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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – I – Medical Bacteriology – SMB3101

1. THE HISTORY OF INFECTIOUS DISEASES

1.1 The Past

Infectious diseases have been known for thousands of years, although accurate information on their etiology has only been available for about a century. In the medical teachings of Hippocrates, the cause of infections occurring frequently in a certain locality or during a certain period (epidemics) was sought in "changes" in the air according to the theory of miasmas. This concept, still reflected in terms such as "swamp fever" or "malaria," was the predominant academic opinion until the end of the 19th century, despite the fact that the Dutch cloth merchant A. van Leeuwenhoek had seen and described bacteria as early as the 17th century, using a microscope he built himself with a single convex lens and a very short focal length. At the time, general acceptance of the notion of "spontaneous generation"—creation of life from dead organic material—stood in the way of implicating the bacteria found in the corpses of infection victims as the cause of the deadly diseases. It was not until Pasteur disproved the doctrine of spontaneous generation in the second half of the 19th century that a new way of thinking became possible. By the end of that century, microorganisms had been identified as the causal agents in many familiar diseases by applying the Henle-Koch postulates formulated by R. Koch in 1890.

The postulates can be freely formulated as follows:

- The microorganism must be found under conditions corresponding to the pathological changes and clinical course of the disease in question.
- It must be possible to cause an identical (human) or similar (animal) disease with pure cultures of the pathogen.
- The pathogen must not occur within the framework of other diseases as an "accidental parasite."

These postulates are still used today to confirm the cause of an infectious disease. However, the fact that these conditions are not met does not necessarily exclude a contribution to disease etiology by a pathogen found in context. In particular, many infections caused by subcellular entities do not fulfill the postulates in their classic form.

1.2 Host–Pathogen Interactions

- The factors determining the genesis, clinical picture and outcome of an infection include complex relationships between the host and invading organisms that differ widely depending on the pathogen involved. Despite this variability, a number of general principles apply to the interactions between the invading pathogen with its aggression factors and the host with its defenses. Since the pathogenesis of bacterial infectious diseases has been researched very thoroughly, the following summary is based on the host–

invader interactions seen in this type of infection. The determinants of bacterial pathogenicity and virulence can be outlined as follows:

- Adhesion to host cells (adhesins).
- Breaching of host anatomical barriers (invasins) and colonization of tissues (aggressins).
- Strategies to overcome nonspecific defenses, especially antiphagocytic mechanisms (impedins).
- Strategies to overcome specific immunity, the most important of which is production of IgA proteases (impedins), molecular mimicry, and immunogen variability.
- Damage to host tissues due to direct bacterial cytotoxicity, exotoxins, and exoenzymes (aggressins).
- Damage due to inflammatory reactions in the macroorganism: activation of complement and phagocytosis; induction of cytokine production (modulins). The above bacterial pathogenicity factors are confronted by the following host defense mechanisms:
- Nonspecific defenses including mechanical, humoral, and cellular systems. Phagocytosis is the most important process in this context.
- Specific immune responses based on antibodies and specific reactions of T lymphocytes. The response of these defenses to infection thus involves the correlation of a number of different mechanisms. Defective defenses make it easier for an infection to take hold. Primary, innate defects are rare, whereas acquired, secondary immune defects occur frequently, paving the way for infections by microorganisms known as “facultative pathogens” (opportunists).

1. 3 Basic Terminology of Infectiology

The terms pathogenicity and virulence are not clearly defined in their relevance to microorganisms. They are sometimes even used synonymously. It has been proposed that pathogenicity be used to characterize a particular species and that virulence be used to describe the sum of the disease-causing properties of a population (strain) of a pathogenic species.

Pathogenicity and virulence in the microorganism correspond to susceptibility in a host species and disposition in a specific host organism, whereby an individual may be anywhere from highly disposed to resistant.

1.4 Determinants of Bacterial Pathogenicity and Virulence

Relatively little is known about the factors determining the pathogenicity and virulence of microorganisms, and most of what we do know concerns the disease-causing mechanisms of bacteria.

There are five groups of potential bacterial contributors to the pathogenesis of infectious diseases:

1. Adhesins. They facilitate adhesion to specific target cells.

2. Invasins. They are responsible for active invasion of the cells of the macroorganism.
3. Impedins. These components disable host immune defenses in some cases.
4. Aggressins. These substances include toxins and tissue-damaging enzymes.
5. Modulins. Substances that induce excess cytokine production (i.e., lipopolysaccharides of Gram-negative bacteria, superantigens, murein fragments).

1.5 Strategies against Nonspecific Immunity

Establishment of a bacterial infection in a host presupposes the capacity of the invaders to overcome the host's nonspecific immune defenses. The most important mechanisms used by pathogenic bacteria are:

a) Antiphagocytosis

— Capsule. Renders phagocytosis more difficult. Capsule components may block alternative activation of complement so that C3b is lacking (ligand for C3b receptor of phagocytes) on the surface of encapsulated bacteria. Microorganisms that use this strategy include Streptococcus pneumonia and Haemophilus influenzae.

— Phagocyte toxins. Examples: leukocidin from staphylococci, streptolysin from streptococci.

— Macrophages may be disabled by the type III secretion system of certain Gram-negative bacteria (for example salmonellae, shigellae, yersiniae, and coli bacteria). This system is used to inject toxic proteins into the macrophages.

— Inhibition of phagosome-lysosome fusion. Examples: tuberculosis bacteria, gonococci, Chlamydia psittaci.

— Inhibition of the phagocytic "oxidative burst." No formation of reactive O2 radicals in phagocytes. Examples: Legionella pneumophilia, Salmonella typhi.

b) Serum resistance.

Resistance of Gram-negative bacteria to complement. A lipopolysaccharide in the outer membrane is modified in such away that it cannot initiate alternative activation of the complement system. As a result, the membrane attack complex (C5b6789), which would otherwise lyse holes in the outer membrane, is no longer produced.

c) Siderophores.

Siderophores (e.g., enterochelin, aerobactin) are low-molecular- weight iron-binding molecules that transport Fe3+ actively into the intracellular space. They complex with iron, thereby stealing this element from proteins containing iron (transferrin, lactoferrin). The intricate iron transport system is localized in the cytoplasmic membrane, and in Gram negative bacteria in the outer membrane as well. To thrive, bacteria require 10^{-5} mol/l free iron ions. The free availability of only about 10–20 mol/l iron in human body fluids thus presents a challenge to them.

Strategies against Specific Immunity

Immunotolerance.

— Prenatal infection. At this stage of development, the immune system is unable to recognize bacterial immunogens as foreign.

— Molecular mimicry. Molecular mimicry refers to the presence of molecules on the surface of bacteria that are not recognized as foreign by the immune system. Examples of this strategy are the hyaluronic acid capsule of Streptococcus pyogenes or the neuraminic acid capsule of Escherichia coli K1 and serotype B Neisseria meningitidis.

Antigen variation.

Some bacteria are characterized by a pronounced variability of their immunogens (= immune antigens) due to the genetic variability of the structural genes coding the antigen proteins. This results in production of a series of antigen variants in the course of an infection that no longer "match" with the antibodies to the "old" antigen. Examples: gonococci can modify the primary structure of the pilin of their attachment pili at a high rate (Fig. 1.2). The borreliae that cause relapsing fevers have the capacity to change the structure of one of the adhesion proteins in their outer membrane (vmp = variable major protein), resulting in the typical "recurrences" of fever. Similarly, meningococci can change the chemistry of their capsule polysaccharides ("capsule switching").

IgA proteases.

Mucosal secretions contain the secretory antibodies of the sIgA1 class responsible for the specific local immunity of the mucosa. Classic mucosal parasites such as gonococci, meningococci and Haemophilus influenza produce proteases that destroy this immunoglobulin.

Defenses against Infection

A macroorganism manifests defensive reactions against invasion by microorganisms in two forms: as specific, acquired immunity and as nonspecific, innate resistance

Primary defenses.

The main factors in the first line of defense against infection are mechanical, accompanied by some humoral and cellular factors. These defenses represent an attempt on the part of the host organism to prevent microorganisms from colonizing its skin and mucosa and thus stave off a generalized invasion.

Secondary defenses.

The second line of defense consists of humoral and cellular factors in the blood and tissues, the most important of which are the professional phagocytes.

Phagocytosis.

“Professional” phagocytosis is realized by polymorphonuclear, neutrophilic, eosinophilic granulocytes—also known as microphages— and by mononuclear phagocytes (macrophages). The latter also play an important role in antigen presentation

Microphages contain both primary granules, which are lysosomes containing lysosomal enzymes and cationic peptides, and secondary granules. Both microphages and macrophages are capable of ameboid motility and chemotactic migration, i.e., directed movement along a concentration gradient toward a source of chemotactic substances, in most cases the complement components C3a and C5a. Other potentially chemotactic substances include secretory products of lymphocytes, products of infected and damaged cells or the N-formyl peptides (fMet-Phe and fMet-Leu-Phe).

Phagocytes are capable of ingestion of both particulate matter (phagocytosis) and solute matter (pinocytosis). Receptors on the phagocyte membrane initiate contact (Fig. 1.6). Particles adhering to the membrane are engulfed, ingested and deposited in a membrane-bound vacuole, the so-called phagosome, which then fuses with lysosomes to form the phagolysosome. The bacteria are killed by a combination of lysosomal factors: — Mechanisms that require no oxygen. Low pH; acid hydrolases, lysozyme; proteases; defensins (small cationic peptides). Mechanisms that require oxygen. Halogenation of essential bacterial components by the myeloperoxidase-H2O2–halide system; production of highly reactive O2 radicals (oxidative burst) such as superoxide anion (O2 –), hydroxyl radical (!OH), and singlet oxygen (1O2). Specific Defense Mechanisms Specific immunity, based on antibodies and specifically reactive T lymphocytes, is acquired in a process of immune system stimulation by the corresponding microbial antigens. Humoral immunity is based on antitoxins, opsonins, microbicidal antibodies, neutralizing antibodies, etc. Cellular immunity is based on cytotoxic T lymphocytes (T killer cells) and T helper cells. See

Defects in Immune Defenses

Hosts with defects in their specific and/or nonspecific immune defenses are prone to infection.

& Primary defects. Congenital defects in the complement-dependent phagocytosis system are rare, as are B and T lymphocyte defects.

& Secondary defects. Such effects are acquired, and they are much more frequent. Examples include malnutrition, very old and very young hosts, metabolic disturbances (diabetes, alcoholism), autoimmune diseases, malignancies (above all lymphomas and leukemias), immune system infections (HIV), severe primary diseases of parenchymatous organs, injury of skin or mucosa, immunosuppressive therapy with corticosteroids, cytostatics and immunosuppressants, and radiotherapy.

One result of progress in modern medicine is that increasing numbers of patients with secondary immune defects are now receiving hospital treatment. Such “problem patients” are frequently infected by opportunistic bacteria that would not present a serious threat to normal immune defenses. Often, the pathogens involved (“problem bacteria”) have developed a resistance to

numerous antibiotics, resulting in difficult courses of antibiotic treatment in this patient category. Normal Flora Commensals are regularly found in certain human microbiotopes. The normal human microflora is thus the totality of these commensals.

Bacteria are the predominant component of the normal flora. They proliferate in varied profusion on the mucosa and most particularly in the gastrointestinal tract, where over 400 different species have been counted to date. The count of bacteria per gram of intestinal content is 10^1–10^5 in the duodenum, 10^3–10^7 in the small intestine, and 10^{10}–10^{12} in the colon. Over 99% of the normal mucosal flora are obligate anaerobes, dominated by the Gram-neg. anaerobes. Although life is possible without normal flora (e.g., pathogen-free experimental animals), commensals certainly benefit their hosts. One way they do so is when organisms of the normal flora manage to penetrate into the host through microtraumas, resulting in a continuous stimulation of the immune system. Commensals also compete for living space with overtly pathogenic species, a function known as colonization resistance. On the other hand, a potentially harmful effect of the normal flora is that they can also cause infections in immunocompromised individuals.

NORMAL HUMAN MICROBIOTA

The term “normal microbial flora” denotes the population of microorganisms that inhabit the skin and mucous membranes of healthy normal persons. The microorganisms that live inside and on humans (now referred to as the normal microbiota) are estimated to outnumber human somatic and germ cells by a factor of 10. The genomes of these microbial symbionts are collectively defined as the microbiome. Research has shown that the “normal microbiota” provides a first line of defense against microbial pathogens, assist in digestion, play a role in toxin degradation, and contribute to maturation of the immune system. Shifts in the normal microbiota or stimulation of inflammation by these commensals may cause diseases such as bacterial vaginosis, periodontitis, and inflammatory bowel disease.

HUMAN MICROBIOME PROJECT

In a broad attempt to understand the role played by resident microbial ecosystems in human health and disease, in 2007, the National Institutes of Health launched the Human Microbiome Project. One of the main goals of this project is to understand the range of human genetic and physiologic diversity, the microbiome, and the factors that influence the distribution and evolution of the constituent microorganisms. One aspect of this project involves having several research groups simultaneously embark upon surveying the microbial communities on human skin and in mucosal areas such as the mouth, esophagus, stomach, colon, and vagina using small-subunit (16S) ribosomal RNA gene sequencing. Among the questions that will be addressed by this project are: How stable and resilient is an individual’s microbiota throughout one day and during his or her lifespan? How similar are the microbiomes between members of a family or members of a community or across communities in different environments? Do all humans have an identifiable “core” microbiome, and if so, how is it acquired and transmitted? What affects the genetic diversity of the microbiome, and how does this diversity affect adaptation by the microorganisms and the host to markedly different lifestyles and to various physiological or pathophysiological states? Numerous observations have already been made.

For example, it has been determined that there are large differences among individuals in terms of the numbers and types of species of microorganisms inhabiting the colon and that obesity may be correlated with the types of microbes involved in specific metabolic pathways in the gastrointestinal tract. Readers should be aware that this field is rapidly evolving, and our understanding of the human microbiota will necessarily change as more information about resident microbial communities becomes available through the Human Microbiome Project.

ROLE OF THE RESIDENT MICROBIOTA

The skin and mucous membranes always harbor a variety of microorganisms that can be arranged into two groups:

(1) the resident microbiota consists of relatively fixed types of microorganisms regularly found in a given area at a given age; if disturbed, it promptly reestablishes itself; and

(2) the transient microbiota consists of nonpathogenic or potentially pathogenic microorganisms that inhabit the skin or mucous membranes for hours, days, or weeks. The transient microbiota is derived from the environment, does not produce disease, and does not establish itself permanently on the surface. Members of the transient microbiota are generally of little significance so long as the normal resident flora remains intact. However, if the resident microbiota is disturbed, transient microorganisms may colonize, proliferate, and produce disease.

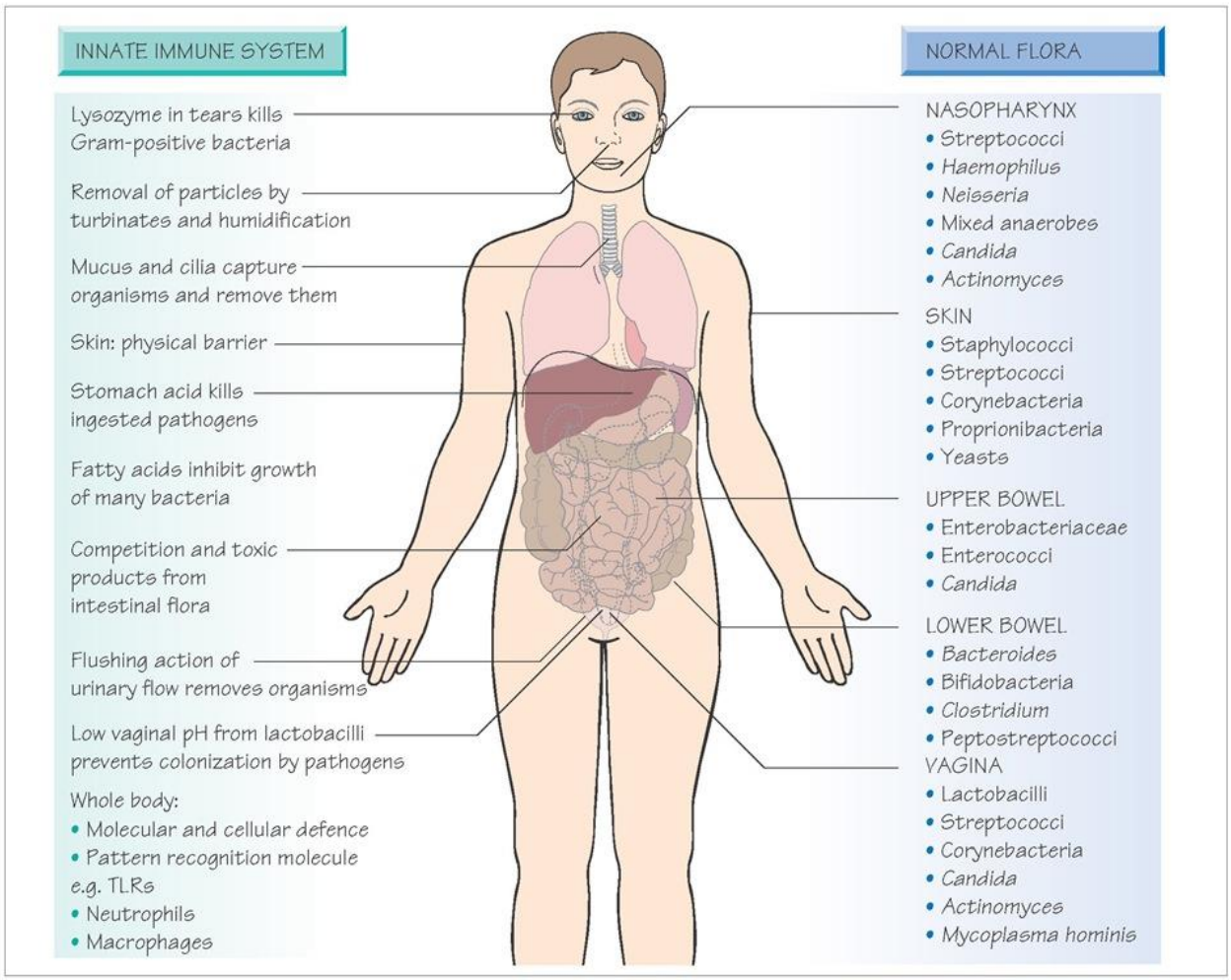

Fig.1.1: Normal flora of human body

Organisms frequently encountered in specimens obtained from various areas of the human body—and considered normal microbiota.

It is likely that microorganisms that can be cultured in the laboratory represent only a fraction of those that are part of the normal resident or transient microbiota. When the broad range polymerase chain reaction (PCR) is used to amplify bacterial 16S rDNA, many previously unidentified bacteria can be detected, as in secretions from patients with bacterial vaginosis. The number of species that make up the normal microbiota has been shown to be much greater than previously recognized. Thus, the understanding of normal microbiota is in transition. As already mentioned, the relationship of previously unidentified microorganisms, which are potentially part of the normal microbiota, to disease is likely to change.

