the levels of deconjugating enzymes. If an antibiotic is given to a patient who is also taking a drug that undergoes enterohepatic circulation, the resulting depression of the enterohepatic circulation will increase the fecal excretion of the drug and thereby lower its plasma level and half life. For example, the blood levels and half life of the estrogen in birth control pills decrease when antibiotics are administered.

The Microflora and Nutrition. Enzymes produced by intestinal bacteria are important in the metabolism of several vitamins. The intestinal microflora synthesizes vitamin K, which is a necessary cofactor in the production of prothrombin and other blood clotting factors. Treatment with antibiotics, particularly in individuals eating a diet low in vitamin K, can result in low plasma prothrombin levels and a tendency to bleed. Intestinal bacteria also synthesize biotin, vitamin B_{12}, folic acid, and thiamine.

The intestinal flora is capable of fermenting indigestible carbohydrates (dietary fiber) to short-chain fatty acids such as acetate, propionate, and butyrate. The major source of such fermentable carbohydrate in the human colon is plant cell wall polysaccharides such as pectins, cellulose, and hemicellulose. The acids produced

from these fiber substrates by bacteria can be an important energy source for the host.

Some people are deficient in intestinal lactase, the mucosal enzyme responsible for hydrolyzing the disaccharide lactose in milk. In these individuals, lactose is not adequately digested and absorbed in the intestine. Lactose that reaches the large bowel undergoes vigorous bacterial fermentation. The result can be distention, flatus, and diarrhea.

The Intestinal Microflora and Infection

Protective Activities of the Flora. Like other complex ecosystems, the intestinal microflora is relatively stable over time, maintaining roughly constant numbers and types of bacteria in each area of the bowel. The stability of normal flora both discourages infection by exogenous pathogens and prevents overgrowth of potentially pathogenic members. New organisms that enter the system in contaminated food or water generally are suppressed by the established flora. This suppression is related to production by members of the resident flora of antimicrobial substances such as bacteriocins or short-chain fatty acids, which inhibit the growth of alien microorganisms. Antibiotics that kill off part of the intestinal flora can upset its balance and may open the door to infection or pathologic overgrowth.

The pathogenesis of *Salmonella* food poisoning illustrates this phenomenon. Normal individuals are quite resistant to *Salmonella*, and a large oral inoculum is required to initiate infection. If the intestinal flora is suppressed by antibiotics, however, the individual becomes much more susceptible and can be infected by a relatively small inoculum.

Diseases Caused by Overgrowth of Potential Pathogens. The normal intestinal flora includes small populations of organisms that cause disease if they overgrow. For example, overgrowth of *Clostridium difficile* produces severe inflammation of the colon with diarrhea (pseudomembranous colitis). Administration of antibiotics initiates the process by suppressing the normal flora.

Peritonitis. Bacteria from the intestinal flora are the prime cause of infection in the peritoneal cavity when the normal barriers of the intestinal wall are violated. The intestinal wall can be perforated by trauma (knife wounds, gunshot wounds, blunt trauma), by disease (appendicitis, penetrating intestinal cancers), or by surgical procedures. Once the mucosal barrier is breached, bacteria penetrate through the intestinal wall into the normally sterile peritoneal cavity and its surrounding structures. Poor circulation, reduced oxygen supply, and dead tissue in the vicinity of the perforation promote the formation of an abscess and particularly favor the growth of anaerobic bacteria. Cultures of a peritoneal abscess generally yield several types of bacteria from the intestinal microflora, particularly species of *Bacteroides*, *Clostridium*, and *Peptostreptococcus* and *E coli*.

Bacterial Diarrheas

Enterotoxin-Mediated Diarrheal Diseases. Several enterotoxin-producing bacteria cause diarrheal diseases. The diarrheal disease caused by *Vibrio cholerae* and enterotoxigenic strains of *E coli* has three main characteristics. First, there is intestinal fluid loss that is related to the action of an enterotoxin on the small bowel epithelial cells. Second, the organism itself does not invade the mucosal surface; rather, it colonizes the upper small bowel, adhering to the epithelial cells and elaborating the enterotoxin. The mucosal architecture remains intact with no evidence of cellular destruction. Bacteremia does not occur. Third, the fecal effluent is watery and often voluminous, so that the diarrhea can result in clinical

dehydration. The fluid originates in the upper small bowel. where the enterotoxin is most active.

Cholera. The paradigm of the enterotoxigenic diarrheal diseases is cholera, in which stool volume can exceed 1 L/h, with daily fecal outputs of 15 to 20 L if the patient is kept hydrated. Cholera is caused by *V cholerae*, which is usually ingested in contaminated water. Vibrios that survive passage through the stomach colonize the surface of the small intestine, proliferate, and elaborate the enterotoxin. Cholera toxin acts via adenylate cyclase to stimulate secretion of water and electrolytes from the epithelial cells into the lumen of the gut. The duodenum and upper jejunum are more sensitive to the toxin than the ileum is. The colon is relatively insensitive to the toxin and may still absorb water and electrolytes normally. Thus, cholera is an "overflow diarrhea," in which the large volumes of fluid produced in the upper intestine overwhelm the resorptive capacity of the lower bowel.

Cholera stool is described as resembling rice water—a clear fluid flecked with mucus—and is isotonic with plasma. Microscopy reveals no inflammatory cells in the fecal effluent; all that can be seen are small numbers of shed mucosal cells.

Enterotoxigenic E coli Diarrhea. Certain strains of *E coli* cause diarrheal disease by elaborating enterotoxins. These strains produce two types of enterotoxin. One, called heat-labile toxin, is similar in structure and in its mechanism of action to cholera toxin. The other, called heat-stable toxin, appears to act via guanylate cyclase. Enterotoxigenic *E coli* strains are the most common cause of travelers' diarrhea

Other Diarrhea-Causing Toxins. Many strains of *Shigella* produce an enterotoxin, called Shiga toxin, that causes secretion of fluid from the small intestine. Shiga toxin has a destructive, cytotoxic effect on the small-bowel epithelium, causing gross

injury to the bowel surface. It does not activate adenylate cyclase. *E coli* 0157:H7, the organism associated with consumption of undercooked chopped meat, also produces a Shiga-like toxin; it causes bloody diarrhea and colitis. An organism that produces a different type of cytotoxin is *Vibrio parahaemolyticus*, a bacterium associated with seafood. Food-poisoning strains of *Staphylococcus aureus* and *Clostridium perfringens* both produce enterotoxins that are cytotoxic. The staphylococcal enterotoxin also has a direct effect on the vomiting center in the brain.

Gastrointestinal Disease Caused by Invasive Bacteria. Unlike the enterotoxigenic organisms, invasive bacteria exert their main impact on the host by causing gross destruction of the epithelial architecture; histologic findings include mucosal ulceration and an inflammatory reaction in the lamina propria. The principal pathogens in this group are *Salmonella, Shigella, Campylobacter*, invasive *E coli*, and *Yersinia*. The enteric viruses also invade intestinal epithelial cells, but the extent of mucosal destruction is considerably less than that caused by invasive bacterial pathogens.

***Salmonella enteritis*.** *Salmonella* species are a common cause of food poisoning. The main site of attack is the lower ileum, where the salmonellae cause mucosal ulceration. They rapidly make their way through the epithelial surface into the lamina propria and enter the lymphatics and bloodstream. At least two virulence factors are associated with intestinal infection: one responsible for mucosal invasion, and the other causing secretion of fluid and electrolytes into the bowel.

***Shigella dysentery*.** *Shigella* organisms cause bacillary dysentery, an invasive diarrheal disease of the lower bowel in which the stool contains an inflammatory exudate composed of polymorphonuclear leukocytes. The bacilli invade the epithelium of the colon and cause superficial ulceration. This invasive process

depends on the presence of two virulence factors. The first mediates the initial penetration of the mucosal surface by destroying the brush border; the bacteria are subsequently engulfed by invagination of the plasma membrane. The second virulence factor allows the organism to multiply within the mucosal tissue. Mucosal ulceration results, accompanied by an intense inflammatory response in the lamina propria. The infection is usually restricted to the mucosa; lymph node involvement and bacteremia are uncommon.

Fluid Production in Invasive Diarrheal Diseases. The mechanism(s) by which the fluid that causes watery diarrhea is produced in the invasive diarrheal diseases is under debate. Three mechanisms have been proposed. First, *Shigella* and possibly *Salmonella* strains apparently produce an enterotoxin that stimulates the mucosa to secrete water and electrolytes. Second, there is evidence that invasive organisms stimulate prostaglandin synthesis at the site of inflammation and that the prostaglandins induce fluid secretion. In experimental animals, fluid secretion can be blocked by prostaglandin inhibitors such as indomethacin and aspirin. Third, some evidence suggests that damage to the colonic epithelium causes diarrhea by prevention of normal resorption of fluid.

Viral Diarrheas

Two viruses—rotavirus and Calicivirus (Norwalk virus)—have been identified as major enteric pathogens in humans. The rotaviruses are a very important cause of infantile diarrhea, which in undeveloped countries can be fatal. Adults may be infected and shed virus, but clinical disease appears almost exclusively in children younger than 2 years. Calicivirus, in contrast, can produce gastroenteritis in all age groups and is a cause of major epidemics. The initial lesion forms in the proximal small bowel. The mucosal architecture is damaged, with shortening of the villi and

hyperplasia of the crypts. An inflammatory exudate then appears in the lamina propria.

The mechanisms responsible for fluid secretion in viral diarrheas have not been elucidated. It is known that infection with Calicivirus can produce steatorrhea and xylose malabsorption and causes direct damage to brush border enzymes. The activity of adenylate cyclase in the epithelial cells is not altered in the acute illness.

Parasitic Diarrheas

Several species of protozoa and helminths can cause diarrheal disease. Some of these infections can be acquired in the United States, although exposure to enteric parasites is far more common in tropical and developing countries. Some of the more common causes of parasitic diarrhea are *Entamoeba histolytica, Giardia lamblia, Strongyloides stercoralis,* and the intestinal flukes.

Clinical Diagnosis of Diarrheal Disease

An understanding of pathophysiology can be used to make a presumptive diagnosis in patients with infectious diarrhea. Perhaps the most convenient approach is to separate pathogens that involve the small intestine from those that attack the large bowel. Enterotoxigenic bacteria (*E coli, V cholerae*), viruses, and the parasite *Giardia* are examples of small-bowel pathogens. These organisms produce watery diarrhea, which may lead to dehydration. Abdominal pain, although often diffuse and poorly defined, is generally periumbilical. Microscopic examination of the stool fails to reveal formed cellular elements such as erythrocytes and leukocytes.

The large-bowel pathogens (the major ones being *Shigella* and *Campylobacter*) are invasive organisms and cause the clinical syndrome known as dysentery. Involvement of the colon is strongly suggested by the characteristic rectal pain known as tenesmus. Although the fecal effluent may be watery at first, by the

second or third day of illness the stool is scanty and often bloody or mucoid. Microscopic examination almost invariably reveals abundant erythrocytes and leukocytes. Proctoscopy shows a diffusely ulcerated, hemorrhagic, and friable colonic mucosa. Salmonella food poisoning does not fit into this simple scheme, because the disease can display features typical of both small- and large-bowel disease. The organism is invasive for the mucosa of the small intestine, particularly the lower ileum, and can cause voluminous fluid secretion. In additional, septicemia and metastatic spread of the pathogen to other organs sometimes occur.

Microbiology of the Nervous System

General Concepts

The anatomy of the brain and meninges determines the special character of central nervous system (CNS) infections. Epidural abscesses remain localized, whereas subdural abscesses spread over a hemisphere. Subarachnoid space infections spread widely over the brain and spinal cord. The blood-brain barrier formed by the tight junctions between cells of the cerebral capillaries, choroid plexus, and arachnoid largely prevents macromolecules from entering the brain parenchyma. As a result, immunoglobulins and immune-competent cells are scarce in the brain except at foci of inflammation. The space between cells in the brain parenchyma is too small to permit passage even of a virus. However, tetanus toxin and some viruses travel through the CNS by axoplasmic flow.

Meningitis

Etiology. Major bacterial causes are *Haemophilus influenzae, Streptococcus pneumoniae,* and *Neisseria meningitidis.* Major viral causes are enteroviruses, mumps virus and lymphocytic choriomeningitis virus.

Pathogenesis. Most agents invade from blood. Bacteria grow rapidly in cerebrospinal fluid; viruses infect meningeal and ependymal cells.

Clinical Manifestations. Headache, fever and stiff neck are the symptoms of meningitis. Untreated bacterial meningitis is usually fatal; viral meningitis is benign. Cerebrospinal fluid findings are critical in differential diagnosis.

Treatment. Antibiotics are used to treat bacterial and fungal meningitis. Viral meningitis is treated symptomatically.

Brain Abscess.

Etiology. Brain abscesses often exhibit a mixed flora of aerobic and anaerobic bacteria. Fungi are uncommon.

Pathogenesis. Abscesses begin when bacteria seed sites of necrosis, caused usually by infarction.

Clinical Manifestations. Headache, focal signs and seizures indicate a brain abscess. There are also characteristic computed tomography (CT) and magnetic resonance image (MRI) findings.

Treatment. Treatment consists of surgical drainage and appropriate antibiotics.

Encephalitis

Etiology. Many viruses cause mild meningoencephalitis; herpes simplex viruses and arboviruses are the major causes of potentially fatal disease.

Pathogenesis. Herpes simplex virus causes acute diffuse encephalitis in neonates. Herpes simplex type 1 causes focal temporal and frontal encephalitis in children and adults probably owing to invasion along olfactory or sensory nerves in the immune host. Arboviruses invade from the blood and cause diffuse predominantly neuronal infection. Rabies invades along peripheral nerves.

Clinical Manifestations. Encephalitis causes headache, fever, CNS depression, seizures, and mononuclear cells in cerebrospinal fluid. Focal temporal lobe signs occur in herpes simplex virus encephalitis.

Treatment. Acyclovir is used to treat herpes simplex encephalitis. Some arboviruses can be prevented by mosquito control or vaccines.

Slow and Chronic CNS Infections

Spirochetes. Untreated syphilis and Lyme disease can cause varied later CNS disease.

Retroviruses. Human immunodeficiency virus can cause acute and progressive CNS disease. HTLV-I causes chronic spastic paraparesis in a small number of infected persons.

Conventional Viruses. Persistent measles and rubella virus infections can cause subacute encephalitis with dementia. JC virus, a papovavirus, can cause progressive demyelinating disease in immunodeficient patients.

Unconventional Agents. Kuru and Creutzfeldt-Jakob disease are chronic noninflammatory, degenerative diseases of the brain that are caused by unconventional agents called prions.

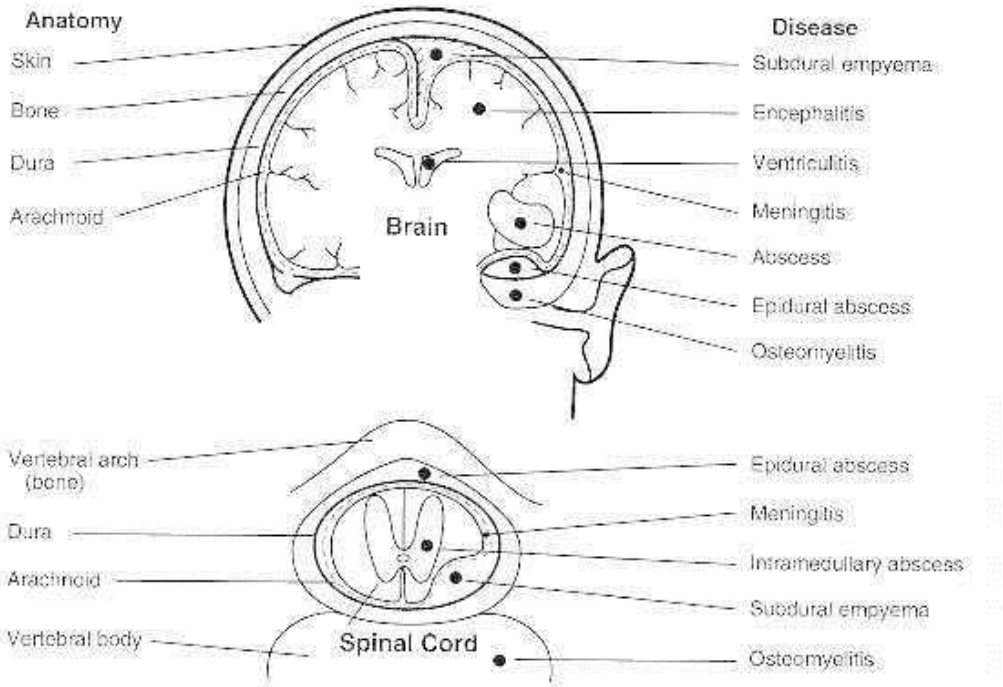
Parasites. Parasites may cause acute meningitis or encephalitis, chronic encephalopathy, and cerebral granulomas. Neurocysticercosis is the most common parasitic neurologic disease.

Introduction

Infections of the nervous system are rare but life-threatening complications of systemic infections. The central nervous system (CNS) presents a special milieu for bacterial, fungal, viral and parasitic infections: the brain and spinal cord are protected by bone and meningeal coverings that compartmentalize infection; they are divided by barriers from the systemic circulation; they lack an intrinsic immune system; and they have a unique compact structure.

Gross Anatomy

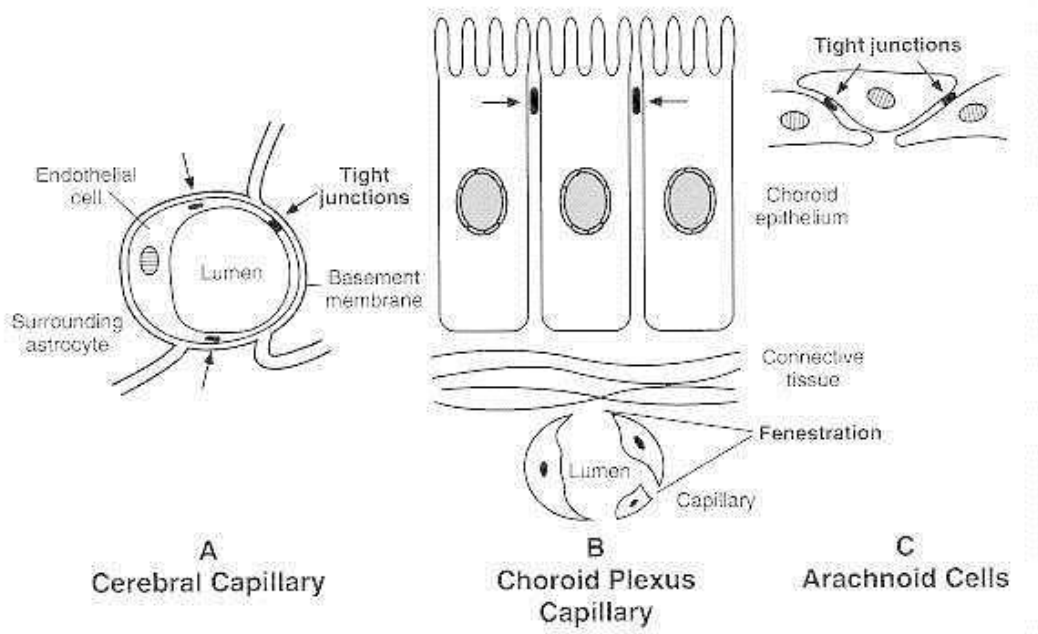
The brain is protected by the bony calvaria, and the outer meningeal covering, the dura, is tightly bound to the bone. Epidural infections usually arise from bone infection (osteomyelitis) and remain localized. At the foramen magnum the dura becomes free, forming a true epidural space around the spinal cord. The dura and arachnoid are not adherent to each other. Consequently, when bacteria penetrate the dura into the subdural space, infection can spread rapidly over a cerebral hemisphere. However, subdural empyema is usually confined to one hemisphere by the dural reflexions along the falx and tentorium. The subarachnoid space is a true space, containing cerebrospinal fluid (CSF) that flows from the ventricles to the basilar cisterns over the convexities of the hemispheres and through the spinal subarachnoid space. The CSF contains little antibody or complement and few phagocytic cells. Therefore, bacteria that enter this space undergo an initial phase of logarithmic growth, accounting for the often explosive onset of acute bacterial meningitis.



Anatomy and site of infection of the brain and spinal cord. (Modified from Butler IJ, Johnson RT: Central nervous system infections.

Blood-Brain Barrier

Dyes such as trypan blue injected into the systemic circulation stain virtually all tissues, with the exception of the brain and spinal cord. This blood-brain barrier, which excludes most macromolecules and microorganisms, is due to the cellular configuration of the cerebral capillaries, the choroid plexus, and arachnoid cells. This barrier excludes not only most microbes, but most immunocompetent cells and antibodies. Therefore, although the barrier deters invasion of infectious agents, it hampers their clearance once it is penetrated.



Tight junctions envelop the CNS between capillary endothelial cells, choroid plexus epithelial cells, and arachnoid cells. The cerebral capillaries (A) lack fenestrations, have a dense basement membrane, and have tightly apposed footplates of astrocytes. The capillaries in the choroid plexus (B) are fenestrated, lack tight junctions, and have loose surrounding connective tissue. The choroid epithelial cells are joined by tight junctions at their apices. Tight junctions join arachnoid cells (C).

Meningitis

Meningitis is an inflammation of the pia-arachnoid meninges. It can be caused by growth of bacteria, fungi, or parasites within the subarachnoid space or by the growth of bacteria or viruses within the meningeal or ependymal cells. Meningitis is a diffuse infection caused by a variety of different agents.

Etiology. Approximately 20,000 cases of bacterial meningitis occur in the United States each year. Seventy percent of these are in children younger than 10 years old. Infants are particularly susceptible because of their predisposition to bacterial

infection, possible lower integrity of barriers, and immature defense mechanisms. In neonates younger than 28 days old, meningitis is usually due to enteric bacilli (especially *Escherichia coli*), group B streptococci, or *Listeria*. Neonatal meningitis represents fewer than 10 percent of cases of meningitis, but more than 50 percent of meningitis deaths. In the postnatal period, *Haemophilus influenzae* is the most common cause of bacterial meningitis, but this infection is largely limited to childhood. Significant reductions in some countries are occurring due to use of capsular polysaccharide-protein conjugate vaccines during infancy. Adult bacterial meningitis is predominantly due to *Neisseria meningitidis* and *Streptococcus pneumoniae,* except in cases where there had been a penetrating wound to the skull, surgery, or immunosuppression in the host. *Neisseria meningitidis* causes epidemic disease, all other forms of pyogenic meningitis are sporadic. Tuberculosis and fungi usually cause subacute meningitis. *Cryptococcus neoformans* often causes meningitis in immunosuppressed patients, but can cause indolent meningitis in immunocompetent individuals. *Coccidioides immitis* and, rarely, other fungi also cause subacute meningitis.

Viral meningitis occurs more frequently than bacterial meningitis, with over 50,000 cases each year in the United States. The disease is benign and tends to be seasonal. Enteroviruses (echoviruses and coxsackieviruses) cause disease, primarily in the late summer and early fall; mumps virus spreads predominantly in the spring; and lymphocytic choriomeningitis virus is more common in winter, since this virus is acquired from mice, which move indoors during cold weather and increase human exposure.

Pathogenesis. Most bacteria and viruses invade the CNS from the blood, and the risk of CNS invasion has been shown to be related to the magnitude and duration of the bacteremia or viremia. Particles in the blood, including bacteria or viruses, are normally cleared by the reticuloendothelial system, and speed of removal is

proportional to size. The bacteria that maintain a bacteremia (and incidentally cause meningitis) are largely those which elaborate capsid polysaccharides that increase their resistance to phagocytosis. Intracellular bacteria and a variety of viruses elude clearance by growing within blood cells. Enteroviruses and some arthropod-borne viruses (arboviruses) are cleared less effectively from serum because of their small size. Some viruses enter the CNS by infecting endothelial cells or choroid plexus epithelium. Indeed, in mumps virus meningitis, choroid plexus cells containing viral nucleocapsids are frequently found within the CSF.

Clinical Manifestations. The primary clinical manifestations of meningitis are headache, fever, and nuchal rigidity (stiffness of the neck on passive forward flexion due to stretching of the inflamed meninges). Flexion of the neck may also cause reflex flexion of the legs (Brudzinski sign), and meningeal irritation may limit extension of the leg when flexed at the knee (Kernig sign). Meningeal inflammation may also cause some degree of obtundation (reduced consciousness), and seizures are common in children. If bacterial meningitis is not promptly treated, purulent material collects around the base of the brain, which may cause cranial nerve palsies and obstruct the flow of CSF, resulting in hydrocephalus. Vasculitis develops, with infarction of the brain and multifocal neurological deficits. Untreated bacterial meningitis is a uniformly fatal disease. Viral meningitis, on the other hand, is benign and self-limited.

Systemic clinical signs sometimes suggest the agent (e.g., the rash or herpangina of enterovirus infections, the parotitis of mumps, or the multiple petechiae of meningococcemia). Examination of the CSF provides the most important diagnostic information. Acute bacterial infections evoke a polymorphonuclear cell response in the CSF and profound reductions of CSF sugar content. Bacteria can usually be seen on smears of the CSF and can be cultured if antibiotics have not been given. Subacute tuberculous or fungal meningitis is more difficult to diagnose. The

inflammatory response is usually composed of mononuclear cells, and the reduction of CSF sugar evolves slowly. Organisms are difficult to see on direct smears, although cryptococci may be identified by mixing India ink with the CSF to outline the capsule of the organism and differentiate it from mononuclear inflammatory cells. In general, viruses produce a modest mononuclear cell response, and although the CSF protein may be elevated, CSF sugar is normal or only mildly depressed. Viruses, such as enteroviruses and mumps virus, can be grown from the CSF, but this requires special viral cultures. A rapid diagnosis may be achieved by demonstrating antigen of various bacterial and fungal agents or the presence of IgM against specific viral agents.