The microorganisms that are constantly present on body surfaces are frequently described as commensals (ie, one partner benefits, while the other seems unaffected). However, in some sites (eg, gut), mutualistic (ie, both parties derive benefit) may be a better description of this relationship. Their flourishing in a given area depends on physiologic factors of temperature, moisture, and the presence of certain nutrients and inhibitory substances. Their presence is not essential to life because "germ-free" animals can be reared in the complete absence of a normal microbiota. Yet the resident flora of certain areas plays a definite role in maintaining health and normal function. Members of the resident microbiota in the intestinal tract synthesize vitamin K and aid in the absorption of nutrients. On mucous membranes and skin, the resident microbiota may prevent colonization by pathogens and possible disease through "bacterial interference." The mechanism of bacterial interference may involve competition for receptors or binding sites on host cells, competition for nutrients, mutual inhibition by metabolic or toxic products, mutual inhibition by antibiotic materials or bacteriocins, or other mechanisms. Suppression of the normal microbiota clearly creates a partial local void that tends to be filled by organisms from the environment or from other parts of the body. Such organisms behave as opportunists and may become pathogens. On the other hand, members of the normal microbiota may themselves produce disease under certain circumstances. These organisms are adapted to a noninvasive mode of life defined by the limitations of the environment. If forcefully removed from the restrictions of that environment and introduced into the bloodstream or tissues, these organisms may become pathogenic. For example, streptococci of the viridans group are the most common resident organisms of the upper respiratory tract. If large numbers of them are introduced into the bloodstream (eg, after tooth extraction or oral surgery), they may settle on deformed or prosthetic heart valves and produce infective endocarditis. Small numbers occur transiently in the bloodstream with minor trauma (eg, dental scaling or vigorous brushing).

Bacteroides species are the most common resident bacteria of the large intestine and are quite harmless in that location. However, if introduced into the peritoneal cavity or into pelvic tissues along with other bacteria as a result of trauma, they cause suppuration and bacteremia. There are many other examples, but the important point is that the normal resident microbiota is harmless and may be beneficial in their normal location in the host and in the absence of coincident abnormalities. They may produce disease if introduced into foreign locations in large numbers and if predisposing factors are present.

NORMAL MICROBIOTA OF THE SKIN

The skin is the human body's largest organ, colonized by a diverse array of microorganisms, most of which are harmless or even beneficial to the host. Because of its constant exposure to and contact with the environment, the skin is particularly apt to contain transient microorganisms. Nevertheless, there is a constant and well-defined resident flora, modified in different anatomic areas by secretions, habitual wearing of clothing, or proximity to mucous membranes (mouth, nose, and perineal areas)

The predominant resident microorganisms of the skin are aerobic and anaerobic diphtheroid bacilli (eg, Corynebacterium, Propionibacterium); nonhemolytic aerobic and anaerobic staphylococci (Staphylococcus epidermidis and other coagulase-negative staphylococci, occasionally Staphylococcus aureus, and Peptostreptococcus species); grampositive, aerobic, spore-forming bacilli that are ubiquitous in air, water, and soil; α-hemolytic streptococci (viridans streptococci) and enterococci (Enterococcus species); and gram-negative coliform bacilli and Acinetobacter. Fungi and yeasts are often present in skin folds; acid-fast, nonpathogenic mycobacteria occur in areas rich in sebaceous secretions (genitalia, external ear). Among the factors that may be important in eliminating nonresident microorganisms from the skin are the low pH, the fatty acids in sebaceous secretions, and the presence of lysozyme. Neither profuse sweating nor washing and bathing can eliminate or significantly modify the normal resident flora. The number of superficial microorganisms may be diminished by vigorous daily scrubbing with soap containing hexachlorophene or other disinfectants, but the flora is rapidly replenished from sebaceous and sweat glands even when contact with other skin areas or with the environment is completely excluded. Placement of an occlusive dressing on the skin tends to result in a large increase in the total microbial population and may also produce qualitative alterations in the flora.

Anaerobes and aerobic bacteria often join to form synergistic infections (gangrene, necrotizing fasciitis, and cellulitis) of skin and soft tissues. The bacteria are frequently part of the normal microbial flora. It is usually difficult to pinpoint one specific organism as being responsible for the progressive lesion because mixtures of organisms are usually involved. In addition to being a

physical barrier, the skin is an immunologic barrier. Keratinocytes continuously sample the microbiota colonizing the skin surface through pattern recognition receptors (eg, Toll-like receptors, mannose receptors, NOD-like receptors). The activation of keratinocyte pattern recognition receptors by pathogen-associated molecular patterns initiates the innate immune response, resulting in the secretion of antimicrobial peptides, cytokines, and chemokines. Despite being constantly exposed to large numbers of microorganisms, the skin can distinguish between harmless commensals and harmful pathogenic microorganisms. The mechanism for this selectivity is unclear.

NORMAL MICROBIOTA OF THE MOUTH AND UPPER RESPIRATORY TRACT

The flora of the nose consists of prominent corynebacteria, staphylococci (S epidermidis, S aureus), and streptococci. In direct contrast to the highly differentiated communities of their mothers, neonates harbored bacterial communities that were undifferentiated across multiple body habitats, regardless of delivery mode. Thus, at its earliest stage of community development (<5 minutes postdelivery), the human microbiota is homogeneously distributed across the body. Vaginally delivered infants harbor bacterial communities (in all body habitats) that are most similar in composition to the vaginal communities of the mothers; C-section babies lack bacteria from the vaginal community (eg, Lactobacillus, Prevotella, Atopobium, and Sneathia spp.). Infants delivered via C-section harbor bacterial communities (across all body habitats) that are most similar to the skin communities of the mothers (eg, Staphylococcus, Corynebacterium, or Propionibacterium spp.).

Within 4–12 hours after birth, viridans streptococci become established as the most prominent members of the resident flora and remain so for life. These organisms probably originate in the respiratory tracts of the mother and attendants. Early in life, aerobic and anaerobic staphylococci, gram-negative diplococci (neisseriae, Moraxella catarrhalis), diphtheroids, and occasional lactobacilli are added. When teeth begin to erupt, the anaerobic spirochetes, Prevotella species (especially Prevotella melaninogenica), Fusobacterium species, Rothia species, and Capnocytophaga species establish themselves along with some anaerobic vibrios and lactobacilli. Actinomyces species are normally present in tonsillar tissue and on the gingivae in adults, and various protozoa may also be present. Yeasts (Candida species) occur in the mouth.

In the pharynx and trachea, a similar flora establishes itself, but few bacteria are found in normal bronchi. Small bronchi and alveoli are normally sterile.

The predominant organisms in the upper respiratory tract, particularly the pharynx, are nonhemolytic and α-hemolytic streptococci and neisseriae. Staphylococci, diphtheroids, haemophili, pneumococci, mycoplasmas, and prevotellae are also encountered. More than 600

different species have been described from the human oral cavity, but only limited information is available on the normal microbiota of healthy individuals. The human oral microbiome, as represented by the human salivary microbiome, has recently been characterized in samples obtained from 120 healthy individuals from 12 worldwide locations by 16S rRNA sequencing. There is considerable diversity in the saliva microbiome, both within and among individuals; however, it does not vary substantially around the world. The 16S rRNA sequences could be assigned to 101 known bacterial genera, of which 39 were not previously reported from the human oral cavity; phylogenetic analysis suggests that an additional 64 unknown genera are also present. Infections of the mouth and respiratory tract are usually caused by mixed oronasal flora, including anaerobes. Periodontal infections, perioral abscesses, sinusitis, and mastoiditis may involve predominantly P melaninogenica, Fusobacteria, and Peptostreptococci. Aspiration of saliva (containing up to 102 of these organisms and aerobes) may result in necrotizing pneumonia, lung abscess, and empyema.

The Role of the Normal Mouth Microbiota in Dental Plaque and Caries

Dental plaque, which has come to be viewed and managed as a complex biofilm, can be defined simplistically as an adherent dental deposit that forms on the tooth surface composed almost entirely of bacteria derived from the normal flora of the mouth (Figure 10-2). Dental plaque is the most prevalent and densest of human biofilms. The advantages for the microbes in the biofilm include protection from environmental hazards (including antimicrobials) and optimization of spatial arrangements that maximize energy through movement of nutrients. Organisms within the biofilm interact dynamically at multiple metabolic and molecular levels. The biofilm first forms in relation to the dental pellicle, which is a physiologic thin organic film covering the mineralized tooth surface composed of proteins and glycoproteins derived from saliva and other oral secretions . As the plaque biofilm evolves, it does so in relation to the pellicle and not the mineralized tooth itself. Plaque formation takes place in stages and layers at two levels. The first is the anatomical location of the plaque in relation to the gingival line; the earliest plaque is supragingival, which may then extend to subgingival plaque. The second level is the layering within the plaque, the bacterial species involved, and the bacteria–pellicle and bacteria–bacteria binding mechanisms involved. The initial colonizing organisms are mainly grampositive bacteria that use specific ionic and hydrophobic interactions as well as lectin-like surface structures to adhere to the pellicle and to each other. The prototype early colonizer is Streptococcus sanguis, but other streptococci (Streptococcus mutans, Streptococcus mitis, Streptococcus salivarius, Streptococcus oralis, Streptococcus gordonii), lactobacilli, and Actinomyces species are usually present. Late colonizers can appear in the biofilm in as little as 2–4 days and consist primarily of gram-negative anaerobes (eg, Porphyromonas, Prevotella, Fusobacterium, Veillonella species), including anaerobic spirochetes (eg, Treponema denticola), and more Actinomyces species. These bacteria use similar

mechanisms to bind to the early colonizers and to each other. High-molecular-weight extracellular glucan polymers are synthesized, which act like a cement binding the plaque biofilm together. The carbohydrate polymers (glucans) are produced mainly by streptococci (S mutans), perhaps in association with Actinomyces species. In all, there are thought to be 300–400 bacterial species present in mature dental plaque.

Caries is a disintegration of the teeth beginning at the surface and progressing inward. First the surface enamel, which is entirely noncellular, is demineralized. This has been attributed to the effect of acid products of glycolytic metabolic activity when the plaque bacteria are fed the right substrate. Subsequent decomposition of the dentin and cementum of the exposed root surface involves bacterial digestion of the protein matrix. S mutans is considered to be the dominant organism for the initiation of caries; however, multiple members of the plaque biofilm participate in the evolution of the lesions. These include other streptococci (S salivarius, S sanguis, Streptococcus sobrinus), lactobacilli (Lactobacillus acidophilus, Lactobacillus casei), and actinomycetes (Actinomyces viscosus, Actinomyces naeslundii). The large amounts of organic acid products produced from carbohydrates by the interaction of S mutans with these other species in plaque are the underlying cause of caries.

The accumulation of these acid products causes the pH of the plaque to drop to levels sufficient to react with the hydroxyapatite of the enamel, demineralizing it to soluble calcium and phosphate ions. Production of acid and decreased pH is maintained until the substrate is depleted after which the plaque pH returns to its more neutral pH resting level and some recovery can take place. Dietary monosaccharides (eg, glucose, fructose) and disaccharides (eg, sucrose, lactose, and maltose) provide an appropriate substrate for bacterial glycolysis and acid production to cause tooth demineralization. Foods with high sugar content, particularly sucrose, which adhere to the teeth and have long oral clearance times, are more cariogenic than less retentive food stuffs such as sugar-containing liquids. A possible edge for S mutans is its ability to metabolize sucrose more efficiently than other oral bacteria. An additional factor is that sucrose is also used for the synthesis of extracellular polyglycans such as dextrans and levans by transferase enzymes on the bacterial cell surface. Polyglycan production contributes to aggregation and accumulation of S mutans on the tooth surface and may also serve as an extracellular storage form of substrate for other plaque bacteria.

Periodontal pockets in the gingiva are particularly rich sources of organisms, including anaerobes that are rarely encountered elsewhere. Plaque-induced periodontal disease encompasses two separate disease entities, gingivitis and chronic periodontitis. Both conditions are caused by

bacteria in the subgingival dental plaque found within the gingival crevice or the sulcus around the necks of the teeth. Periodontitis is a biofilm-induced chronic inflammatory disease which affects the tooth-supporting tissues. Although the toothassociated biofilm plays a crucial role in the initiation and progression of periodontitis, it is primarily the host inflammatory response that is responsible for the damage to the periodontium, leading to tooth loss in some cases. It has been hypothesized that Porphyromonas gingivalis impairs innate immunity in ways that alter the growth and development of the entire biofilm, triggering a breakdown in the normally homeostatic host–microbiota interplay in the periodontium. Although the microorganisms within the biofilm may participate in periodontal disease and tissue destruction, attention is drawn to them when they are implanted elsewhere (eg, producing infective endocarditis or bacteremia in a granulocytopenic host). Examples are Capnocytophaga species and Rothia dentocariosa. Capnocytophaga species are fusiform, gram-negative, gliding anaerobes; Rothia species are pleomorphic, aerobic, gram-positive rods. In granulocytopenic immunodeficient patients, they can lead to serious opportunistic lesions in other organs.

Control of caries involves physical removal of plaque, limitation of sucrose intake, good nutrition with adequate protein intake, and reduction of acid production in the mouth by limitation of available carbohydrates and frequent cleansing. The application of fluoride to teeth or its ingestion in water results in enhancement of acid resistance of the enamel. Control of periodontal disease requires removal of calculus (calcified deposit) and good mouth hygiene.

Normal Microbiota of the Intestinal Tract

The human gastrointestinal tract is divided into sections, allowing digestion and nutrient absorption in the proximal region to be separate from the vast microbial populations in the large intestine. At birth, the intestine is sterile, but organisms are soon introduced with food. The environment (eg, maternal vaginal, fecal, or skin microbiota) is a major factor in determining the early microbial profile. Many early studies reported that the intestinal microbiota of breastfed children is dominated by Bifidobacteria. However, recent studies employing microarrays and quantitative PCR suggested that in most babies, Bifidobacteria did not appear until several months after birth and thereafter persisted as a minority population. In bottle-fed children, a more mixed flora exists in the bowel, and lactobacilli are less prominent. As food habits develop toward the adult pattern, the bowel flora changes. Diet has a marked influence on the relative composition of the intestinal and fecal flora. For example, individuals on an animal-based diet have been shown to have an increased abundance of bile-tolerant microorganisms (Alistipes, Bilophilia, and Bacteroides) and decreased levels of Firmicutes that metabolize dietary plant polysaccharides (Roseburia, Eubacterium rectale, and Ruminococcus bromii). Bowels of newborns in intensive

care nurseries tend to be colonized by Enterobacteriaceae, such as Klebsiella, Citrobacter, and Enterobacter.

In normal adults, the esophagus contains microorganisms arriving with saliva and food. The stomach's acidity keeps the number of microorganisms at a minimum (102–103/ mL of contents) unless obstruction at the pylorus favors the proliferation of gram-positive cocci and bacilli. From the hundreds of phylotypes detected in the human stomach, only Helicobacter pylori persists in this environment. The normal acid pH of the stomach markedly protects against infection with some enteric pathogens (eg, Vibrio cholerae). Administration of antacids, H2-receptor antagonists, and proton pump inhibitors for peptic ulcer disease and gastroesophageal reflux disease leads to a great increase in microbial flora of the stomach, including many organisms usually prevalent in feces. As the pH of intestinal contents becomes alkaline, the resident flora gradually increases. In the adult duodenum, there are 103–104 bacteria/mL of effluent; with higher populations in the jejunum, 10^4–10^5 bacteria/mL, and ileum, 108 bacteria/mL; and in the cecum and transverse colon, 10^{11}–10^{12} bacteria/mL, which is the highest recorded for any microbial habitat. In the upper intestine, the bacterial population associated with the mucosa include the phylum Bacteroidetes and members of the Clostridiales, and those of the lumen can include members of the Enterobacteriales and enterococci. In the sigmoid colon and rectum, the bacteria constitute about 60% of the fecal mass. Anaerobes outnumber facultative organisms by 1000-fold. In diarrhea, the bacterial content may diminish greatly, but in intestinal stasis, the count rises. In a normal adult colon, 96–99% of the resident bacterial flora consists of anaerobes. Six major phyla predominate; these are Bacteroidetes, Firmicutes, Actinobacteria, Verrucomicrobiota, Fusobacteria, and Proteobacteria. More than 100 distinct types of organisms, which can be cultured routinely in the laboratory, occur regularly in normal fecal flora. Archae are represented primarily by the methane producer Methanobrevibacter smithii, a low abundance microorganism that may play an important role in stabilizing gut microbial communities. There probably are more than 500 species of bacteria in the colon, including many that are likely unidentified. In addition to Bacteria and Archae, other types of microbes are present, such as protozoans and fungi, whose functions are less well understood. Viruses, mostly phages whose hosts are prominent members of the microbiota, are remarkably common in the colon. Minor trauma (eg, sigmoidoscopy, barium enema) may induce transient bacteremia in about 10% of procedures.

The first of these are protective functions in which the resident bacteria displace and inhibit potential pathogens indirectly by competing for nutrients and receptors or directly through the production of antimicrobial factors, such as bacteriocins and lactic acid. Second, commensal organisms are important for the development and function of the mucosal immune system. They induce the secretion of IgA, influence the development of the intestinal humoral immune system,

and modulate local T-cell responses and cytokine profiles. The third category consists of a broad range of metabolic functions. The microbiota of the small intestine can contribute to the amino acid requirements of the host if they are not provided by the diet itself. Intestinal bacteria produce short-chain fatty acids that control intestinal epithelial cell differentiation. They synthesize vitamin K, biotin, and folate and enhance ion absorption. Certain bacteria metabolize dietary carcinogens and assist with fermentation of nondigestible dietary residue. There is now evidence that gut bacteria can influence fat deposition in the host, leading to obesity.

Methanogenic archae are minor components of the gut microbiota. However, their ability to reduce small organic compounds (eg, CO2, acetic acid, formic acid, and methanol) into methane in the presence of H2 has significant consequences because the removal of excess hydrogen through methanogenesis prevents the inhibition of bacterial NADH dehydrogenase. This will, in turn, lead to an increased yield of ATP from bacterial metabolism and a greater harvest of energy from the diet. Antimicrobial drugs taken orally can, in humans, temporarily suppress the drug-susceptible components of the fecal flora. The acute effects of antibiotic treatment on the native gut microbiota range from self-limiting diarrhea to life-threatening pseudomembranous colitis. Intentional suppression of the fecal flora is commonly done by the preoperative oral administration of insoluble drugs. For example, neomycin plus erythromycin can in 1–2 days suppress part of the bowel flora, especially aerobes. Metronidazole accomplishes that for anaerobes. If lower bowel surgery is performed when the counts are at their lowest, some protection against infection by accidental spill can be achieved. However, soon thereafter, the counts of fecal flora rise again to normal or higher than normal levels, principally of organisms selected out because of relative resistance to the drugs used. The drug-susceptible microorganisms are replaced by drug-resistant ones, particularly staphylococci, enterobacters, enterococci, protei, pseudomonads, Clostridium difficile, and yeasts.

The feeding of large quantities of L acidophilus may result in the temporary establishment of this organism in the gut and the concomitant partial suppression of other gut microflora.

Fecal microbiota transplantation (FMT) also known as stool transplant is the process of transplantation of fecal bacteria from a healthy individual into a recipient. It has been used successfully as a treatment for patients suffering from C difficile infection. The hypothesis behind the success of FMT rests on the concept of bacterial interference, ie, using harmless bacteria to displace pathogenic bacteria. FMT restores the colonic microbiota to its natural state by replacing missing Bacteroidetes and Firmicutes species. However, recent studies suggest that other factors may be important.

The anaerobic flora of the colon, including *Bacteroides fragilis*, clostridia, and peptostreptococci, plays a main role in abscess formation originating in perforation of the bowel. Prevotella bivia and Prevotella disiens are important in abscesses of the pelvis, originating in the female genital organs. Similar to B fragilis, these species are penicillin resistant; therefore, another agent should be used. Although the intestinal microbiota is normally an asset for the host, in genetically susceptible individuals, some components of the flora can result in disease. For example, inflammatory bowel diseases are believed to be associated with a loss of immune tolerance to bacterial antigens. This leads to intense inflammation caused by an exuberant immune response. Similar mechanisms may be important in intestinal malignancy such as colon cancer.

NORMAL MICROBIOTA OF THE URETHRA

The anterior urethras of both sexes contain small numbers of the same types of organisms found on the skin and perineum. These organisms regularly appear in normal voided urine in numbers of 10^2–10^4/mL.

NORMAL MICROBIOTA OF THE VAGINA

Soon after birth, aerobic lactobacilli appear in the vagina and persist as long as the pH remains acidic (several weeks). When the pH becomes neutral (remaining so until puberty), a mixed flora of cocci and bacilli is present. At puberty, aerobic and anaerobic lactobacilli reappear in large numbers and contribute to the maintenance of acid pH through the production of acid from carbohydrates, particularly glycogen. This appears to be an important mechanism in preventing the establishment of other, possibly harmful microorganisms in the vagina. If lactobacilli are suppressed by the administration of antimicrobial drugs, yeasts or various bacteria increase in numbers and cause irritation and inflammation (vaginitis). Bacterial vaginosis is a syndrome marked by dramatic shifts in the types and relative proportions of the vaginal microbiota as the vaginal ecosystem changes from a healthy state, characterized by the presence of lactobacilli, to a diseased state characterized by the presence of organisms belonging to phylotaxa such as Actinobacteria and Bacteroidetes species. A recent study on the vaginal microbiome of 396 asymptomatic reproductive-age women found variations in the vaginal pH and vaginal microbiome of different ethnic groups (ie, white, black, Hispanic, and Asian), suggesting the need to consider ethnicity as an important factor in assessing normal or abnormal flora.

After menopause, lactobacilli again diminish in number, and a mixed flora returns. The normal vaginal flora includes group B streptococci in as many as 25% of women of childbearing age. During the birth process, a baby can acquire group B streptococci, which subsequently may cause neonatal sepsis and meningitis. The normal vaginal flora often also includes α-hemolytic streptococci, anaerobic streptococci (peptostreptococci), Prevotella species, clostridia, Gardnerella

vaginalis, Ureaplasma urealyticum, and sometimes Listeria or Mobiluncus species. The cervical mucus has antibacterial activity and contains lysozyme. In some women, the vaginal introitus contains a heavy flora resembling that of the perineum and perianal area. This may be a predisposing factor in recurrent urinary tract infections. Vaginal organisms present at time of delivery may infect the newborn (eg, group B streptococci).

NORMAL MICROBIOTA OF THE CONJUNCTIVA

The predominant organisms of the conjunctiva are diphtheroids, S epidermidis, and nonhemolytic streptococci. Neisseriae and gram-negative bacilli resembling haemophili (Moraxella species) are also frequently present. The conjunctival flora is normally held in check by the flow of tears, which contain antibacterial lysozyme.

THE STAPHYLOCOCCI

The staphylococci are gram-positive spherical cells, usually arranged in grapelike irregular clusters. They grow readily on many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. Some are members of the normal microbiota of the skin and mucous membranes of humans; others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia. The pathogenic staphylococci often hemolyze blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins. The most common type of food poisoning is caused by a heat-stable staphylococcal enterotoxin. Staphylococci rapidly develop resistance to many antimicrobial agents, which consequently presents difficult therapeutic problems. The genus Staphylococcus has at least 45 species. The four most frequently encountered species of clinical importance are Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus lugdunensis, and Staphylococcus saprophyticus. S aureus is coagulase positive, which differentiates it from the other species. S aureus is a major pathogen for humans. Almost every person will have some type of S aureus infection during a lifetime, ranging in severity from food poisoning or minor skin infections to severe life-threatening infections. The coagulase-negative staphylococci (CoNS) are normal human microbiota and sometimes cause infection, often associated with implanted devices, such as joint prostheses, shunts, and intravascular catheters, especially in very young, old, and immunocompromised patients. Approximately 75% of these infections caused by coagulase-negative staphylococci are caused by S epidermidis; infections caused by S lugdunensis, Staphylococcus warneri, Staphylococcus hominis, and other species are less common. S saprophyticus is a relatively common cause of urinary tract infections in young women, although it rarely causes infections in hospitalized patients. Other species are important in veterinary medicine.

Morphology and Identification

A. Typical Organisms

Staphylococci are spherical cells about 1 μm in diameter arranged in irregular clusters (Figure 13-1). Single cocci, pairs, tetrads, and chains are also seen in liquid cultures. Young cocci stain strongly gram positive; on aging, many cells become gram negative. *Staphylococci* are nonmotile and do not form spores. Under the influence of drugs such as penicillin, staphylococci are lysed. Micrococcus species often resemble staphylococci. They are found free living in the environment and form regular packets of four (tetrads) or eight cocci. Their colonies can be yellow, red, or orange. Micrococci are rarely associated with disease.

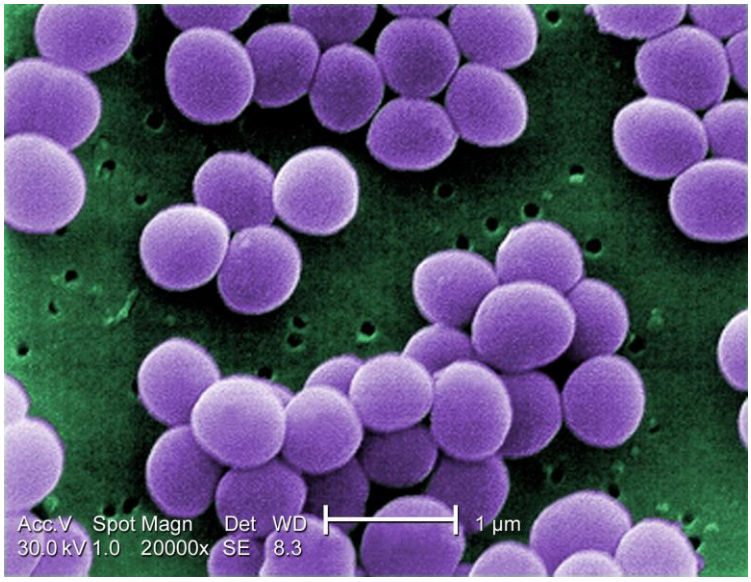

Fig:1.2 Microscopic image of Staphylococcus

B. Culture

Staphylococci grow readily on most bacteriologic media under aerobic or microaerophilic conditions. They grow most rapidly at 37°C but form pigment best at room temperature (20–25°C). Colonies on solid media are round, smooth, raised, and glistening (Figure 13-2). S aureus usually forms gray to deep golden yellow colonies. S epidermidis colonies usually are gray to white on primary isolation; many colonies develop pigment only upon prolonged incubation. No pigment is produced anaerobically or in broth. Various degrees of hemolysis are produced by S aureus and occasionally by other species. Peptostreptococcus and Peptoniphilus species, which are anaerobic cocci, often resemble staphylococci in morphology. The genus Staphylococcus contains two species, Staphylococcus saccharolyticus and S aureus subsp. anaerobius, which initially grow only under anaerobic conditions but become more aerotolerant on subcultures. This may be seen on rare occasions with some strains of S epidermidis as well.

C. Growth Characteristics

The staphylococci produce catalase, which differentiates them from the streptococci. Staphylococci slowly ferment many carbohydrates, producing lactic acid but not gas. Proteolytic activity varies greatly from one strain to another. Pathogenic staphylococci produce many extracellular substances, which are discussed below.

Staphylococci are relatively resistant to drying, heat (they withstand 50°C for 30 minutes), and 10% sodium chloride but are readily inhibited by certain chemicals (eg, 3% hexachlorophene). Staphylococci are variably susceptible to many antimicrobial drugs. Resistance is caused by several mechanisms:

1. β-Lactamase production is common, is under plasmid control, and makes the organisms resistant to many penicillins (penicillin G, ampicillin, ticarcillin, piperacillin, and similar drugs). The plasmids are transmitted by transduction and perhaps also by conjugation.

2. Resistance to nafcillin (and to methicillin and oxacillin) is independent of β-lactamase production. Resistance to nafcillin is encoded and regulated by a sequence of genes found in a region of the chromosome called the staphylococcal cassette chromosome mec (SCCmec). Specifically, the mecA and newly described mecC genes on this locus encode a low-affinity penicillin-binding protein (PBP2a) that is responsible for the resistance. There are 12 different SCCmec types. Types I, II, III, VI, and VIII are associated with hospital-acquired infections (HA-MRSA) and may contain genes that encode resistance to other antimicrobials as well. SCCmec type IV has principally been found in community-acquired methicillin-resistant S aureus (CA-MRSA) strains that tend to be less resistant, more transmissible, and responsible for outbreaks over the past decade in the United States and some countries in Europe. Types IX and X are associated with animals (livestock-associated MRSA [LA-MRSA]) of which type IX contains mecC. The other types have been limited to various geographic locations around the world.

3. In the United States, S aureus and S lugdunensis are considered to be susceptible to vancomycin if the minimum inhibitory concentration (MIC) is 2 μg/mL or less; of intermediate susceptibility if the MIC is 4–8 μg/mL; and resistant if the MIC is 16 μg/mL or greater. Strains of S aureus with intermediate susceptibility to vancomycin have been isolated in Japan, the United States, and several other countries. These are often known as vancomycin-intermediate S aureus (VISA). They generally have been isolated from patients with complex infections who have received prolonged vancomycin therapy. Often there has been vancomycin treatment failure. The mechanism of resistance is associated with increased cell wall synthesis and alterations in the cell wall and is not caused by the van genes found in enterococci. S aureus strains of intermediate susceptibility to vancomycin usually are nafcillin resistant but generally are susceptible to oxazolidinones and to quinupristin–dalfopristin.

4. Since 2002, several isolates of vancomycin-resistant S aureus (VRSA) strains (MICs ≥ 16 μg/mL) were isolated from patients in the United States. The isolates contained the vancomycin resistance gene vanA likely derived from enterococci and the nafcillin resistance gene mecA Both of the initial VRSA strains were susceptible to other antibiotics. Vancomycin resistance in S aureus is of major concern worldwide.

5. Plasmid-mediated resistance to tetracyclines, erythromycins, aminoglycosides, and other drugs is frequent in staphylococci.

6. “Tolerance” implies that staphylococci are inhibited by a drug but not killed by it—that is, there is great difference between minimal inhibitory and minimal lethal concentrations of an antimicrobial drug. Patients with endocarditis caused by a tolerant S aureus may have a prolonged clinical course compared with patients who have endocarditis caused by a fully susceptible S

aureus. Tolerance can at times be attributed to lack of activation of autolytic enzymes in the cell wall.

D. Variation

A culture of staphylococci contains some bacteria that differ from the bulk of the population in expression of colony characteristics (colony size, pigment, hemolysis), in enzyme elaboration, in drug resistance, and in pathogenicity. In vitro, the expression of such characteristics is influenced by growth conditions: When nafcillin-resistant S aureus is incubated at 37°C on blood agar, one in 107 organisms expresses nafcillin resistance; when it is incubated at 30°C on agar containing 2–5% sodium chloride, one in 103 organisms expresses nafcillin resistance. Some isolates may develop alterations in phenotypes such as smaller size (pin point colonies) and loss of hemolysis. These are referred to as small colony variants (SCVs) and the variations in phenotypic characteristics enable better survival under intracellular conditions, facilitating persistence and leading to chronic infections.

Antigenic Structure

S aureus has amazing adaptive capacity. Full genome sequencing of numerous isolates (www.ncbi.nlm.nih.gov/genome/ genomes/154) has elucidated the evolution of various structures, toxins, and enzymes that this organism has developed over time. S aureus has acquired many mobile genetic elements (eg, insertion sequences, transposons, etc) that determine both pathogenicity and antimicrobial resistance. Staphylococci contain antigenic polysaccharides and proteins as well as other substances important in cell wall structure. Peptidoglycan, a thick polysaccharide polymer containing linked subunits, provides the rigid exoskeleton of the cell wall and anchors the adhesins. Peptidoglycan is destroyed by strong acid or exposure to lysozyme. It is important in the pathogenesis of infection: It elicits production of interleukin-1 (endogenous pyrogen) and opsonic antibodies by monocytes, and it can be a chemoattractant for polymorphonuclear leukocytes, have endotoxin-like activity, and activate complement. Peptidoglycan assembly is a target of β-lactam and glycopeptide antimicrobial agents. Teichoic acids, which are polymers of polyribitol–phosphate, are cross-linked to the peptidoglycan and can be antigenic. They are important in cell wall metabolism. Antiteichoic acid antibodies detectable by gel diffusion may be found in patients with active endocarditis caused by S aureus. Protein A is a cell wall component of S aureus strains and is a bacterial surface protein that has been characterized among a group of adhesins called microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). Bacterial attachment to host cells is mediated by MSCRAMMs, and these are important virulence factors. Protein A binds to the Fc portion of IgG molecules except IgG3. The Fab portion of the IgG bound to protein A is free to combine with a specific antigen. Protein A has become an important reagent in immunology and diagnostic

laboratory technology; for example, protein A with attached IgG molecules directed against a specific bacterial antigen agglutinates bacteria that have that antigen ("coagglutination"). Another important MSCRAMM is clumping factor on the cell wall surface; clumping factor binds nonenzymatically to fibrinogen and platelets, yielding aggregation of the bacteria. The remaining MSCRAMMs, too numerous to mention here, play important roles in establishing S aureus colonization and invasion in major infections such as endocarditis. Most S aureus strains of clinical importance have polysaccharide capsules, which inhibit phagocytosis by polymorphonuclear leukocytes unless specific antibodies are present. At least 11 serotypes have been identified, with types 5 and 8 responsible for the majority of infections. These capsule types are targets for a conjugate vaccine. Serologic tests have limited usefulness in identifying staphylococci.

Enzymes and Toxins

Staphylococci can produce disease both through their ability to multiply and spread widely in tissues and through their production of many extracellular substances. Some of these substances are enzymes; others are considered to be toxins, although they may function as enzymes. Many of the toxins are under the genetic control of plasmids; some may be under both chromosomal and extrachromosomal control; and for others, the mechanism of genetic control is not well defined.

A. Catalase

Staphylococci produce catalase, which converts hydrogen peroxide into water and oxygen. The catalase test differentiates the staphylococci, which are positive, from the streptococci, which are negative.

B. Coagulase and Clumping Factor

S aureus produces an extracellular coagulase, an enzymelike protein that clots oxalated or citrated plasma. Coagulase binds to prothrombin; together they become enzymatically active and initiate fibrin polymerization. Coagulase may deposit fibrin on the surface of staphylococci, perhaps altering their ingestion by phagocytic cells or their destruction within such cells. Coagulase production is considered synonymous with invasive pathogenic potential.

Clumping factor is cell wall bound and is another example of an MSCRAMM that is responsible for adherence of the organisms to fibrinogen and fibrin. When mixed with plasma, S aureus forms clumps. Clumping factor is distinct from coagulase. Because clumping factor induces a strong immunogenic response in the host, it has been the focus of vaccine efforts. However, no human vaccines against this factor are available to date. C. Other Enzymes Other enzymes produced by staphylococci include a hyaluronidase, or spreading factor—a staphylokinase resulting in fibrinolysis but acting much more slowly than streptokinase, proteinases, lipases, and β-lactamase.

D. Hemolysins

S aureus possesses four hemolysins that are regulated by agr. α-Hemolysin is a heterogeneous protein that acts on a broad spectrum of eukaryotic cell membranes. The β-toxin degrades sphingomyelin and therefore is toxic for many kinds of cells, including human red blood cells. The δ-toxin is heterogeneous and dissociates into subunits in nonionic detergents. It disrupts biologic membranes and may have a role in S aureus diarrheal diseases. The γ-hemolysin is a leukocidin that lyses white blood cells and is composed of two proteins designated S and F. γ-Hemolysin can interact with the two proteins comprising the Panton– Valentine leukocidin (PVL; see later discussion) to form six potential two-component toxins. All six of these protein toxins are capable of efficiently lysing white blood cells by causing pore formation in the cellular membranes that increase cation permeability. This leads to massive release of inflammatory mediators such as IL-8, leukotriene, and histamine, which are responsible for necrosis and severe inflammation.

E. Panton–Valentine Leukocidin

This toxin of S aureus has two components, and unlike the chromosomally encoded hemolysins above, PVL is encoded on a mobile phage. It can kill white blood cells of humans and rabbits. The two components designated as S and F act synergistically on the white blood cell membrane as described for γ-toxin. This toxin is an important virulence factor in CA-MRSA infections.

F. Exfoliative Toxins

These epidermolytic toxins of S aureus are two distinct proteins of the same molecular weight. Exfoliative toxin A is encoded by eta located on a phage and is heat stable (resists boiling for 20 minutes). Exfoliative toxin B is plasmid mediated and heat labile. These epidermolytic toxins yield the generalized desquamation of the staphylococcal scalded skin syndrome by dissolving the mucopolysaccharide matrix of the epidermis. The toxins are superantigens.

G. Toxic Shock Syndrome Toxin

Most S aureus strains isolated from patients with toxic shock syndrome produce a toxin called toxic shock syndrome toxin-1 (TSST-1), which is the same as enterotoxin F. TSST-1 is the prototypical superantigen. TSST-1 binds to major histocompatibility class (MHC) class II molecules, yielding T-cell stimulation, which promotes the protean manifestations of the toxic shock syndrome. The toxin is associated with fever, shock, and multisystem involvement, including a desquamative skin rash. The gene for TSST-1 is found in about 20% of S aureus isolates, including MRSA.

H. Enterotoxins

There are 15 enterotoxins (A–E, G–P) that, similar to TSST-1, are superantigens. Approximately 50% of S aureus strains can produce one or more of them. The enterotoxins are heat stable and resistant to the action of gut enzymes. Important causes of food poisoning, enterotoxins are produced when S aureus grows in carbohydrate and protein foods. Ingestion of 25 μg of enterotoxin B results in vomiting and diarrhea. The emetic effect of enterotoxin is probably the result of central nervous system stimulation (vomiting center) after the toxin acts on neural receptors in the gut. The exfoliative toxins, TSST-1, and the enterotoxin genes are on a chromosomal element called a pathogenicity island. It interacts with accessory genetic elements—bacteriophages— to produce the toxins.

Pathogenesis

Staphylococci, particularly S epidermidis, are members of the normal microbiota of the human skin and respiratory and gastrointestinal tracts. Nasal carriage of S aureus occurs in 20–50% of humans. Staphylococci are also found regularly on clothing, bed linens, and other fomites in human environments.

The pathogenic capacity of a given strain of S aureus is the combined effect of extracellular factors and toxins together with the invasive properties of the strain. At one end of the disease spectrum is staphylococcal food poisoning, attributable solely to the ingestion of preformed enterotoxin; at the other end are staphylococcal bacteremia and disseminated abscesses in all organs. Pathogenic, invasive S aureus produces coagulase and tends to produce a yellow pigment and to be hemolytic. Nonpathogenic, noninvasive staphylococci such as S epidermidis are coagulase negative and tend to be nonhemolytic. Such organisms rarely produce suppuration but may infect orthopedic or cardiovascular prostheses or cause disease in immunosuppressed persons. They may be refractory to treatment because of the formation of bioflims. S lugdunensis has emerged as a virulent organism causing a disease spectrum similar to S aureus with whom it shares phenotypic characteristics such as hemolysis and clumping factor. S saprophyticus is typically non-pigmented, novobiocin resistant, and nonhemolytic; it causes urinary tract infections in young women.

Regulation of Virulence Determinants

The expression of staphylococcal virulence determinants is regulated by several systems that sense and respond to environmental signals. The first of these systems consists of two proteins (two-component systems), an example of which is accessory gene regulator (agr). The other two systems consist of DNA-binding proteins (eg, Sar proteins) and small regulatory RNAs, respectively (eg, RNAIII), the latter of which have become more appreciated as having major roles in regulation of gene expression. Binding of sensors to specific extracellular ligands, or to a receptor, results in a phosphorylation cascade that leads to binding of the regulator to specific DNA sequences. This

ultimately leads to activation of transcription regulating functions. There are several well-described two component regulatory systems in S aureus. These include agr, the best described, sae RS, srrAB, arlSR, and lytRS.