TABLE 96-2 CSF Findings in Nervous System Infections

Infection	Pressure	Type (Number) of Cells	Protein	Sugar	Culture
Meningitis					
Viral	Normal	Mononuclear (10–1,000)	↑	Normal	Special tests
Bacterial	Normal or ↑	Polymorphonuclear (>100)	↑↑	↓↓↓	+++
Subacute	Normal	Mononuclear	↑↑	Normal to ↓	±
Brain abscess	↑	Polymorphonuclear (small numbers)	↑	Normal	0
Encephalitis	↑	Mononuclear	↑	Normal	Special tests in some forms only

CSF Findings in Nervous System Infections.

Treatment. Early diagnosis of bacterial and fungal meningitis and treatment with appropriate antimicrobial agents are crucial. The mortality rate due to untreated disease approaches 100 percent. Even with treatment, the death rate of individuals with acute bacterial meningitis remains approximately 15%; it is as high as 30% for pneumococcal meningitis. Sequelae are frequent in survivors. This mortality and morbidity have remained relatively unchanged since the introduction of antibiotics. Further reduction of death and disability rests primarily on the physician's early suspicion, diagnosis, and treatment of the disease. Viral meningitis requires only symptomatic treatment since the disease is self-limited; the prime management

problem is to rule out nonviral, treatable illnesses that can mimic acute viral meningitis (partially treatable bacterial meningitis, tuberculous or fungal meningitis, syphilis, Lyme disease, etc.).

Infection of the Brain Parenchyma

Abscess. An abscess is a focus of purulent infection and is usually due to bacteria. Brain abscesses develop from either a contiguous focus of infection (such as the ears, the sinuses, or the teeth) or hematogenous spread from a distant focus (such as the lungs or heart, particularly with chronic purulent pulmonary disease, subacute bacterial endocarditis, or cyanotic congenital heart disease). In many cases the source is undetected.

Etiology. Many brain abscesses have a mixed flora of aerobic and anaerobic bacteria. Approximately 60 to 70 percent contain streptococci; and *Staphylococcus aureus,* enterobacteria and *Bacteroides* are frequently present. Fungi cause fewer than 10 percent of brain abscesses.

Pathogenesis. Abscesses in the brain parenchyma are thought to result from a bacterial seeding of already devitalized tissue. In experimental animals, direct injection of bacteria into the carotid arteries does not lead to brain abscess, whereas injection of microspheres that occlude small vessels, followed by injection of bacteria does lead to abscess formation. With chronic purulent ear or sinus infection, infection extending along the veins may cause infarction of brain tissue; a bacterial abscess may then evolve. In cyanotic congenital heart disease (right-to-left shunt), emboli cause small infarcts of the brain which are then seeded by bacteria from the blood.

Clinical Manifestations. The primary clinical manifestations of abscess are headache, focal signs, and seizures. The headache may not be severe, however, and the development of signs may be insidious. There may be no fever. If focal signs are present computed tomography (CT) or magnetic resonance imaging (MRI) is performed rather than CSF examination. An abscess is identified by a hypodense area representing pus surrounded by an enhancing area representing the neovascularization and edema around the fibrous abscess wall. The CSF is usually sterile, and bacteriologic diagnosis can only be obtained by culturing an aspirate of the abscess cavity.

Treatment. If a poorly defined area of cerebritis is found, treatment is begun with multiple antibiotics to cover the multiple common organisms. If there is encapsulation, the abscess should be drained to determine specific bacterial flora and prevent catastrophic rupture of the abscess into the ventricles.

In contrast, epidural abscesses usually cause local pain and tenderness. Pressure against a localized area of the brain may lead to focal signs. Spinal epidural and cerebral or spinal subdural abscesses are surgical emergencies. Spinal epidural abscesses have a rapid course, starting with segmental pain along nerve roots, followed by paresthesias of the body below the abscess level, and finally irreversible paraplegia. Subdural abscesses (subdural empyema) spread rapidly over a wider area. Subdural empyema causes septic thrombosis of bridging veins, leading to hemiplegia and seizures.

Encephalitis

Encephalitis is defined as inflammation of the brain. Unlike an abscess, which is a localized area of bacterial or fungal growth, encephalitis is usually due to viruses that produce more widespread intracellular infections.

Etiology. Many viruses, including enteroviruses, mumps, and lymphocytic choriomeningitis viruses, cause mild forms of encephalitis. Life-threatening viral encephalitis is due primarily to herpes simplex viruses and arboviruses. Rabies virus causes uniformly fatal infection, but no more than six cases have occurred in any year since 1979 in the United States.

Pathogenesis. The pathogenesis of encephalitis due to herpes simplex virus, arboviruses, and rabies virus is different for each virus. Herpes simplex viruses, both types 1 and 2 (HSV-1 AND HSV-2), cause encephalitis. In neonates, the disease is predominantly due to HSV-2 virus, and irrespective of serotype, the acute generalized necrotizing encephalitis is often accompanied by evidence of systemic infection of the liver, adrenals, and other organs. In children and adults, however, encephalitis is caused by HSV-1 virus and is usually localized. This virus, which is acquired in childhood, remains latent within the trigeminal and other ganglia. It may reactivate to cause cold sores. Encephalitis in an immune host results either from the entry of a new virus, possibly across the olfactory mucosa, or from reactivation of latent virus in the trigeminal ganglia, which spread along sensory nerve fibers to the base of the anterior and middle fossa. In either case, infection is localized to the orbital frontal and medial temporal lobes. Because the host is immune, virus presumably spreads from cell to cell over a contiguous localized area, infecting neurons and glial cells.

In contrast, arboviruses (mainly togaviruses, flaviviruses, and bunyaviruses) spread to the brain from the blood. The systemic infection causes few, if any, symptoms. Depending on the virus, between 1 in 20 and 1 in 1000 infections are complicated by CNS infection. The encephalitis is diffuse, but is localized largely to neurons.

Rabies, in contrast, is usually acquired through the bite of a rabid warm-blooded animal. This virus spreads by axonal transport from the inoculated skin or muscle to the corresponding dorsal root ganglion or anterior horn cells and then to populations

of neurons throughout the CNS. The early involvement of neurons of the limbic system cause the typical behavioral changes of clinical rabies. Polioviruses also show a selective infection of specific motor neuron populations which explains the asymmetrical flaccid motor paralysis of poliomyelitis.

Clinical Manifestations. Herpes simplex virus-1 encephalitis in the non-neonate typically causes focal signs that may evolve over a period of up to 1 or 2 weeks. In addition to headache and fever, hallucinations and bizarre behavior are common, and these are sometimes confused with psychiatric illness. Focal seizures and hemiparesis are frequent, and aphasia develops if the disease is localized to the dominant temporal lobe.

Arbovirus infections cause a more diffuse and acute disease, with a rapid depression of consciousness, greater frequency of generalized seizures, and multifocal signs. At times, however, this or any other form of encephalitis may localize to the temporal areas, producing signs very similar to those of herpes simplex virus encephalitis.

The CSF examination in acute encephalitis may or may not show an increase in pressure, but usually reveals an inflammatory response of mononuclear cells. Examination early in disease may show no cellular response or a predominance of polymorphonuclear cells. Red blood cells are frequently found in herpes encephalitis because of the necrotizing pathology of the disease, but they are not universally present nor are they specific to the disease. The CSF protein level is usually elevated and the CSF sugar level remains normal. Cultures for herpes simplex virus are usually negative. Polymerase chain reaction tests for herpesvirus sequences are highly sensitive and specific in experienced laboratories. Intrathecal antiherpesvirus antibody may be detected late in the course of the disease, but too late to instigate therapy. In most arbovirus infections, virus-specific IgM is present in spinal fluid, for specific diagnosis at the time of initial presentation.

The electroencephalogram (EEG) is helpful in the diagnosis of herpes simplex virus encephalitis because periodic spikes and slow waves often localize to the infected temporal lobe. In other forms of encephalitis slowing is more diffuse. Computerized tomography in cases of herpes simplex virus encephalitis usually shows an attenuated area in the medial temporal lobes and sometimes a mass effect, but these findings, like the CSF and EEG changes, are not diagnostic. A prompt, definitive diagnosis of HSV-1 encephalitis requires brain biopsy of the area where typical encephalitis with inclusion bodies is seen, and the diagnosis is confirmed by either immunocytochemical staining of herpes simplex virus antigens in brain cells or virus isolation.

Treatment. Rapid diagnosis of herpes simplex virus encephalitis is important because a specific antiviral therapy, acyclovir (acycloguanosine), reduces the mortality from 70 percent without treatment to 25 percent if treatment is initiated prior to the onset of coma. Other forms of viral encephalitis are treated primarily with supportive care, although some arboviral encephalitides, such as Japanese encephalitis, can be prevented by vaccines, and others can be reduced by mosquito control.

Slow and Chronic Infection and Chronic Neurologic Disease

Chronic nervous system infections, such as those that occur with syphilis, persist over many years with the unpredictable appearance of varied neurologic complications. In contrast, slow infections, such as Creutzfeldt-Jakob disease, have more predictable incubation periods with a progressive buildup of infectivity, followed by a disease of predictable course lasting months or years. The slow infections resemble acute infections, with a predictable incubation period and disease course, but extend over months or years. Chronic or slow neurologic diseases due to persistent infection must be differentiated from chronic diseases that

represent the static sequelae of acute bacterial meningitis or viral encephalitis; the former are progressive and depend on the ongoing replication of the infectious agent in the nervous system.

Spirochetes. Syphilis can cause varied neurologic diseases over the lifetime of the untreated patient. During secondary syphilis, 6 weeks to 3 months after primary infection, a benign mild meningitis may accompany the primary CNS invasion that occurs in approximately 25 percent of untreated patients. Later complications include acute meningovascular inflammatory disease leading to stroke (meningovascular syphilis) 3 to 5 years after the primary infection, progressive dementia (general paresis) 8 to 10 years later, or a chronic arachnoiditis involving primarily the posterior roots of the spinal cord (tabes dorsalis) 10 to 20 years after infection. This development of vasculitis, parenchymal involvement and chronic arachnoiditis parallel the complications that occur over weeks during untreated bacterial meningitis. Lyme disease also may be complicated by early and late neurologic involvement. Mild meningitis and facial palsy often accompany the initial rash and systemic symptoms following the tickbite. In 15 percent of untreated patients, subacute or recurrent meningitis, encephalitis, cranial nerve palsies, and peripheral neuropathies develop 1 to 9 months later, and rarely a chronic meningoencephalitis has been described years later.

Retroviruses. Two human retroviruses cause chronic neurological diseases. Human immunodeficiency virus (HIV) infects the CNS soon after systemic infection in most patients. An acute meningitis or acute demyelinating polyneuritis (Guillain-Barré syndrome) occasionally occurs at the time of seroconversion and a recurrent meningitis and motor neuropathies can occur during the long, otherwise asymptomatic seropositive period. Years later at the time of clinical AIDS, dementia, myelopathy and a painful sensory neuropathy are frequent.

In contrast, most persons infected with human T-cell lymphotropic virus type 1 (HTLV-I) suffer no neurologic disease. Less than 1 percent of those infected develop a slowly progressive myelopathy called tropical spastic paraparesis or HTLV-associated myelopathy. This inflammatory disease of the spinal cord usually develops during the fourth or fifth decade of life even though HTLV-1 infection is most frequently acquired from breast feeding during the neonatal period.

In chronic spirochetal and retroviral infections the CSF often has a mild mononuclear cell inflammatory response, mild elevation of protein levels, and elevated IgG in an oligoclonal pattern, suggesting an ongoing infection.

Conventional Viruses. Some conventional viruses occasionally produce chronic disease. This outcome may result from defective replication of the virus or a defect in the host. Following uncomplicated measles, approximately one per million children develop subacute sclerosing panencephalitis (SSPE) 6 to 8 years later. This chronic dementing illness with myoclonic movements is due to a defective measles virus infection in the CNS. Progressive multifocal leukoencephalopathy, in contrast, is due to a ubiquitous papovavirus, JC virus, which infects almost all children without recognized symptoms. In immunodeficient patients, this virus may cause a subacute or chronic demyelinating disease of the brain with multifocal signs, leading to death usually in less than 6 months. Rubella virus has been associated with chronic encephalitis after congenital infection, and, in very rare cases, there has been a relapse of a disease in adolescence resembling SSPE. In these infections the precise location of virus and the virus-host relationship during the long incubation period is not known.

Unconventional Agents. Unconventional agents called prions or spongiform encephalopathy agents are transmissible but have no identified nucleic acid. Kuru, the first of these to be described, has been limited to an isolated population in New

Guinea. Creutzfeldt-Jakob disease, however, occurs worldwide. It is a presenile dementia with histopathologic abnormalities limited to the CNS; the brain shows vacuolization of neurons and glia, but no inflammatory response. The disease has a course of rapidly progressive cognitive deficits with myoclonic movements. Death usually occurs in less than 6 months. In experimental infection with these agents, infectivity in the brain and extraneural tissues slowly accumulates during the long incubation period, but no immune response to the agent is found in natural or experimental infection.

Parasites. Parasitic infections such as malaria, amebiasis with free-swimming amoebas and trichinosis can produce acute encephalopathy or meningitis. Others are associated with chronic disease, such as the chronic sleeping sickness of African trypanosomiasis, the chronic cerebral granulomas caused by *Schistosoma japonicum*, or abscesses caused by *Toxoplasma gondii* in immunodeficient patients. The commonest parasitic neurologic disease is cysticercosis caused by the larvae form of *Taenia solium*. The parasitic cysts and resulting basilar arachnoiditis are the most common causes of epilepsy and hydrocephalus in many areas of South America and Asia.

Microbiology of the Genitourinary System

General Concepts

Clinical Presentations. In women, genital infections may cause a vaginal discharge, mucosal ulceration producing local discomfort and pain on intercourse, or pelvic inflammatory disease. Ongoing infection of the upper genital tract leads to infertility, ectopic pregnancies and chronic pelvic pain. In men, genital infection may cause urethral discharge, pain on voiding, and painful scrotal swellings. Genital ulcers are usually painful. Some diseases cause enlarged inguinal lymph nodes.

Etiology. Primary genitourinary infections are usually sexually transmitted; common pathogens include parasites (*Trichomonas vaginalis*), bacteria (*Treponema pallidum, Neisseria gonorrhoeae, Chlamydia trachomatis*, *Haemophilus ducreyi*), and viruses (herpes simplex virus, human papillomavirus, human immunodeficiency virus). Members of the normal flora, such as the fungus *Candida albicans*, may cause opportunistic infections.

Pathogenesis. Pathogens may enter the genital tract by local invasion or ascending infection. *Treponema pallidum*, *H ducreyi*, herpes simplex virus, etc., locally invade the skin and mucous membranes. *T pallidum* and Human Immunodeficiency Virus disseminate via the bloodstream to distant sites. Other pathogens such as *N gonorrhoeae* cause ascending infection through the urethra and cervix. Infants born through a genital tract infected with some of these pathogens may become infected.

Microbiologic Diagnosis. The organisms responsible for genital infections are generally fastidious and often difficult to culture. Specimens must be correctly collected and transported. Dark-field examination and serologic studies are necessary to diagnose syphilis; specialized tissue culture or antigen detection techniques are used for *C trachomatis*, viral culture for herpes simplex virus, and specialized media for culturing *N gonorrhoeae* and *H ducreyi*.

Prevention and Treatment. Education to modify sexual behavior and use of condoms are essential. Screening asymptomatic individuals in some populations and case contact tracing are also effective measures. Effective drug therapies exist for all bacterial genital infections and for herpes simplex.

Urinary Tract Infections

Clinical Manifestations. Urinary tract infections in adults may cause painful, frequent urination with a feeling of incomplete emptying of the bladder, perineal

pain, fever, chills, and back pain. Most elderly patients are asymptomatic, and in small children, the symptoms are nonspecific.

Etiology. Most urinary infections are caused by bacteria from the intestinal flora. *Eschericia coli* causes about 70 percent of all infections. *Staphylococcus saprophyticus* causes about 10 percent of infections in young women. *Pseudomonas aeruginosa*, *Serratia marcescens*, *Enterococcus faecalis*, and *Staphylococcus epidermidis* are common hospital-acquired pathogens. Yeasts and, in some parts of the world, protozoa are occasional pathogens.

Pathogenesis. Organisms can ascend through the urethra to infect the bladder and renal pelvis. Occasionally, they may interfere with renal function or produce abscesses within renal tissue. Because of the shorter urethra, intercourse can facilitate urinary tract infections in women. Pyuria is almost always present. Hydrolysis of urea by bacteria (e.g., *Proteus mirabilis*) can cause the formation of struvite stones.

Microbiologic Diagnosis. Presumptive diagnosis can be made by demonstrating pyuria. Quantitative urine culture is essential for diagnosis. Specimens should be refrigerated until cultured to prevent bacterial replication. Properly submitted urine that contains $>10^5$ or 10^8 organisms/ml indicates significant infection. However, with acute cystitis, bacterial counts may be lower. Blood cultures may be positive in patients with pyelonephritis.

Prevention and Treatment. Antimicrobial agents cure most urinary tract infections. Recurrence is common, and may be prevented by prolonged therapy. Prolonged use of a urinary catheter greatly increases the likelihood of a urinary tract infection.

Introduction

Genitourinary infections fall into two main categories: (1) primary infections due to sexually transmitted pathogenic microorganisms and (2) infections due to members of the resident flora. Genital infections are uncommon in children and increase dramatically in sexually active adults, in whom sexually transmitted diseases are the second most prevalent group of reportable communicable illness in North America. Sexually transmitted pathogens include parasites (*Trichomonas vaginalis*), bacteria (*Treponema pallidum*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Haemophilus ducreyi*), and viruses (Herpes simplex virus, human papillomavirus, human immunodeficiency virus). Genital infections due to the fungus *Candida albicans* or to members of the endogenous bacterial flora (*Bacteroides fragilis* and members of the family Enterobacteriaceae) are not known to be sexually transmitted. Bacterial vaginosis occurs when the balance of vaginal flora is upset.

The urinary tract and urine are normally sterile. Numerous mechanical and biologic processes ensure that microorganisms do not enter the urinary tract. Women are more susceptible to urinary infections because the female urethra is short and because the area around the urethral opening is colonized with potential pathogens (e.g. *E coli* and *E faecalis*).

Urethritis and Epididymitis

Clinical Manifestations. Urethritis (inflammatory disease of the urethra) is characterized by urethral discharge (Table 97-1). The incubation time varies, averaging 3 days for gonococcal urethritis and 7 days for nongonococcal urethritis. The clinical symptoms range from mild to severe. In both men and women, dysuria is common. Discharge and dysuria are seen in 70 percent of patients with gonococcal urethritis, whereas patients with nongonococcal urethritis are more

likely to have one or the other of these symptoms but not both. Other symptoms include itching, frequency, urgency, or a feeling of heaviness in the genitals. Polyarthralgia, involving large joints, characteristic rash, and low-grade fever are typically present in patients with disseminated gonococcal infection.

TABLE 97-1 Gential Infections in Males

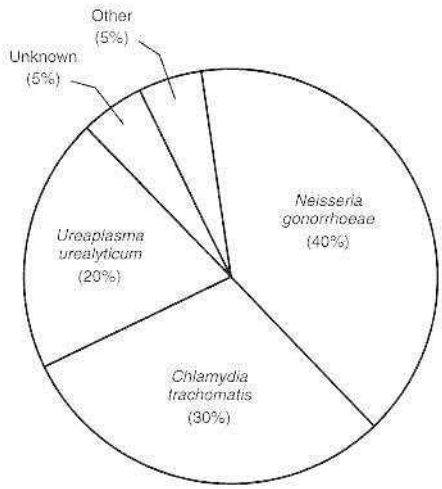
	Urethritis		Genital Ulcers		
	Gonococcal	Nongonococcal	Herpes Simplex	Chancroid	Syphillis
Incubation time (days)	2-5	5-10	3-10	3-10	21
Ulcer	–	–	Vesicle or ulcer	Deeply eroded, purulent	Painless, papule/ulcer
Urethral discharge	+++	++	+	+	+
Microscopy	Gram stain (intracellular Gram-negative diplococci)	Gram stain (PMN, no intracellular Gram-negative diplococci)	N[a]	Gram stain insensitive (Gram negative rod "school of fish")	Dark-field (spirochetes)
Culture	Y[b]	Y[b]	Y	Y	N
Antigen detection	n[b]	Y (*C. trachomatis*)	N	n	N
Serology	N	N	n	n	Y

[a]N, Test not used for diagnosing this disease.
[b]Y, Test routinely performed; n, test can be done but not routine.
[c]Culture is the "gold standard" for *C. trachomatis* but, because of the length of time required, direct fluorescent-antibody or antigen detection methods are usually used. Recently, DNA amplification methods have gained more widespread acceptance.

Gential Infections in Males. Orchitis and epididymitis are complications of both *N gonorrhoeae* and *C trachomatis* infections that present as a painful, swollen mass in the scrotum. Testicular atrophy follows in some patients with orchitis.

Etiology. *Neisseria gonorrhoeae* and *C trachomatis* account for most cases of urethritis in men. *Chlamydia trachomatis* is responsible for 40 to 60 percent of cases of nongonococcal urethritis. The etiology of *chlamydia*-negative nongonococcal urethritis is uncertain. *Ureaplasma urealyticum* can cause nongonococcal urethritis;

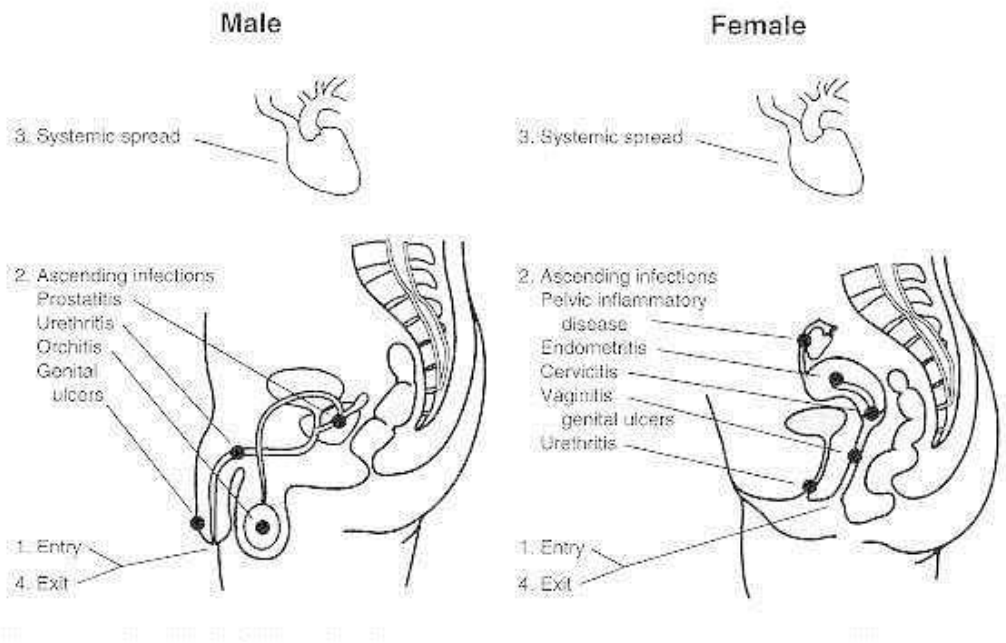
however, the high incidence of this organism in the normal genital flora makes it difficult to interpret its role in nongonococcal urethritis. Less common agents isolated from nongonococcal urethritis include herpes simplex virus and *Trichomonas vaginalis*. Although gonococcal urethritis is a more acute disease than nongonococcal urethritis, overlap in the symptoms mandates laboratory confirmation. Patients often have multiple sexually transmitted pathogens. Approximately 1/3 of heterosexual men infected with *Neisseria gonorrhoeae* are concurrently infected with *Chlamydia trachomatis*. If only the gonococci are treated, these patients develop a nongonococcal urethritis called postgonococcal urethritis. Therefore, patients with gonorrhea should also be treated with agents that will effectively eradicate *C trachomatis*.



Major causes of urethritis.

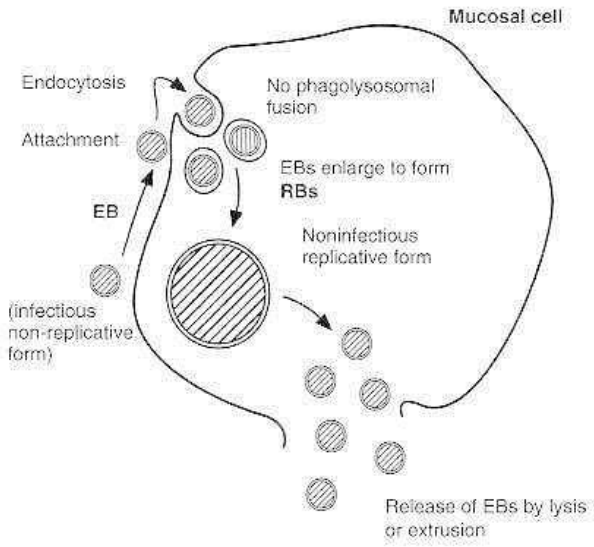
Pathogenesis. *Neisseria gonorrhoeae* and *C trachomatis* are transmitted by sexual intercourse. *Neisseria gonorrhoeae* attaches to mucosal cells via pili and other surface proteins. The organism then is phagocytosed and passes through the mucosal

epithelium. Proliferation occurs with subsequent influx of polymorphonuclear neutrophils (PMN), which produce the exudate that is the hallmark of gonorrhea. *Neisseria gonorrhoeae* spreads to cause disseminated gonococcal infection in approximately 1 to 3 percent of patients with gonorrhea. Disseminated gonococcal infection is more prevalent in women than in men. Up to 80 percent of patients with disseminated gonococcal infection have had an asymptomatic local infection for 7 to 30 days prior to dissemination.