The accessory gene regulator (agr) is essential in quorumsensing control of gene expression. It controls the preferential expression of surface adhesins (protein A, coagulase, and fibronectin-binding protein) and production of exoproteins (toxins such as TSST-1) depending upon the growth phase (and hence bacterial density). At low cell density, the promoter P2 is off, and transcriptions of transmembrane protein, AgrB, peptide precursor, AgrD, transmembrane sensor, AgrC, and transcription regulator, Agr A, are at low levels. As cell density increases during stationary growth phase, the AgrC sensor activates the regulator AgrA. AgrA is a DNA-binding protein that activates promoter P2 and promoter P3. Promoter P3 initiates transcription of δ-hemolysin and an effector called RNAIII, which downregulates the expression of surface adhesins and activates secretion of exoproteins at both the transcriptional and translational levels. Agr is also positively controlled by a DNA-binding protein called SarA (encoded by sar) and possibly by other regulatory systems.

At least 10 two-component regulatory systems have been shown to affect virulence gene expression and are also involved in metabolic control. Those involved in virulence include: sae, S aureus exoproteins; srrAB, staphylococcal respiratory response; arlS, autolysis-related locus sensor; and lytRS. Sae regulates gene expression at the transcriptional level and is essential for production of α-toxin, β-hemolysins, and coagulase. Its activity is independent from that of agr. SsrAB is important for regulation of virulence factor expression that is influenced by environmental oxygen. The arlSR locus is important to the control of autolysis and decreases the activation of the agr locus. The lytRS locus is also involved in autolysis.

Pathology

The prototype of a staphylococcal lesion is the furuncle or other localized abscess. Groups of S aureus established in a hair follicle lead to tissue necrosis (dermonecrotic factor). Coagulase is produced and coagulates fibrin around the lesion and within the lymphatics, resulting in formation of a wall that limits the process and is reinforced by the accumulation of inflammatory cells and, later, fibrous tissue. Within the center of the lesion, liquefaction of the necrotic tissue occurs (enhanced by delayed hypersensitivity), and the abscess “points” in the direction of least resistance. Drainage of the liquid center necrotic tissue is followed by slow filling of the cavity with granulation tissue and eventual healing. Focal suppuration (abscess) is typical of staphylococcal infection. From any one focus, organisms may spread via the lymphatics and bloodstream to other parts of the body. Suppuration within veins, associated with thrombosis, is a

common feature of such dissemination. In osteomyelitis, the primary focus of S aureus growth is typically in a terminal blood vessel of the metaphysis of a long bone, leading to necrosis of bone and chronic suppuration. S aureus may cause pneumonia, meningitis, empyema, endocarditis, or sepsis with suppuration in any organ. Staphylococci of low invasiveness are involved in many skin infections (eg, acne, pyoderma, or impetigo). Anaerobic cocci (Peptostreptococcus species) participate in mixed anaerobic infections. Staphylococci also cause disease through the elaboration of toxins without apparent invasive infection. Bullous exfoliation, the scalded skin syndrome, is caused by the production of exfoliative toxins. Toxic shock syndrome is associated with TSST-1.

Clinical Findings

A localized staphylococcal infection appears as a "pimple," hair follicle infection, or abscess. There is usually an intense, localized, painful inflammatory reaction that undergoes central suppuration and heals quickly when the pus is drained. The wall of fibrin and cells around the core of the abscess tend to prevent spread of the organisms and should not be broken down by manipulation or trauma.

S aureus infection can also result from direct contamination of a wound, such as a postoperative staphylococcal wound infection or infection after trauma (chronic osteomyelitis subsequent to an open fracture, meningitis after skull fracture). If S aureus disseminates and bacteremia ensues, endocarditis, acute hematogenous osteomyelitis, meningitis, or pulmonary infection can result. The clinical presentations resemble those seen with other bloodstream infections. Secondary localization within an organ or system is accompanied by the symptoms and signs of organ dysfunction and intense focal suppuration.

Food poisoning caused by staphylococcal enterotoxin is characterized by a short incubation period (1–8 hours); violent nausea, vomiting, and diarrhea; and rapid convalescence. There is no fever.

Toxic shock syndrome is manifested by an abrupt onset of high fever, vomiting, diarrhea, myalgias, a scarlatiniform rash, and hypotension with cardiac and renal failure in the most severe cases. It often occurs within 5 days after the onset of menses in young women who use high-absorbency tampons, but it also occurs in children and men with staphylococcal wound infections. The syndrome can recur. Toxic shock syndrome–associated S aureus can be found in the vagina, on tampons, in wounds or other localized infections, or in the throat but virtually never in the bloodstream.

Diagnostic Laboratory Tests

A. Specimens

Surface swab pus or aspirate from an abscess, blood, endonasotracheal aspirate, expectorated sputum, or spinal fluid for culture, depending on the localization of the process, are all appropriate specimens for testing. The anterior nares are frequently swabbed to determine nasal colonization, either by culture or by nucleic acid amplification tests, for epidemiological purposes.

B. Smears

Typical staphylococci appear as gram-positive cocci in clusters in Gram-stained smears of pus or sputum. It is not possible to distinguish nonaureus (eg, S epidermidis) from the pathogenic S aureus organisms on smears.

C. Culture

Specimens planted on blood agar plates give rise to typical colonies in 18 hours at 37°C, but hemolysis and pigment production may not occur until several days later and are optimal at room temperature. S aureus but not other staphylococci ferment mannitol. Specimens contaminated with a mixed microbiota can be cultured on media containing 7.5% NaCl; the salt inhibits most other normal microbiota, but not S aureus. Mannitol salt agar or commercially available chromogenic media are used to screen for nasal carriers of S aureus and to recover S aureus from respiratory specimens of patients with cystic fibrosis.

D. Catalase Test

This test is used to detect the presence of cytochrome oxidase enzymes. A drop of 3% hydrogen peroxide solution is placed on a slide, and a small amount of the bacterial growth is placed in the solution. The formation of bubbles (the release of oxygen) indicates a positive test result.

E. Coagulase Test

Citrated rabbit (or human) plasma diluted 1:5 is mixed with an equal volume of broth culture or growth from colonies on agar and incubated at 37°C. A tube of plasma mixed with sterile broth is included as a control. If clots form in 1–4 hours, the test result is positive. Rapid latex and agglutination assays are more timely and in some cases more sensitive in the differentiation between S aureus and CoNS. These assays detect protein A and clumping factor, and some have monoclonal antibodies against capsular polysaccharides. Coagulase-positive staphylococci are considered pathogenic for humans; however, coagulase-positive staphylococci of dogs (*Staphylococcus intermedius*) and dolphins (*Staphylococcus delphini*) rarely cause disease in humans. Infections of prosthetic devices can be caused by organisms of the coagulase- negative S epidermidis group.

F. Susceptibility Testing

Clinical laboratories adopt methods recommended by the Clinical and Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) for

the performance of susceptibility testing of staphylococci. Broth microdilution using manual or automated commercial methods, or disk diffusion susceptibility testing should be done routinely on staphylococcal isolates from clinically significant infections. Resistance to penicillin G can be predicted by a positive test result for β-lactamase; approximately 90% of S aureus produce β-lactamase. Resistance to nafcillin (and oxacillin and methicillin) occurs in about 65% of S aureus and approximately 75% of *S epidermidis* isolates. Nafcillin resistance correlates with the presence of mecA or mecC, the genes that encode for a penicillin-binding protein (PBP2a) not affected by these drugs. These genes can be detected using the polymerase chain reaction (PCR) or other nucleic acid amplification test. Several FDA-cleared systems combine identification and mecA resistance marker detection directly from positive blood cultures. The Verigene R assay (Nanosphere, Inc., Northbrook, IL) and the Bio- Fire FilmArrayR BCID assay (BioFire Diagnostics, Inc., Salt Lake City, UT) are two examples of such tests but many more are in development. Alternatively, an assay for the mecA gene product, PBP2a, is commercially available and is much more rapid than PCR for mecA or than testing for resistance using traditional phenotypic methods.

When using disk diffusion to detect nafcillin resistance, the cefoxitin disk test is recommended for testing S aureus, S lugdunensis, and S saprophyticus. Zone sizes < 22 mm indicate resistance. When using broth microdilution, either oxacillin or cefoxitin may be used to detect oxacillin resistance. If the latter drug is tested, then 2% NaCl is added to the media and the test must be incubated for a full 24 hours at 35°C. An organism that is mecA or mecC positive or phenotypically is nafcillin, oxacillin, or methicillin resistant is also resistant to all extended spectrum penicillins, carbapenems, and cephalosporins with the exception of ceftaroline, a new cephalosporin with activity against MRSA.

G. Serologic and Typing Tests

Serologic tests for diagnosis of S aureus infections have little practical value.

Antibiotic susceptibility patterns may be helpful in tracing S aureus infections and in determining if multiple S epidermidis isolates from blood cultures represent bacteremia caused by the same strain, seeded by a nidus of infection. Molecular typing techniques have been used to document the spread of epidemic disease-producing clones of S aureus. Pulsed-field gel electrophoresis and multilocus sequence typing are highly discriminatory. Spa typing is less discriminatory but easier to perform.

Treatment

Most persons harbor staphylococci on the skin and in the nose or throat. Even if the skin can be cleared of staphylococci (eg, in eczema), reinfection by droplets will occur almost immediately.

Because pathogenic organisms are commonly spread from one lesion (eg, a furuncle) to other areas of the skin by fingers and clothing, scrupulous local antisepsis is important to control recurrent furunculosis. Serious multiple skin infections (acne, furunculosis) occur most often in adolescents. Similar skin infections occur acne, lipases of staphylococci and corynebacteria liberate fatty acids from lipids and thus cause tissue irritation. Tetracyclines are used for long-term treatment. Abscesses and other closed suppurating lesions are treated by drainage, which is essential, and antimicrobial therapy. Many antimicrobial drugs have some effect against staphylococci in vitro. However, it is difficult to eradicate pathogenic staphylococci from infected persons because the organisms rapidly develop resistance to many antimicrobial drugs and the drugs cannot act in the central necrotic part of a suppurative lesion.

It may also be difficult to eradicate the S aureus nasal carrier state. Some success has been reported with treatment of colonized individuals with intranasal mupirocin. Literature demonstrates success in reducing postsurgical wounds infections and prevention of bacteremia when treating identified hospitalized patients with 5 days of mupirocin with or without bathing using chlorhexidine, a topical antiseptic. Acute hematogenous osteomyelitis responds well to antimicrobial drugs. In chronic and recurrent osteomyelitis, surgical drainage and removal of dead bone is accompanied by long-term administration of appropriate drugs, but eradication of the infecting staphylococci is difficult. Hyperbaric oxygen and the application of vascularized myocutaneous flaps have aided healing in chronic osteomyelitis. Bacteremia, endocarditis, pneumonia, and other severe infections caused by S aureus require prolonged intravenous therapy with a β-lactamase-resistant penicillin.

Vancomycin is often reserved for use with nafcillin-resistant staphylococci. In recent years, an increase in MICs to vancomycin among many MRSA strains recovered from hospitalized patients has led physicians to seek alternative therapies. Alternative agents for the treatment of MRSA bacteremia and endocarditis include newer antimicrobials such as daptomycin, linezolid, and quinupristin–dalfopristin. Also, these agents may be bactericidal and offer alternatives when allergies preclude the use of other compounds or the patient’s infection appears to be failing clinically. However, the use of these agents should be discussed with infectious diseases physicians or pharmacists because the side effect profiles and pharmacokinetics are quite unique to each agent. Recently, a novel cephalosporin called ceftaroline, which has activity against MRSA and other gram-positive and some gramnegative bacteria, has been approved for the treatment of skin and soft tissue infections and community-acquired pneumonia. This drug does not yet have an indication for bacteremia. If the infection is found to be caused by non–β-lactamaseproducing S aureus, penicillin G is the drug of choice, but these S aureus strains are rarely encountered.

S epidermidis infections are difficult to cure because they occur in prosthetic devices where the bacteria can sequester themselves in a biofilm. S epidermidis is more often resistant to antimicrobial drugs than is S aureus; approximately 75% of S epidermidis strains are nafcillin resistant. Several newer agents that have activity against CoNS and MSSA and MRSA have recently been FDA-cleared for treatment of skin and skin structure infections. These include dalbavancin, a longacting intravenous lipoglycopeptide; tedizolid phosphate, an intravenous and oral oxazolidinone, similar to linezolid; and oritavancin, a semisynthetic glycopeptide. Because of the frequency of drug-resistant strains, meaningful staphylococcal isolates should be tested for antimicrobial susceptibility to help in the choice of systemic drugs. Resistance to drugs of the erythromycin group tends to emerge so rapidly that these drugs should not be used singly for treatment of chronic infection. Drug resistance (to penicillins, tetracyclines, aminoglycosides, erythromycins, and so on) determined by plasmids can be transmitted among staphylococci by transduction and perhaps by conjugation. Penicillin G–resistant S aureus strains from clinical infections always produce penicillinase. They constitute more than 95% of S aureus isolates in communities in the United States. They are often susceptible to β-lactamase–resistant penicillins, cephalosporins, or vancomycin. Nafcillin resistance is independent of β-lactamase production, and its clinical incidence varies greatly in different countries and at different times. The selection pressure of β-lactamase–resistant antimicrobial drugs may not be the sole determinant for resistance to these drugs: For example, in Denmark, nafcillinresistant S aureus comprised 40% of isolates in 1970 and only 10% in 1980 without notable changes in the use of nafcillin or similar drugs. In the United States, nafcillin-resistant S aureus accounted for only 0.1% of isolates in 1970 but in the 1990s constituted 20–30% of isolates from infections in some hospitals. Currently, about 60% of nosocomial S aureus among intensive care patients in the United States are resistant to nafcillin. Fortunately, S aureus strains of intermediate susceptibility to vancomycin have been relatively uncommon, and the isolation of vancomycin-resistant strains has been rare.

Epidemiology and Control

Staphylococci are ubiquitous human pathogens. The chief sources of infection are shedding human lesions, fomites contaminated from such lesions, and the human respiratory tract and skin. Contact spread of infection has assumed added importance in hospitals, where a large proportion of the staff and patients may carry antibiotic-resistant staphylococci in the nose or on the skin. Although cleanliness, hygiene, and aseptic management of lesions can control the spread of staphylococci from lesions, few methods are available to prevent the wide dissemination of staphylococci from carriers. Aerosols (eg, glycols) and ultraviolet irradiation of air have little effect.

In hospitals, the areas at highest risk for severe staphylococcal infections are newborn nurseries, intensive care units, operating rooms, and cancer chemotherapy wards. Massive introduction of

“epidemic” pathogenic S aureus into these areas may lead to serious clinical disease. Personnel with active S aureus lesions and carriers may have to be excluded from these areas. In such individuals, the application of topical antiseptics such as mupirocin to nasal or perineal organisms. Rifampin coupled with a second oral antistaphylococcal drug sometimes provides long-term suppression and possibly cure of nasal carriage; this form of therapy is usually reserved for major problems of staphylococcal carriage because staphylococci can rapidly develop resistance to rifampin. To diminish transmission within the hospital setting, high-risk patients, such as those in intensive care units and patients transferred from chronic care facilities where prevalence is high, are frequently surveyed for anterior nares colonization. Patients who test positive by culture or PCR are placed on contact precautions to minimize spread on the hands of health care workers. Health care workers should strictly adhere to infection control policies by wearing gloves and washing hands before and after patient contact. Until relatively recently, MRSA was confined primarily to the hospital setting. Worldwide dissemination of a few distinct clones of CA-MRSA and now LA-MRSA has resulted in an increase in skin and soft tissue infections and necrotizing pneumonia, primarily in younger patients without known risk factors for MRSA acquisition. These strains appear to be more virulent. CA-MRSA isolates are characterized by the presence of PVL and the presence of staphylococcal cassette chromosome mec type IV which may explain the increased susceptibility to other antimicrobial agents compared with health care–associated MRSA strains.

THE STREPTOCOCCI

The streptococci, enterococci, and related organisms are gram-positive spherical bacteria that characteristically form pairs or chains during growth. They are widely distributed in nature. Some are members of the normal human microbiota; others are associated with important human diseases attributable to the direct effects of infection or in other cases to an immunologic response to them. Streptococci elaborate a variety of extracellular substances and enzymes. The streptococci are a large and heterogeneous group of bacteria, and no one system suffices to classify them. Yet, understanding their taxonomy is key to understanding their medical importance.

CLASSIFICATION OF STREPTOCOCCI

The classification of streptococci into major categories has been based on a series of observations over many years: (1) colony morphology and hemolytic reactions on blood agar, (2) serologic specificity of the cell wall group-specific substance (Lancefield antigens) and other cell wall or capsular antigens, (3) biochemical reactions and resistance to physical and chemical factors, and (4) ecologic features. More recently, molecular genetics have replaced phenotypic methods in the taxonomic assignment of these organisms.

A. Hemolysis

Many streptococci are able to hemolyze red blood cells in vitro in varying degrees. Complete disruption of erythrocytes with clearing of the blood around the bacterial growth is called β-hemolysis. Incomplete lysis of erythrocytes with reduction of hemoglobin and the formation of green pigment is called α-hemolysis. Other streptococci are nonhemolytic (sometimes called γ-[gamma-] hemolysis). The classification of hemolytic patterns is used primarily with the streptococci although other bacteria that cause disease may also typically produce a variety of hemolysins.

B. Group-Specific Substance (Lancefield Classification)

This carbohydrate is contained in the cell wall of many streptococci and forms the basis of serologic grouping into Lancefield groups A–H and K–U. The serologic specificity of the groupspecific carbohydrate is determined by an amino sugar. For group A streptococci, this is rhamnose-N-acetylglucosamine; for group B, it is rhamnose-glucosamine polysaccharide; for group C, it is rhamnose-N-acetylgalactosamine; for group D, it is glycerol teichoic acid containing d-alanine and glucose; and for group F, it is glucopyranosyl-N-acetylgalactosamine. Extracts of group-specific antigen for grouping streptococci are prepared by a variety of methods, including extraction of centrifuged culture treated with hot hydrochloric acid, nitrous acid, or formamide; by

enzymatic lysis of streptococcal cells (eg, with pepsin or trypsin); or by autoclaving of cell suspensions. These extracts contain the carbohydrate group–specific substance that yields precipitin reactions specific antisera. This permits arrangement of many streptococci into groups A–H and K–U. Typing is generally done only for groups A, B, C, F, and G, which cause disease in humans and for which reagents are available that allow typing using simple agglutination or color reactions.

C. Capsular Polysaccharides

The antigenic specificity of the capsular polysaccharides is used to classify Streptococcus pneumoniae into more than 90 types and to type the group B streptococci (*Streptococcus agalactiae*).

D. Biochemical Reactions

Biochemical tests include sugar fermentation reactions, tests for the presence of enzymes, and tests for susceptibility or resistance to certain chemical agents. Biochemical tests are most often used to classify streptococci after the colony growth and hemolytic characteristics have been observed. Biochemical tests are used for species that typically do not react with the commonly used antibody preparations for the group-specific substances, groups A, B, C, F, and G.

For example, the viridans streptococci are α-hemolytic or nonhemolytic and do not react with the antibodies commonly used for the Lancefield classification. Speciation of the viridans streptococci requires a battery of biochemical tests. However, because biochemical reactions are labor intensive and often unreliable, laboratories with molecular capabilities, such as gene sequencing or that have implemented mass spectrometry for organism identification (matrix-assisted laser desorption ionization-time of flight mass spectrometry [MALDI-TOF MS]), are replacing phenotypic tests with these methods when identification of viridians streptococci is required.