Pathogenesis of genital tract infections.

Chlamydia trachomatis is an obligate intracellular parasite with a dimorphic life cycle. Urethral infection is asymptomatic in about 30 percent of men. If left untreated, the infection can progress to cause epididymitis. Proctitis also can occur in homosexual males.



Replication cycle of *Chlamydia* (EB, elementary body; RB, reticulate body).

Microbiologic Diagnosis

Neisseria gonorrhoeae. Diagnosis of gonorrhea is based on microscopic examination of exudate, culturing of the organism, and rapid methods such as antigen or nucleic acid detection. Urethral exudates should be examined by Gram stain for leukocytes and Gram-negative intracellular diplococci. The Gram stain has good sensitivity (90 percent) and specificity (95 percent) in males. Ideally, urethral exudate should be planted directly onto a split plate containing modified Thayer-Martin and chocolate agar. The Thayer-Martin agar contains vancomycin, colistin, nalidixic acid and trimethoprim. Approximately 5 percent of strains of *N gonorrhoeae* are susceptible to vancomycin, thus necessitating the use of a non-inhibitory medium such as chocolate agar. The medium is incubated in 5 percent CO_2 in a moist environment for 48 hours. *Neisseria gonorrhoeae* grows as translucent gray colonies that are oxidase-positive. The identity of the cultures is

confirmed by carbohydrate utilization: glucose is fermented, but lactose, maltose and sucrose are negative. Fluorescent-antibody analysis using monoclonal antibodies may also be used to confirm the identity of cultures. Culture confirmation can also be performed using DNA probes that detect specific sequences of the rRNA of *N gonorrhoeae*.

Chlamydia trachomatis. Diagnosis of *C. trachomatis* infection involves growing the organism in tissue culture or using direct bacterial antigen detection techniques. Since the organism is an intracellular parasite, infected urethral cells must be collected on the swab sample. The best transport media are 2-sucrose-phosphate or sucrose-glutamate-phosphate. If swab specimens are not processed immediately, they should be stored at -70°C prior to culturing. The swabs are then used to inoculate cell culture lines that have been treated with cyclohexamide. After 48 to 72 hours, the monolayer is fixed and, after staining, is examined for chlamydial inclusion bodies. Because of the labor intensiveness of tissue culture, direct antigen detection methods have been developed for *C trachomatis*. These include either direct visualization of the organism using fluorescein-conjugated antibodies or enzyme-like immunosorbent assay (ELISA) methods which detect solubilized chlamydial LPS. Serologic tests based on either complement fixation or microimmunofluorescence are available in specialized laboratories. Nucleic acid detection systems using amplification steps are now commercially available.

Prevention and Treatment. No vaccines exist for *N gonorrhoeae* or *Chlamydia trachomatis*. Control requires modification of sexual behavior and cure of the patient and their contacts.

For gonorrhea, ceftriaxone is the drug of choice. Resistance to penicillin due to β-lactamase has been increasing; therefore, all isolates of *N gonorrhoeae* should be tested for β-lactamase production and susceptibility testing performed if the strain is

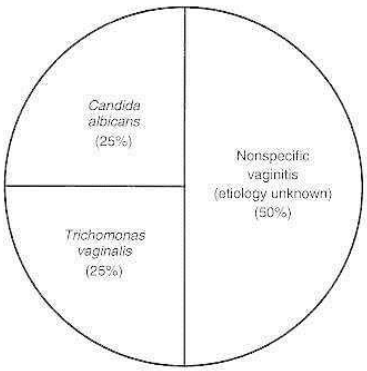
β-lactamase positive. Gonorrhea is a reportable disease, and contact tracing is one strategy to contain the spread of infection. As mentioned previously, all patients being treated for gonorrhea should be concomittantly treated for the agents of nongonococcal urethritis such as *C trachomatis*. Thus, ceftriaxone combined with tetracycline is an optimal regime. Recently azithromycin given as a single oral dose has been recommended for treatment of *C trachomatis*. This approach leads to better compliance.

Vaginitis, Cervicitis, Endometritis, and Pelvic Inflammatory Disease

Clinical Presentation. Endometritis and pelvic inflammatory disease usually result from ascending vaginal or cervical infections. Vaginal infections seldom have systemic manifestations; they present as an abnormal vaginal discharge that may have an unusual odor. Pruritus may also be present. The consistency of the discharge often reflects the nature of the disease. A thin watery discharge that sticks to the anterior and lateral vaginal walls is seen in bacterial or non-specific vaginosis; the vaginal walls appear normal. In candidiasis the vaginal walls are erythematous, and in trichomoniasis they have a strawberry appearance and the vaginal discharge is green and frothy. A yellow, purulent discharge suggests cervicitis or other forms of vaginitis. In prepubertal girls, gonorrhea causes an inflammatory response in the vagina, whereas following puberty, the infection is primarily a cervicitis. Mucosal ulceration of the vagina results in local discomfort and pain on intercourse. Abdominal discomfort is rare in vaginitis, and is more suggestive of cystitis or pelvic inflammatory disease.

Etiology. About one-half of the cases of vaginitis are due to *Candida albicans* or *Trichomonas vaginalis*. The rest are classified as bacterial vaginosis. Bacterial vaginosis involves an upset in the distribution of the normal flora that is associated with an increase in the numbers of *Gardnerella vaginalis* mobilencus sp. and

anaerobes of the *Bacteroides* group. Since 40 to 50 percent of normal individuals may carry *Gardnerella vaginalis*, isolation of the organism does not reliably predict bacterial vaginosis. However, the absence of this organism almost rules out bacterial vaginosis.



Major causes of vaginitis.

Neisseria gonorrhoeae and *C. trachomatis* are responsible for the majority of cervical infections. Sexually transmitted infections with more than one pathogen are common. Due to the overlap in clinical features, accurate diagnosis requires laboratory confirmation. About two-thirds of women infected with *N gonorrhoeae* or *C trachomatis* do not exhibit purulent cervical discharge. Therefore, for both gonococcal and chlamydial cervicitis, physical examination is not adequate to exclude these infections. Herpes simplex is an occasional cause of cervicitis in women and can be isolated from approximately 90 percent of women with primary herpes simplex virus infection.

Pathogenesis. Vaginitis due to *T. vaginalis* and *C. albicans* remains localized, producing vaginal discharge and itching. Yeasts are part of the normal flora in 50

percent of adult women of child-bearing age. Antibiotics such as tetracyclines disturb the balance of the flora and cause overgrowth by *C. albicans*,with resulting vulvovaginal candidiasis. *Trichomonas vaginalis* infection is superficial; penetration of the vaginal epithelial cells has not been described. Inflammation of the vaginal walls and exocervix includes erythema, punctate hemorrhages, and small ulcerations. The pH of the vaginal discharge is often greater than 4.5.

Ascending infection may take the form of salpingo-oophoritis or pelvic inflammatory disease. The onset of ascending infection frequently coincides with menses. Ascending genital infection occurs in approximately 10 to 20 percent of women with *Neisseria gonorrhoeae* with endocervical infection.

Gonococcal ophthalmia neonatorum occurs as a result of the passage of the newborn through the birth canal of infected mothers.

The organism spreads by ascending infection from the vagina and endocervix to the endometrium, fallopian tubes and other contiguous structures. Pelvic inflammatory disease can be an acute or a chronic complication of endocervitis. Infection results in scarring of the fallopian tubes; this accounts for the ten-fold increase in risk of ectopic pregnancies in women with a history of pelvic inflammatory disease. Each episode of pelvic inflammatory disease also increases the incidence of sterility.

Microbiologic Diagnosis. A wet mount is the optimal method for detecting *T vaginalis* or *C albicans* in patients with vaginitis. However, the wet mount is negative in 30 percent of symptomatic women with trichomoniasis and therefore does not rule out this infection. Active vaginal candidiasis is characterized by many yeast cells with active budding. Low numbers of *Gardnerella vaginalis* are often present in women who do not have nonspecific vaginitis. Gram-stained smears that show no lactobacilli but many curved, Gram-negative rods are very suggestive of

nonspecific vaginitis. According to current recommendations, a diagnosis of nonspecific vaginitis should be based on a positive Gram-stained smear in conjunction with a vaginal pH greater than 4.5 and a positive "whiff test".

Microbiologic diagnosis of *N gonorrhoeae* involves examining Gram-stained cervical exudate, as well as culturing the organism on selected media such as modified Thayer-Martin agar. The sensitivity of the Gram stain is substantially lower for exudates from women than from men: it is positive in only about 80 percent of women with cervical gonorrhea. If pelvic inflammatory disease or pelvic abscesses are suspected, material should, if possible, be aspirated from the infected site, smeared, and cultured. Microbiologic diagnosis for *C trachomatis* is performed by either tissue culture or antigen detection techniques. Cervical mucus should be cleared, and then a second sample taken by vigorously rubbing the cervical orifice to ensure that cervical cells are sampled, since *C trachomatis* is an intracellular parasite. The methods used for culturing and antigen detection are described above.

Prevention and Treatment. There are no vaccines for any of these sexually transmitted diseases. Preventive measures are directed at education with emphasis on safe sexual behavior. Barrier methods such as condoms are essential to decreasing the spread of genital infections. Contact tracing of the reportable infections has also been invaluable. *Trichomonas* infection is treated with metronidazole either as a single oral dose of 2 g or as 250 mg orally three times a day for 7 days. Treatment of candidal infection requires local application of an antifungal agent. The imadiazoles (e.g. clotrimazole or miconazole) are usually more effective than the polyenes (e.g. nystatin). The usual length of treatment is 3 days. Women who have frequent recurrences may be left on longer courses of systemic or local therapy.

Genital Ulcer Disease

Clinical Manifestations. Genital ulcers are transmitted by sexual intercourse and can be caused by a variety of microorganisms. However, some clinical features are found more commonly with a particular etiologic agent. Up to one-third of the episodes of genital ulceration have a clinical diagnosis that did not agree with the microbiologic diagnosis. This indicates the importance of microbiologic confirmation of diagnosis for genital ulcers. In developed countries, the most common genital ulcer diseases are herpes simplex, followed by syphilis. In developing countries, the most common causes of genital ulceration are chancroid, followed by syphilis, lymphogranuloma venereum and granuloma inguinale. The reason for this difference in prevalence between different geographical locations is not understood. The variable incubation period for genital ulcers and the fact that initial lesions are often overlooked are two factors that lead to the increased dissemination of these diseases. Genital ulcers increase the risk of heterosexual transmission of AIDS.

The incubation period for chancroid ranges from 3 to 10 days. The ulcers begin as small, inflammatory papules that develop into ulcers that have an undermined edge, contain purulent exudate and bleed easily. Chancroid is a sexually transmitted disease that is more prevalent in males than females, with a ratio of approximately 8:1. In men, the ulcer is located primarily on the prepuce and around the coronal sulcus. In women, the forchette, labia and perianal area can be involved. Multiple "kissing" ulcers (ulcers in direct opposition to each other) are common. Approximately one-third of the patients with chancroid also have enlarged inguinal lymph nodes that are extremely tender. These lymph nodes may coalesce and rupture and drain, forming buboes.

Syphilis usually presents as a small papule that develops into a painless, eroded, indurated ulcer. The mean incubation period is 21 days. If untreated, the genital ulcer disappears and, after a variable length of time, the patient may develop

secondary syphilis with widespread, protean symptoms usually involving the skin with disseminated diffuse papules. The final stage of the disease, tertiary syphilis, can remain latent or appear as late syphilis consisting of neurosyphilis, cardiovascular syphilis, or gummatous syphilis. Lymphogranuloma venereum, a disease due to restricted types of *C trachomatis*, usually begins as a painless papule that frequently goes unnoticed. The second stage of the disease involves enlargement of regional lymph nodes and the third stage manifests as rectal strictures and fibrosis. Granuloma inguinale also begins as a painless papule that ultimately develops into a painless, raised, beefy-red lesion. The lesions of herpes simplex initially begin as small papules that develop into extremely painful vesicles or ulcers.

Etiology. *Haemophilus ducreyi* is the etiologic agent of chancroid. This organism is a fastidious, Gram-negative rod that requires hemin. The incidence of chancroid has been increasing in the United States. *Treponema pallidum*, a spirochete, is the agent of syphilis.

Chlamydia trachomatis serovars L1, L2, and L3 are the agents of lymphogranuloma venereum. These strains of *C trachomatis* differ from ocular and nongonococcal urethritis in being more invasive in a mouse model and more resistant to trypsin treatment. Granuloma inguinale is caused by *Calmatobacterium granulomatis*, which is usually demonstrated as intracellular Donovan bodies visualized by Warthin-Starry silver stain or Giemsa stain of histologic sections. The disease is most prevalent in India, Papua, New Guinea, and the Caribbean.

Pathogenesis. *Treponema pallidum* invades intact mucosa and, after entering the lymphatics, can disseminate throughout the body to almost any organ. Obliterative endarteritis is the pathologic hallmark of syphilis and is found in all stages of the disease. *Treponema pallidum* can infect the fetus of a syphilitic mother, producing

congenital syphilis. The pathogenesis of chancroid is poorly understood, but the organism is thought to gain access through minute breaks in the mucosal epithelium. The organism is drained to the regional lymph nodes but does not disseminate further in the body. The organisms remain localized in the superficial layers of the ulcer. *Chlamydia trachomatis* serovars L1, L2 and L3 and *Calmatobacterium granulomatis* both remain localized at the site of ulceration but sometimes may show contiguous spread.

Microbiologic Diagnosis. Because the clinical symptoms of the genital ulcer diseases overlap, and because two or more pathogens may be present simultaneously, culture is critical to confirming the diagnosis. Chancroid is diagnosed by culturing either ulcer or bubo exudate. The organism is fastidious but can be grown in 48 hours by culturing on Mueller-Hinton or GC medium enriched with IsoVitaleX, fetal bovine serum, and vancomycin. The cultures grow best in a water saturated environment at 37°C with 5 percent CO_2. Even under optimal culture conditions, the isolation rates are between 50 and 80 percent. The organism is identified by hemin requirement, alkaline phosphatase production, ability to reduce nitrate and a positive oxidase test.

Treponema pallidum cannot be grown in vitro. Diagnosis is made by dark-field examination of ulcer material for spirochetes and by serologic detection of an antibody response. Herpes simplex is diagnosed by submitting appropriate vesicular fluid for a viral culture or antigen detection. Lymphogranuloma venereum is usually diagnosed by culturing *C trachomatis* and examination for inclusion bodies in the inoculated cell line. Antigen detection methods using fluorescein-conjugated antibody have also proved useful. *Calmatobacterium granulomatis* is usually detected by staining histologic sections with Warthin-Starry silver stain and observing Donovan bodies within the host cells.

Prevention and Treatment. As with other sexually transmitted diseases, rapid diagnosis, treatment and contact tracing help contain the spread of the disease. Use of condoms and modification of sexual behavior are important preventitive measures. Effective treatment is available for all the genital ulcer diseases. Chancroid is treated with a seven day course of erythromycin. Other useful antibiotics include ceftriaxone, trimethoprim, either with or without sulfonamides, and ciprofloxacin. Syphilis is treated with penicillin. If the patient is allergic to penicillin, tetracycline is an effective alternative. Early treatment of pregnant women with syphilis is crucial to prevent congenital syphilis. The susceptibility profiles of Lymphogranuloma venereum strains are similar to those of other *C trachomatis* serovars. Tetracyclines, erythromycin and sulfonamides are all effective. Granuloma inguinale has been successfully treated with trimethoprim-sulfamethoxazole, tetracycline and erythromycin. Treatment should be continued for 3 weeks.

Urinary Tract Infections

Cystitis, Pyelonephritis, Asymptomatic Bacteriuria, Renal Abscess

Clinical Manifestations. Acute cystitis is a superficial inflammation of the bladder and urethra which leads to urinary frequency, painful urination, a feeling of fullness following voiding, and suprapubic discomfort. Acute pyelonephritis is due to bacterial invasion of the renal tissue with inflammation and swelling, leading to fever, back pain, and sometimes renal dysfunction. Acute cystitis occurs together with acute pyelonephritis in about one-third of patients. Acute prostatitis occurs when bacteria invade the prostate, causing perineal pain and fever.

Infection can spread within the urinary tract and patients often have recurrences of cystitis, sometimes interspersed with episodes of pyelonephritis. Symptoms that persist and recur are often referred to as chronic cystitis, chronic pyelonephritis or

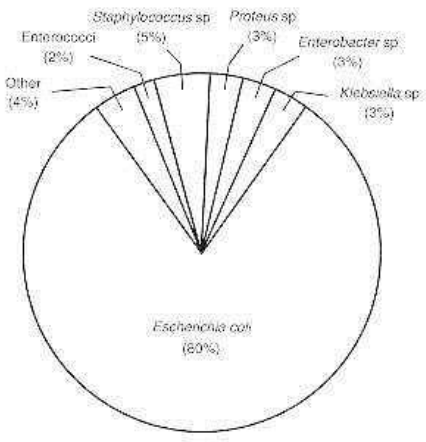
chronic prostatitis. However, these chronic conditions are much more difficult to define. Recurring kidney infection in childhood sometimes leads to renal damage and ultimate kidney failure. Hypertension is also an occasional outcome of chronic renal infection.

Asymptomatic infections of the urinary tract—asymptomatic bacteriuria—are common. In childhood, about 1 percent of girls have asymptomatic bacteriuria. The prevalence increases to 3 to 5 percent among adult women and 10 to 50 percent in elderly men and women. Occasionally, individuals do have symptoms such as incontinence or ongoing malaise that are not recognized as due to bacteriuria until it is diagnosed and treated. Some patients have what are called "complicated urinary tract infections." This includes individuals who have congenital or acquired anatomic abnormalities of the urinary tract. Obstruction of the urinary tract as a result of either a stone or a malfunctioning bladder secondary to a neural injury also predisposes to infection and makes infections more difficult to treat. Kidney and bladder stones can be the consequence of infection, and management of the infection is usually only successful if the stone is also removed. Many individuals cared for by urologists have underlying abnormalities of the urinary tract that make infections complicated and difficult to cure without surgical restoration of normal urine flow. Urinary catheters, used to drain the urinary tract in cases of obstruction or incontinence, bypass normal host defenses, and individuals with indwelling catheters are very prone to infections. Nosocomial urinary infections due to catheterization account for almost one-half of all infections acquired in hospital and can lead to invasive, life-threatening sepsis.

Urinary infections are the commonest type of bacterial infection that causes women to seek medical care. In a given year, about 1 in 20 women have acute cystitis. Acute pyelonephritis is one of the most common infections that require hospital admission for intravenous antibacterial therapy.

Urinary infections are recurrent in about 5 percent of women, and the recurring cystitis and pyelonephritis cause substantial morbidity. Recurrent urinary infection in women can be due to a number of underlying causes. Sexual intercourse, the syndrome referred to as "honeymoon cystitis", is responsible for about one-half of urinary infections in sexually active adult women. These infections are not acquired from the sexual partner but rather are due to the mechanical irritation associated with intercourse. Unfortunately, women who acquire frequent infections that are associated with intercourse often have difficulties developing healthy, normal sexual relations and this may require special attention. The use of a diaphragm for contraception is also a major risk factor, increasing the risk of cystitis threefold.

Etiology. Organisms normally present in the intestinal tract cause most urinary tract infections. The commonest of these by far is *Escherichia coli*, which is responsible for 80 percent of infections that are acquired outside of hospitals. Other Gram-negative rods such as *Klebsiella*, *Enterobacter*, and *Proteus* spp are relatively common, each accounting for 3 to 5 percent of infections. Within the hospital environment, *Pseudomonas aeruginosa*, *Serratia marscesens*, and other, more resistant, hospital-associated pathogens account for many infections.



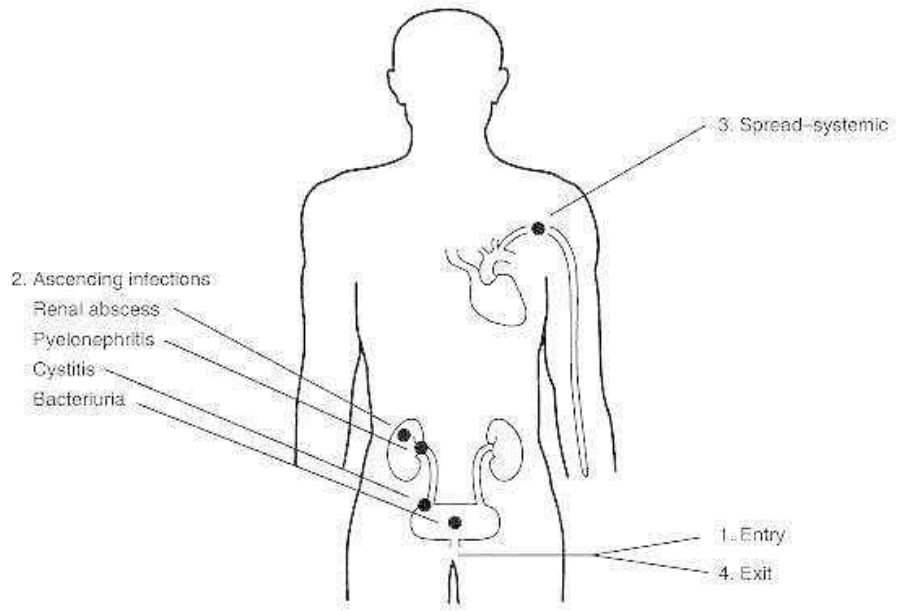
Major causes of urinary tract infections.

Gram-positive organisms, particularly coagulase-negative staphylococci and enterococci, cause some infections. *Staphylococcus saprophyticus* causes about 10 percent of urinary tract infections in young women. *Candida albicans* is also a frequent pathogen in hospitalized patients, particularly if diabetes is present.

Anaerobes and fastidious organisms rarely cause urinary infections. A number of viruses, particularly mumps virus, cytomegalovirus, and coxsackieviruses, can be present in the kidneys and urine, but rarely cause symptoms or any consequences.

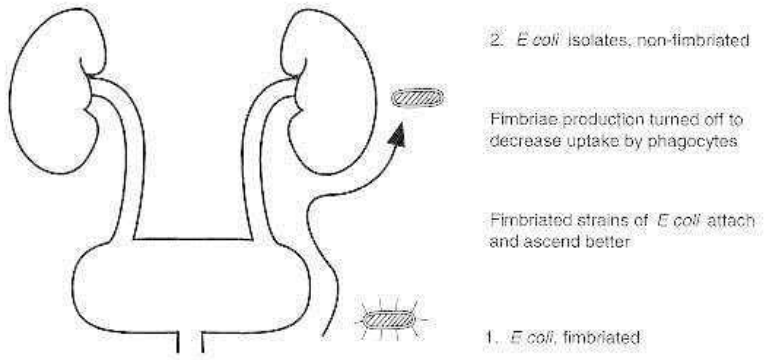
A number of sexually transmitted pathogens (e.g., *Neisseria gonorrhoeae*) may invade the urethra. *Chlamydia trachomatis* and herpes simplex can present with symptoms that mimic acute cystitis in both men and women.

Pathogenesis. Bacteria invade the urinary tract by ascending or hematogenous routes. The ascending route is the most common, with hematogenous spread causing kidney abscesses.



Pathogenesis of urinary tract infections.

Escherichia coli serogroups 01, 02, 04, 06, 07 and 075 are the most common agents of urinary tract infections. The most important virulence factor for these bacteria is the enhanced ability to adhere to uroepithelial cells. This attachment is mediated by specific pilus adhesins on the surface of *E coli*. The mucosal epithelial cells of women and children with recurrent urinary tract infections have been shown to have an increased avidity for attachment of *E. coli*. However, phasic variation occurs after the organism ascends to the kidney or pelvis, and pili are no longer produced. Motility has been shown to facilitate ascending infection and bacterial endotoxins can decrease urethral peristalsis.



Phasic variation of *E coli* in urinary tract infections.

Alterations of urine flow by scarring, obstruction due to stones, or catheterization greatly enhances the risk of acquiring a urinary tract infection.

Microbiologic Diagnosis. The diagnosis of a urinary tract infection is confirmed by culturing the organism from urine. Most bacteria that cause urinary infection grow readily, and the clinical diagnosis of urinary tract infection is usually confirmed within 24 hours. Because urine is an excellent culture media for a variety of microorganisms, considerable effort must be made to ensure that a urine sample is not contaminated during collection and that organisms are not permitted to grow before the urine is cultured. Patients suspected of urinary infection are usually asked to collect a mid-stream sample after cleaning the perineum or glans penis with soap and water. An early-morning collection is best because the concentration of bacteria in the urine is greatest prior to the morning voiding. The urine is then refrigerated or taken to the laboratory for immediate culture. Urine can be stored in a refrigerator for up to 24 hours without any loss of bacterial viability.

In the laboratory, a quantitative loop that samples 0.001 ml is usually used to inoculate both a nonselective medium (blood agar) and a selective medium

(MacConkey agar). The cultures are incubated at 37°C. By the following day, the organism can be identified and quantitated. Cultures that represent true infection rather than specimen contamination are identified quantitatively. Approximately 65 percent of patients with acute cystitis have $>10^5$ organisms/ml of urine and almost 90 percent of patients with acute pyelonephritis and asymptomatic bacteriuria also have counts in excess of 10^5 organisms/ml. Unless contaminated or overgrown, most negative urines have $<10^4$ organisms/ml. Antibacterial susceptibility profiles of the pathogens are also analyzed. Examination of urine to demonstrate the presence of pus cells is an important part of diagnosing urinary infection. Most infections of the urinary tract are associated with an inflammatory response. Thus, quantitative urine culture and the presence of pyuria are straightforward, reliable means of confirming the clinical diagnosis of urinary tract infection.