STREPTOCOCCUS PYOGENES

Most streptococci that contain the group A antigen are S pyogenes. It is a prototypical human pathogen. It is used here to illustrate general characteristics of streptococci and specific characteristics of the species. S pyogenes is the main human pathogen associated with local or systemic invasion and poststreptococcal immunologic disorders. S pyogenes typically produces large (1 cm in diameter) zones of β-hemolysis around colonies greater than 0.5 mm in diameter. They are PYR-positive (hydrolysis of l-pyrrolidonyl-β-naphthylamide) and usually are susceptible to bacitracin.

Morphology and Identification

A. Typical Organisms Individual cocci are spherical or ovoid and are arranged in chains (Figure 14-1). The cocci divide in a plane perpendicular to the long axis of the chain. The members of the chain often have a striking diplococcal appearance, and rod-like forms are occasionally seen. The lengths of the chains vary widely and are conditioned by environmental factors. Streptococci are gram positive; however, as a culture ages and the bacteria die, they lose their gram positivity and can appear to be gram negative; for some streptococci, this can occur after overnight incubation.

Most group A strains produce capsules composed of hyaluronic acid. The capsules are most noticeable in very young cultures. They impede phagocytosis. The hyaluronic acid capsule likely plays a greater role in virulence than is generally appreciated and together with M protein was believed to be an important factor in the resurgence of rheumatic fever (RF) in the United States in the 1980s and 1990s. The capsule binds to hyaluronic-acid-binding protein, CD44, present on human epithelial cells. Binding induces disruption of intercellular junctions allowing microorganisms to remain extracellular as they penetrate the epithelium. Capsules of other streptococci (eg, S agalactiae and S pneumoniae) are different. The S pyogenes cell wall contains proteins (M, T, R antigens), carbohydrates (group specific), and peptidoglycans. Hair like pili project through the capsule of group A streptococci. The pili consist partly of M protein and are covered with lipoteichoic acid. The latter is important in the attachment of streptococci to epithelial cells.

B. Culture

Most streptococci grow in solid media as discoid colonies, usually 1–2 mm in diameter. S pyogenes is β-hemolytic; other species have variable hemolytic characteristics.

C. Growth Characteristics

Energy is obtained principally from the utilization of glucose with lactic acid as the end product. Growth of streptococci tends to be poor on solid media or in broth unless enriched with blood or tissue fluids. Nutritive requirements vary widely among different species. The human pathogens

are most exacting, requiring a variety of growth factors. Growth and hemolysis are aided by incubation in 10% CO2. Most pathogenic hemolytic streptococci grow best at 37°C. Most streptococci are facultative anaerobes and grow under aerobic and anaerobic conditions.

D. Variation

Variants of the same Streptococcus strain may show different colony forms. This is particularly marked among S pyogenes strains, giving rise to either matte or glossy colonies. Matte colonies consist of organisms that produce much M protein and generally are virulent. The S pyogenes in glossy colonies tend to produce little M protein and are often not virulent.

Antigenic Structure

A. M Protein This substance is a major virulence factor of S pyogenes. M protein is a filamentous structure anchored to the cell membrane that penetrates and projects from the streptococcal cell wall. When M protein is present, the streptococci are virulent, and in the absence of M type-specific antibodies, they are able to resist phagocytosis by polymorphonuclear leukocytes by inhibiting activation of the alternate complement pathway. S pyogenes that lack M protein are not virulent. Immunity to infection with group A streptococci is related to the presence of type-specific antibodies to M protein. Because there are more than 150 types of M protein, a person can have repeated infections with S pyogenes of different M types. Both groups C and G streptococci have genes homologous to the genes for M protein of group A, and M proteins similar to those of group A have been found on groups C and G streptococci. The M protein molecule has a rodlike coiled structure that separates functional domains. The structure allows for a large number of sequence changes while maintaining function, and the M protein immunodeterminants, therefore, can readily change. There are two major structural classes of M protein, classes I and II.

It appears that M protein and perhaps other streptococcal cell wall antigens have an important role in the pathogenesis of rheumatic fever. Purified streptococcal cell wall membranes induce antibodies that react with human cardiac sarcolemma; the characteristics of the cross-reactive antigens are not clear. A component of the cell wall of selected M types induces antibodies that react with cardiac muscle tissue. Conserved antigenic domains on the class I M protein crossreact with human cardiac muscle, and the class I M protein may be a virulence determinant for rheumatic fever.

Toxins and Enzymes

More than 20 extracellular products that are antigenic are elaborated by S pyogenes, including the following.

A. Streptokinase (Fibrinolysin)

Streptokinase is produced by many strains of group A β-hemolytic streptococci. It transforms the plasminogen of human plasma into plasmin, an active proteolytic enzyme that digests fibrin and other proteins, allowing the bacteria to escape from blood clots. This process of digestion may be interfered with by nonspecific serum inhibitors and by a specific antibody, antistreptokinase. Streptokinase has been given intravenously for treatment of pulmonary emboli, coronary artery, and venous thromboses.

B. Deoxyribonucleases

Streptococcal deoxyribonucleases A, B, C, and D degrade DNA (DNases) and similar to streptokinase facilitate the spread of streptococci in tissue by liquefying pus. The enzymatic activity can be measured by the decrease in viscosity of known DNA solutions. Purulent exudates owe their viscosity largely to deoxyribonucleoprotein. Mixtures of streptokinase and DNases are used in "enzymatic debridement." They help to liquefy exudates and facilitate removal of pus and necrotic tissue; antimicrobial drugs thus gain better access, and infected surfaces recover more quickly. An antibody to DNAse develops after streptococcal infections (normal limit, 100 units), especially after skin infections.

C. Hyaluronidase Hyaluronidase splits hyaluronic acid, an important component of the ground substance of connective tissue. Thus, hyaluronidase aids in spreading infecting microorganisms (spreading factor). Hyaluronidases are antigenic and specific for each bacterial or tissue source. After infection with hyaluronidase-producing organisms, specific antibodies are found in the serum.

D. Pyrogenic Exotoxins (Erythrogenic Toxin)

Pyrogenic exotoxins are elaborated by S pyogenes. There are three antigenically distinct streptococcal pyrogenic exotoxins (Spe): A, B, and C. SpeA has been most widely studied. It is produced by group A streptococci that carry a lysogenic phage. The streptococcal pyrogenic exotoxins have been associated with streptococcal toxic shock syndrome and scarlet fever. Most strains of group A streptococci isolated from patients with streptococcal toxic shock syndrome either produce Spe A of group A streptococci isolated from other patients have the gene. Spe C, also encoded by a phage, may contribute to the syndrome. Spe B, a potent protease, interferes with phagocytosis. The group A streptococci associated with toxic shock syndrome are primarily of M protein types 1 and 3. The pyrogenic exotoxins act as superantigens, which stimulate T cells by binding to the class II major histocompatibility complex in the Vβ region of the T-cell receptor. The activated T cells release cytokines that mediate shock and tissue injury. The mechanisms of action appear to be similar to those caused by staphylococcal toxic shock syndrome toxin-1 and the staphylococcal enterotoxins.

E. Hemolysins

The β-hemolytic group A S pyogenes elaborates two hemolysins (streptolysins) that not only lyse the membranes of erythrocytes but also damage a variety of other cell types. Streptolysin O is a protein (molecular weight [MW], 60,000) that is hemolytically active in the reduced state (available– SH groups) but rapidly inactivated in the presence of oxygen. Streptolysin O is responsible for some of the hemolysis seen when growth occurs in cuts made deep into the medium in blood agar plates. It combines quantitatively with antistreptolysin O (ASO), an antibody that appears in humans after infection with any streptococci that produce streptolysin O. This antibody blocks hemolysis by streptolysin O. This phenomenon forms the basis of a quantitative test for the antibody. An ASO serum titer in excess of 160–200 units is considered abnormally high and suggests either recent infection with S pyogenes or persistently high antibody levels caused by an exaggerated immune response to an earlier exposure in a hypersensitive person. Streptolysin S is the agent responsible for the hemolytic zones around streptococcal colonies growing on the surface of blood agar plates. It is elaborated in the presence of serum—hence the name streptolysin S. It is not antigenic. Most isolates of S pyogenes produce both of these hemolysins. Up to 10% produce only one.

Pathogenesis and Clinical Findings

A variety of distinct disease processes are associated with S pyogenes infections. The infections can be divided into several categories.

A. Diseases Attributable to Invasion by **S pyogenes**, β-Hemolytic Group A Streptococci The portal of entry determines the principal clinical picture. In each case, however, there is a diffuse and rapidly spreading infection that involves the tissues and extends along lymphatic pathways with only minimal local suppuration. From the lymphatics, the infection can extend to the bloodstream.

1. Erysipelas—If the portal of entry is the skin, erysipelas results. Lesions are raised and characteristically red. There is massive brawny edema and a rapidly advancing, sharply demarcated margin of infection.
2. Cellulitis—Streptococcal cellulitis is an acute, rapidly spreading infection of the skin and subcutaneous tissues. It follows infection associated with mild trauma, burns, wounds, or surgical incisions. Pain, tenderness, swelling, and erythema occur. Cellulitis is differentiated from erysipelas by two clinical findings: In cellulitis, the lesion is not raised, and the line between the involved and uninvolved tissue is indistinct.
3. Necrotizing fasciitis (streptococcal gangrene)— There is extensive and very rapidly spreading necrosis of the skin, tissues, and fascia. Bacteria other than S pyogenes can also cause necrotizing fasciitis. The group A streptococci that cause necrotizing fasciitis have sometimes been termed flesh-eating bacteria.

4. Puerperal fever—If the streptococci enter the uterus after delivery, puerperal fever develops, which is essentially a septicemia originating in the infected wound (endometritis).
5. Bacteremia or sepsis—Infection of traumatic or surgical wounds with streptococci results in bacteremia, which can rapidly be fatal. S pyogenes bacteremia can also occur with skin infections, such as cellulitis and rarely pharyngitis.

B. Diseases Attributable to Local Infection With S pyogenes and Their Byproducts

1. Streptococcal sore throat

The most common infection caused by β-hemolytic S pyogenes is streptococcal sore throat or pharyngitis. S pyogenes adheres to the pharyngeal epithelium by means of lipoteichoic acid–covered surface pili and by means of hyaluronic acid in encapsulated strains. The glycoprotein fibronectin (MW, 440,000) on epithelial cells probably serves as lipoteichoic acid ligand. In infants and small children, the sore throat occurs as a subacute nasopharyngitis with a thin serous discharge and little fever but with a tendency of the infection to extend to the middle ear and the mastoid. The cervical lymph nodes are usually enlarged. The illness may persist for weeks. In older children and adults, the disease is more acute and is characterized by intense nasopharyngitis, tonsillitis, and intense redness and edema of the mucous membranes, with purulent exudate; enlarged, tender cervical lymph nodes; and (usually) a high fever. Twenty percent of infections are asymptomatic. A similar clinical picture can occur with infectious mononucleosis, diphtheria, gonococcal infection, and adenovirus infection.

S pyogenes infection of the upper respiratory tract does not usually involve the lungs. Pneumonia, when it does occur, is rapidly progressive and severe and is most commonly a sequela to viral infections, such as influenza or measles, which seem to greatly enhance the predisposition to bacterial superinfection with this and other pathogens, such as S pneumoniae.

2. Streptococcal pyoderma

Local infection of superficial layers of skin, especially in children, is called impetigo. It consists of superficial vesicles that break down and eroded areas whose denuded surface is covered with pus and later is encrusted. It spreads by continuity and is highly communicable, especially in hot, humid climates. More widespread infection occurs in eczematous or wounded skin or in burns and may progress to cellulitis. Group A streptococcal skin infections are often attributable to M types 49, 57, and 59–61 and may precede glomerulonephritis (GN) but do not lead to rheumatic fever. A clinically identical infection can be caused by Staphylococcus aureus and sometimes both S pyogenes and S aureus are present.

C. Invasive Group A Streptococcal Infections, Streptococcal Toxic Shock Syndrome, and Scarlet Fever

Fulminant, invasive S pyogenes infections with streptococcal toxic shock syndrome are characterized by shock, bacteremia, respiratory failure, and multiorgan failure. Death occurs in about 30% of patients. The infections tend to occur after minor trauma in otherwise healthy persons with several presentations of soft tissue infection. These include necrotizing fasciitis, myositis, and infections at other soft tissue sites; bacteremia occurs frequently. In some patients, particularly those infected with group A streptococci of M types 1 or 3, the disease presents with focal soft tissue infection accompanied by fever and rapidly progressive shock with multiorgan failure. Erythema and desquamation may occur. The S pyogenes of the M types 1 and 3 (and types 12 and 28) that make pyrogenic exotoxin A or B are associated with the severe infections. Pyrogenic exotoxins A–C also cause scarlet fever in association with S pyogenes pharyngitis or with skin or soft tissue infection. The pharyngitis may be severe. The rash appears on the trunk after 24 hours of illness and spreads to involve the extremities. Streptococcal toxic shock syndrome and scarlet fever are clinically overlapping diseases.

D. Poststreptococcal Diseases (Rheumatic Fever, Glomerulonephritis)

After an acute S pyogenes infection, there is a latent period of 1–4 weeks (mean 7 days), after which nephritis or rheumatic fever occasionally develops. The latent period suggests that these poststreptococcal diseases are not attributable to the direct effect of disseminated bacteria but instead represent a hypersensitivity response. Nephritis is more commonly preceded by infection of the skin; rheumatic fever is more commonly preceded by infection of the respiratory tract.

1. Acute glomerulonephritis

This sometimes develops 1–5 weeks (mean 7 days) after S pyogenes skin infection (pyoderma, impetigo) or pharyngitis. Some strains are particularly nephritogenic, principally with M types 2, 42, 49, 56, 57, and 60 (skin). Other nephritogenic M types associated with throat infections and glomerulonephritis are 1, 4, 12, and 25. After random streptococcal skin infections, the incidence of nephritis is less than 0.5%. Glomerulonephritis may be initiated by antigen— antibody complexes on the glomerular basement membrane. The most important antigens are thought to be SpeB and a nephritis-associated plasmin receptor. In acute nephritis, the patient has blood and protein in the urine, edema, high blood pressure, and urea nitrogen retention; serum complement levels are also low. A few patients die, some develop chronic glomerulonephritis with ultimate kidney failure, and the majority recovers completely.

2. Rheumatic fever

This is the most serious sequela of S pyogenes because it results in damage to heart muscle and valves. Certain strains of group A streptococci contain cell membrane antigens that cross-react with human heart tissue antigens. Sera from patients with rheumatic fever contain antibodies to these antigens. The onset of acute rheumatic fever (ARF) is often preceded by S pyogenes pharyngitis 1–5 weeks (mean 19 days) earlier, although the infection may be mild and may not be detected. In general, however, patients with more severe streptococcal sore throats have a greater chance of developing rheumatic fever. Rheumatic fever is not associated with cutaneous streptococcal infections. In the 1950s, untreated streptococcal infections were followed by rheumatic fever in up to 3% of military personnel and 0.3% of civilian children. In the 1980s through 2000 a resurgence of ARF occurred in the United States. M types 1, 3, 5, 6, and 18 were most frequently involved. Since that time, the incidence has once again declined. Rheumatic fever occurs up to 100 times more frequently in tropical countries and is the most important cause of heart disease in young people in developing countries.

Typical symptoms and signs of rheumatic fever include fever, malaise, a migratory nonsuppurative polyarthritis, and evidence of inflammation of all parts of the heart (endocardium, myocardium, and pericardium). The carditis characteristically leads to thickened and deformed valves and to small perivascular granulomas in the myocardium (Aschoff bodies) that are finally replaced by scar tissue. Patients may develop severe and progressive congestive heart failure. Sydenham's chorea is another manifestation of ARF and is characterized by involuntary, uncoordinated movements and associated muscle weakness. It has been hypothesized that other types of neurobehavioral conditions may also follow streptococcal infections. These are referred to as PANDAS— post-streptococcal autoimmune, neuropsychiatric disorders associated with streptococci. More research is required to definitely establish a link to S pyogenes infections. levels, electrocardiograms, and other tests are used to estimate rheumatic activity.

Whereas rheumatic fever has a marked tendency to be reactivated by recurrent streptococcal infections, nephritis does not. The first attack of rheumatic fever usually produces only slight cardiac damage, which, however, increases with each subsequent attack. It is therefore important to protect such patients from recurrent S pyogenes infections by prophylactic penicillin administration.

Diagnostic Laboratory Tests

A. Specimens

Specimens to be obtained depend on the nature of the streptococcal infection. A throat swab, pus, cerebrospinal fluid or other sterile body fluid, or blood is obtained for culture. Serum is obtained for antibody determinations.

B. Smears

Smears from pus often show single cocci or pairs rather than definite chains. Cocci are sometimes gram negative because the organisms are no longer viable and have lost their ability to retain the blue dye (crystal violet) and be gram positive. If smears of pus show streptococci but cultures fail to grow, anaerobic organisms must be suspected. Smears of throat swabs are rarely contributory because viridans streptococci are always present and have the same appearance as group A streptococci on stained smears.

C. Culture

Specimens suspected of containing streptococci are cultured on blood agar plates. If anaerobes are suspected, suitable anaerobic media must also be inoculated. Incubation in 10% CO2 often speeds hemolysis. Slicing the inoculum into the blood agar has a similar effect because oxygen does not readily diffuse through the medium to the deeply embedded organisms, and it is oxygen that inactivates streptolysin O. Blood cultures will grow hemolytic group A streptococci (eg, in sepsis) within hours or a few days. Certain α-hemolytic streptococci and enterococci may grow slowly, so blood cultures in cases of suspected endocarditis may not turn positive for a few days.

The degree and kind of hemolysis (and colonial appearance) may help place an organism in a definite group. S pyogenes can be identified by rapid tests specific for the presence of the group A-specific antigen and by the PYR test. Streptococci belonging to group A may be presumptively identified by inhibition of growth by bacitracin, but this should be used only when more definitive tests are not available.

D. Antigen Detection Tests

Several commercial kits are available for rapid detection of group A streptococcal antigen from throat swabs. These kits use enzymatic or chemical methods to extract the antigen from the swab, then use enzyme immunoassay (EIA) or agglutination tests to demonstrate the presence of the antigen. The tests can be completed in minutes to hours after the specimen is obtained. They are 60–90% sensitive, depending on the prevalence of the disease in the population, and 98–99% specific compared with culture methods. More sensitive assays that use DNA probes or nucleic acid amplification techniques are now available and are beginning to replace the earlier antigen detection tests, although they remain more costly.

E. Serologic Tests

A rise in the titer of antibodies to many group A streptococcal antigens can be estimated. Such antibodies include ASO, particularly in respiratory disease; anti-DNase B and antihyaluronidase, particularly in skin infections; antistreptokinase; anti-M type-specific antibodies; and others. Of these, the anti-ASO titer is most widely used.

Immunity

Resistance against streptococcal diseases is M type specific. Thus, a host who has recovered from infection by one group A streptococcal M type is relatively immune to reinfection by the same type but fully susceptible to infection by another M type. Anti-M type-specific antibodies can be demonstrated in a test that exploits the fact that streptococci are rapidly killed after phagocytosis. M protein interferes with phagocytosis, but in the presence of type-specific antibody to M protein, streptococci are killed by human leukocytes. Antibody to streptolysin O develops after infection; it blocks hemolysis by streptolysin O but does not indicate immunity. High titers (>250 units) indicate recent or repeated infections and are found more often in rheumatic individuals than in those with uncomplicated streptococcal infections.