Prevention and Treatment. Most antimicrobial agents are excreted in the urine and, therefore many different treatment regimens can be used to cure urinary infections. The most commonly prescribed agents for *acute cystitis* are oral regimens of nitrofurantoin, a sulfonamide-trimethoprim combination, amoxicillin, cephalexin, and ciprofloxacin or other quinolones. Each of these regimens cure 90 to 95 percent of females with acute cystitis. Amoxacillin which previously was the drug of choice now fails in 10 to 20 percent of patients due to widespread resistance among *E coli*. Long courses are not necessary, and many studies have shown that single dose therapy or therapy prescribed for 3 days is as effective as longer courses. Intravenous antimicrobials are usually prescribed for patients acutely ill with *acute pyelonephritis* or *acute prostatitis*. Aminoglycosides and cephalosporins are frequently chosen. Longer courses of oral antibacterial agents are useful to prevent recurring infections in women who are susceptible to frequent reinfections; women need not live in fear of their next infection. If they wish to not take continuous

preventive therapy, the next treatment regimens should be carried for self-initiation with the onset of acute symptoms.

Microbial Infections of Skin and Nails

General Concepts

Etiology. Skin diseases can be caused by viruses, bacteria, fungi, or parasites. The most common bacterial skin pathogens are *Staphylococcus aureus* and group A β-hemolytic streptococci. Herpes simplex is the most common viral skin disease. Of the dermatophytic fungi, *Trichophyton rubrum* is the most prevalent cause of skin and nail infections.

Pathogenesis. *Primary Infections*: Primary skin infections have a characteristic clinical picture and disease course, are caused by a single pathogen, and usually affect normal skin. Impetigo, folliculitis, and boils are common types. The most common primary skin pathogens are *S aureus*, β-hemolytic streptococci, and coryneform bacteria. These organisms usually enter through a break in the skin such as an insect bite. Many systemic infections involve skin symptoms caused either by the pathogen or by toxins; examples are measles, varicella, gonococcemia, and staphylococcal scalded skin syndrome. Dermatophytic fungi have a strong affinity for keratin and therefore invade keratinized tissue of the nails, hair, and skin.

Secondary Infections: Secondary infections occur in skin that is already diseased. Because of the underlying disease, the clinical picture and course of these infections vary. Intertrigo and toe web infection are examples.

Clinical Manifestations. Most skin infections cause erythema, edema, and other signs of inflammation. Focal accumulations of pus (furuncles) or fluid (vesicles, bullae) may form. Alternatively, lesions may be scaling with no obvious

inflammation. Nail infections cause discoloration of the nail and thickening of the nail plate.

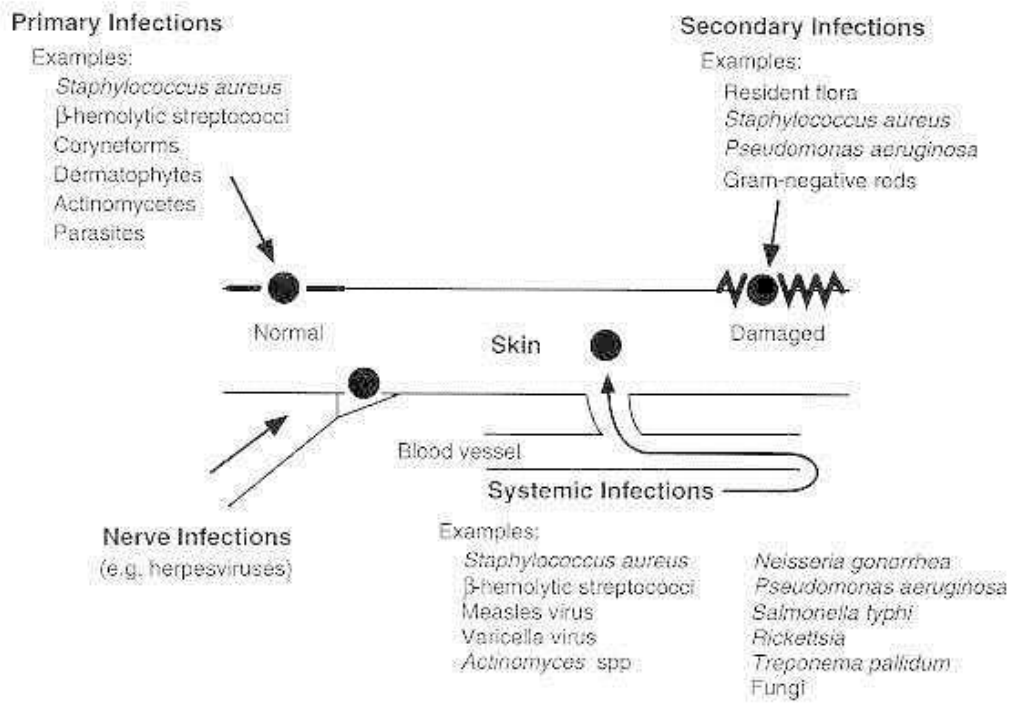
Microbiologic Diagnosis. Clinical examination and staining and/or culturing of a specimen of pus or exudate are often adequate for diagnosis. Ultraviolet light (Wood's lamp) is helpful in diagnosing erythrasma and some toe web and fungal infections. Microscopic examination of a KOH preparation of skin scales, nail scrapings, or loose hair is useful for fungal infections. For viral infections, stained smears of vesicle fluid are examined under the microscope for typical cytopathology.

Prevention and Treatment. Cleansing and degerming the skin with a soap or detergent containing an antimicrobial agent may be useful. Drying agents, such as aluminum chloride, and keratinolytic agents, such as topical salicylate, are also helpful. Topical antimicrobial agents can be used for some infections, but systemic therapy may be necessary for patients with extensive disease.

Introduction. Skin diseases are caused by viruses, rickettsiae, bacteria, fungi, and parasites. This chapter focuses on the common bacterial diseases of skin. Viral infections are also described, but of the cutaneous fungal diseases, only nail infections are included. The other fungal diseases are described in the Mycology section.

Skin Infections. Skin infections may be either primary or secondary (Fig. 98-1). Primary infections have characteristic morphologies and courses, are initiated by single organisms, and usually occur in normal skin. They are most frequently caused by *Staphylococcus aureus*, *Streptococcus pyogenes*, and coryneform bacteria. Impetigo, folliculitis, boils, and erythrasma are common examples. Systemic infections may also have skin manifestations. Secondary infections originate in

diseased skin as a superimposed condition. Intertrigo and toe web infections are examples of secondary infections.



Spread of infections to skin.

Clinical manifestations vary from disease to disease. Most skin diseases involve erythema, edema, and other signs of inflammation. Focal accumulations of pus (furuncles) or fluid (vesicles and bullae) may form, but lesions may also be scaling without obvious inflammation.

Methods for Laboratory Diagnosis

Specimen Collection.

Bacteria. Specimens are collected with a blade or by swabbing the involved areas of the skin. When pustules or vesicles are present, the roof or crust is removed with a sterile surgical blade. The pus or exudate is spread as thinly as possible on a clear glass slide for Gram staining.

For actinomycetes, pus is collected from closed lesions by aspirations with a sterile needle and syringe. Material is collected from draining sinuses by holding a sterile test tube at the edge of the lesion and allowing the pus and granules to run into the tube. Granules are aggregates of inflammatory cells, debris, proteinaceous material and delicate branching filaments. Pus and other exudates are examined microscopically for the presence of granules.

Viruses. Vesicles are cleaned with 70 percent alcohol followed by sterile saline. Viruses are obtained by unroofing a vesicle with a needle or a scalpel blade. The fluid is collected with a swab or with a tuberculin syringe with a 26- to 27-gauge needle. The fluid obtained from fresh vesicles may contain enough viruses for culture. Direct smears are prepared by scraping cells from the base of the lesions. The cells are smeared on a slide, fixed, and stained with Giemsa or Wright stain or with specific antibodies conjugated to fluorescein or peroxidase.

Fungi. Cutaneous samples are obtained by scraping skin scales or infected nails into a sterile Petri dish or a clean envelope. For suppurative lesions of deep skin and subcutaneous tissues, aspiration with a sterile needle and syringe is recommended. Direct mounts are made by mixing a small portion of the sample in two or three drops of physiologic saline or KOH on a microscopic slide. A glass coverslip is placed over the preparation before microscopic examination.

Cultures. Most pathogenic skin bacteria grow on artificial media, and selection of the medium is important. For general use, blood agar plates (preferably 5 percent

defibrinated sheep blood) are recommended. In many situations, a selective medium combined with a general-purpose medium is recommended. For example, *Staphylococcus aureus* may overgrow *Streptococcus pyogenes* in blood agar medium when both organisms are present. When crystal violet (1 μg/ml) is added to blood agar, *S pyogenes* is selected over *S aureus*. Cultures for meningococci, gonocci, and brucellae must be incubated in a CO_2 atmosphere. If tuberculosis or fungal infection is suspected, specimens are collected on appropriate media and incubated aerobically. Viruses are cultured on tissue cultures selected for the virus that may be contained in the specimen.

Bacterial Skin Infections

The classification of bacterial skin infections (pyodermas) is an attempt to integrate various clinical entities in an organized manner.

Primary Infections

Impetigo. Three forms of impetigo are recognized on the basis of clinical, bacteriologic, and histologic findings. The lesions of common or superficial impetigo may contain group A β-hemolytic streptococci, *S aureus*, or both, and controversy exists about which of these organisms is the primary pathogen. The lesions have a thick, adherent, recurrent, dirty yellow crust with an erythematous margin. This form of impetigo is the most common skin infection in children. Impetigo in infants is highly contagious and requires prompt treatment.

The lesions in bullous (staphylococcal) impetigo, which are always caused by *S aureus*, are superficial, thin-walled, and bullous. When a lesion ruptures, a thin, transparent, varnish-like crust appears which can be distinguished from the stuck-on crust of common impetigo. This distinctive appearance of bullous impetigo results

from the local action of the epidermolytic toxin (exfoliation). The lesions most often are found in groups in a single reglon.

Ecthyma is a deeper form of impetigo. Lesions usually occur on the legs and other areas of the body that are generally covered, and they often occur as a complication of debility and infestation. The ulcers have a punched-out appearance when the crust or purulent materials are removed. The lesions heal slowly and leave scars.

Cellulitis and Erysipelas. *Streptococcus pyogenes* is the most common agent of cellulitis, a diffuse inflammation of loose connective tissue, particularly subcutaneous tissue. The pathogen generally invades through a breach in the skin surface, and infection is fostered by the presence of tissue edema. Cellulitis may arise in normal skin. However, the lesion of cellulitis is erythematous, edematous, brawny, and tender, with borders that are poorly defined.

No absolute distinction can be made between streptococcal cellulitis and erysipelas. Clinically, erysipelas is more superficial, with a sharp margin as opposed to the undefined border of cellulitis. Lesions usually occur on the cheeks.

Staphylococcal Scalded Skin Syndrome. Staphylococcal scalded skin syndrome (SSSS), also called Lyell's disease or toxic epidermal necrolysis, starts as a localized lesion, followed by widespread erythema and exfoliation of the skin. This disorder is caused by phage group II staphylococci which elaborate an epidermolytic toxin. The disease is more common in infants than in adults.

Folliculitis. Folliculitis can be divided into two major categories on the basis of histologic location: superficial and deep.

The most superficial form of skin infection is staphylococcal folliculitis, manifested by minute erythematous follicular pustules without involvement of the surrounding

skin. The scalp and extremities are favorite sites. Gram-negative folliculitis occurs mainly as a superinfection in acne vulgaris patients receiving long-term, systemic antibiotic therapy. These pustules are often clustered around the nose. The agent is found in the nostril and the pustules. *Propionibacterium acnes* folliculitis has been misdiagnosed as staphylococcal folliculitis. The primary lesion is a white to yellow follicular pustule, flat or domed. Gram stain of pus reveals numerous intracellular and extracellular Gram-positive pleomorphic rods. The lesions are more common in men than in women. The process may start at the age when acne usually appears, yet most cases occur years later.

In deep folliculitis, infection extends deeply into the follicle, and the resulting perifolliculitis causes a more marked inflammatory response than that seen in superficial folliculitis. In sycosis barbae (barber's itch), the primary lesion is a follicular pustule pierced by a hair. Bearded men may be more prone to this infection than shaven men.

A furuncle (boil) is a staphylococcal infection of a follicle with involvement of subcutaneous tissue. The preferred sites of furuncles are the hairy parts or areas that are exposed to friction and macerations. A carbuncle is a confluence of boils, a large indurated painful lesion with multiple draining sites.

Erysipeloid. Erysipeloid, a benign infection that occurs most often in fishermen and meat handlers, is characterized by redness of the skin (usually on a finger or the back of a hand), which persists for several days. The infection is caused by *Erysipelothrix rhusiopathiae*.

Pitted Keratolysis. Pitted keratolysis is a superficial infection of the plantar surface, producing a punched-out appearance. The pits may coalesce into irregularly shaped areas of superficial erosion. The pits are produced by a lytic process that spreads

peripherally. The areas most often infected are the heels, the ball of the foot, the volar pads, and the toes. Humidity and high temperature are frequent aggravating factors. Gram-positive coryneform bacteria have been isolated from the lesions.

Erythrasma. Erythrasma is a chronic, superficial infection of the pubis, toe web, groin, axilla, and inframammary folds. Most lesions are asymptomatic, but some are mildly symptomatic with burning and itching. The patches are irregular, dry and scaly; initially pink and later turning brown. The widespread, generalized form is more common in warmer climates. *Corynebacterium minutissimum* is the agent. Because of its small size, the organism is difficult to observe in KOH preparations of infected scales; however, it is readily demonstrable by Gram staining of the stratum corneum. Coral red fluorescence of the infected scales under Wood's light is diagnostic.

Trichomycosis. Trichomycosis involves the hair in the axillary and pubic regions and is characterized by development of nodules of varying consistency and color. The condition is generally asymptomatic and not contagious. Underlying skin is normal. Infected hairs obtained for microscopic examination are placed on a slide in a drop of 10 percent KOH under a coverslip. The nodules on the hairs are composed of short bacillary forms. Three types of coryneforms are associated with trichomycosis; one resembles *C minutissimum*, one is lipolytic, and the third is *C tenuis*.

Secondary Infections

Intertrigo. Intertrigo is most commonly seen in chubby infants or obese adults. In the skin fold, heat, moisture, and rubbing produce erythema, maceration, or even erosions. Overgrowth of resident or transient flora may produce this problem.

Acute Infectious Eczematoid Dermatitis. Acute infectious eczematoid dermatitis arises from a primary lesion such as a boil or a draining ear or nose, which are sources of infectious exudate. A hallmark of this disease is a streak of dermatitis along the path of flow of the discharge material. Coagulase-positive staphylococci are the organisms most frequently isolated.

Pseudofolliculitis of the Beard. Pseudofolliculitis of the beard, a common disorder, occurs most often in the beard area of black people who shave. The characteristic lesions are usually erythematous papules or, less commonly, pustules containing buried hairs. This occurs when a strongly curved hair emerging from curved hair follicles reenters the skin to produce an ingrown hair. Gram-positive microorganisms that belong to the resident flora are associated with this disorder—a clear illustration of the opportunism of nonpathogenic bacteria when the host defense is impaired.

Toe Web Infection. The disease commonly referred to as athlete's foot has traditionally been regarded as strictly a fungal infection. This assumption has been revised, however, because fungi often cannot be recovered from the lesions throughout the disease course. Researchers now believe that the dermatophytes, the first invaders, cause skin damage that allows bacterial overgrowth, which promotes maceration and hyperkeratosis. The fungi, through the production of antibiotics, then create an environment that favors the growth of certain coryneform bacteria and *Brevibacterium*. Proteolytic enzymes, which are produced by some of these bacteria, may aggravate the condition. If the feet become superhydrated, resident Gram-negative rods become the predominant flora, and the toe webs incur further damage. The fungi are then eliminated either by the action of antifungal substances of bacterial origin or by their own inability to compete for nutrients with the vigorously growing bacteria.

Other Bacterial Skin Diseases

Skin Tuberculosis (Localized Form). Localized skin tuberculosis may follow inoculation of *Mycobacterium tuberculosis* into a wound in individuals with no previous immunologic experience with the disease. The course starts as an inflammatory nodule (chancre) and is accompanied by regional lymphangitis and lymphadenitis. The course of the disease depends on the patient's resistance and the effectiveness of treatment. In an immune or partially immune host, two major groups of skin lesions are distinguished: tuberculosis verrucosa and lupus vulgaris.

***Mycobacterium marinum* Skin Disease.** Many cases of *M marinum* skin disease occur in children and adolescents who have a history of using swimming pools or cleaning fish tanks. Often, there is a history of trauma, but even in the absence of trauma the lesions appear frequently on the sites most exposed to injury. The usually solitary lesions are tuberculoid granulomata that rarely show acid-fast organisms. The skin tuberculin test is positive.

***Mycobacterium ulcerans* Skin Disease.** Lesions in *M ulcerans* skin disease occur most often on the arms or legs and occasionally elsewhere, but not on the palms or soles. Most patients have a single, painless cutaneous ulcer with characteristic undermined edges. Geographic association of the disease with swamps and watercourses has been reported. In some tropical areas, chronic ulcers caused by this organism are common.

In scrofuloderma, tuberculosis of lymph nodes or bones is extended into the skin, resulting in the development of ulcers.

A disseminated form of the disease occurs when bacteria are spread through the bloodstream in patients who have fulminating tuberculosis of the skin. When

hypersensitivity to tubercle bacilli is present, hematogenously disseminated antigen produces uninfected tuberculous skin lesions such as lichen scrofillosllls.

Actinomycetoma. There are several agents of actinomycetoma. About half of the cases are due to actinomycetes (actinomycetoma); the rest are due to fungi (eumycetoma). The most common causes of mycetoma in the United States are *Pseudallescheria (Petriel lidium) boydii* (a fungus) and *Actinomyces israelii* (a bacterium). Regardless of the organism involved, the clinical picture is the same. Causative organisms are introduced into the skin by trauma. The disease is characterized by cutaneous swelling that slowly enlarges and becomes softer. Tunnel-like sinus tracts form in the deeper tissues, producing swelling and distortion, usually of the foot. The draining material contains granules of various sizes and colors, depending on the agent.

Actinomycosis. *Actinomyces israelii* usually is the agent of human actinomycosis; *Arachnia propionica (Actinomyces propinicus)* is the second most common cause. The characteristic appearance of the lesion is a hard, red, slowly developing swelling. The hard masses soften and eventually drain, forming chronic sinus tracts with little tendency to heal. The sinus tracts discharge purulent material containing "sulfur" granules. In about 50 percent of cases, the initial lesion is cervicofacial, involving the tissues of the face, neck, tongue, and mandible. About 20 percent of cases show thoracic actinomycosis, which may result from direct extension of the disease from the neck or from the abdomen or as a primary infection from oral aspiration of the organism. In abdominal actinomycosis, the primary lesion is in the cecum, the appendix, or the pelvic organs.

Treatment of the Pyodermas

General Considerations. Debriding superficial pyoderma and then repeatedly cleansing the exposed lesions with topical antiseptics such as chlorhexidine removes the source of infection and minimizes its spread to adjacent skin sites or to other patients. Many secondary superficial skin infections, such as the web infections, will clear with simple twice-daily cleansing. For foot infections, the patient should wear open shoes or sandals, which permit air circulation. Aluminum chloride, a drying agent, inhibits overgrowth of opportunistic bacteria in foot, perineal, and axillary areas. Keratinolytic agents (e.g., topical salicylates) remove hyperkeratotic lesions that harbor pathogens, improving the exposure of the infected skin surface to other topical treatments.

Topical Treatment. Topical antibiotics contain a combination of neomycin, bacitracin, and polymyxin. Some newer preparations contain mupirocin, gramicidin, or erythromycin, and others combine these antibiotics with steroids. For an informed, cooperative patient suffering only minimal disease, topical antibiotics are often preferred to oral antibiotics because of the adverse reactions associated with systemic therapy.

Systemic Therapy. Systemic treatment with antibiotics is mandatory for extensive pyoderma. Systemic antibiotics can be administered orally or parenterally. Oral therapy is sufficient for most extensive dermal infections, but the parenteral route is preferred for severe infections.

A wide range of antibiotics for systemic therapy of pyoderma is available. The choice of a specific antibiotic should be based on two factors: isolation and identification of the pathogen, and the depth and extent of infection. In this costconscious world one must also relate efficacy to consumer cost. Many less expensive antibiotics are just as effective against a given pathogen as the most expensive drugs with wider spectra.

Viral Skin Diseases

Viral skin diseases can produce both localized and generalized skin infections. Viruses from several major groups cause skin lesions.

Herpes Simplex Virus. Herpes simplex virus infection is probably the most common viral skin disease. Almost the entire adult population has had herpes simplex at one time or another. Herpes simplex virus, a DNA virus, is the agent. There are two types of herpes simplex virus. Type 1 is usually associated with nongenital lesions, whereas type 2 is recovered from genital lesions. The incidence of type 1 genital infections in young patients has recently increased.

Poxviruses. The viruses that cause smallpox, vaccinia, and cowpox are closely related; all are large DNA viruses. The smallpox virus is now extinct. Cowpox virus causes an infection of cattle that is acquired by handling infected animals. Vaccinia viruses are vaccine strains developed in the laboratory and adapted to grow in the skin of humans, rabbits, and calves. Several clinical manifestations may occur in individuals who were vaccinated against smallpox with vaccinia virus. The main problem with vaccinia virus arose when it became desirable to vaccinate a person already suffering from eczema or other skin diseases. Vaccination may produce eczema vaccinatum. Molluscum contagiosum also is caused by a poxvirus and is characterized by numerous small, pink nodules, most often on the face, genitalia, or the rectal area. Lesions also occur on the back, arms, buttocks, and inner thighs. The disease is generally harmless and self-limiting.

Papillomaviruses. Human papillomaviruses cause warts. Verruca vulgaris occurs commonly on hands and fingers as single or multiple lesions. These warts are generally painless, firm, dry, and rough. They may remain stable or regress

spontaneously. Verruca plantaris (plantar wart) is a clinical variety of verruca vulgaris that occurs on the sole of the foot.

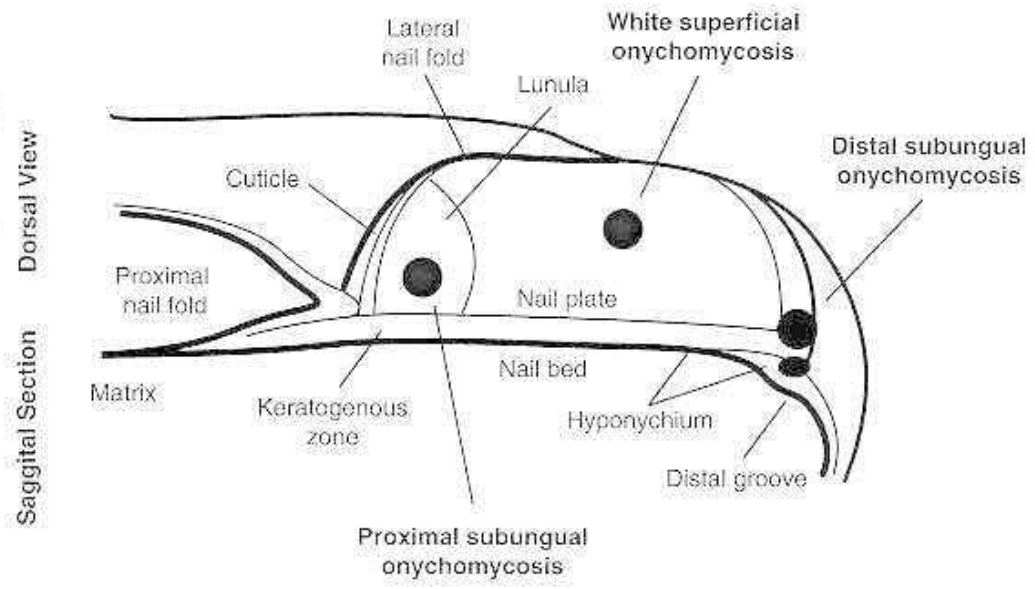
During standing, walking, and running, these warts push into the skin and may be painful. Genital warts appear as large lesions of red, soft masses that may coalesce. Verruca plana juvenilis (also known as juvenile flat warts) occurs most commonly in children. The lesions are in groups and may appear on the face, neck, back of the hands, and arms. These warts may also occur in adults.

Treatment. Because of the limited number of effective antiviral agents, prevention is important. Oral and intravenous acyclovir is effective for treatment of primary herpesvirus infection and for recurrent genital herpes and herpes zoster in immunosuppressed persons.

Fungal Skin Diseases. Several genera of fungi are responsible for diseases of the skin. This group of fungi, known collectively as dermatophytes, is discussed in the chapters on mycology. Some nondermatophytes, including yeasts, can also cause skin infections.

Nail

The nail consists of four epidermal components: the matrix, proximal nailfold, nailbed, and hyponychium. The matrix is close to the bony phalanx. The horny end product of the matrix is the nail plate, which migrates distally over the nailbed. The distal portion of the matrix, the lunula, is visible as a white, crescent-shaped structure. The proximal nailfold is a modified extension of the epidermis of the dorsum of the finger, which forms a fold over the matrix; its horny end product is the cuticle. The nailbed is an epidermal structure that begins at the distal margin of the lunula and terminates in the hyponychium, which is the extension of the volar epidermis under the nail plate. It ends adjacent to the nailbed.



Longitudinal section (diagrammatic sketch) of fingernail.

Fungal Infections of the Nails. Onychomycoses are infections of the nails by fungi. Universally recognized agents of these diseases are species of *Trichophyton, Microsporum* (rarely), and *Epidermophyton*. These dermatophytes are commonly called ringworm fungi. Nondermatophytic fungi also occasionally cause onychomycoses, but usually cause only toenail problems; they rarely affect the fingernails.

Treatment of Nail Diseases

Onychomycosis. Superficial types of onychomycosis may be successfully treated. Mechanical scraping of the chalky white material on the nail plate and application of topical antifungal agents such as miconazole, ciclopirox olamine, or clotrimazole are recommended. Newer therapeutic nail lacquers are being tested in the United States. Distal subungual and proximal subungual onychomycosis infections are much more

difficult to treat. Oral griseofulvin may be required to bring about clearing of the fingernail. For toenails with extensive involvement, oral itraconazole, fluconazole and terbinafine are effective. No oral or topical medication is effective in eliminating nondermatophyte mold infection of the nails.

Bacterial Nail Infections. *Pseudomonas aeruginosa* is associated with green nail syndrome, which is essentially a greenish discoloration of the nail plate. Attempts to culture *Pseudomonas* from the deep section of the nail have not been successful; however, *P aeruginosa* has been isolated on cultures of specimens from the paronychia (inflammatory lesion around the margin of a nail). Whether there is true invasion of the nail plate by the bacteria or just diffusion of the pigment into the nail plate is not certain. Black paronychia is associated with *Proteus* species. Staphylococci and streptococci may be found as secondary invaders.

Microbiology of Dental Decay and Periodontal Disease

General Concepts

Bacteriology of Dental Infections. The mouth is colonized by 200 to 300 bacterial species, but only a limited number of these species participate in dental decay (caries) or periodontal disease.

Dental Decay. Dental decay is due to the irreversible solubilization of tooth mineral by acid produced by certain bacteria that adhere to the tooth surface in bacterial communities known as dental plaque.

Etiology. *Streptococcus mutans* is the main cause of dental decay. Various lactobacilli are associated with progression of the lesion.

Pathogenesis. The tooth surface normally loses some tooth mineral from the action of the acid formed by plaque bacteria after ingestion of foods containing fermentable carbohydrates. This mineral is normally replenished by the saliva between meals. However, when fermentable foods are eaten frequently, the low pH in the plaque is sustained and a net loss of mineral from the tooth occurs. This low pH selects for aciduric organisms, such as *S mutans* and lactobacilli, which (especially *S mutans*) store polysaccharide and continue to secrete acid long after the food has been swallowed.

Clinical Manifestations. Caries become intensely painful when the lesion approaches the tooth pulp.

Microbiologic Diagnosis. New, chair-side culture procedures allow for an estimate of the number of *S mutans* organisms in saliva.

Prevention and Treatment. The widespread use of fluoride in the water supply, in dentifrices, and in local applications by the dentist has reduced the prevalence of caries by 30 to 50 percent among young people in many industrialized countries. In clinical trials, the use of topical antimicrobial agents to eradicate diagnosed *S mutans* infections usually significantly reduces decay.

Periodontal Disease

Definition. Periodontal disease can establish itself when the gums detach from the teeth as a result of an inflammatory response to plaque.

Etiology. Periodontal infections are usually mixed, most often involving anaerobes such as *Treponema denticola* and *Porphyromonas gingivalis*. The microaerophile *Actinobacillus actinomycetemcomitans* causes a rare form known as localized juvenile periodontitis.

Pathogenesis. Plaque bacteria elaborate various compounds (H_2S, NH_3, amines, toxins, enzymes, antigens, etc.) that elicit an inflammatory response that is protective but also is responsible for loss of periodontal tissue, pocket formation, and loosening and loss of teeth.

Clinical Manifestations. There is no apparent pain until very late when abscesses may occur. Bleeding gums and bad breath may occur.

Microbiologic Diagnosis. Microbiologic diagnosis is usually not sought. Spirochetes and other motile organisms are found upon dark-field microscopic examination. Immunologic reagents, DNA probes and enzyme assays have been developed for *P gingivalis, T denticola, Bacteroides forsythus, A actinomycetemcomitans* and other organisms.

Prevention and Treatment. Daily toothbrushing and regular professional cleanings by the dentist appear to be adequate to prevent periodontal disease. Rigorous debridement of tooth surfaces is the standard treatment. Often, some form of surgery is used to improve access to root surfaces. Recent studies suggest that short-term use of antimicrobial agents, especially metronidazole and doxycycline, is beneficial.

Introduction. The tooth surfaces are unique in that they are the only body part not subject to metabolic turnover. Once formed, the teeth are, under the correct conditions, essentially indestructible, as witnessed by their importance in fossil records and forensic medicine. Yet in the living individual, the integrity of the teeth is assaulted by a microbial challenge so great that dental infections rank as the most universal affliction of humankind. The discomfort caused by these infections and their enormous cost (dental infections rank third in medical costs, behind heart disease and cancer, in the United States) gives dental diseases prominence despite their non-life-threatening nature.

This chapter reviews the bacterial aspects of dental caries and periodontal disease and suggests that, in the future, treatment will be directed toward eliminating or suppressing certain bacterial species that appear to be overt pathogens in the dental plaque.

Dental Caries (Decay). Dental decay is due to the dissolution of tooth mineral (primarily hydroxyapatite, Ca_{10} $(P0_4)_6(0H)_2$) by acids derived from bacterial fermentation of sucrose and other dietary carbohydrates. These bacteria live in bacterial communities known as dental plaque which accumulates on the tooth surface. For almost a century it was believed that any bacterial community on the tooth surface could cause decay, and treatment was almost exclusively the mechanical cleaning of these surfaces by toothbrushing, using some type of mild abrasive. Such treatments based upon debridement and, in extreme cases, upon dietary carbohydrate restriction, were singularly unsuccessful in reducing dental decay. In fact, the prevalence of dental decay was so high among young men that it was the major cause of rejection from military service in World Wars I, II, and the Korean War. This staggering amount of dental morbidity led to the formation of dentistry as a separate health profession in the late 19th century; to the expectation that all people would, if they lived long enough, be edentulous (toothless); and to a dental health bill to the public of approximately 34 billion dollars per year in 1990.

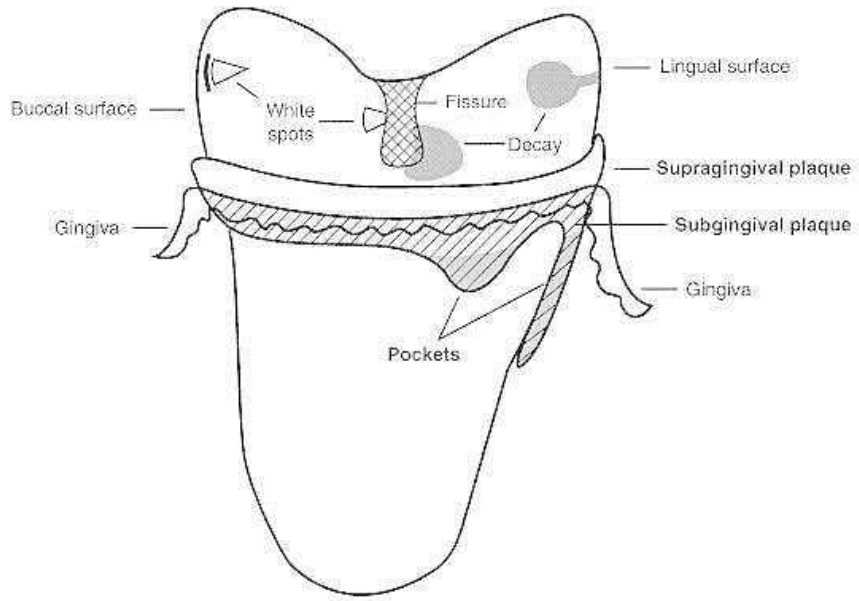
Things have changed. Water fluoridation has proven to be a most cost-effective way of reducing decay; fluoride dentifrices were even more effective than initially projected; and research findings indicate that most carious lesions actually reflect a sucrose-dependent *Streptococcus mutans* infection. Individuals at risk for this infection can be diagnosed and treated by frequent mechanical intervention, by intensive application of prescription levels of fluorides or other antimicrobials (such as chlorhexidine), by restriction of ingestion of sucrose between meals, or by use of products that contain sucrose substitutes (such as xylitol). The net result is that

dental decay in the late 20th century is a controllable infection and should be preventable in many individuals. Almost 50% of young children are caries-free, and the level of edentia among individuals over 65, has dropped from 50% to about 20%.

Etiology. Dental decay has been known since recorded history, but was not an important health problem until sucrose became a major component of the human diet. When sucrose is consumed frequently, an organism known as *Streptococcus mutans* emerges as the predominant organism, and it is this organism that has been uniquely associated with dental decay.

In 1924 *S mutans* was isolated from human carious lesions, but subsequently was not thoroughly studied until the 1960s when it was re-identified as the etiologic agent of a transmissible caries infection in rodent models. In these studies, all of Koch's postulates for infectivity were fulfilled in animal models. However, it proved difficult to show that *S mutans* was a human dental pathogen, because *S mutans* appears to be a member of the normal flora on the teeth, and it was difficult to show that an increase in *S mutans* actually preceded and/or coincided with the earliest clinical lesion.

Dental decay is measured clinically as a cavitation on the tooth surface. However, cavitation is a late event in the pathogenesis of decay, being preceded by a clinically detectable subsurface lesion known as a white spot, and prior to that by subsurface demineralization that can only be detected microscopically. From a diagnostic and treatment perspective, the lesion should be detected at the white spot stage. This usually cannot be done without rigorous descriptive criteria (not all white spots are due to the decay process) and because the white spot stage in the caries-prone fissures and approximal surfaces of the tooth cannot be directly visualized during a dental examination.



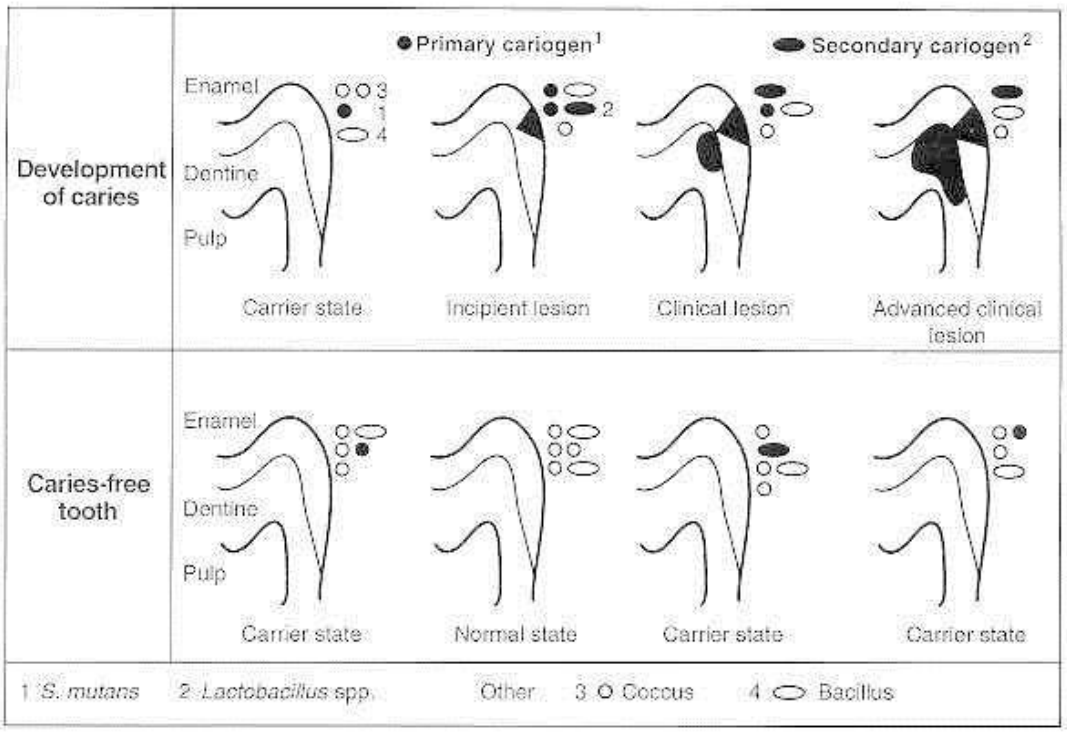
Schematic drawing of a cross-section of tooth showing decay and white spot lesions.

The prevalence of dental morbidity is documented in terms of the number of teeth (T) or tooth surfaces (S) that have obvious decay (D), contain a dental restoration or filling (F), or are missing (M). These DMF teeth (DMFT) and DMF surface (DMFS) scores do not discriminate as to the relative proportions of the score due to decay, versus fillings and extractions. This insensitivity of the DMFT and DMFS scores in quantitating the actual decay, independent of morbidity led in early clinical studies to unimpressive associations between *S mutans* and DMFT or DMFS scores. However, when the comparison was limited to individuals with decayed teeth or when the plaque samples were taken from a decayed tooth site, a significant association between *S mutans* and decay was evident.

This association is clearly seen in individuals who developed xerostomia secondary to radiation treatment of head and neck cancer. *S mutans* and lactobacilli are normally present in low numbers in the plaque of these individuals. When the

salivary flow decreases, the pH in the plaque drops, leading to a selection for aciduric (acid-tolerant) bacteria, such as *S mutans* and lactobacilli. New decayed lesions become obvious within 3 months after radiotherapy and the patient may average one or more new decayed surfaces per post-radiation month. During the development of decay, the proportions of, first, *S mutans* and then lactobacilli increased significantly. This sequence of events indicated that *S mutans* was involved with the initiation of decay, whereas the lactobacilli were associated with the progression of the lesion.

This bacterial succession is illustrated in the following figure, which shows the sequence of events occurring on the surface of a caries-free tooth that either becomes carious or remains caries free. In either case, the tooth surface initially represents a carrier state relative to harboring a primary cariogen, such as *S mutans*, in the plaque on a smooth surface. The proportion of the cariogen in the flora is similar in both cases, but the location of *S mutans* differs within the plaques. In the tooth destined to develop decay, *S mutans* is located on the enamel surface, whereas in the tooth destined to remain caries free, *S mutans* is confined to the saliva-plaque interface. Debriding procedures, such as toothbrushing and flossing, might remove most plaque organisms, but could leave untouched those bacteria either firmly attached to the enamel surface or sequestered in defects in the enamel surface. In surfaces destined to become carious, the residual organisms would include *S mutans*, whereas in surfaces destined to remain caries free, *S mutans* would be absent. Over time these caries-free surfaces might alternately acquire and lose *S mutans*, thereby having an intermittent carrier-state status. However, in those surfaces in which caries will eventually develop, *S mutans* becomes a dominant member of the flora, undoubtedly secondary to frequent sucrose ingestion.



Relationship between location of cariogenic bacteria and development of dental caries.

The incipient or white spot lesion occurs when the acidogenic activity of the cariogen causes tooth mineral to be mobilized from the subsurface enamel to buffer the pH at the plaque-enamel interface. Bacteriologic sampling at this stage should reveal both a proportional and an absolute increase in the levels of *S mutans*. When the lesion progresses to the stage of cavitation, the organisms penetrate into the enamel crystals. Also, secondary cariogens, such as the lactobacilli, appear as a result of the selection for aciduric organisms in the plaque. When the lesion reaches the advanced clinical stage, conditions may be such that *S mutans* can no longer survive, and only secondary cariogens like the lactobacilli and opportunistic organisms can be found. This model predicts that a bacterial succession occurs during the progression of a carious lesion and that the flora of the advanced lesion may bear little resemblance to the flora of the incipient lesion. Thus it was necessary

to sample the plaque during the initial lesion or white spot stage to find the etiologic agents of decay. When this was done, *S mutans* dominated in the flora. However, for the lesion to progress to the stage of cavitation, lactobacilli seem necessary. Thus while *S mutans* could be isolated from both progressive and nonprogressive lesions, *L casei* could only be isolated from progressive lesions.

Pathogenesis. These clinical studies indicated that of the 200 to 300 species which can be isolated from plaque, only *S mutans*, and to a lesser extent the lactobacilli, can be consistently associated with dental decay. What makes these organisms cariogenic relative to all other bacterial types found in the plaque?

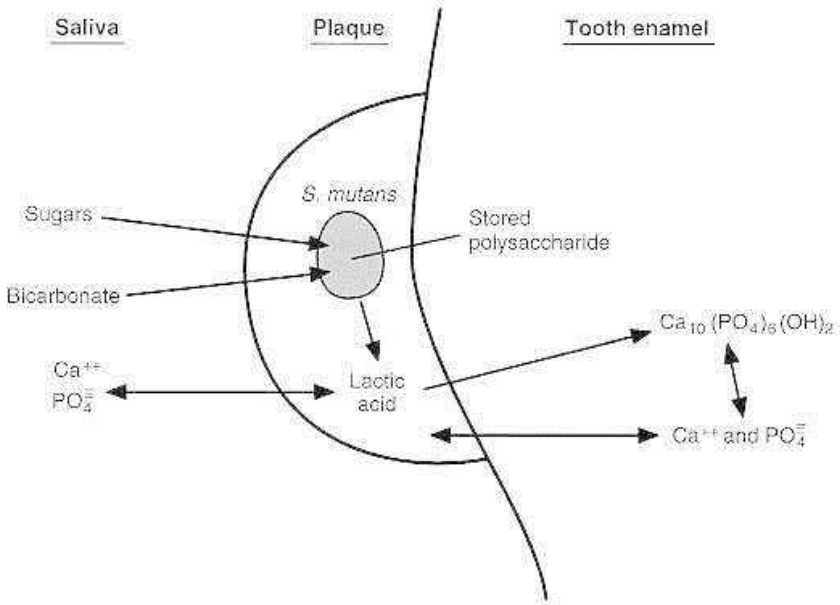
In the 19th century, microbial acid production from dietary substrates was linked to the etiology of dental decay in what was called the chemoparasitic theory of decay. But researchers were not able to associate any single acidogenic species with decay, and concluded that decay was bacteriologically nonspecific and due to the increased amounts of acid formed when bacteria accumulated in plaque on the tooth surfaces. It was noted that decay occurred at retentive sites on the teeth and recommended mechanical debridement of these sites as the best method of reducing decay. While the clinical observations were correct, there was no way of determining that the retentive sites were caries prone because they provide the micro-environment which selects for *S mutans* and lactobacilli. In this section we shall examine those attributes of *S mutans* and the lactobacilli that enable them to be successful on retentive sites and show that these attributes constitute, in effect, the virulence factors which make these organisms specific odontopathogens.

Sucrose in the diet. Considerable evidence from epidemiologic observations and animal experiments indicates that, shortly after sucrose is introduced into the diet, a notably higher incidence of decay occurs. The relationship between sucrose ingestion and dental caries is reasonably well understood. The supragingival plaque

flora derives its nutrients from various sources that include diet, saliva, sloughed epithelial cells, dead microbes, and gingival crevice fluid or exudate. All sources, except the foods in the diet provide only small amounts of nutrients. Dietary components are normally high-molecular-weight polymers (such as starch and proteins) that are in the mouth for short periods. They have a minimal effect on plaque growth except in those instances when food is retained between and on the teeth. Sucrose, however, changes this pattern because it is a low-molecular-weight disaccharide that can be rapidly sequestered and utilized by the plaque flora. Plaque organisms capable of fermenting sucrose have a decided advantage over the non-sucrose fermenters in that they can proliferate during periods of sucrose ingestion and thereby become the dominant plaque organisms.

Sucrose fermentation produces a rapid drop in the pH, to 5.0 or lower, at the point of interface between plaque and enamel. When sucrose is ingested during meals, sufficient saliva is secreted to buffer the plaque pH and decay does not occur. In fact, studies show that as much as one-half of a pound of sucrose consumed daily at meals for two years was not associated with an increase in dental decay; however, when the same or lesser amounts of sucrose were ingested between meals, subjects developed new decay at the rate of about three to four tooth surfaces per year. The frequent ingestion of sucrose has been shown to increase the lengths of time that sucrose could be detected in the saliva. This means that if this sucrose were available for microbial fermentation in the plaque, low plaque pHs would be present for long periods each day. When the plaque pH value falls below 5.0-5.2, the salivary buffers are overwhelmed and as lactic acid diffuses into the tooth, enamel begins to dissolve, releasing Ca and PO_4 ions from sites beneath the surface enamel. Normally, the bathing saliva replenishes these minerals, but if the length of the flux from the enamel is great, repair does not occur and cavitation results. Thus, sucrose

consumption per se does not cause decay, but the frequent ingestion of sucrose by prolonging the time period by which the plaque is acidic, is cariogenic.



Pathogenesis of dental decay.

Plaque bacteria that ferment sucrose produce acids, which *in vitro* lower the pH value to below 5.0. However, only *S mutans* of all these species reliably caused decay in germ-free animals fed a high-sucrose diet. This suggested that microbial acid production was not the exclusive determinant of decay and that *S mutans* had to possess other attributes which were responsible for its virulence. *S mutans* was subsequently shown to metabolize sucrose in a remarkably diverse fashion that is not matched by any other known plaque organism. The major pathway is concerned with energy metabolism; in this process, the enzyme invertase splits sucrose into its component glucose and fructose molecules, which are then converted to lactic acid by the glycolytic pathway. Other enzymes, called glucosyltransferases, split sucrose but transfer the glucose moiety to a glucose polymer known as a glucan. *S mutans*

forms several complex glucans that differ in their core linkage, amount of branching, and molecular weight. The first glucan identified had a core linkage consisting of an a1–6 bond that classified it as a dextran. Later, a unique glucan having an a1–3 core linkage was identified and given the name mutan. *S mutans* also has enzymes that split sucrose and transfer the fructose moiety to a fructose polymer known as a fructan. Other plaque bacteria can use sucrose to synthesize one or more of these polymers, with the exception of mutan. Only *S mutans* can form all of them, a fact that led to an inquiry into the relationship between polymer production and caries formation.

A series of *in vitro* experiments showed that the glucans enable *S mutans* to adhere to surfaces. This suggested that *in vivo* these adhesive polymers would enable *S mutans* to adhere tenaciously to the tooth surface and to accumulate on these surfaces, thereby causing decay in the underlying surface.

Animal experiments in which rodents were infected with mutants of *S mutans* that lacked the ability to form either dextran or mutan, indicated that the absence of mutan was associated with a greater reduction in smooth surface decay than was the absence of dextran. In each instance, the amount of pit and fissure decay was not significantly affected by these mutations. Decay on smooth surfaces seems to depend on the retentive polymers formed by *S mutans*, whereas in sites where retention is provided by the anatomy of the teeth (pits, fissures, and contact points between teeth), these polymers are not as important. Accordingly, pit and fissure decay may be caused simply by any acidogenic organism that can survive in these retentive sites.

This nonspecific explanation does not seem completely satisfactory, because in animal models and in human caries, *S mutans,* again, is the dominant organism involved or associated with pit and fissure decay. A few other organisms, such as

Lactobacillus casei and *Streptococcus faecalis*, can cause fissure decay in germ-free rats. These three organisms are all relatively aciduric compared to other plaque bacteria; that is, they not only produce acids, but they are relatively resistant to the resulting low pH caused by acid accumulation. Lactobacilli are the most aciduric of the plaque bacteria, but these organisms only predominate by the time the carious lesion has extended into the dentin. At the time the earliest carious lesion is detected, only *S mutans* has reached significant levels and proportions. When *S mutans*, lactobacilli, and other plaque species were compared *in vitro* for their ability to ferment sucrose at different pH values, *S mutans* was found to be more active than the other bacteria at pH 5.0, and thus, it is probably most active *in vivo* at the very pH at which the teeth begin to demineralize.

This aciduricity best explains the involvement of both *S mutans* and lactobacilli in human decay. A retentive site is colonized by those organisms present in saliva. *S mutans*, although scarce in the initial inoculum (fewer than 0.1% of the initial colonizers), is selected for if the average pH value in the site is not well buffered by saliva. Frequent ingestion of sucrose-containing products predisposes toward lower pH values and thus selects for *S mutans*. When the pH remains in the vicinity of 5.0–5.5, tooth mineral is solubilized, thereby buffering the plaque and maintaining an environment suitable for growth of *S mutans*. Eventually, enough mineral is lost so that a cavitation occurs in the enamel, and if this enlarges so that it extends into the dentin, a semiclosed system is formed in which the pH value drops below 5.0. Under these acidic conditions, growth of lactobacilli is favored, and these organisms succeed as the predominant flora in the carious lesion.

Clinical Manifestation. Dental decay occurs at discrete sites on the surface of the enamel. Progress through the enamel is usually slow because of the remineralizing action of the saliva, and is asymptomatic. When decay spreads into the dentin, the process accelerates, most likely because the very low pH that can arise in this

semiclosed environment denatures the collagen scaffold that holds the hydroxyapatite salts in place and rapidly solubilizes them. When the dentinal decay approaches the innervated tooth pulp, the pain can be intermittent or continuous, and dull or excruciating. Pain is the chief complaint of the patient.

Microbiologic Diagnosis. A microbiologic diagnosis for a *S mutans*/lactobacilli infection is rarely sought, primarily because the acute pain that brings the patient to the dentist is almost always relieved by a dental restoration or extraction. Thus, the knowledge of an underlying *S mutans* infection would not change the treatment. However, microbiologic diagnosis would be advantageous in the management of the patient to prevent or minimize future decay. Such situations would occur whenever an expensive treatment is planned, such as orthodontic treatment, or the placement of dental crowns and bridges to replace missing teeth. Microbiologic examination would also be useful at the end of any restorative treatment to determine the residual level of *S mutans* and lactobacilli colonization on the teeth.

Scandinavian investigators have empirically determined that 10^6 CFU *S mutans* per milliliter of stimulated saliva can be associated with future caries activity. Accordingly, they have recommended active intervention with fluoride, dietary counseling, and antimicrobial agents in individuals so infected. They have designed simple chairside tests that can, in a semiquantitated manner provide information on the salivary levels of *S mutans*. All of these tests rely on the fact that *S mutans* is resistant to 5 ug/ml of bacitracin and that it will grow in the presence of 20% sucrose. In liquid media containing these additives, *S mutans* will form adherent colonies on the side of glass, plastic strips, or any other solid surfaces that are present.