Treatment

All S pyogenes are susceptible to penicillin G. Macrolides, such as erythromycin and clindamycin, have often been recommended for penicillin-allergic patients and for patients with necrotizing fasciitis. However, resistance to macrolide antibiotics has been increasing in Europe and the United States. Some are resistant to tetracyclines. Antimicrobial drugs have no effect on established glomerulonephritis and rheumatic fever. In acute streptococcal infections, however, every effort must be made to rapidly eradicate streptococci from the patient, eliminate the antigenic stimulus (before day 8), and thus prevent poststreptococcal disease. Doses of penicillin or erythromycin that result in effective tissue levels for 10 days usually accomplish this. Antimicrobial drugs are also very useful in preventing reinfection with β-hemolytic group A streptococci in patients with rheumatic fever.

Epidemiology, Prevention, and Control

Although humans can be asymptomatic nasopharyngeal or perineal carriers of S pyogenes, the organism should be considered significant if it is detected by culture or other means. The ultimate source of group A streptococci is a person harboring these organisms. The individual may have a clinical or subclinical infection or may be a carrier distributing streptococci directly to other persons via droplets from the respiratory tract or skin. The nasal discharges of a person harboring S pyogenes are the most dangerous source for spread of these organisms.

Many other streptococci (eg, viridans streptococci, enterococci) are members of the normal microbiota of the human body. They produce disease only when established in parts of the body where they do not normally occur (eg, heart valves). To prevent such accidents, particularly in the course of surgical procedures on the respiratory, gastrointestinal, and urinary tracts that result in temporary bacteremia, antimicrobial agents are often administered prophylactically to persons

with known heart valve deformity and to those with prosthetic valves or joints. Guidelines published by the American Heart Association and other professional societies have clarified some of these recommendations.

Control procedures are directed mainly at the human source:

1. Detection and early antimicrobial therapy of respiratory and skin infections with group A streptococci. Prompt eradication of streptococci from early infections can effectively prevent the development of poststreptococcal disease. This requires maintenance of adequate penicillin levels in tissues for 10 days (eg, benzathine penicillin G given once intramuscularly). Erythromycin is an alternative drug, although many S pyogenes are now resistant.

2. Antistreptococcal chemoprophylaxis in persons who have suffered an attack of rheumatic fever. This involves giving one injection of benzathine penicillin G intramuscularly every 3–4 weeks or daily oral penicillin or oral sulfonamide. The first attack of rheumatic fever infrequently causes major heart damage; however, such persons are particularly susceptible to reinfections with streptococci that precipitate relapses of rheumatic activity and give rise to cardiac damage. Chemoprophylaxis in such individuals, especially children, must be continued for years. Chemoprophylaxis is not used in glomerulonephritis because of the small number of nephritogenic types of streptococci. An exception may be family groups with a high rate of poststreptococcal nephritis.

3. Eradication of S pyogenes from carriers. This is especially important when carriers are in areas such as obstetric delivery rooms, operating rooms, classrooms, or nurseries. Unfortunately, it is often difficult to eradicate β-hemolytic streptococci from permanent carriers, and individuals may occasionally have to be shifted away from “sensitive” areas for some time.

CLOSTRIDIUM SPECIES

The clostridia are large anaerobic, gram-positive, motile rods. Many decompose proteins or form toxins, and some do both. Their natural habitat is the soil, marine sediments, sewage, or the intestinal tract of animals and humans, where they live as saprophytes. The clostridia continue to increase in number as new species are discovered and several species have been sequenced. There are 19 clusters based on 16SrRNA gene sequence analysis. Most clinically related species are in RNA Cluster I. Among the pathogens in this cluster are the organisms causing botulism, tetanus, gas gangrene, and pseudomembranous colitis.

Morphology and Identification

A. Typical Organisms Spores of clostridia are usually wider than the diameter of the rods in which they are formed. In the various species, the spore is placed centrally, subterminally, or terminally. Most species of clostridia are motile and possess peritrichous flagella.

A Gram stain of a Clostridium species with terminal spores is shown in Figure 11-2.

B. Culture

Clostridia are anaerobes and grow under anaerobic conditions; a few species are aerotolerant and also grow in ambient air. In general, the clostridia grow well on the blood-enriched media or other media used to grow anaerobes.

C. Colony Forms

Some clostridia produce large raised colonies (eg, C perfringens); others produce smaller colonies (eg, C tetani). Some clostridia form colonies that spread or swarm on the agar surface (Clostridium septicum). Many clostridia produce a zone of β-hemolysis on blood agar. C perfringens characteristically produces a double zone of β-hemolysis around colonies.

D. Growth Characteristics

Clostridia can ferment a variety of sugars (saccharolytic) and many can digest proteins (proteolytic); some species do both. These metabolic characteristics are used to divide the clostridia into groups. Milk is turned acid by some and digested by others and undergoes “stormy fermentation” (ie, clot torn by gas) with a third group (eg, C perfringens). Various enzymes are produced by different species.

CLOSTRIDIUM BOTULINUM

C botulinum, which causes the disease botulism, is worldwide in distribution; it is found in soil and occasionally in animal feces. Types of C botulinum are distinguished by the antigenic type of toxin they produce. Spores of the organism are highly resistant to heat, withstanding 100°C for several hours. Heat resistance is diminished at acid pH or high salt concentration.

Toxins

During the growth of C botulinum and during autolysis of the bacteria, toxin is liberated into the environment. Seven antigenic varieties of toxin (serotypes A–G) are known. Types A, B, E, and F are the principal causes of human illness. Types A and B have been associated with a variety of foods and type E predominantly with fish products. Type C produces limberneck in birds; type D causes botulism in mammals. Type G is not associated with disease. Botulinum toxins have three domains. Two of the domains facilitate binding to and entry of toxin into the nerve cell. The third domain is the toxin which is a 150 kDa protein that is cleaved into a heavy chain (H, 100 kDa) and a light chain (L, 50 kDa) that are linked by a disulfide bond. Botulinum toxin is absorbed from the gut, enters the blood circulation, and binds to receptors of presynaptic membranes of motor neurons of the peripheral nervous system and cranial nerves. The toxin does not cross the blood brain barrier or affect the central nervous system. Proteolysis— by the L chain of botulinum toxin—of the target SNARE proteins (soluble-N-ethyl maleimide-sensitive factor attachment protein) in the neurons inhibits the release of acetylcholine at the synapse, resulting in lack of muscle contraction and paralysis. The SNARE proteins are synaptobrevin (also known as vesicle-associated membrane protein or VAMP), SNAP 25, and syntaxin. The toxins of C botulinum types A, C, and E cleave the 25,000 kDa SNAP 25. Type C also cleaves syntaxin. Types B, D, F, and G toxins cleave only synaptobrevin. C botulinum toxins are among the most toxic substances known: The lethal dose for a human is probably about 1–2 μg/kg. The toxins are destroyed by heating for 20 minutes at 100°C. Rare strains of Clostridium butyricum and Clostridium baratii have also been shown to produce botulinum neurotoxin and cause botulism in humans. Strains that produce toxins A, B, or F are associated with infant botulism.

Pathogenesis

Resurgence of wound botulism caused by types A or B toxin has occurred recently in the United States, the United Kingdom, and Germany in association with skin-popping using contaminated “black tar” heroin. However, most cases of botulism represent an intoxication resulting from the ingestion of food in which C botulinum has grown and produced toxin. The most common offenders are spiced, smoked, vacuum packed, or canned alkaline foods that are eaten without cooking. In such foods, spores of C botulinum germinate; that is, under anaerobic conditions,

vegetative forms grow and produce toxin. In infant botulism, honey is the most frequent vehicle of infection. The pathogenesis differs from the way that adults acquire infection. The infant ingests the spores of C botulinum (or C butyricum or C baratii), and the spores germinate within the intestinal tract. The vegetative cells produce toxin as they multiply; the neurotoxin then gets absorbed into the bloodstream. In rare instances, adults with gastrointestinal anatomical abnormalities or functional disorders may develop "infant botulism." Wound botulism is the result of tissue contamination with spores and is seen primarily in injection drug users. Very rarely, inhalational botulism occurs when toxin enters the respiratory tract. The toxin acts by blocking release of acetylcholine at synapses and neuromuscular junctions. The result is flaccid paralysis. The electromyogram and edrophonium strength test results are typical.

Clinical Findings

Symptoms begin 18–24 hours after ingestion of the toxic food, with visual disturbances (incoordination of eye muscles, double vision), inability to swallow, and speech difficulty; signs of bulbar paralysis are progressive, and death occurs from respiratory paralysis or cardiac arrest. Gastrointestinal symptoms are not prominent. There is no fever. The patient remains fully conscious until shortly before death. The mortality rate is high. Patients who recover do not develop antitoxin in the blood. In the United States, infant botulism is as common as or more common than the classic form of paralytic botulism associated with the ingestion of toxin-contaminated food. The infants in the first months of life develop poor feeding, weakness, and signs of paralysis (floppy baby). Infant botulism may be one of the causes of sudden infant death syndrome. C botulinum and botulinum toxin are found in feces but not in serum.

Diagnostic Laboratory Tests

Clinicians who suspect a case of botulism should contact the appropriate public health authorities before submitting specimens to the laboratory. Detection of toxin and not the organism is required for definitive diagnosis. Toxin can often be demonstrated in serum, gastric secretions, or stool from the patient, and toxin may be found in leftover food. Clinical swabs or other specimens obtained from patients should be transported using anaerobe containers. Suspect foods should be left in their original containers. Mice injected intraperitoneally with such specimens from these patients die rapidly. The antigenic type of toxin is identified by neutralization with specific antitoxin in mice. This mouse bioassay is the test of choice for the confirmation of botulism. C botulinum may be grown from food remains and tested for toxin production, but this is rarely done and is of questionable significance. In infant botulism, C botulinum and toxin can be demonstrated in bowel contents but not in serum. Other methods used to detect toxin include ELISAs and PCR, but the latter may detect organisms that carry the gene but do not express toxin.

Treatment

Supportive care, especially intensive care, is key in the management of patients with botulism. Adequate respiration must be maintained by mechanical ventilation if necessary and in severe cases may need to be maintained for up to 8 weeks. These measures have reduced the mortality rate from 65% to below 25%. Potent antitoxins to three types of botulinum toxins have been prepared in horses. Because the type responsible for an individual case is usually not known, trivalent (A, B, E) antitoxin must be promptly administered intravenously with customary precautions. Antitoxin does not reverse the paralysis, but if administered early, it can prevent its advancement. Although most infants with botulism recover with supportive care alone, treatment with humanderived botulinum immune globulin (BIG) is recommended.

Epidemiology, Prevention, and Control

Because spores of C botulinum are widely distributed in soil, they often contaminate vegetables, fruits, and other materials. A large restaurant-based outbreak was associated with sautéed onions. When such foods are canned or otherwise preserved, they either must be sufficiently heated to ensure destruction of spores or must be boiled for 20 minutes before consumption. Strict regulation of commercial canning has largely overcome the danger of widespread outbreaks, but commercially prepared foods have caused deaths. A chief risk factor for botulism lies in home-canned foods, particularly string beans, corn, peppers, olives, peas, and smoked fish or vacuum-packed fresh fish in plastic bags. Toxic foods may be spoiled and rancid, and cans may “swell,” or the appearance may be innocuous. The risk from home-canned foods can be reduced if the food is boiled for more than 20 minutes before consumption. Botulinum toxin is considered to be a major potential agent for bioterrorism and biologic warfare.

CLOSTRIDIUM TETANI

C tetani, which causes tetanus, is worldwide in distribution in the soil and in the feces of horses and other animals. Several types of C tetani can be distinguished by specific flagellar may be masked, and all produce the same antigenic type of neurotoxin, tetanospasmin.

Toxin

The vegetative cells of C tetani produce the plasmid-encoded toxin tetanospasmin (150 kDa) that is cleaved by a bacterial protease into two peptides (50 and 100 kDa) linked by a disulfide bond. The larger peptide initially binds to receptors on the presynaptic membranes of motor neurons. It then migrates by the retrograde axonal transport system to the cell bodies of these neurons to the spinal cord and brainstem. The toxin diffuses to terminals of inhibitory cells, including both glycinergic interneurons and γ-aminobutyric acid (GABA)– secreting neurons from the brainstem. The smaller peptide degrades synaptobrevin (also called VAMP2, see above under C botulinum toxin), a protein required for docking of neurotransmitter vesicles on the presynaptic membrane. Release of the inhibitory glycine and GABA is blocked, and the motor neurons are not inhibited. Hyperreflexia, muscle spasms, and spastic paralysis result. Extremely small amounts of toxin can be lethal for humans.

Pathogenesis

C tetani is not an invasive organism. The infection remains strictly localized in the area of devitalized tissue (wound, burn, injury, umbilical stump, surgical suture) into which the spores have been introduced. The volume of infected tissue is small, and the disease is almost entirely a toxemia. Germination of the spore and development of vegetative organisms that produce toxin are aided by (1) necrotic tissue, (2) calcium salts, and (3) associated pyogenic infections, all of which aid establishment of low oxidation-reduction potential. The toxin released from vegetative cells reaches the central nervous system and rapidly becomes fixed to receptors in the spinal cord and brainstem and exerts the actions described.

Clinical Findings

The incubation period may range from 4 to 5 days up to 3 weeks. The disease is characterized by tonic contraction of voluntary muscles. Muscular spasms often involve first the area of injury and infection and then the muscles of the jaw (trismus, lockjaw), which contract so that the mouth cannot be opened. Gradually, other voluntary muscles become involved, resulting in tonic spasms. Any external stimulus may precipitate a tetanic generalized muscle spasm. The patient is fully

conscious, and pain may be intense. Death usually results from interference with the mechanics of respiration. The mortality rate in generalized tetanus is very high.

Diagnosis

The diagnosis rests on the clinical picture and a history of injury, although only 50% of patients with tetanus have an injury for which they seek medical attention. The primary differential diagnosis of tetanus is strychnine poisoning. Anaerobic culture of tissues from contaminated wounds may yield C tetani, but neither preventive nor therapeutic use of antitoxin should ever be withheld pending such demonstration. Proof of isolation of C tetani must rest on production of toxin and its neutralization by specific antitoxin.

Prevention and Treatment

The results of treatment of tetanus are not satisfactory. Therefore, prevention is all important. Prevention of tetanus depends on (1) active immunization with toxoids, (2) aggressive wound care, (3) prophylactic use of antitoxin, and (4) administration of penicillin.

The intramuscular administration of 250–500 units of human antitoxin (tetanus immune globulin) gives adequate systemic protection (0.01 unit or more per milliliter of serum) for 2–4 weeks. It neutralizes the toxin that has not been fixed to nervous tissue. Active immunization with tetanus toxoid should accompany antitoxin prophylaxis. Patients who develop symptoms of tetanus should receive muscle relaxants, sedation, and assisted ventilation. Sometimes, they are given very large doses of antitoxin (3000–10,000 units of tetanus immune globulin) intravenously in an effort to neutralize toxin that has not yet been bound to nervous tissue. However, the efficacy of antitoxin for treatment is doubtful except in neonatal tetanus, in which it may be lifesaving.

Surgical debridement is vitally important because it removes the necrotic tissue that is essential for proliferation of the organisms. Hyperbaric oxygen has no proven effect. Penicillin strongly inhibits the growth of C tetani and stops further toxin production. Antibiotics may also control associated pyogenic infection. When a previously immunized individual sustains a potentially dangerous wound, an additional dose of toxoid should be injected to restimulate antitoxin production. This “recall” injection of toxoid may be accompanied by a dose of antitoxin if the patient has not had current immunization or boosters or if the history of immunization is unknown.

Control

Tetanus is a totally preventable disease. Universal active immunization with tetanus toxoid should be mandatory. Tetanus toxoid is produced by detoxifying the toxin with formalin and then concentrating it. Aluminum salt–adsorbed toxoids are used. Three injections comprise the initial

course of immunization followed by another dose about 1 year later. Initial immunization should be carried out in all children during the first year of life. A “booster” injection of toxoid is given upon entry into school. Thereafter, “boosters” can be spaced 10 years apart to maintain serum levels of more than 0.01 unit antitoxin per milliliter. In young children, tetanus toxoid is often combined with diphtheria toxoid and acellular pertussis vaccine. Environmental control measures are not possible because of the wide dissemination of the organism in the soil and the long survival of its spores.

CLOSTRIDIA THAT PRODUCE INVASIVE INFECTIONS

Many different toxin-producing clostridia (C perfringens and related clostridia) (Figure 11-3) can produce invasive infection (including myonecrosis and gas gangrene) if introduced into damaged tissue. About 30 species of clostridia may produce such an effect, but the most common in invasive disease is C perfringens (90%). An enterotoxin of C perfringens is a common cause of food poisoning.

Toxins

The invasive clostridia produce a large variety of toxins and enzymes that result in a spreading infection. Many of these toxins have lethal, necrotizing, and hemolytic properties. In some cases, these are different properties of a single substance; in other instances, they are attributable to different chemical entities. The alpha toxin of C perfringens type A is a lecithinase, and its lethal action is proportionate to the rate at which it splits lecithin (an important constituent of cell membranes) to phosphorylcholine and diglyceride. Alpha toxin also aggregates platelets, thereby leading to formation of thrombi in small blood vessels and adding to poor tissue profusion and extending the consequences of anaerobiosis, namely, destruction of viable tissue (gas gangrene). The theta toxin has similar hemolytic and necrotizing effects but is not a lecithinase. It is a member of the cholesterol-dependent cytolysins that act by forming pores in cell membranes. Epsilon toxin is a protein that causes edema, and hemorrhage is very potent. DNase and hyaluronidase, a collagenase that digests collagen of subcutaneous tissue and muscle, are also produced.

Some strains of C perfringens produce a powerful enterotoxin (C perfringens enterotoxin, CPE), especially when grown in meat dishes. When more than 108 vegetative cells are ingested and sporulate in the gut, CPE is formed. CPE is a protein (35 kDa) that may be a nonessential component of the spore coat; it is distinct from other clostridial toxins. It induces intense diarrhea in 7–30 hours. The action of C perfringens enterotoxin involves marked hypersecretion in the jejunum and ileum, with loss of fluids and electrolytes in diarrhea. Much less frequent symptoms include nausea, vomiting, and fever. This illness is similar to that produced by B cereus and tends

to be self-limited. Enterotoxin-producing strains of C perfringens may also play a role in antibioticassociated diarrhea and necrotizing enterocolitis in infants.

Pathogenesis

In invasive clostridial infections, spores reach tissue either by contamination of traumatized areas (soil, feces) or from the intestinal tract. The spores germinate at low oxidationreduction potential; vegetative cells multiply, ferment carbohydrates present in tissue, and produce gas. The distention of tissue and interference with blood supply, together with the secretion of necrotizing toxins and hyaluronidase, favor the spread of infection. Tissue necrosis extends, providing an opportunity for increased bacterial growth, hemolytic anemia, and, ultimately, severe toxemia and death.

In gas gangrene (clostridial myonecrosis), a mixed infection is the rule. In addition to the toxigenic clostridia, proteolytic clostridia and various cocci and gram-negative organisms are also usually present. C perfringens occurs in the genital tracts of 5% of women. Before legalization of abortion in the United States, clostridial uterine infections followed instrumented abortions. Clostridium sordellii has many of the properties of C perfringens. C sordellii has been reported to cause a toxic shock syndrome after medical abortion with mifepristone and intravaginal misoprostol. Endometrial infection with C sordellii is implicated. Clostridial bacteremia, especially that caused by C septicum, is a frequent occurrence in patients with neoplasms. In New Guinea, C perfringens type C produces necrotizing enteritis (pigbel) that can be highly fatal in children. Immunization with type C toxoid appears to have preventive value.

Clinical Findings

From a contaminated wound (eg, a compound fracture, postpartum uterus), the infection spreads in 1–3 days to produce crepitation in the subcutaneous tissue and muscle, foul-smelling discharge, rapidly progressing necrosis, fever, hemolysis, toxemia, shock, and death. Treatment is with early surgery amputation) and antibiotic administration. Until the advent of specific therapy, early amputation was the only treatment. At times, the infection results only in anaerobic fasciitis or cellulitis. C perfringens food poisoning usually follows the ingestion of large numbers of clostridia that have grown in warmed meat dishes. The toxin forms when the organisms sporulate in the gut, with the onset of diarrhea—usually without vomiting or fever—in 7–30 hours. The illness lasts only 1–2 days.

Diagnostic Laboratory Tests

Specimens consist of material from wounds, pus, and tissue. The presence of large gram-positive rods in Gram-stained smears suggests gas gangrene clostridia; spores are not regularly present. Material is inoculated into chopped meat–glucose medium and thioglycolate medium and onto

blood agar plates incubated anaerobically. After pure cultures have been obtained by selecting colonies from anaerobically incubated blood plates, they are identified by biochemical reactions (various sugars in thioglycolate, action on milk), hemolysis, and colony morphology. Lecithinase activity is evaluated by the precipitate formed around colonies on egg yolk media. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a rapid and sensitive method for identification of invasive Clostridium species recovered in culture. C perfringens rarely produces spores when cultured on agar in the laboratory.

Treatment

The most important aspect of treatment is prompt and extensive surgical debridement of the involved area and excision of all devitalized tissue, in which the organisms are prone to grow. Administration of antimicrobial drugs, particularly penicillin, is begun at the same time. Hyperbaric oxygen may be of help in the medical management of clostridial tissue infections. It is said to "detoxify" patients rapidly.

Antitoxins are available against the toxins of C perfringens, Clostridium novyi, Clostridium histolyticum, and C septicum, usually in the form of concentrated immune globulins. Polyvalent antitoxin (containing antibodies to several toxins) has been used. Although such antitoxin is sometimes administered to individuals with contaminated wounds containing much devitalized tissue, there is no evidence for its efficacy. Food poisoning caused by C perfringens enterotoxin usually requires only symptomatic care.

Prevention and Control

Early and adequate cleansing of contaminated wounds and surgical debridement, together with the administration of antimicrobial drugs directed against clostridia (eg, penicillin), are the best available preventive measures. Antitoxins should not be relied on. Although toxoids for active immunization have been prepared, they have not come into practical use.

CLOSTRIDIUM DIFFICILE AND DIARRHEAL DISEASE

Pseudomembranous Colitis

Pseudomembranous colitis is diagnosed by detection of one or both C difficile toxins in stool and by endoscopic observation of pseudomembranes or microabscesses in patients who have diarrhea and have been given antibiotics. Plaques and microabscesses may be localized to one area of the bowel. The diarrhea may be watery or bloody, and the patient frequently has associated abdominal cramps, leukocytosis, and fever. Although many antibiotics have been associated with pseudomembranous colitis, the most common are ampicillin and clindamycin and, more recently, the fluoroquinolones. The disease is treated by discontinuing administration of the offending

antibiotic and orally giving metronidazole, vancomycin, or fidaxomicin. Fecal transplantation has become a successful and routine method for recurrent and refractory disease. This usually involves administration of the feces of a healthy related donor by way of colonoscopy or less commonly via a nasogastric tube into the gastrointestinal tract of the patient. Administration of antibiotics results in proliferation of drug-resistant C difficile that produces two toxins. Toxin A, a potent enterotoxin that also has some cytotoxic activity, binds to the brush border membranes of the gut at receptor sites.

Toxin B is a potent cytotoxin. C difficile toxins have glycosyltransferase activity and act by modifying signaling molecules that control various cellular functions. This results in apoptosis, capillary leakage, cytokine stimulation, and other consequences that lead to colitis. Both toxins are usually found in the stools of patients with pseudomembranous colitis. However, toxin A–negative, toxin B–positive infections have been described. Not all strains of C difficile produce the toxins, and the toxin genes are found on a large, chromosomal pathogenicity island along with three other genes that regulate toxin expression.

Diagnosis is made clinically and supported by demonstration of toxin in the stool by a variety of methods that includes anaerobic toxigenic culture, enzyme immunoassay, and molecular tests that detect the genes that encode toxins A or B. See the reference by Burnham for a more complete discussion of C difficile diagnosis.

The surge in C difficile infections since the beginning of the 21st century is believed to be related to a combination of host and organism factors. The responsible host factors include the aging population, the increase in survival of immunocompromised susceptible individuals, and the increase in administration of antibiotics and gastric acid suppressant agents. Organism factors relate primarily to emergence of certain strain types that are more virulent due to mutations in the pathogenicity locus.

Antibiotic-Associated Diarrhea

The administration of antibiotics frequently leads to a mild to moderate form of diarrhea, termed antibiotic-associated diarrhea. This disease is generally less severe than the classic form of pseudomembranous colitis. As many as 25% of cases of antibiotic-associated diarrhea are caused by C difficile infection. Other Clostridium species such as C perfringens and C sordellii have also been implicated. The latter two species are not associated with pseudomembranous colitis.

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UNIT – II – Medical Bacteriology – SMB3101

2. The Neisseriae

The family Neisseriaceae includes the genera Neisseria, Kingella, Eikenella, Simonsiella, and Alysiella. The neisseriae are gram-negative cocci that usually occur in pairs (diplococci). Neisseria gonorrhoeae (gonococci) and Neisseria meningitidis (meningococci) are pathogenic for humans and typically are found associated with or inside polymorphonuclear cells. Some neisseriae are normal inhabitants of the human respiratory tract, rarely if ever cause disease, and occur extracellularly.

Gonococci and meningococci are closely related, with 70% DNA homology, and are differentiated by a few laboratory tests and specific characteristics. Meningococci have polysaccharide capsules but gonococci do not, and meningococci rarely have plasmids but most gonococci do. Most importantly, the two species are differentiated by the usual clinical presentations of the diseases they cause: Meningococci typically are found in the upper respiratory tract and cause meningitis, but gonococci cause genital infections. However, the clinical spectra of the diseases caused by gonococci and meningococci do overlap.

2.1 Morphology and Identification

A. Typical Organisms

The typical Neisseria is a gram-negative, nonmotile diplococcus, approximately 0.8 μm in diameter. Individual cocci are kidney bean shaped; when the organisms occur in pairs, the flat or concave sides are adjacent.

B. Culture

In 48 hours on enriched media (eg, modified Thayer-Martin, Martin-Lewis, GC-Lect, and New York City), gonococci and meningococci form convex, glistening, elevated, mucoid colonies 1–5 mm in diameter. Colonies are transparent or opaque, nonpigmented, and nonhemolytic. Neisseria flavescens, Neisseria cinerea, Neisseria subflava, and Neisseria lactamica may have yellow pigmentation. Neisseria sicca produces opaque, brittle, wrinkled colonies. Moraxella catarrhalis produces nonpigmented or pinkish gray opaque colonies.

C. Growth Characteristics

The neisseriae grow best under aerobic conditions, but some grow in an anaerobic environment. They have complex growth requirements. Most neisseriae oxidize carbohydrates, producing acid but not gas, and their carbohydrate patterns are a means of distinguishing them. The neisseriae produce oxidase and give positive oxidase reactions; the oxidase test is a key test for identifying them. When bacteria are spotted on a filter paper soaked with tetramethylparaphenylenediamine hydrochloride (oxidase), the neisseriae rapidly turn dark purple. Meningococci and gonococci grow best on media containing complex organic substances such as heated blood, hemin, and animal proteins and in an atmosphere containing 5% CO_2 (eg, candle jar). Growth is inhibited by some toxic constituents of the medium (eg, fatty acids or salts). The organisms are rapidly killed by drying, sunlight, moist heat, and many disinfectants. They produce autolytic enzymes that result in rapid swelling and lysis in vitro at 25°C and at an alkaline pH.

2.2 NEISSERIA GONORRHOEAE

Gonococci oxidize only glucose and differ antigenically from the other neisseriae. Gonococci usually produce smaller colonies than those of the other neisseriae. Gonococci that require arginine, hypoxanthine, and uracil (Arg−, Hyx−, and Ura− auxotype) tend to grow most slowly on primary culture. Gonococci isolated from clinical specimens or maintained by selective subculture have typical small colonies containing piliated bacteria. On nonselective subculture, larger colonies containing nonpiliated gonococci are also formed. Opaque and transparent variants of both the small and large colony types also occur; the opaque colonies are associated with the presence of a surface-exposed protein, Opa.

Antigenic Structure

N gonorrhoeae is antigenically heterogeneous and capable of changing its surface structures in vitro—and presumably in vivo—to avoid host defenses. Surface structures include the following.

A. Pili (Fimbriae)

Pili are the hairlike appendages that extend up to several micrometers from the gonococcal surface. They enhance attachment to host cells and resistance to phagocytosis. They are made up of stacked pilin proteins (molecular weight [MW], 17–21 kDa). The amino terminal of the pilin molecule, which contains a high percentage of hydrophobic amino acids, is conserved. The amino acid sequence near the midportion of the molecule also is conserved; this portion of the molecule serves in attachment to host cells and is less prominent in the immune response. The amino acid sequence near the carboxyl terminal is highly variable; this portion of the molecule is most prominent in the immune response. The pilins of almost all strains of N gonorrhoeae are antigenically different, and a single strain can make many antigenically distinct forms of pilin.

B. Por

Por protein extends through the gonococcal cell membrane. It forms pores in the surface through which some nutrients enter the cell. Por proteins may impact intracellular killing of gonococci within neutrophils by preventing phagosome– lysosome fusion. In addition, variable resistance of gonococci to killing by normal human serum depends on whether Por protein selectively binds to complement components C3b and C4b. The MW of Por varies from 32 to 36 kDa. Each strain of gonococcus expresses only one of two types of Por, but the Por of different strains is antigenically different. Serologic typing of Por by agglutination reactions with monoclonal antibodies was a useful method for studying the epidemiology of N gonorrhoeae. However, this method has been replaced by genotypic methods such as pulsed-field gel electrophoresis, Opa typing, and DNA sequencing.

C. Opa Proteins

These proteins function in adhesion of gonococci within colonies and in attachment of gonococci to host cell receptors such as heparin-related compounds and CD66 or carcinoembryonic antigen–related cell adhesion molecules. One portion of the Opa molecule is in the gonococcal outer membrane, and the rest is exposed on the surface. The MW of Opa ranges from 20 to 28 kDa. A strain of gonococcus can express no, one, two, or occasionally three types of Opa, but each strain has 11–12 genes for different Opas. PCR of the opa genes followed by restriction endonuclease digestion, and analysis of subsequent fragments by gel electrophoresis is a useful method of strain typing performed by reference laboratories.

D. Rmp (Protein III)
This protein (MW, 30–31 kDa) is antigenically conserved in all gonococci. It is a reduction-modifiable protein (Rmp) and changes its apparent MW when in a reduced state. It associates with Por in the formation of pores in the cell surface.

E. Lipooligosaccharide
In contrast to the enteric gram-negative rods gonococcal lipopolysaccharide (LPS) does not have long O-antigen side chains and is called a lipooligosaccharide (LOS). Its MW is 3–7 kDa. Gonococci can express more than one antigenically different LOS chain simultaneously. Toxicity in gonococcal infections is largely attributable to the endotoxic effects of LOS. Specifically, in the fallopian tube explant model, LOS causes ciliary loss and mucosal cell death. In a form of molecular mimicry, gonococci make LOS molecules that structurally resemble human cell membrane glycosphingolipids. The gonococcal LOS and the human glycosphingolipid of the same structural class react with the same monoclonal antibody, indicating the molecular mimicry. The presence on the gonococcal surface of the same surface structures as human cells helps gonococci evade immune recognition. The terminal galactose of human glycosphingolipids is often conjugated with sialic acid. Sialic acid is a nine-carbon, 5-N-acetylated ketulosonic acid also called N-acetylneuraminic acid (NANA).

Gonococci do not make sialic acid but do make a sialyltransferase that functions to take NANA from the human nucleotide sugar cytidine 5′-monophospho-N-acetylneuraminic acid (CMPNANA) and place the NANA on the terminal galactose of a gonococcal acceptor LOS. This sialylation affects the pathogenesis of gonococcal infection. It makes the gonococci resistant to killing by the human antibody–complement system and interferes with gonococcal binding to receptors on phagocytic cells. N meningitidis and Haemophilus influenzae make many but not all of the same LOS structures as N gonorrhoeae. The biology of the LOS for the three species and for some of the nonpathogenic Neisseria species is similar. Four of the various serogroups of N meningitidis make different sialic acid capsules, indicating that they also have biosynthetic pathways different from those of gonococci. These four serogroups sialylate their LOS using sialic acid from their endogenous pools.

F. Other Proteins
Several antigenically constant proteins of gonococci have poorly defined roles in pathogenesis. Lip (H8) is a surfaceexposed protein that is heat modifiable like Opa. The Fbp (ferric-binding protein), similar in MW to Por, is expressed when the available iron supply is limited, such as in human infection. Gonococci elaborate an IgA1 protease that splits and inactivates IgA1, a major mucosal immunoglobulin of humans. Meningococci, H influenzae, and Streptococcus pneumoniae elaborate similar IgA1 proteases.

Genetics and Antigenic Heterogeneity
Gonococci have evolved mechanisms for frequently switching from one antigenic form (pilin, Opa, or LPS) to another antigenic form of the same molecule. This switching takes place in one in every 102.5–103 gonococci, an extremely rapid rate of change for bacteria. Because pilin, Opa, and LPS are surface-exposed antigens on gonococci, they are important in the immune response to infection. The molecules' rapid switching from one antigenic form to another helps the

gonococci elude the host immune system. The switching mechanism for pilin, which has been the most thoroughly studied, is different from the mechanism for Opa.

Gonococci have multiple genes that code for pilin, but only one gene is inserted into the expression site. Gonococci can remove all or part of this pilin gene and replace it with all or part of another pilin gene. This mechanism allows gonococci to express many antigenically different pilin molecules over time. The switching mechanism of Opa involves, at least in part, the addition or removal from the DNA of one or more of the pentameric coding repeats preceding the sequence that codes for the structural Opa gene. The switching mechanism of LPS is unknown. Gonococci contain several plasmids; 95% of strains have a small, "cryptic" plasmid (MW, 2.6 mDa) of unknown function. Two other plasmids (MW, 3.4 and 4.7 mDa) contain genes that code for TEM-1 type (penicillinases) β-lactamases, which cause resistance to penicillin. These plasmids are transmissible by conjugation among gonococci; they are similar to a plasmid found in penicillinase-producing Haemophilus species and may have been acquired from Haemophilus or other gram-negative organisms. About 5–20% of gonococci contain a plasmid (MW, 24.5 × 106 kDa) with the genes that code for conjugation; the incidence is highest in geographic areas where penicillinase-producing gonococci are most common. High-level tetracycline resistance (minimum inhibitory concentrations [MICs] of ≥16 mg/L) has developed in gonococci by the insertion of a streptococcal gene tetM coding for tetracycline resistance into the conjugative plasmid.

Pathogenesis, Pathology, and Clinical Findings

Gonococci exhibit several morphologic types of colonies, but only piliated bacteria appear to be virulent. Opa protein expression varies depending on the type of infection. Gonococci that form opaque colonies are isolated from men with symptomatic urethritis and from uterine cervical cultures at midcycle. Gonococci that form transparent colonies are frequently isolated from men with asymptomatic urethral infection, from menstruating women, and from patients with invasive forms of gonorrhea, including salpingitis and disseminated infection. Antigenic variation of surface proteins during infection allows the organism to circumvent host immune response.

Gonococci attack mucous membranes of the genitourinary tract, eye, rectum, and throat, producing acute suppuration that may lead to tissue invasion; this is followed by chronic inflammation and fibrosis. Men usually have urethritis, with yellow, creamy pus and painful urination. The process may extend to the epididymis. As suppuration subsides in untreated infection, fibrosis occurs, sometimes leading to urethral strictures. Urethral infection in men can be asymptomatic. In women, the primary infection is in the endocervix and extends to the urethra and vagina, giving rise to mucopurulent discharge. It may then progress to the uterine tubes, causing salpingitis, fibrosis, and obliteration of the tubes. Infertility occurs in 20% of women with gonococcal salpingitis. Chronic gonococcal cervicitis and proctitis are often asymptomatic.

Gonococcal bacteremia leads to skin lesions (especially hemorrhagic papules and pustules) on the hands, forearms, feet, and legs and to tenosynovitis and suppurative arthritis, usually of the knees, ankles, and wrists. Gonococci can be cultured from blood or joint fluid of only 30% of patients with gonococcal arthritis. Gonococcal endocarditis is an uncommon but severe infection. Gonococci sometimes cause meningitis and eye infections in adults; these have manifestations similar to those caused by meningococci. Complement deficiency is frequently found in patients

with gonococcal bacteremia. Patients with bacteremia, especially if recurrent, should be tested for total hemolytic complement activity. Gonococcal ophthalmia neonatorum, an infection of the eye in newborns, is acquired during passage through an infected birth canal. The initial conjunctivitis rapidly progresses and, if untreated, results in blindness. To prevent gonococcal ophthalmia neonatorum, instillation of tetracycline, erythromycin, or silver nitrate into the conjunctival sac of newborns is compulsory in the United States. Gonococci that produce localized infection are often serum sensitive (ie, killed by antibody and complement).

Diagnostic Laboratory Tests

A. Specimens

Pus and secretions are taken from the urethra, cervix, rectum, conjunctiva, throat, or synovial fluid for culture and smear. Blood culture is necessary in systemic illness, but a special culture system is helpful because gonococci (and meningococci) may be susceptible to the polyanethol sulfonate present in standard blood culture media. Proprietary swabs may be required for diagnostic molecular assays. Clinicians should check with clinical laboratories regarding the appropriate collection devices for the assays used in a particular institution.

B. Smears

Gram-stained smears of urethral or endocervical exudates reveal many diplococci within pus cells. These give a presumptive diagnosis. Stained smears of the urethral exudate from men have a sensitivity of about 90% and a specificity of 99%. Stained smears of endocervical exudates have a sensitivity of about 50% and a specificity of about 95% when examined by an experienced microscopist. Additional diagnostic testing of urethral exudates from men is not necessary when the stain result is positive, but nucleic acid amplification tests (NAATs) or cultures should be done for women. Stained smears of conjunctival exudates can also be diagnostic, but those of specimens from the throat or rectum are generally not helpful.

C. Culture

Immediately after collection, pus or mucus is streaked on enriched selective medium (eg, modified Thayer-Martin medium [MTM]) and incubated in an atmosphere containing 5% CO_2 (candle extinction jar) at 37°C. To avoid overgrowth by contaminants, the selective medium contains antimicrobial drugs (eg, vancomycin, 3 μg/mL; colistin, 7.5 μg/mL; amphotericin B, 1 μg/mL; and trimethoprim, 3 μg/mL). If immediate incubation is not possible, the specimen should be placed in a CO_2-containing transport-culture system. Fortyeight hours after culture, the organisms can be quickly identified by their appearance on a Gram-stained smear; by oxidase positivity; and by coagglutination, immunofluorescence staining, or other laboratory tests. The species of subcultured bacteria may be determined by oxidation of specific carbohydrates. Matrix-assisted laser desorption/ ionization time of flight mass spectrometry (MALDI-TOF MS) has potential to provide rapid (same-day) identification of cultured isolates. The gonococcal isolates from anatomic sites other than the genital tract or from children should be identified as to species using two different confirmatory tests because of the legal and social implications of a positive culture result. Most laboratories have abandoned culture in favor of NAATs. Because of this, it may be difficult to monitor for increasing multidrug resistance. Culture should be considered in a patient who appears to have failed standard treatment.