In a practical application of these tests, the clinician would not place orthodontic bands on an individual with 10^6 CFU of *S mutans,* because this individual would be

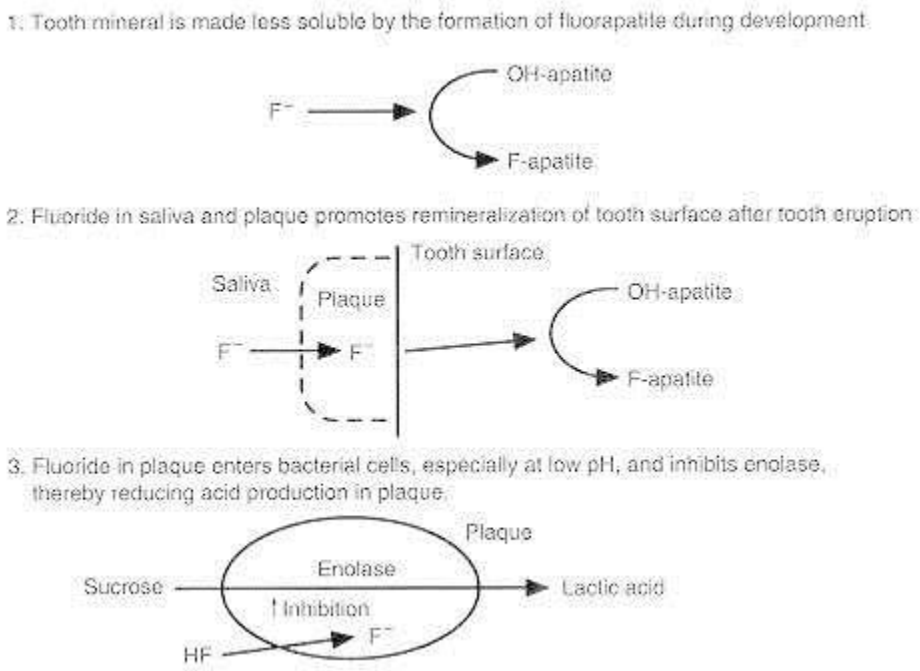
apt to develop decay around the margins of the bands. Likewise an individual who is having extensive bridgework (the placing of dental restorations across an edentulous space) would be at risk of developing new decay around the margins of these restorations. In both instances, the patient needs to be treated for *S mutans* infection prior to the placement of the dental devices or restorations.

Prevention and Treatment. Conventional dental therapy has not yet incorporated any microbiologically-based strategy into its armamentarium. Instead, a treatment based on response to symptoms has prevailed. The bankruptcy of this approach, which depends on a turn-of-the-century biologic base, has been demonstrated in the Scandinavian countries, where a socialized dental delivery system has made quality dentistry available to everyone. Because of the emphasis on treatment rather than prevention, the results have only prolonged the life span of the tooth by about 10 years, a rather poor therapeutic result. In England, where the health care system also emphasized treatment rather than prevention, one-half of the people over 35 years of age were edentulous in the 1970s. The Scandinavians, especially in Sweden, have changed their approach and have instituted plaque prophylactic programs for children and adults. Thorough dental cleaning with a 5% fluoride paste given at 2–4 week intervals combined with oral hygiene education, has lowered dental decay in children by about 80%–90%, compared to youngsters receiving symptomatic treatment. (Symptomatic treatment involves placing dental restorations in an obviously carious tooth, and pulling teeth.) Similar success has been achieved in adults with and without periodontal disease.

Thorough cleaning with fluoride apparently selects for the more desirable bacterial types, such as *S sanguis* and *S mitis*, which are capable of rapidly colonizing the tooth surfaces. *S mutans* presumably does not have an opportunity to become dominant, because the frequent debridement neutralizes its ability to be selected for

by the low pH values that characterize an undisturbed plaque. Also, the 5% fluoride paste has an immediate bacteriostatic effect on the plaque organisms.

Fluoride as an Antimicrobial Agent in Plaque. The mechanisms by which fluoride prevents decay are multiple, and the relative contributions of each mechanism are not fully understood. The 30%–50% reduction in decay that follows water fluoridation is generally attributed to the fluoride replacing hydroxyl groups in the tooth crystal, thereby forming fluorapatite. Fluorapatite is less soluble in acid than hydroxyapatite, which means that a tooth containing fluorapatite dissolves slowly in the low pH value found in plaque, and accordingly, remineralizes faster in the intervals between sugar ingestion. These explanations do not completely account for the proved efficacy of topically-applied fluorides and raises questions about other modes of fluoride action.



Anti-caries mechanisms of fluoride.

The fluoride ion (F^-) inhibits the bacterial enzyme enolase, thereby interfering with production of phosphoenolpyruvate (PEP). PEP is a key intermediate of the glycolytic pathway and, in many bacteria, is the source of energy and phosphate needed for sugar uptake. The presence of 10–100 ppm of F^-, inhibits acid production by most plaque bacteria. These levels are delivered easily by most prescription fluoride preparations, such as were used in the Swedish studies. Of equal interest is the finding that at acidic pH values (5.5 or below), low levels of F^- (1–5 ppm) inhibit the oral streptococci. These levels are found in plaque, especially in individuals who drink fluoridated water or who use fluoridated dentifrices. If this plaque fluoride is derived from the tooth, an antibacterial mode of action, which involves a depot effect, can be postulated for systemic (water) and topical fluoride administration.

The depot effect comes about in this manner. Water fluoridation promotes the formation of fluorapatite, whereas topical fluorides cause a net retention by the enamel of fluoride as fluorapatite or as more labile calcium salts. Microbial acid production in the plaque may solubilize this enamel-bound fluoride, which at the prevailing low pH in the plaque microenvironment could become lethal for the acid-producing microbes. Such a sequence would discriminate against *S mutans* and lactobacilli because they, as a result of their aciduric nature, are most likely the numerically dominant acid producers at the plaque-enamel interface. The fluoridated tooth thus contains a depot of a potent antimicrobial agent that is not only released at an acid pH value but is most active at this pH value. This hypothesis, then, attributes some of the success of water fluoridation and topical fluorides to an antimicrobial effect. It further suggests that judicious use of topical fluorides would be effective in patients with highly active caries.

The most effective dose schedule and fluoride preparation have not been determined. Neutral 1.0% sodium fluoride given daily to adults, who normally

would experience rampant caries secondary to a xerostomia following irradiation for jaw cancer, has resulted in few or no caries. Controls, who were given a placebo as well as the best available hygiene instruction, averaged over two new decayed surfaces per post-radiation month. When the control patients were placed on the daily fluoride regimen, their decay rate dropped almost to zero. In another study, 5- to 6-year-old children, who had 10 or more carious tooth surfaces, were given the necessary dental restorations and either 1.2% F^- as a neutral sodium fluoride gel or a placebo gel. The gels were taken unsupervised at home, twice a day for 1 week. After 2 years, the fluoride group had about 40% less decay than the placebo group. Eleven of 20 of these formerly rampant caries children had no new decay in their permanent teeth. In these xerostomia and pediatric patients, the initially high proportions of *S mutans* were decreased by the fluoride treatments which resulted in reduced decay.

Sucrose Substitutes that Aid in Caries Control. Eating foods that contain sucrose between meals can be highly cariogenic. Dietary counseling, that instructs patients to avoid between meal snacks may help to decrease the incidence of dental decay, but only if the patients are compliant. Another dietary approach to caries control is to recommend that patients eat snack foods that contain compounds that provide the hedonistic appeal of sucrose, but are not fermented by the plaque flora to the low pH levels associated with enamel demineralization.

The least acidogenic sucrose substitutes are the polyols, such as sorbitol, mannitol, and xylitol. Few plaque bacteria can ferment these substances, and those that can (*S mutans* and *L. casei* ferment sorbitol and mannitol) exhibit a slow fermentation, because glucose catabolite repression keeps the necessary degradative enzymes at minimum levels. Xylitol, the only polyol with a sweet taste comparable to that of sucrose, and the only one that cannot be fermented by *S mutans*, has been shown to be anticariogenic when substituted for sucrose in either foods or chewing gum. In a

chewing gum study, young adults who consumed about 6 to 7 g of xylitol gum per day had, after one year, an 80% reduction in caries increment compared to a control group who consumed 6 to 7 g of sucrose gum per day. In later studies, this type of intensive use of a xylitol chewing gum was shown to decrease salivary and plaque levels of *S mutans.* When the between-meal sucrose supply is reduced, the levels of *S mutans* will decline, as the low plaque pH values that selected for them are not as dominant a factor in the plaque microecology. Thus, xylitol can satisfy the craving for sweets, discriminate against *S mutans*, and significantly reduce the incidence of dental decay.

Periodontal Disease. Periodontal disease is the general description applied to the inflammatory response of the gingiva and surrounding connective tissue to the bacterial or plaque accumulations on the teeth. These inflammatory responses are divided into two general groupings: *gingivitis* or *periodontitis*. Gingivitis is extremely common, and is manifested clinically as bleeding of the gingival or gum tissues without evidence of bone loss or deep periodontal pockets. Pocketing is the term given to the pathologic loss of tissue between the tooth and the gingiva, creating spaces that are filled by dental plaque. Periodontitis occurs when the plaque-induced inflammatory response in the tissue results in actual loss of collagen attachment of the tooth to the bone, to loss of bone, and to deep periodontal pockets which, in some cases, can extend the entire length of the tooth root (15 to 20 mm).

Periodontitis is usually graded according to the severity of the tissue loss and the number of teeth involved. Periodontitis is not as prevalent as once thought: a recent survey of American adults revealed that only 8% of the population surveyed had one tooth site with attachment loss measuring 6 mm or more. This finding was surprising, given past surveys, which indicated that almost everyone would experience advanced forms of periodontal disease as they aged, but is in agreement

with recent population surveys in other countries which show that from 5 to 15% of the population has periodontitis.

Etiology and Pathogenesis. The most important new finding concerning periodontal disease is the realization that these clinical entities are really specific infections. These infections are unusual in that massive or even obvious bacterial invasion of the tissues is rarely encountered. Rather, bacteria in the plaque touching the tissue elaborate various compounds, such as H_2S, NH_3, amines, endotoxins, enzymes (such as collagenases) and antigens, all of which penetrate the gingiva and elicit an inflammatory response. This inflammatory response, although overwhelmingly protective, appears to be responsible for a net loss of periodontal supporting tissue, and leads to periodontal pocket formation, loosening of the teeth, and eventual tooth loss. As will be described subsequently, neutrophils are extremely important in this inflammatory response and, if they are absent, as in various neutropenias, or compromised as a result of chemotherapy, an aggressive form of periodontitis is encountered. T4 helper cells play a role in this defense, as witnessed by the periodontitis encountered in patients with acquired immune deficiency syndrome.

Gingivitis. The simplest form of gingivitis is associated with the accumulation of supragingival plaque along the gingival margins of the teeth. This form of gingivitis has been extensively studied in human volunteers, and the sequence of events is well described. In these studies, individuals are brought to a state of health and then refrain from all forms of oral hygiene for a 3- to 4-week period. The initial colonizers of the teeth are streptococci, which proliferate and in turn become colonized by other bacteria present in saliva, such as various *Actinomyces* species and *Veillonella*. The greatest growth of the plaque occurs at the gingival margin, where plaque accumulations usually are visible after several days. This plaque may, in some instances, provoke a bleeding gingivitis in which spirochetes and

Actinomyces viscosus are prominent members of the plaque flora. If this plaque remains undisturbed, the flora gradually shifts toward an anaerobic, Gram-negative flora that includes black pigmented bacteroides and several types of spirochetes. The increase in these anaerobic organisms can be explained by the low oxidation-reduction potential of the aged plaque and by nutrients derived from the inflammatory exudate at the site.

The gingivitis may resolve itself or fester subclinically for an indeterminate period; however, the potential for the formation of a periodontal pocket (periodontitis) exists at any time. When pockets are detected clinically, they usually are associated with calcified plaque deposits, called calculus, present on the tooth surfaces. For many years, calculus was thought to be the etiologic agent of periodontitis, because inflammation usually subsided when it was removed and the tooth surfaces were mechanically cleaned. However, calculus is always covered by plaque, and removal of calculus would be synonymous with debridement of plaque. The subgingival plaque flora associated with periodontitis is dominated by an anaerobic, Gram-negative flora in all cases but one, and that is a unique clinical entity formerly known as periodontosis, and now as localized juvenile periodontitis (LJP). LJP is an important clinical entity because of the understanding it has provided of the complex and dynamic interactions between the host and the flora in the pocket ecosystem.

Localized Juvenile Periodontitis (LJP). LJP is different from all other periodontal infections, as it is not associated with plaque accumulations or calculus (in fact the absence of such led early investigators to consider it as a degenerative condition), is localized to certain anterior or front teeth and first molars, and is seen following puberty. It is a rather rare entity, occurring in about 0.1 to 0.5% of teenagers, but when found, is often clustered within families. This familial background suggested a genetic predisposition, which subsequently has been identified as a neutrophil defect associated with reduced chemotaxis. Bacterial examinations of subgingival plaque

from affected teeth and adjacent healthy teeth, revealed that the diseased teeth were colonized by an essentially Gram-negative flora dominated by organisms subsequently identified as various *Capnocytophaga* and *Wolinella* species and *Actinobacillus actinomycetemcomitans*. It is *A actinomycetemcomitans* that appears to be the etiologic agent of LJP, and the arguments for its involvement are illustrative of the arguments made to implicate other species in other forms of periodontitis.

Once LJP has been recognized clinically, most of the tissue damage has already occurred, thereby permitting only a retrospective diagnosis of an *A actinomycetemcomitans* infection. *A actinomycetemcomitans* is found at a higher prevalence in tooth sites associated with LJP and at a lower prevalence in healthy sites in the same mouth, or at sites in periodontally healthy individuals. It is often found among other family members in a household with an LJP individual, and indeed among siblings at risk to LJP, there is suggestive data that colonization by *A actinomycetemcomitans* precedes the development of a pocket and subsequent bone loss. But what has been the most important reason for implicating *A actinomycetemcomitans* as a periodontopathogen, is its killing effect on neutrophils.

A actinomycetemcomitans produces a leukotoxin that kills neutrophils *in vitro*. It is clear that this leukotoxin is expressed *in vivo,* because patients with LJP have developed circulating antibodies which can neutralize this toxin *in vitro*. From this finding, a scenario can be developed that explains the localized nature of LJP. Children with a neutrophil chemotactic defect become colonized by *A actinomycetemcomitans* in early life, presumably by contact with infected household members. The colonization spreads to those permanent teeth that erupted at ages 5 to 7, but remains quiescent as an infection during the time that the primary or baby teeth are lost, and new permanent teeth appear at about ages 11 to 13. The individual entering puberty, has a dentition composed of first molars and incisors that are

colonized by *A actinomycetemcomitans* and newly erupted teeth that either are not colonized or only minimally colonized.

Something then triggers the relative overgrowth of *A actinomycetemcomitans* in the subgingival plaque, and some of these organisms invade the gingival tissue and cause attachment and bone loss in the absence of an obvious inflammatory response. The latter can be explained by both a sluggish neutrophil response to the bacteria and by the leukotoxin inhibiting the neutrophils, and thereby preventing a protective host response in the pocket microenvironment. The leukotoxin is antigenic and elicits an antibody response which may neutralize the leukotoxin at other tooth sites, thereby limiting the infection to the originally colonized molars and incisors.

This scenario, while incomplete, does explain the localized nature of LJP, partially explains the absence of an inflammatory response in the tissue, and demonstrates the dynamic role of neutrophils and circulating antibodies in defending the periodontium. Presumably, this theme is operating in the more commonly found cases of adult periodontitis. Certainly, the central role of the neutrophils in host defense is unquestioned, as individuals with neutropenias, chronic granulomatous disease and various leukemias often present with advanced forms of periodontal disease.

Early-Onset Periodontitis (EOP) and Adult Periodontitis (AP). The more common forms of periodontitis comprise at least two clinical entities, an early onset form in mainly young individuals and a chronic form seen in older adults. The early-onset periodontitis (EOP) is more aggressive looking, while the adult periodontitis (AP) may reflect a stable, but tenuous, stand-off between the host's defensive systems and the plaque bacteria. It is not clear whether these entities represent multiple types of infections with two clinical manifestations, or a single mixed anaerobic infection with different levels of host containment.

The inability to distinguish microbiologically between these two general patterns reflects methodologic procedures relating to the sampling of the subgingival plaque and the inability of any one culture medium and/or technique to give the total picture of the 200 to 300 bacterial species found in the plaque flora. For example, the spirochetes cannot be quantitatively cultured and may account for more than 40% of the flora in EOP and AP. They can be enumerated by microscopic examination of the plaque but would be ignored in cultural studies. These cultural studies, in turn, reveal a bewildering array of species, many of them either newly-described or as yet unspeciated. None of these cultivable species predominates in all disease-associated plaques. For example, *Bacteroides forsythus*, a nonpigmenting fusiform organism has been associated with the active periodontal lesion. *B forsythus* is present in 13% of the active sites and at 8% of the inactive sites, a difference which is hardly indicative of etiologic association. Yet the authors concluded that *B forsythus* is a probable periodontal pathogen because its levels, when present, were on the average 4 times higher in the active sites than in the inactive sites, i.e., 2.5% *vs* 0.6%. This difference is well within the error of the methods used to isolate the organisms.

Despite these problems in assigning virulence to any one species, it is clear that the bacterial communities at disease sites are different from the communities at healthy and successfully-treated periodontal sites. The diseased sites are dominated by anaerobes, and in particular, by spirochetes and black-pigmented bacteroides species, such as *Porphyromonas gingivalis* and *Prevotella intermedia*. Among the latter, *P gingivalis* most often is associated with EOP, whereas *P intermedia* is found in both EOP and AP. No species, except the ubiquitous spirochetes, are consistently found in all lesions. Among the spirochetes, *Treponema denticola* is the only species that can be reliably cultured. It has been shown to possess a wide array of enzymes, such as a collagenase, peptidases, hyaluronidase, and a keratinolytic enzyme, and to produce noxious end products, such as butyrate, NH_3, H_2S, and

endotoxin, that could cause, if they entered the periodontal tissue, an inflammatory response. However, comparable enzymes occur in *P gingivalis* and other anaerobic species found in the plaque, so that it would be difficult to assign etiologic significance to any one of these organisms based on the production of these enzymes. This being the case, it may be best to consider that the collective overgrowth of all these anaerobic species in the plaque causes a mixed infection that is responsible for tissue loss in EOP and AP.

Clinical Manifestations. Periodontal disease is usually painless until late in the disease process, when the teeth are so loose that some discomfort may appear upon chewing. Retention of food in a pocket site may provoke a sudden burst of microbial growth which could result in a painful abscess. At other times, the anterior teeth may become so loose that they separate and the patient visits a dentist because of the resulting poor aesthetics. However, under ordinary circumstances, it is bleeding upon brushing and/or concern over halitosis that brings the patient to the dentist. A thorough dental examination should find any pockets which may exist. If these pockets bleed upon probing, such bleeding is synonymous with tissue inflammation and warrants therapeutic intervention.

Microbiological Diagnosis. Microbiologic diagnosis is not commonly used in the management of periodontal disease. The oldest method is the use of darkfield and phase contrast microscopy to identify spirochetes and other motile organisms in plaque samples. However, as spirochetes are detectable in most plaques, it is necessary to establish some critical value above which a spirochetal infection can be diagnosed. Our experience suggests that $\geq$ 20% spirochetes in any plaque sample permits the diagnosis of an anaerobic infection.

A microscopic examination cannot distinguish the species of bacteria present unless one uses an immunologic staining reagent specific for the organism in question. Such immunodiagnostic reagents have been used to detect and quantitate the levels of *P gingivalis*, *P intermedia*, *T denticola*, and *A actinomycetemcomitans* in the plaque. Cultural methods can, if the appropriate nonselective and selective media are used, provide information on the levels of *A actinomycetemcomitans*, black pigmented species, *Campylobacter* species, and other periodontopathogens. Also, because viable organisms are available, antibiotic sensitivities of the isolated organisms can be determined, which may be useful in certain instances.

Other diagnostic reagents are being developed to detect, in plaque, specific microbes or metabolites or enzymes unique to inflammation or infection. For example, specific microbes can be demonstrated in plaque by the use of DNA probes. Probes for *A actinomycetemcomitans*, *P intermedia* and *P gingivalis* are commercially available for testing via a reference laboratory. Future diagnostic procedures may rely on the detection of hydroxyproline, a collagen degradation product; prostaglandin, an inflammatory mediator; and enzymes, derived from either the host or the microbes. A trypsin-like enzyme is present in *T denticola*, *P gingivalis*, and *B forsythus* and is absent from at least 60 other subgingival plaque organisms. This enzyme can be detected by the hydrolysis of the trypsin substrate benzoyl-DL-arginine naphthylamide (BANA). The ability of subgingival plaque to hydrolyze BANA was associated with elevated levels and proportions of spirochetes and with probing depths greater than 6 mm. Subsequently, BANA hydrolysis was shown to be related to the *T denticola* and *P gingivalis* content of the plaque and to the clinical diagnosis of health or disease. As *T denticola*, *P gingivalis* and *B forsythus* are anaerobes, a positive BANA test may be useful in the diagnosis of an anaerobic plaque infection.

When these identification procedures were performed on the same plaque samples, the DNA probes and immunological reagents were significantly more likely to detect *P gingivalis, B forsythus, A actinomycetemcomitans* and *T denticola* than was the traditional cultural approach. In fact, this study suggested that culturing may be the least accurate detection procedure for these plaque species. When the probes and immunological reagents were compared to the BANA test, the probes and antibodies were slightly more accurate, i.e., 88% vs. 83%. All three approaches were essentially comparable indicating that reliable non-cultural methods are available to aid in the microbiological diagnosis of periodontal infections. Because the BANA test detects an enzyme(s) found in three anaerobic species, it may be used to detect an anaerobic periodontal infection.

Prevention and Treatment. Gingivitis can be prevented by good oral hygiene and professional surveillance. Gingivitis can be effectively treated by debridement of the teeth, and, if needed, by short-term use of products containing chlorhexidine, stannous fluoride, or other antimicrobial agents. Mouth rinses, gels, and toothpastes, when used in conjunction with toothbrushing and flossing, are probably adequate to deliver any antimicrobial agents to subgingival sites that are 1 to 3 mm in depth. At probing depths greater than 3 mm, there may not be sufficient penetration of the agent to the bottom of the pocket, and infection may persist. Subgingival scaling (debridement) by a professional is indicated, and additional benefits can usually be obtained by the use of irrigating devices containing an antimicrobial agent.

There is rarely any need to use systemic antimicrobial agents to treat gingivitis associated with pocket depths of 1 to 4 mm, with the exception of an increasingly rare and painful condition known today as acute necrotizing ulcerative gingivitis (ANUG) and formerly as trench mouth. Cases of ANUG that are refractory to mechanical debridement and topical antimicrobial agents respond, quickly and dramatically, to systemic metronidazole. The recognition of metronidazole's efficacy

in ANUG led to the discovery that metronidazole has bactericidal activity against anaerobes. ANUG is characterized by tissue invasion by spirochetes and possibly other anaerobes and by elevated plaque levels of spirochetes and *P intermedia*. ANUG thus resembles periodontitis in being an anaerobic infection.

Clinical dentistry has been about 80 to 85% successful in treating periodontitis by debridement and surgical procedures. However, surgery is labor intensive, and therefore costly. This limits the number of individuals who can be treated in a cost-efficient manner. However, if the majority of clinical cases of periodontitis represent specific bacterial infections, then an alternate treatment strategy would be to diagnose and treat the infection. It would seem that the crucial determination for the clinician in his treatment plan will be the diagnosis of either a micro-aerophilic infection, due to *A actinomycetemcomitans,* or an anaerobic infection characterized by the overgrowth of spirochetes and other anaerobic species.

A actinomycetemcomitans is sensitive to tetracycline, and early uncontrolled studies showed that tetracycline, scaling and root planing, periodontal flap surgery, and topical treatment with chlorhexidine were able, to save hopeless teeth in LJP patients. Additional studies, but none of a double-blind nature, confirm the usefulness of tetracycline in the treatment of LJP. Subsequently it was shown that tetracycline is concentrated in the fluid that seeps out of the periodontium into the pocket micro-environment. This fact, combined with the demonstration that *A actinomycetemcomitans* can be found in some plaques associated with EOP and AP, has led to the use of tetracycline in those clinical entities. Results have been equivocal, but this has not detracted from the popularity of tetracycline as a treatment for periodontitis.

Most bacteriologic studies implicate anaerobes as the etiologic agent(s) of EOP and AP, and this would point to the use of a drug such as metronidazole. However, early

animal studies that employed lifetime feeding of extremely high dosages of metronidazole suggested that the drug might be tumorigenic. These studies have not been confirmed and, indeed, in 1981 the FDA approved metronidazole for treatment of anaerobic infections. In dentistry, this concern has caused a reluctance to use metronidazole, but has also allowed time for well controlled clinical trials of metronidazole.

Six double-blind studies have demonstrated that metronidazole, given for periods of time as short as 1 week, can significantly improve periodontal health. In all cases, the metronidazole was given in conjunction with professional debriding of the teeth. Maximal benefits were obtained when the metronidazole was given after the debridement. The best clinical response was often noted in patients with more advanced disease, in which the pocket depths were ≥ 6 mm, whereas there was only a moderate benefit when the pocket depths were from 4 to 6 mm. In these advanced cases some teeth, that were initially scheduled for extraction upon reexamination, were found no longer to need extraction and thus, in a sense, were saved.

Metronidazole has subsequently been evaluated to determine whether it can reduce the need for periodontal surgery. In three double-blind studies, the unsupervised usage of metronidazole for one week, when combined with the standard debridement procedures, was able to significantly reduce the number of teeth needing surgery when compared to the debridement procedures plus placebo treatment. This sparing effect on surgery has lasted for several years after the one-week period of systemic treatment.

The localized nature of the periodontal infection and the easy access of the teeth has prompted the development of delivery systems which release the antimicrobial agent directly into the periodontal pocket. The first of these delivery systems that is commercially available is a tetracycline impregnated cord which can be wrapped

around the tooth below the gingival margin. This cord releases over 100μg of tetracycline per ml of gingival crevicular fluid during the entire period that it is *in situ*. In this manner, patient compliance is assured and the plaque microbes are constantly exposed to therapeutic levels of the agent.

These data from the double-blind metronidazole studies indicate that EOP and AP respond to treatment as if they were anaerobic infections and would seem to presage the more frequent usage of anti-anaerobic agents, such as metronidazole, in the future treatment of periodontal disease. Further developments of delivery systems which release antimicrobials directly into the periodontal pocket should assure that in the future, most periodontal infections will be medically managed.

Bone, Joint, and Necrotizing Soft Tissue Infections

General Concepts

Necrotizing Soft Tissue Infections

Etiology. Anaerobic microorganisms such as *Bacteroides* species, *Peptostreptococcus* species, and *Clostridium* species are largely responsible for these infections. Mixed infections by aerobic and facultative anaerobic organisms are common.

Pathogenesis. Susceptible persons have experienced trauma or surgery and frequently have diabetes and/or vascular insufficiency. Organisms gain entry via direct inoculation. Local hypoxia and decreased oxygen-reduction potentials favor anaerobic growth.

Clinical Manifestations. This signs of disease include production of tissue gas, a putrid discharge, tissue necrosis, fever, (occasionally) systemic toxicity, and absence of classic signs of inflammation.

Microbiologic Diagnosis. These infections are usually diagnosed by clinical presentation. Aerobic and anaerobic wound cultures help identify the major pathogens.

Prevention and Treatment. Immediate surgical debridement of all necrotic tissue is vital. High-dose parenteral antibiotic therapy should be started immediately. Hyperbaric oxygen therapy may be indicated.

Joint Infections

Etiology. *Neisseria gonorrhoeae* and S *taphylococcus aureus* are responsible for most cases of bacterial arthritis.

Pathogenesis. Joint infections are usually a result of hematogenous spread, but may also arise from traumatic inoculation or by extension from an adjacent focus of infection. Proteolytic enzymes of polymorphonuclear leukocytes, bacterial toxins, and pressure from joint swelling all contribute to the damage of articular surfaces.

Clinical Manifestations. Joint swelling. pain, warmth (inflammation), decreased range of motion, and fever are the classic symptoms. Disseminated gonococcal infections may also cause migratory polyarthritis, dermatitis, and tenosynovitis.

Microbiologic Diagnosis. Aspiration and culture of synovial fluid usually provides the definite diagnosis.

Prevention and Treatment. Gonococcal arthritis may be prevented by techniques used to decrease the risk for sexually transmitted disease. The treatment for all septic

arthritides is administration of parenteral antibiotics. Some cases may require aspiration and/or surgical debridement.

Bone Infections

Etiology. *Staphylococcus aureus* is the most commonly isolated pathogen. Polymicrobic infections are frequent in contiguous-focus osteomyelitis.

Pathogenesis. Organisms may reach the bones by hematogenous spread, by direct extension from a contiguous focus of infection, or as a result of trauma. A cycle of increased pressure from infection, inflammation, local ischemia, and bone necrosis may establish itself and lead to a chronic infection.

Clinical Manifestations. Hematogenous osteomyelitis classically presents with high fever and pain around the involved bone. Sinus tracts with purulent drainage are evidence of chronic osteomyelitis.

Microbiologic Diagnosis. Bone biopsy and/or debridement cultures are mandatory with rare exceptions. Sinus tract cultures are unreliable.

Prevention and Treatment. Treatment consists of surgical debridement and long-term, culture-directed antimicrobial therapy. Hematogenous osteomyelitis in children may be treated with antibiotics alone.

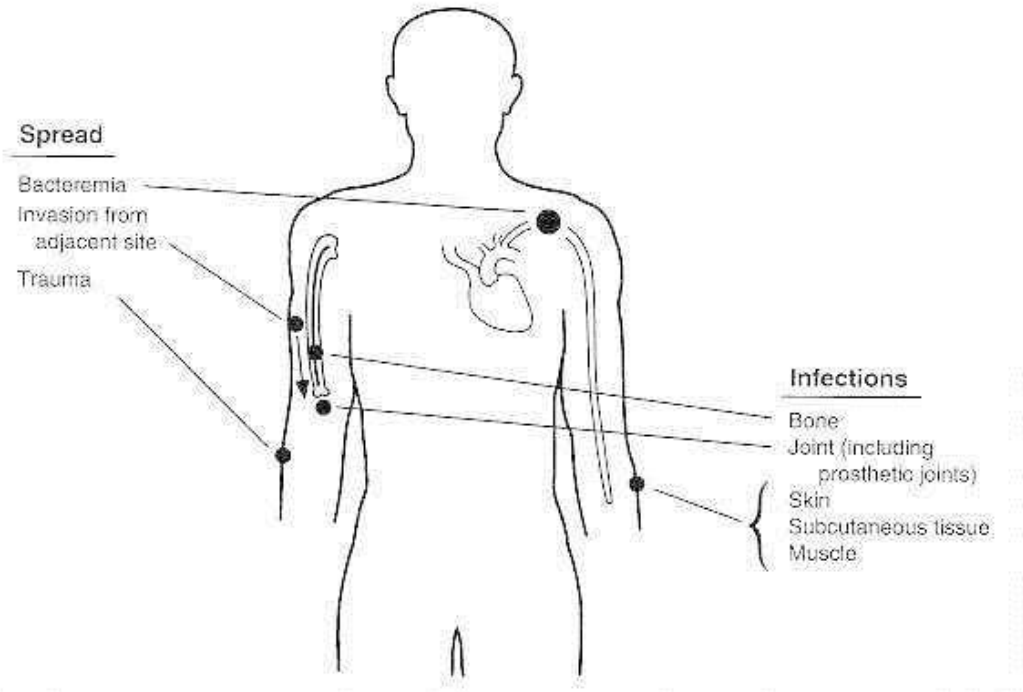
Introduction

Necrotizing infections of the soft tissues are characterized by extensive tissue necrosis and production of tissue gas. These infections may extend through tissue planes and are not well contained by the usual inflammatory mechanisms. They may develop and progress with dramatic speed, and extensive surgery and systemic antibiotic therapy are required to eradicate them.

Arthritis or inflammation of a joint space may be caused by a wide variety of infectious or noninfectious processes. Non-infectious arthritis is the more common type of arthritis and is usually secondary to degenerative, rheumatoid, or posttraumatic changes within the joint. Infectious arthritis, although less common, is often accompanied by a striking polymorphonuclear inflammatory response and can cause severe destruction of the articular cartilage if not properly diagnosed and treated.

Bone infections are called osteomyelitis (from *osteo* [bone], plus *myelitis* [inflammation of the marrow]). Hematogenous osteomyelitis and contiguous-focus osteomyelitis are the two major types of bone infections. Both types can progress to a chronic bone infection characterized by large areas of dead bone.

Bone, joint, and soft tissues, with the exception of the skin, are normally sterile areas. Bacteria may reach these sites by either hematogenous spread or spread from an exogenous or endogenous contiguous focus of infection. Host defenses are important in containing necrotizing soft tissue infections. A systemically or locally compromised host is more likely to develop these types of infections and to be unable to contain them.



Bacterial spread to bone, joints, and soft tissue.

Necrotizing Soft Tissue Infections. An exact classification of necrotizing subcutaneous, fascial, and muscle infections is difficult because the distinctions between many of the clinical entities are blurred. Clinical classification is as follows: (1) crepitant anaerobic cellulitis, (2) necrotizing fasciitis, (3) nonclostridial myonecrosis, (4) clostridial myonecrosis, (5) fungal necrotizing cellulitis, and (6) miscellaneous necrotizing infections in the immunocompromised host. These types of infections usually occur in traumatic or surgical wounds or around foreign bodies and in patients who are medically compromised by diabetes mellitus, vascular insufficiency, or both. In the traumatically, surgically, or medically compromised patient, local tissue conditions, hypoxia, and decreased oxidation-reduction potential (E_h) promote the growth of anaerobes. Most necrotizing soft tissue infections have an endogenous anaerobic component. Since anaerobes are the predominant members

of the microflora on most mucous membranes, there are many potential pathogens. Hypoxic conditions also allow proliferation of facultative aerobic organisms, since polymorphonuclear leukocytes function poorly under decreased oxygen tensions. The growth of aerobic organisms further lowers the E_h, more fastidious anaerobes become established, and the disease process rapidly accelerates.

Discernible quantities of tissue gas are present in most of these infections. Carbon dioxide and water are the natural end products of aerobic metabolism. Carbon dioxide rapidly dissolves in aqueous media and rarely accumulates in tissues. Incomplete oxidation of energy sources by anaerobic and facultative aerobic bacteria can result in the production of gases that are less water soluble and therefore accumulate in tissues. Hydrogen is presumably the major tissue gas in mixed aerobic-anaerobic soft tissue infections. Its presence indicates rapid bacterial multiplication at a low E_h.

Clinically, the hallmarks of mixed aerobic-anaerobic soft tissue infections are tissue necrosis, a putrid discharge, gas production, the tendency to burrow through soft tissue and fascial planes, and the absence of classic signs of tissue inflammation.

Crepitant Anaerobic Cellulitis. Nonclostridial and clostridial cellulitides have a similar clinical picture and are discussed together under the term, crepitant anaerobic cellulitis. Crepitant anaerobic cellulitis appears as a necrotic soft tissue infection with abundant connective tissue gas. The condition usually occurs after local trauma in patients with vascular insufficiency of the lower extremities. Multiple aerobic and anaerobic organisms have been isolated, including *Bacteroides* species, *Peptostreptococcus* species, *Clostridium* species, and members of the family Enterobacteriaceae. Crepitant anaerobic cellulitis can be differentiated from more

serious soft tissue infections by the abundance of soft tissue gas, lack of marked systemic toxicity, gradual onset, less severe pain, and absence of muscle involvement.

Necrotizing Fasciitis. Necrotizing fasciitis is a relatively rare infection with a high mortality (40 percent). The infection was originally called hemolytic streptococcal gangrene by Meleney in 1924. Although his clinical description was accurate, better culture techniques have demonstrated that organisms other than *Streptococcus pyogenes* more commonly cause these infections. Clinical manifestations include extensive dissection and necrosis of the superficial and often the deep fascia. The infection undermines adjacent tissue and leads to marked systemic toxicity. Thrombosis of subcutaneous blood vessels leads to necrosis of the overlying skin. Initial local pain is replaced by numbness or analgesia as the infection involves the cutaneous nerves. Most cases of fasciitis follow surgery or minor trauma. The highest incidence is seen in patients with small vessel diseases such as diabetes mellitus. When careful bacteriologic techniques are used, anaerobes, particularly *Peptostreptococcus*, *Bacteroides*, and *Fusobacterium* species, are found in 50 to 60 percent of cases. Aerobic organisms, especially *Streptococcus pyogenes*, *Staphylococcus aureus*, and members of the Enterobacteriaceae have also been isolated. Most infections are mixed aerobic-anaerobic infections, but a type of necrotizing fasciitis caused solely by *Streptococcus pyogenes* has been reported and is referred to by the lay press as "flesh eating bacteria."

Nonclostridial Myonecrosis. Nonclostridial myonecrosis, called synergistic necrotizing cellulitis by Stone and Martin, is a particularly aggressive soft tissue infection. It is similar to clostridial myonecrosis in that there is widespread involvement of soft tissue with necrosis of muscle tissue and fascia. The prominent involvement of muscle tissue differentiates this infection from necrotizing fasciitis. Subcutaneous tissue and skin are secondarily involved. Clinically, there is exquisite

local tenderness, with minimal skin changes, and drainage of foul-smelling "dish-water" pus from small skin surface ulcers. Severe systemic toxicity is found in most patients. Nonclostridial myonecrosis occurs most frequently in the perineal area, as a result of an extension of a perirectal abscess, and in the lower extremities of patients with vascular insufficiency. Multiple organisms have been isolated, including *Peptostreptococcus* and *Bacteroides* species and members of the Enterobacteriaceae. Mortality approaches 75 percent.

Clostridial Myonecrosis. Clostridial myonecrosis, or gas gangrene, is a clostridial infection primarily of muscle tissue. *Clostridium perfringens* is isolated in 90 percent of these infections. Other clostridial species frequently isolated are *C novyi* (4 percent), *C septicum* (2 percent), *C histolyticum*, *C fallax*, and *C bifermentans*. Classically, clostridial myonecrosis has an acute presentation and a fulminant clinical course. The infection usually occurs in areas of major trauma or surgery or as a complication of thermal burns. However, it also has been reported following minor trauma, including intravenous administration of drugs, intramuscular injections of epinephrine, insect bites, and nail punctures. Moreover, it may occur in the absence of recent trauma by activation of dormant clostridial spores in old scar tissue. Finally, clostridial myonecrosis may occur in the absence of trauma, by bacteremic spread of the organism from a gastrointestinal or genitourinary site. *Clostridium septicum* is the major cause of spontaneous, nontraumatic gas gangrene and is often associated with a lesion in the colon such as an adenocarcinoma.

Clostridial myonecrosis is diagnosed mainly on a clinical basis. The infection may be so rapidly progressive that any delay in recognition or treatment may be fatal. The onset is sudden, often within 4 to 6 hours after an injury. Sudden, severe pain in the area of infection is an early clinical finding. Early in the course of infection, the skin overlying the wound appears shiny and tense and then becomes dusky. Within hours, the skin color may progress from dusky to a bronze discoloration, which can

advance at a rate of 1 inch per hour. Vesicles or hemorrhagic bullae appear near the wound. A thin, brownish, often copious fluid exudes from the wound. Bubbles occasionally appear in the drainage. This exudate has often been described as having a sweet "mousy" odor. Swelling and edema in the area of infection is pronounced. Within hours the skin overlying the lesion can rupture and the muscle herniate. At surgery, the infected muscle is dark red to black, is noncontractile, and does not bleed when cut. Crepitus, although not prominent, is sometimes detected. Radiographs may show tissue gas outlining fascial planes and muscle bundles.

The rapid tissue necrosis in clostridial myonecrosis is caused by the clostridial toxins. Clostridial species are capable of producing multiple toxins, each with its own mode of action. *Clostridium perfringens* produces at least 12 different extracellular toxins. The most common of these, a lecithinase called alpha toxin, is hemolytic, histotoxic, and necrotizing. Other toxins act as collagenases, proteinases, deoxyribonucleases (DNases), fibrinolysins, and hyaluronidases. The systemic toxic reaction cannot be fully explained by a single circulating exotoxin. The "toxic factor" may be produced by interaction of the clostridial toxins with infected tissue. The mortality from clostridial myonecrosis ranges from 15 to 30 percent.

Fungal Necrotizing Cellulitis. *Phycomyces* and *Aspergillus* species may cause a gangrenous cellulitis in compromised hosts. The hallmark of these infections is the invasion of blood vessels by hyphae, followed by thrombosis and subsequent necrosis extending to all soft tissue compartments. Spores from these fungi are ubiquitous.

The *Phycomyces* species are characterized by broad-based nonseptate hyphae. *Rhizopus*, *Mucor*, and *Absidia* are the major pathogenic genera within the family Mucoraceae. Serious rhinocerebral, pulmonary, or disseminated infections have been found in patients with diabetes, lymphoma, or leukemia. Phycomycotic

gangrenous cellulitis usually occurs in patients with severe burns or diabetes. The characteristic dermal lesion is a black, anesthetic ulcer or an area of necrosis with a purple edematous margin. There is no gas or exudate, and the infection may progress rapidly.

Aspergillus species are characterized histologically by branching septate hyphae. These fungi can cause serious pulmonary or disseminated infections in compromised hosts. *Aspergillus* gangrenous cellulitis may be primary or from a disseminated infection. The dermal lesion is an indurated plaque that leads to a necrotic ulcer. Gas and exudate are not present.

Joint Infections. Infectious arthritis may arise either from hematogenous spread or by direct extension from an adjacent bone or soft tissue infection. The infection is usually a localized suppurative process. Although any joint can become infected, the knee is most commonly involved (53 percent), followed by the hip (20 percent), shoulder (11 percent), wrist (9 percent), ankle (8 percent), and elbow (7 percent). The infection is monarticular almost 90 percent of the time. However, a bacterial polyarthritis may be seen.

In the normal host, polymorphonuclear leukocytes respond rapidly to the infection and release proteolytic enzymes, which can cause extensive destruction of the articular cartilage within 3 days. The joint may also be damaged directly by the release of bacterial toxins and lysosomal enzymes. Furthermore, an effusion is almost always present and is confined within the joint capsule; this increases intra-articular pressure and interferes with blood supply and nutrition. These complications may occur with almost any type of septic arthritis, but are most common in nongonococcal bacterial infections. Children are especially vulnerable since extension to the epiphyseal growth plate may stunt bone growth.

Several conditions are known to predispose joints to the development of septic arthritis. Corticosteroid therapy, rheumatoid arthritis, and degenerative joint disease are the most common underlying factors. Total joint arthroplasties are susceptible to hematogenous infections. Patients with diabetes mellitus, leukemia, cancer, cirrhosis, chronic granulomatous diseases, or hypogammaglobulinemia or those undergoing cytotoxic chemotherapy or practicing substance abuse also have an increased incidence of infectious arthritis.

Gonococcal Arthritis. The most common cause of bacterial arthritis in healthy young adults in North America is *Neisseria gonorrhoeae*. Gonococcal arthritis typically follows primary infection of a mucosal site and is thought to spread hematogenously to the joint. Females are affected four times as often as males, and about one-half of all affected females are either pregnant or menstruating. This association supports the theory that endocrine factors play a role in gonococcal arthritis, although the exact mechanism has not been elucidated. Strains of *N gonorrhoeae* that cause disseminated gonococcal infections differ phenotypically from those that cause simple mucosal infections and are thought to be more virulent.

The disease may manifest itself as part of a disseminated gonococcal infection or as a monarticular joint infection. The presenting symptoms in disseminated gonococcal infections may be mixed, with migratory polyarthralgias, fever, chills, dermatitis, and tenosynovitis. Most of these patients have asymptomatic genital, anal, or pharyngeal gonococcal infections. Skin lesions, when present, begin as small erythematous papules but usually progress to vesicular or pustular stages. Tenosynovitis is characterized by pain, swelling, and periarticular redness. Patients with monarticular disease often have a history of polyarthralgias, and some authorities believe that this represents a continuum from disseminated gonococcal infection.

Nongonococcal Arthritis. Nongonococcal bacterial arthritis is a serious infection with significant sequelae. Mortality as high as 12 percent has been reported, and up to 75 percent of survivors suffer some type of functional loss in the involved joint. Classically, patients present with fever and pain, swelling, warmth, and decreased range of motion in the involved joint. The joint effusion should be aspirated and cultured to determine the exact etiologic agent. There are variations among age groups, but the most common cause of nongonococcal bacterial arthritis is *Staphylococcus aureus*. In adults, all Gram-negative bacilli together account for about 20 percent of cases. It is generally accepted that Gram-negative infections are the most virulent, with *Pseudomonas aeruginosa* and *Escherichia coli* being the most common. Intravenous drug abusers have a significant incidence of infection with Gram-negative organisms. Streptococcal species engender a small but significant proportion of infections (10 to 15 percent). About 10 percent of patients with nongonococcal arthritis have polymicrobial infections. In addition, there are frequent microbiologic associations with concomitant disease states. For example, bacterial arthritis following infectious diarrhea may be caused by *Shigella*, *Salmonella*, *Campylobacter*, or *Yersinia* species. *Streptobacillus moniliformis* may cause a migrating polyarthritis; however, this is rare. In children, *Haemophilus influenzae* is a cause of septic arthritis.

Diagnosis of Bacterial Arthritis. Several laboratory tests are used to diagnose infectious arthritis. The definitive test involves culturing the fluid from the involved joint after aspiration or incision and drainage. Gram stains are often unreliable, although they may provide initial clues. Synovial fluid analysis usually reveals a turbid fluid with leukocyte counts greater than 100,000/mm^3 in 30 to 50 percent of cases. In bacterial arthritis, the level of polymorphonuclear leukocytes often approaches 90 percent. Low joint fluid glucose levels and high lactate levels are indicative of septic arthritis, but are nonspecific. Peripheral blood leukocyte counts

are usually elevated in children, but are often within normal limits in adults. Finally, radiography may show joint space widening and soft tissue swelling in infections more than 2 weeks old.

Granulomatous Arthritis. Infectious arthritis may be caused by mycobacteria and certain fungi. This disease may be very insidious and may progress for several months before infection is even considered. These organisms usually produce a chronic monarticular arthritis with a granulomatous inflammatory response. *Mycobacterium tuberculosis* infections of the musculoskeletal system are the most common extrapulmonary manifestation of tuberculosis and result from hematogenous dissemination. Atypical mycobacteria, especially *M fortuitum*, *M chelonae*, and *M marinum*, may cause septic arthritis by inoculation or extension from a contiguous focus of infection. The most common cause of fungal arthritis is *Sporothrix schenckii*. This infection usually follows traumatic inoculation, but may also result from pulmonary dissemination. Because of its relative rarity and indolent course, the diagnosis is often missed or delayed. Coccidiomycosis, histoplasmosis, and blastomycosis may all affect the joint. In addition, *Cryptococcus*, *Aspergillus*, and *Candida* species may cause infectious arthritis in the immunocompromised host. Diagnosis of all the granulomatous arthritides usually involves a higher index of suspicion and appropriate fungal or mycobacterial cultures.

Bone Infections. On the basis of clinical and pathologic considerations, osteomyelitis may be classified as either hematogenous or secondary to a contiguous focus of infection. Contiguous-focus osteomyelitis can be further subdivided into bone infection with relatively normal vascularity and bone infection with generalized vascular insufficiency. Either major type of osteomyelitis may progress to a chronic bone infection.

Hematogenous Osteomyelitis. Hematogenous osteomyelitis occurs mainly in infants and children but has recently been found with increasing frequency in the adult population. In infants and children the metaphysis of long bones (tibia, femur) is most frequently involved. The anatomy in the metaphyseal region of long bones seems to explain this clinical finding. Nonanastomosing capillary ends of the nutrient artery make sharp loops under the growth plate and enter a system of large venous sinusoids where the blood flow becomes slow and turbulent. Any obstruction of the capillary ends leads to an area of avascular necrosis. Minor trauma probably predisposes the infant or child to infection by producing a small hematoma and subsequent bone necrosis, both of which can be infected by a transient bacteremia. The infection produces a local cellulitis, which results in increased bone pressure, decreased pH, and a breakdown of leukocytes. All of these factors contribute to necrosis of bone. The infection may proceed laterally through the haversian and Volkmann canal system, perforate the cortex, and lift the periosteum. It may also extend into the intramedullary canal. Extension leads to further vascular compromise and bone necrosis. In infants, capillaries penetrate the growth plate. Therefore, the infection may also spread to the epiphysis and into the joint space. In children over 1 year old, the growth plate is no longer penetrated by capillaries, and the epiphysis and joint space are protected from infection. In adults, the growth plate has been resorbed and joint extension of a metaphyseal infection can recur. However, in adults, the diaphysis of the long bones and the lumbar and thoracic vertebral bodies of the axial skeleton are most frequently involved. Adults with axial skeleton osteomyelitis often have a history of preceding urinary tract infection or intravenous drug abuse.

A single pathogenic organism is usually responsible for hematogenous osteomyelitis. Polymicrobic hematogenous osteomyelitis is rare. *Staphylococcus aureus* is the most frequent organism isolated, but *Streptococcus pyogenes* and

Streptococcus agalactiae are responsible for a significant number of bone infections, especially in infants. Aerobic Gram-negative organisms are responsible for an increasing number of bone infections. *Pseudomonas aeruginosa* is often isolated from intravenous drug abusers with vertebral osteomyelitis.

Patients with hematogenous osteomyelitis usually have normal soft tissue around the infected bone. If antimicrobial therapy directed at the pathogen is begun prior to extensive bone necrosis, the patient has an excellent chance of cure.

Contiguous-Focus Osteomyelitis. Osteomyelitis Secondary to a Contiguous Infection with No Generalized Vascular Insufficiency

In contiguous-focus osteomyelitis, the organism either is directly inoculated into the bone by trauma or surgery or reaches the bone from adjacent infected soft tissue. Common predisposing conditions include open fractures, surgical reduction and internal fixation of fractures, and wound infections. In contrast to hematogenous osteomyelitis, multiple bacteria are isolated from the infected bone. The bacteriology is diverse, but *S aureus* remains the most commonly isolated pathogen. In addition, aerobic Gram-negative bacilli and anaerobic organisms are frequently isolated. Bone necrosis, soft tissue damage, and loss of bone stability are all common, making this form of osteomyelitis difficult to manage.

Osteomyelitis Secondary to a Contiguous Infection with Generalized Vascular Insufficiency. The small bones of the feet (principally the metatarsal bones and phalanges) are commonly involved in osteomyelitis secondary to a contiguous infection in patients with generalized vascular insufficiency. Most commonly, the infection develops as an extension of a local infection, either cellulitis or a trophic skin ulcer. The inadequate tissue perfusion favors the infection by blunting the local inflammatory response. Multiple aerobic and anaerobic bacteria are usually isolated

from the infected bone. Although cure is desirable, a more attainable goal of therapy is to suppress the infection and maintain functional integrity of the involved limb. Recurrent or new bone infections occur in many patients. In time, amputation of the infected area is almost always necessary.

Chronic Osteomyelitis. Both hematogenous osteomyelitis and contiguous-focus osteomyelitis can progress to a chronic bone infection. No exact criteria separate acute from chronic osteomyelitis. Clinically, newly recognized bone infections are considered acute, whereas a relapse of the infection represents chronic disease. However, this simplistic classification is clearly inadequate. The hallmark of chronic osteomyelitis is the presence of large areas of dead bone or sequestra. An involucrum (a reactive bony encasement around the sequestrum) and persistent drainage via one or more sinus tracts are usually present. In chronic osteomyelitis, multiple species of bacteria are usually isolated from the necrotic infected bone, except in cases of chronic hematogenous osteomyelitis, which usually yield a single organism. Unless the necrotic infected bone can be removed, antibiotic therapy is usually unsuccessful. The prognosis for arresting the infection is worse if there is poor soft tissue integrity surrounding the infection, sclerosis of the involved bone, or bone instability.

Diagnosis of Bacterial Osteomyelitis. The bacteriologic diagnosis of bacterial osteomyelitis rests on isolation of the agent from the bone or the blood. In hematogenous osteomyelitis, positive blood cultures often obviate the need for a bone biopsy when there is associated radiographic or radionuclide scan evidence of osteomyelitis. In chronic osteomyelitis, sinus tract cultures are not reliable for predicting which organism(s) will be isolated from the infected bone. Antibiotic treatment of osteomyelitis should not be based on the results of sinus tract cultures. In most instances, bone biopsy cultures are mandatory to guide specific antimicrobial therapy.

Skeletal Tuberculosis. Skeletal tuberculosis is the result of hematogenous spread of the tuberculosis bacillus early in the course of a primary infection. Rarely, skeletal tuberculosis develops as a contiguous infection from an adjacent caseating lymph node. Either the primary bone infection or a reactivated quiescent primary bone infection elicits an inflammatory reaction, followed by the development of granulation tissue. The granulation tissues erodes and destroys the cartilage and cancellous bone. Eventually the infection causes bone demineralization and necrosis. Proteolytic enzymes that can destroy cartilage are not produced in skeletal tuberculosis. Cartilage is destroyed slowly by granulation tissue, and the joint or disc space is preserved for considerable periods. Healing involves deposition of fibrous tissue. Pain is the most frequent clinical complaint.

Any bone may be involved by skeletal tuberculosis, but the infection usually involves one site. In children and adolescents, the metaphyses of the long bones are most frequently infected. In adults, the axial skeleton, followed by the proximal femur, knee, and small bones of the hands and feet, are most often involved. In the axial skeleton, the thoracic vertebral bodies are most frequently infected, followed by the lumbar and cervical vertebral bodies. Vertebral infection usually begins in the anterior portion of a vertebral body adjacent to an intervertebral disc. Adjacent vertebral bodies may become involved, and a paravertebral abscess may develop. Sixty percent of patients with skeletal tuberculosis have evidence of extraosseous tuberculosis.

Tissue for culture and histology is almost always required for the diagnosis of skeletal tuberculosis. Cultures for *Mycobacterium tuberculosis* are positive in approximately 60 percent of the cases, but 6 weeks may be required for growth and identification of the organism. Histology showing granulomatous tissue compatible with tuberculosis and a positive tuberculin test are sufficient to begin tuberculosis therapy. However, a negative skin test does not rule out skeletal tuberculosis.

Therapy for skeletal tuberculosis involves prolonged chemotherapy and in some cases surgical debridement.

Fungal Osteomyelitis. Bone infections may be caused by a variety of fungal organisms, including *Coccidioides*, *Blastomyces*, *Cryptococcus*, and *Sporothrix* species. The lesion most often appears as a cold abscess overlying an osteomyelitic lesion. Joint space extension may occur in coccidioidomycosis and blastomycosis. Therapy for fungal osteomyelitis involves surgical debridement and antifungal chemotherapy.

HOST DEFENSES

Host defenses include both physiologic barriers and immunological responses. Some defenses are non-specific, others are highly specific. Host defenses can vary considerably due to many factors including alcohol, drugs, nutrition, immunologic disorders, etc.

Skin and mucous membranes provide the first line of defense through:

1. Mechanical factors: physical barrier to penetration.
2. Chemical factors: gastric acidity, unsaturated fatty acids, lysozyme.
3. Microbial factors: antagonism by normal flora.

Phagocytic cells provide a secondary line of defense by consuming invaders and secreting substance that produce immune responses:

1. Mononuclear cells: monocytes (blood) and macrophages (tissue).
2. Neutrophils: polymorphonuclear (PMN) granulocytes.
3. Inflammation: immune response producing dilation of blood vessels, increased vascular permeability and diapedesis of monocytes.
4. Phagocytosis: cells destroy invaders by i) utilizing specific membrane receptors for attachment (e.g. immunoglobulin Fc and complement C3b receptors) and ii) ingesting invaders into phagocytic vacuoles, leading to

intracellular killing by fusion with lysosome-like granules containing lowered pH, peroxide, enzymes, etc.

Humoral factors: antibody mediated defenses include:

1. Antitoxins: specific antibodies that bind certain exotoxins.
2. Bacteriolytic antibodies: antibodies plus complement can directly lyse Gram-negative cells.
3. Opsonizing antibodies: coat cell surface and enhance phagocytosis (Fc receptors).

Cell-mediated factors: cell-mediated defenses include:

1. Cytotoxic T-lymphocytes: specific cells capable of destroying altered host cells.
2. K and NK cells: lyse altered or transplanted host cells.
3. Activated macrophages: phagocytes that possess a greatly enhanced capacity for intracellular destruction of ingested microorganisms.

THE CONSTITUTIVE DEFENSES OF THE HOST AGAINST MICROBIAL PATHOGENS

Introduction: Host Defense Mechanisms

Although humans are in continuous associations with microorganisms, and some readily colonize the body surfaces (see The Bacterial Flora of Humans), it is relatively rare that these microorganisms cause damage to their host. In part, this is due to the effectiveness of the host defense mechanisms, which restrict invasion by normal flora (some of which may be potential pathogens), and which defend against non-indigenous microorganisms that are overt pathogens.

Just as the outcome of an interaction between the host and a member of the normal flora always depends on specific properties inherent to both the host and the microbe, so does the outcome of an interaction between the host and a parasite.

Sometimes the host tolerates **colonization** by a parasite but restricts it to regions of the body where it can do no harm (e.g. *Staphylococcus aureus* on the nasal membranes or *Streptococcus pneumoniae* in the upper respiratory tract). If the parasite **invades** (i.e., breaches an anatomical barrier or progress beyond the point of colonization), an **infection** is said to have occurred. If, as a result of infection, pathological harm to the host becomes evident, this is called an **infectious disease**. An infectious disease is a consequence of a microbial parasite causing such a degree of harm to its host that it results in a pathological process.

The healthy animal defends itself against pathogens different stages. The host defenses may be of such a degree that infection can be prevented entirely. Or, if infection does occur, the defenses may stop the process before disease is apparent. At other times, the defenses that are necessary to defeat a parasite may not be effective until infectious disease is well into progress.

Typically the host **defense mechanisms are divided into two groups**:

1. **Constitutive Defenses**: Defenses common to all healthy animals. These defenses provide general protection against invasion by normal flora, or colonization, infection, and infectious disease caused by pathogens. The constitutive defenses have also been referred to as "natural" or " innate" resistance, since they are inherent to a specific host, but these terms are better reserved for certain types of constitutive defense (see below).

2. **Inducible Defenses**: Defense mechanisms that must be induced or turned on by host exposure to a pathogen (as during an infection). Unlike the constitutive defenses, they are not immediately ready to come into play until after the host is appropriately exposed to the parasite. The inducible defenses involve the **immune responses** to a pathogen causing an infection.

The inducible defenses are generally quite specifically directed against an invading pathogen. The constitutive defenses are not so specific, and are directed toward general strategic defense.

Constitutive Defenses of the Host

The **constitutive defenses** of the host can be arranged in the following categories:

Differences in susceptibility to certain pathogens

Anatomical defenses

Microbial antagonism

Inflammation (ability to undergo an inflammatory response)

Phagocytosis

Differences in Susceptibility of Animal Hosts to Microbial Pathogens

This type of resistance is also called **innate** and natural **resistance**. There are two aspects innate resistance: (1) natural (genetic) resistance among all members of a species, called **species resistance** and (2) **individual resistance** within the same animal species.

Species resistance

Certain animals are naturally resistant or nonsusceptible to certain pathogens. Certain pathogens infect only humans, not lower animals, e.g. syphilis, gonorrhea, measles, poliomyelitis. On the other hand, certain pathogens (e.g. canine distemper virus) do not infect humans. *Shigella* infects humans and baboons but not chimpanzees. Little information is available to explain these absolute differences in susceptibility to a pathogen but it could be due to:

Absence of specific tissue or cellular receptors for attachment (colonization) by the pathogen. For example, different strains of enterotoxigenic *E. coli* , defined by different fimbrial antigens, colonize human infants, calves and piglets, by recognizing species-specific carbohydrate receptors on enterocytes in the gastrointestinal tract.

Temperature of the host and ability of pathogen to grow. For example, birds do not normally become infected with mammalian strains of *Mycobacterium*

tuberculosis because these strains cannot grow at the high body temperature of birds. The anthrax bacillus (*Bacillus anthracis*) will not grow in the cold-blooded frog (unless the frog is maintained at 37^{o}).

Lack of the exact nutritional requirements to support the growth of the pathogen. Naturally-requiring purine-dependent strains of *Salmonella typhi* grow only in hosts supplying purines. Mice and rats lack this growth factor and pur- strains are avirulent. By injecting purines into these animals, such that the growth factor requirement for the bacterium is satisfied, the organisms prove virulent.

Lack of a target site for a microbial toxin. Most toxins produced by microbial cells exert their toxic activity only after binding to susceptible cells or tissues in an animal. Certain animals may lack an appropriate target cell or specific type of cell receptor for the toxin to bind to, and may therefore be nonsusceptible to the activity of the toxin. For example, injection of diphtheria toxin fails to kill the rat. The unchanged toxin is excreted in the urine. Inject a sample of the urine (or pure diphtheria toxin) into the guinea pig, and it dies of typical lesions caused by diphtheria toxin.

Individual resistance

There are many reasons why individuals of the same animal species may exhibit greater or lesser susceptibility to the same infective agent.

Age: usually this relates to the development and status of the immune system which varies with age. May also be associated with changes in normal flora coincidental to developmental changes in the animal.

Sex: usually linked to the presence and/or development of the sex organs. For example, mastitis and infectious diseases leading to abortion will obviously occur only in the female; orchitis would occur only in males). Could also be due to anatomical structure related to sex (bladder infections are 14-times more common in females than males), and possibly the effects of sex hormones on infections.

Stress. Stress is a complex of different factors and apparently has a real influence on health. Undue exertion, shock, change in environment, climatic change, nervous or muscular fatigue, etc. are factors known to contribute to increases in susceptibility to infection. The best explanation is that in time of stress the output of cortisone from the adrenal cortex is increased. This suppresses the inflammatory processes of the host and the overall effect may be harmful. There are also a number of relationships between stress-related hormones and the functioning of the immune defenses.

Diet, malnutrition. Infections may be linked with vitamin and protein deficiencies and this might explain partly why many infectious diseases are more prevalent and infant mortality rates are highest in parts of the world where malnourishment is a problem. Also, overfed and obese animals are more susceptible to infection. Diets high in sucrose predispose individuals to dental caries.

Intercurrent disease or trauma. The normal defenses of an animal are impaired by organic diseases such as leukemia, diabetes, AIDS, etc. Frequently, inflammatory or immune responses are delayed or suppressed. Colds or influenza may predispose an individual to pneumonia. Smoking tobacco predisposes to infections of the respiratory tract. Burned tissue is readily infected by *Pseudomonas aeruginosas.*

Therapy against other diseases. Modern therapeutic procedures used in some diseases can render an individual more susceptible to infection. Under these conditions, not only pathogens but organisms of the normal flora and nonpathogens in the host's environment may be able to initiate infection. Examples of therapeutic procedures that reduce the efficiency of the host's defenses are treatment with corticosteroids, cytotoxic drugs, antibiotics, or irradiation.

Anatomical Defenses

The structural integrity of the body surfaces, i.e., the skin and mucous membranes, forms an effective barrier to initial lodgement or penetration by microorganisms. The skin is a very effective barrier to bacteria so that no bacterium by itself is known

to be able to penetrate unbroken skin. Of course, a puncture, cut or scrape in the skin could introduce infectious bacteria. The mucous membranes are more vulnerable to penetration by infectious bacteria but still pose a formidable barrier of mucus and antimicrobial substances. Nonetheless, most infectious agents impinge on the skin or mucous membranes of the oral cavity, respiratory tract, GI tract or urogenital tract, and from these sites most infections occur.

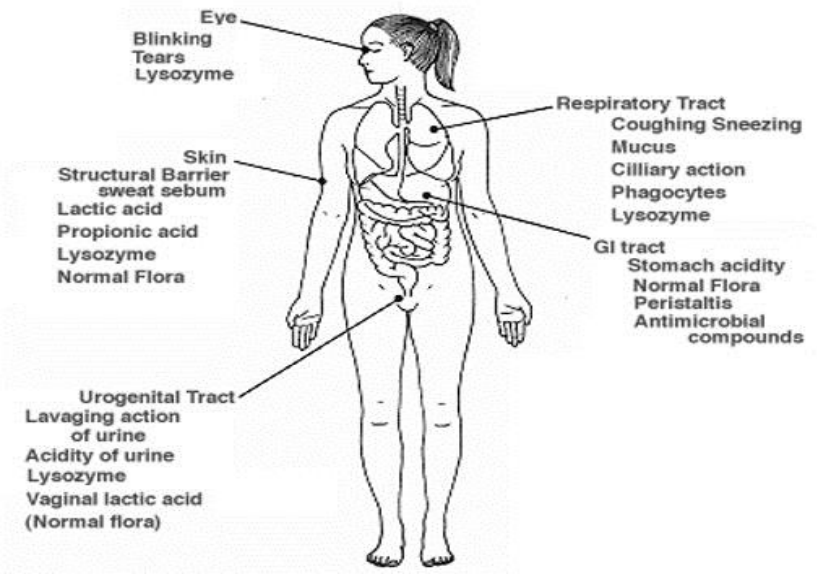


Figure. Anatomical defenses associated with tissue surfaces

Skin. The intact surface of the healthy epidermis seems to be rarely if ever penetrated by bacteria. If the integrity of the epidermis is broken (by the bite of an insect, needle stick, abrasion, cut, etc.) invasive microbes may enter. The normal flora of the skin, which metabolize substances secreted onto the skin, produce end products (e.g. fatty acids) that discourage the colonization of skin by potential pathogens. Perspiration contains lysozyme and other antimicrobial substances.

Mucous membranes. Many are heavily colonized with bacteria in whose moist secretions they survive. These normal flora are restricted from entry and usually occupy any attachment sites that might otherwise be used by pathogens. The normal

flora established on mucous membranes may antagonize non-indigenous species by other means, as well. Typically, mucus contains a number of types of anti-microbial compounds, including lysozyme and secretory antibodies (IgA). Sometimes phagocytes patrol mucosal surfaces (e.g. in the lower respiratory tract). Nonetheless, some pathogens are able to penetrate the mucous membranes, and this is probably the major site from which pathogens invade. Probably, damage to the epithelial cells caused by toxic products of these bacteria plays a role.

Respiratory tract. Fine hairs and baffles of the nares (nasal membranes) entrap bacteria which are inhaled. Those which pass may stick to mucosal surfaces of the trachea or be swept upward by the ciliated epithelium of the lower respiratory tract. Coughing and sneezing also eliminate bacteria. The lower respiratory tract (lung) is well protected by mucus, lysozyme, secretory antibody, and phagocytosis.

Mouth, stomach and intestinal tract. Microorganisms entering by the oral route, more than any other, have to compete with the well-adapted normal flora of the mouth and intestine. Most organisms that are swallowed are destroyed by acid and various secretions of the stomach. Alkaline pH of the lower intestine can discourage other organisms. The peristaltic action of the intestine ultimately flushes out organisms which have not succeeded in colonization. Bile salts and lysozyme are present, which kill or inhibit many types of bacteria.

Urogenital Tract. The flushing mechanisms of sterile urine, and the acidity of urine, maintain the bladder and most of the urethra free of microorganisms. The vaginal epithelium of the female maintains a high population of Doderlein's bacillus (*Lactobacillus acidophilus*) whose acidic end products of metabolism (lactic acid) prevent colonization by most other types of microorganisms including potentially-pathogenic yeast (*Candida albicans*).

Eyes (Conjunctiva). The conjunctiva of the eye is remarkably free of most microorganisms. Blinking mechanically removes microbes, the lavaging action of

tears washes the surface of the eye, and lachrymal secretions (tears) contain relatively large amounts of lysozyme.

Microbial Antagonism

This refers to the protection of the surfaces afforded by an intact normal flora in a healthy animal, and it has already been discussed in several contexts. There are **three main ways that the normal flora protect the surfaces where they are colonized:**

Competition with non-indigenous species for binding (colonization) sites. The normal flora are highly-adapted to the tissues of their host. That is why they are there!

Specific antagonism against non-indigenous species. Members of the normal flora may produce highly specific proteins called bacteriocins which kill or inhibit other (usually closely-related) species of bacteria.

Nonspecific antagonism against non-indigenous species. The normal flora produce a variety of metabolites and end products that inhibit other microorganisms. These include fatty acids (lactate, propionate, etc.) and peroxides.

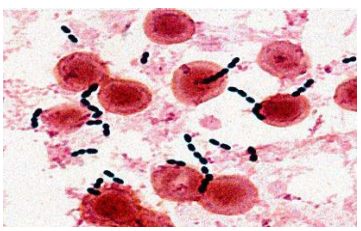


Figure 2. *Enterococcus faecalis*, also classified as *Streptococcus faecalis*. Occassionally there is invasion of the host by the normal flora, as evidenced by this blood culture. *Enterococcus faecalis*, blood culture.

Antimicrobial Substances in Host Tissues

The body fluids and organized tissues of animals naturally contain a variety of antimicrobial agent that kill or inhibit the growth of microbes. The sources and activities of a variety of host antimicrobial substances are summarized in the following Table.

TABLE. ANTIMICROBIAL SUBSTANCES OF HOST ORIGIN PRESENT IN BODY FLUIDS AND ORGANIZED TISSUES.

Substance	Common Sources	Chemical Composition	Activity
Lysozyme	Serum, saliva, sweat, tears	Protein	Bacterial cell lysis
Complement	Serum	Protein-carbohydrate lipoprotein complex	Cell death or lysis of bacteria; participates in inflammation
Basic proteins and polypeptides (histones, ß-lysins and other cationic proteins, tissue polypeptides)	Serum or organized tissues	Proteins or basic peptides	Disruption of bacterial plasma membrane
Lactoferrin and transferrin	Body secretions, serum, organized tissue spaces	Glycoprotein	Inhibit microbial growth by binding (withholding) iron
Peroxidase	Saliva, tissues, cells (neutrophils)	Protein	Act with peroxide to cause lethal oxidations of cells
Fibronectin	Serum and mucosal surfaces	Glycoprotein	Clearance of bacteria (opsonization)
Interferons	Virus-infected cells, lymphocytes	Protein	Resistance to virus infections
Interleukins	Macrophages, lymphocytes	Protein	Cause fever; promote activation of immune system

Antimicrobial Chemotherapy

CLASSIFICATION

Antimicrobials can be classified by at least three different schemes:

1. Effects on cells
2. Range of activity

3. Sites of activity

This page will examine these different classification schemes and describe several examples of each type of antimicrobial. The mechanisms by which organisms become resistant to these agents will also be discussed. Finally, toxicologic properties of antimicrobial chemotherapy will be described.

1. Antimicrobial Effects on Cells

Antimicrobials can be divided into two classifications based upon their effects on target cells. Drugs that actually kill microorganisms are termed *bactericidal*. Drugs that only inhibit the growth of microorganisms are termed *bacteriostatic*. The decision to use a bactericidal or bacteriostatic drug to treat infection depends entirely upon the type of infection. For example, bactericidal drugs will only kill cells that are actively growing. Bacteriostatic drugs, in comparison, will only inhibit the growth of cells; ultimate elimination of the organisms is dependent upon host phagocytic activity. Some examples of bactericidal and bacteriostatic drugs are listed below.

Bactericidal Drugs	Bacteriostatic Drugs
Streptomycin	Sulfonamides
Aminoglycosides	Tetracycline
Penicillin	Chloramphenicol

2. Range of Activity

Antimicrobials can also be classified by their range of activity. In general, five classifications can be described. The first of these is termed *narrow spectrum*. Narrow spectrum drugs, as the name implies, are only active against a relatively small number of organisms. In general, narrow spectrum antibiotics are effective against Gram-positive organisms. The second classification is termed *moderate spectrum*. These drugs are generally effective against the Gram-positives and most systemic, enteric and urinary tract Gram-negative pathogens. The beta-lactam antibiotics (penicillin, ampicillin, cephalosporins, etc.) belong in a third classification, *narrow and moderate spectrum* because some members are only

effective against Gram-positive organisms while other members can also kill certain Gram-negative bacteria. A fourth classification is termed *broad spectrum*. These drugs are effective against all prokaryotes with two exceptions: *Mycobacteria* (see below) and *Pseudomonas*. The fifth group includes those drugs that are effective against *Mycobacteria*. The following table details some examples of these antimicrobials.

Range of Activity	Organisms Affected	Example Antibiotics
Narrow Spectrum	Gram-positives (*Actinomyces*, *Corynebacteria*, *Bacillus*, *Clostridium*, Pyogenic cocci, Spirochetes)	Macrolides (Erythromycin) Polypeptides (Polymyxin)
Moderate Spectrum	Gram-positives plus systemic, enteric and urinary tract Gram-negatives	Sulfonamides Aminoglycosides (Streptomycin, Gentamycin, Tobramycin)
Narrow/Moderate Spectrum	Gram-positives plus Gram-negatives	Beta-lactams (Penicillin, Ampicillin, Cephalosporins)
Broad Spectrum	All prokaryotes except *Mycobacteria* and *Pseudomonas*	Chloramphenicol Tetracycline
Anti-mycobacterial	*Mycobacteria*	Isoniazid Ethambutol Streptomycin Rifampin

3. Sites of Activity

A third means of classifying antimicrobials is by their site of activity within the target cell. Further, antimicrobials may affect either the **integrity** or the **synthesis** of these sites. The various cellular targets include the cell wall, the plasma membrane, the nucleic acids and proteins. The following table lists these sites and gives examples of antimicrobials acting against them.

Site of Activity	Example Antibiotics
Inhibition of cell wall integrity	Lysozyme
Inhibition of cell wall synthesis	
1. Biosynthetic enzymes (cytoplasmic)	Fosfomycin, Cycloserine

2. Membrane-bound phospholipid carrier	Bacitracin
3. Polymerization of subunits	Beta-lactams
4. Combine with wall substrates	Vancomycin
Inhibition of membrane integrity	Surfacants, Polyenes, Polypeptides
Inhibition of membrane synthesis	None
Inhibition of nucleic acid integrity	Alkylating, Intercalating agents (mitomycin, chloroquin)
Inhibition of nucleic acid synthesis	
1. Metabolism of DNA	5-Fluorocytosine, Acyclovir, NTP analogs
2. Replication of DNA	Nalidixic acid, Novobiocin, Nitroimadazoles
3. Synthesis of RNA	Rifampin
Protein integrity	Phenolics, Heavy metals
Protein synthesis	
1. 30S Subunit	Streptomycin, Kanamycin, Tetracycline
2. 50S Subunit	Chloramphenicol, Macrolides (Clindamycin, Erythromycin)
3. Folate metabolism	Sulfonamides, Trimethoprim

RESISTANCE MECHANISMS

The problem of antibiotic resistance is becoming increasingly apparent as more and more strains of pathogenic microorganisms are untreatable with commonly used antimicrobials. This problem can be attributed to a variety of factors including overuse of antibiotics in agriculture and medicine and misuse of antibiotics by consumers. In addition, antibiotic resistance is often plasmid-borne, which means that resistance can be readily transferred from one organism to another. There are several mechanisms for antibiotic resistance and these relate to the sites of antimicrobial activity. These mechanisms include:

1. Altered receptors for the drug
2. Decreased entry into the cell
3. Destruction or inactivation of the drug

These mechanisms and some examples are outlined in the following table.

Altered Receptors	
1. Beta-lactams	Altered Penicillin Binding Proteins
2. Macrolides	Methylation of 2 adenine residues in 23S RNA of the 50S subunit
3. Rifampin	Single amino acid change in RNA polymerase ß-subunit
4. Sulfonamide/trimethoprim	Altered synthetase binds pABA preferentially/altered reductase for TMP
5. Nalidixic acid	Altered gyrase
6. Streptomycin	Altered S12 protein in 30S subunit
Decreased Entry	
1. Tetracycline	Normally biphasic, active transport reduced
2. Fosfomysin (chromosomal)	Glucose-6-phosphate transport reduced
Destruction/Inactivation	
1. Chloramphenicol acetyltransferase	Acetylates chloramphenicol
2. Beta-lactamase	Cleaves ß-lactam ring
3. Aminoglycosides	Acetylation or phosphorylation as drug passes membrane

رؤية الكلية

التميز في تعليم العلوم الأساسية والبحث العلمي للمساهمة فى التنمية المستدامة.

رسالة الكلية

تقديم تعليم مميز في مجالات العلوم الأساسية وإنتاج بحوث علمية تطبيقية للمساهمة فى التنمية المستدامة من خلال إعداد خريجين متميزين طبقا للمعايير الأكاديمية القومية، وتطوير مهارات وقدرات الموارد البشرية، وتوفير خدمات مجتمعية وبيئية تلبي طموحات مجتمع جنوب الوادي، وبناء الشراكات المجتمعية الفاعلة.

ALL MY BEST WISHES

Prof. Dr. Wesam Salem

2023/ 2024