D. Nucleic Acid Amplification Tests

Several Food and Drug Administration–cleared nucleic acid amplification assays are available for direct detection of N gonorrhoeae in genitourinary specimens, and these are the preferred tests from these sources. In general, these assays have excellent sensitivity and specificity in symptomatic, high-prevalence populations. Advantages include better detection, more rapid results, and the ability to use urine as a specimen source. Disadvantages include poor specificity of some assays because of cross-reactivity with nongonococcal Neisseria species. Some of these assays are not approved for use in the diagnosis of extragenital gonococcal infections or for infection in children. NAATs are not recommended as tests of cure because nucleic acid may persist in patient specimens for up to 3 weeks after successful treatment. Patients who are believed to have failed treatment are best reevaluated using culture so that the organism can be tested for resistance.

E. Serology

Serum and genital fluid contain IgG and IgA antibodies against gonococcal pili, outer membrane proteins, and LPS. Some IgM of human sera is bactericidal for gonococci in vitro. In infected individuals, antibodies to gonococcal pili and outer membrane proteins can be detected by immunoblotting, radioimmunoassay, and enzyme-linked immunosorbent assay (ELISA) tests. However, these tests are not useful as diagnostic aids for several reasons, including gonococcal antigenic heterogeneity, the delay in development of antibodies in acute infection, and a high background level of antibodies in the sexually active population.

Immunity

Repeated gonococcal infections are common. Protective immunity to reinfection does not appear to develop as part of the disease process, because of the antigenic variety of gonococci. Although antibodies can be demonstrated, including the IgA and IgG on mucosal surfaces, they either are highly strain specific or have little protective ability.

Treatment

Since the development and widespread use of penicillin, gonococcal resistance to penicillin has gradually risen, owing to the selection of chromosomal mutants and to increased prevalence of penicillinase-producing N gonorrhoeae (PPNG). Chromosomally mediated resistance to tetracycline (MIC $\geq$2 μg/mL) is common. High-level resistance to tetracycline (MIC $\geq$ 32 μg/mL) also occurs. Spectinomycin resistance as well as resistance to fluoroquinolones has been noted. Single-dose fluoroquinolone treatment was recommended for treatment of gonococcal infections from 1993 until 2006. Since 2006, rates of quinolone resistance among gonococcal isolates have exceeded 5% in men who have sex with men and in heterosexual men. Because of the problems with antimicrobial resistance in N gonorrhoeae, the Centers for Disease Control and Prevention (CDC) recommended that patients with uncomplicated genital or rectal infections be treated with ceftriaxone (250 mg) given intramuscularly as a single dose or 400 mg of oral cefixime as a single dose. Additional therapy with 1 g of azithromycin orally in a single dose or with 100 mg of doxycycline orally twice a day for 7 days is recommended for possible concomitant chlamydial infections. Unfortunately, new data from CDC's Gonococcal Isolate Surveillance Project (GISP) have noted an increase in the percentage of isolates exhibiting elevated MICs to both oral cefixime and ceftriaxone. This observation, combined with reports of cefixime treatment failures in other countries, has resulted in revised treatment guidelines. Since ceftriaxone is more potent than cefixime, the CDC no longer recommends cefixime as an effective treatment. Injectable

ceftriaxone 250 mg IM once plus either azithromycin or doxycycline as written above is recommended for treatment of uncomplicated urethritis, cervicitis, and proctitis. Azithromycin has been found to be safe and effective in pregnant women, but doxycycline is contraindicated. Modifications of these therapies are recommended for other types of N gonorrhoeae infection.

Epidemiology, Prevention, and Control

Gonorrhea is worldwide in distribution. In the United States, its incidence rose steadily from 1955 until the late 1970s, when the incidence was between 400 and 500 cases per 100,000 population. Between 1975 and 1997, there was a 74% decline in the rate of reported gonococcal infections. Thereafter, the rates plateaued for 10 years and decreased from 2006 to 2009, but since 2009 rates have once again increased slightly each year. Gonorrhea is exclusively transmitted by sexual contact, often by women and men with asymptomatic infections. The infectivity of the organism is such that the chance of acquiring infection from a single exposure to an infected sexual partner is 20–30% for men and even greater for women.

The infection rate can be reduced by avoiding multiple sexual partners, rapidly eradicating gonococci from infected individuals by means of early diagnosis and treatment, and finding cases and contacts through education and screening of populations at high risk. Mechanical prophylaxis (condoms) provides partial protection. Chemoprophylaxis is of limited value because of the rise in antibiotic resistance of the gonococcus.

Gonococcal ophthalmia neonatorum is prevented by local application of 0.5% erythromycin ophthalmic ointment or 1% tetracycline ointment to the conjunctiva of newborns. Although instillation of silver nitrate solution is also effective and is the classic method for preventing ophthalmia neonatorum, silver nitrate is difficult to store and causes conjunctival irritation; its use has largely been replaced by use of erythromycin or tetracycline ointment.

2.3 NEISSERIA MENINGITIDIS

Antigenic Structure

At least 13 serogroups of meningococci have been identified by immunologic specificity of capsular polysaccharides. The most important serogroups associated with disease in humans are A, B, C, X, Y, and W-135. In contrast to the other capsular serogroups in which the capsule is composed of sialic acid moieties, the group A polysaccharide is a polymer of N-acetyl-mannosamine-1-phosphate. Incorporation of human sialic acid derivatives such as NANA into the meningococcal capsules allows the organism to be overlooked by the host immune system (often referred to as "molecular mimicry"). Meningococcal antigens are found in blood and cerebrospinal fluid of patients with active disease.

Outbreaks and sporadic cases in the Western hemisphere in the past decade have been caused mainly by groups B, C, W-135, and Y; outbreaks in southern Finland and São Paulo, Brazil, were caused by groups B and C; outbreaks in New Zealand have been caused by a particular B strain; and those in Africa were mainly caused by group A. Group C and, especially, group A are associated with epidemic disease. The outer membrane of N meningitidis consists of proteins and LPS that play major roles in organism virulence. There are two porin proteins (Por A and Por B) that are important in controlling nutrient diffusion into the organism and also interact with host cells. These porins have been targets of interest in vaccine development. The opacity proteins (Opa) are comparable to Opa of the gonococci and play a role in attachment.

Meningococci are piliated and these structures initiate binding to nasopharyngeal epithelial cells and other host cells such as endothelium and erythrocytes. The lipid A disaccharide of meningococcal LPS is responsible for many of the toxic effects found in meningococcal disease. The highest levels of endotoxin measured in sepsis have been found in patients with meningococcemia (50- to 100-fold greater than with other gram-negative infections). Collectively, these structures and proteins are responsible for the devastating clinical features so characteristic of meningococcal infections.

Pathogenesis, Pathology, and Clinical Findings

Humans are the only natural hosts for whom meningococci are pathogenic. The nasopharynx is the portal of entry. There, the organisms attach to epithelial cells with the aid of pili; they may form part of the transient microbiota without producing symptoms. From the nasopharynx, organisms may reach the bloodstream, producing bacteremia; the symptoms may be similar to those of an upper respiratory tract infection. Fulminant meningococcemia is more severe, with a high fever and a hemorrhagic rash; the patient may have disseminated intravascular coagulation and circulatory collapse (Waterhouse-Friderichsen syndrome). Meningitis is the most common complication of meningococcemia. It usually begins suddenly with an intense headache, vomiting, and stiff neck and progresses to coma within a few hours.

During meningococcemia, there is thrombosis of many small blood vessels in many organs, with perivascular infiltration and petechial hemorrhages. There may be interstitial myocarditis, arthritis, and skin lesions. In meningitis, the meninges are acutely inflamed, with thrombosis of blood vessels and exudation of polymorphonuclear leukocytes, so that the surface of the brain is covered with a thick purulent exudate.

It is not known what transforms an asymptomatic infection of the nasopharynx into meningococcemia and meningitis, but this can be prevented by specific bactericidal serum antibodies against the infecting serotype. Neisseria bacteremia is favored by the absence of bactericidal antibody (IgM and IgG), inhibition of serum bactericidal action by a blocking IgA antibody, or a complement component deficiency (C5, C6, C7, or C8). Meningococci are readily phagocytosed in the presence of a specific opsonin.

Diagnostic Laboratory Tests

A. Specimens

Specimens of blood are taken for culture, and specimens of spinal fluid are taken for smear, culture, and even some laboratories molecular testing. Nasopharyngeal swab cultures are suitable for carrier surveys. Puncture material from petechiae may be taken for smear and culture.

B. Smears

Gram-stained smears of the sediment of centrifuged spinal fluid or of petechial aspirate often show typical neisseriae within polymorphonuclear leukocytes or extracellularly.

C. Culture

Culture media without sodium polyanethol sulfonate are helpful in culturing blood specimens. Cerebrospinal fluid specimens are plated on chocolate agar and incubated at 37°C in an atmosphere of 5% CO2. A MTM with antibiotics (vancomycin, colistin, amphotericin) favors the growth of neisseriae, inhibits many other bacteria, and is used for nasopharyngeal cultures. Presumptive colonies of neisseriae on solid media, particularly in mixed culture, can be identified by Gram stain and the oxidase test. Spinal fluid and blood generally yield pure cultures that can be further identified by carbohydrate oxidative reactions and agglutination with type-specific or polyvalent serum.

D. Serology

Antibodies to meningococcal polysaccharides can be measured by latex agglutination or hemagglutination tests or by their bactericidal activity. These tests are done only in reference laboratories.

Immunity

Immunity to meningococcal infection is associated with the presence of specific, complement-dependent, bactericidal antibodies in the serum. These antibodies develop after subclinical infections with different strains or injection of antigens and are group specific, type specific, or both. The immunizing antigens for groups A, C, Y, and W-135 are the capsular polysaccharides. For group B, a specific antigen suitable for use as a vaccine has not been defined; however, group B vaccines with mixtures of antigens have been used in many parts of the world. Recently, one such vaccine 4CMenB (BexseroR) was licensed in the European Union. Currently, there are three vaccines against serogroups A, C, Y, and W-135 and one that contains only C and Y available in the United States. A polysaccharide tetravalent vaccine (MenomuneR, Sanofi Pasteur) in which each dose consists of four purified bacterial capsular polysaccharides is poorly immunogenic in children younger than age 18 months, does not confer longlasting immunity, and does not cause a sustainable reduction in nasopharyngeal carriage. This is approved as a single dose for individuals ≥ 2 years. A tetravalent conjugate vaccine approved in 2005 (Menactra™, Sanofi Pasteur) is

licensed for use in persons 9 months to 55 years of age. It contains capsular polysaccharide conjugated to diphtheria toxoid. In children aged 9–23 months, two doses are required. Menveo (Novartis) is another tetravalent conjugate vaccine in which A, C, Y, W135 oligosaccharide is conjugated to diphtheria CRM197. This vaccine is approved for use in individuals 2–55 years of age. The Hib-MenCy-TT conjugate vaccine (GlaxoSmith- Kline) is a four-dose series vaccine approved for children 6 weeks to 18 months old. A quadrivalent meningococcal vaccine in which tetanus toxoid is the conjugate protein (MenACWY-tt; NimenrixR) is available in Europe. The advantage of the conjugate vaccines is that a T cell–dependent response to vaccine is induced. This enhances primary response among infants and substantially reduces asymptomatic carriage.
Routine vaccination of all young adolescents (ages 11–12 years) before high school with a booster dose at age 16 years using an approved conjugate vaccine is now recommended. Vaccination is also recommended for persons 2 months of age or older who are among the following at-risk groups: persons with functional or surgical asplenia, and persons with complement deficiencies. Persons aged 9 months or older who are travelers to or residents of highly endemic areas (eg, sub-Saharan Africa), "closed populations" such as college freshman living in dorms and the military, populations experiencing a community outbreak, and clinical laboratory workers (microbiologists) are other at-risk groups who should routinely be vaccinated.

Treatment

Penicillin G is the drug of choice for treating patients with meningococcal disease. Either chloramphenicol or a thirdgeneration cephalosporin such as cefotaxime or ceftriaxone is used in persons who are allergic to penicillins.

Epidemiology, Prevention, and Control

Meningococcal meningitis occurs in epidemic waves (eg, in military encampments, in religious pilgrims, and in sub-Saharan Africa, the so-called "meningitis belt"); and a smaller number of sporadic interepidemic cases. Serogroup A is responsible for the majority of outbreaks in sub-Saharan Africa, whereas serogroup B is most often the cause of sporadic infections. About 5–30% of the normal population may harbor meningococci (often nontypeable isolates) in the nasopharynx during interepidemic periods. During epidemics, the carrier rate goes up to 70–80%. A rise in the number of cases is preceded by an increased number of respiratory carriers.

Treatment with oral penicillin does not eradicate the carrier state. Rifampin, 600 mg orally for adults, 5 mg/ kg for children < 1 month, or 10 mg/kg for children 1 month or older twice daily for 2 days, is recommended as chemoprophylaxis for household and other close contacts following exposure to an index case. Ciprofloxacin in adults, 500 mg as a single dose, and ceftriaxone in children < 15 years, 125 mg IM as a single dose, are alternative agents. Clinical cases of meningitis present only a negligible source of infection, and isolation therefore has only limited usefulness. More important is the reduction of personal contacts in a population with a high carrier rate. This is accomplished by avoidance of crowding or administration of vaccines as discussed.

E COLI

1. **Urinary tract infection**

E coli is the most common cause of urinary tract infection and accounts for approximately 90% of first urinary tract infections in young women. The symptoms and signs include urinary frequency, dysuria, hematuria, and pyuria. Flank pain is associated with upper tract infection. None of these symptoms or signs is specific for E coli infection. Urinary tract infection can result in bacteremia with clinical signs of sepsis. Most of the urinary tract infections that involve the bladder or kidney in an otherwise healthy host are caused by a small number of O antigen types that have specifically elaborated virulence factors that facilitate colonization and subsequent clinical infections. These organisms are designated as uropathogenic E coli. Typically, these organisms produce hemolysin, which is cytotoxic and facilitates tissue invasion. Strains that cause pyelonephritis express K antigen and elaborate a specific type of pilus, P fimbriae, which binds to the P blood group antigen.

Over the last decade, a pandemic clone, E coli O25b/ ST131, has emerged as a significant pathogen. This organism has been successful largely as a result of its acquisition of plasmid-mediated resistance factors that encode resistance to β-lactam antibiotics (elaboration of extended spectrum β-lactamases), fluoroquinolones, and aminoglycosides.

2. **E coli**–associated diarrheal diseases—E coli that cause diarrhea are extremely common worldwide. These E coli are classified by the characteristics of their virulence properties , and each group causes disease by a different mechanism—at least six of which have been characterized. The small or large bowel epithelial cell adherence properties are encoded by genes on plasmids. Similarly, the toxins often are plasmid or phage mediated.

Enteropathogenic E coli (EPEC) are an important cause of diarrhea in infants, especially in developing countries. EPEC adhere to the mucosal cells of the small bowel. Pathogenicity requires two important factors, the bundle forming pilus encoded by a plasmid EPEC adherence factor (EAF) and the chromosomal locus of enterocyte effacement (LEE) pathogenicity island that promote the tight adherence characteristic of EPEC (attachment and effacement). After attachment, there is loss of microvilli (effacement); formation of filamentous actin pedestals or cuplike structures; and, occasionally, entry of the EPEC into the mucosal cells. Characteristic lesions can be seen on electron micrographs of small bowel biopsy lesions. The result of EPEC infection in infants is characterized by severe, watery diarrhea, vomiting, and fever, which are usually self-limited but can be prolonged or chronic. EPEC diarrhea has been associated with multiple specific serotypes of E coli; strains are identified by O antigen and occasionally by H antigen typing. A two-stage infection model using HEp-2 or HeLa cells also can be performed.

Tests to identify EPEC are performed in reference laboratories. The duration of the EPEC diarrhea can be shortened and the chronic diarrhea cured by antibiotic treatment.

Enterotoxigenic E coli (ETEC) are a common cause of "traveler's diarrhea" and a very important cause of diarrhea in children less than 5 years of age in developing countries. ETEC colonization factors (pili known as colonization factor antigens [CFAs]) specific for humans promote adherence of ETEC to epithelial cells of the small bowel. Some strains of ETEC produce a heat-labile enterotoxin (LT) (molecular weight [MW], 80,000) that is under the genetic control of a plasmid and is closely related to cholera toxin. Its subunit B attaches to the GM1 ganglioside in the apical membrane of enterocytes and facilitates the entry of subunit A (MW, 26,000) into the cell, where the latter activates adenylyl cyclase. This markedly increases the local concentration of cyclic adenosine monophosphate (cAMP) after which ensues a complex cascade that involves the cystic fibrosis transmembrane conductance regulator. The end result is an intense and prolonged hypersecretion of water and chlorides and inhibition of the reabsorption of sodium. The gut lumen is distended with fluid, and hypermotility and diarrhea ensue, lasting for several days. LT is antigenic and cross-reacts with the enterotoxin of Vibrio cholerae, which has an identical mechanism of action. LT stimulates the production of neutralizing antibodies in the serum (and perhaps on the gut surface) of persons previously infected with enterotoxigenic E coli. Persons residing in areas where such organisms are highly prevalent (eg, in some developing countries) are likely to possess antibodies and are less prone to develop diarrhea on reexposure to the LT-producing E coli.
Assays for LT include
(1) fluid accumulation in the intestines of laboratory animals,
(2) typical cytologic changes in cultured Chinese hamster ovary cells or other cell lines,
(3) stimulation of steroid production in cultured adrenal tumor cells,
(4) binding and immunologic assays with standardized antisera to LT, and
(5) detection of the genes that encode the toxins. These assays are done only in reference laboratories.

Some strains of ETEC produce the heat-stable enterotoxin STa (MW, 1500–4000), which is under the genetic control of a heterogeneous group of plasmids. STa activates guanylyl cyclase in enteric epithelial cells and stimulates fluid secretion. Many STa-positive strains also produce LT. The strains with both toxins produce a more severe diarrhea. The plasmids carrying the genes for enterotoxins (LT, ST) also may carry genes for the CFAs that facilitate the attachment of E coli strains to intestinal epithelium. Recognized colonization factors occur with particular frequency in some serotypes. Certain serotypes of ETEC occur worldwide; others have a limited recognized distribution. It is possible that virtually any E coli may acquire a plasmid encoding for enterotoxins.

There is no definite association of ETEC with the EPEC strains causing diarrhea in children. Likewise, there is no association between enterotoxigenic strains and those able to invade intestinal epithelial cells. Care in the selection and consumption of foods potentially contaminated with ETEC is highly recommended to help prevent traveler's diarrhea. Antimicrobial prophylaxis can be effective but may result in increased antibiotic resistance in the bacteria and probably should not be uniformly recommended. When diarrhea develops, antibiotic treatment effectively shortens the duration of disease.

Shiga toxin-producing E coli (STEC) are named for the cytotoxic toxins they produce. There are at least two antigenic forms of the toxin referred to as Shiga-like toxin 1 and Shigalike toxin 2. STEC has been associated with mild non-bloody diarrhea, hemorrhagic colitis, a severe form of diarrhea, and with hemolytic uremic syndrome, a disease resulting in acute renal failure, microangiopathic hemolytic anemia, and thrombocytopenia. Shiga-like toxin 1 is identical to the Shiga toxin of Shigella dysenteriae type 1, and Shiga-like toxin 2 also has many properties that are similar to the Shiga toxin; however, the two toxins are antigenically and genetically distinct. A low infectious dose (< 200 CFU) is associated with infection. Of the more than 150 E coli serotypes that produce Shiga toxin, O157:H7 is the most common and is the one that can be identified most readily in clinical specimens. STEC O157:H7 does not use sorbitol, unlike most other E coli, and is negative (clear colonies) on sorbitol MacConkey agar (sorbitol is used instead of lactose); O157:H7 strains also are negative on MUG tests. Many of the non-O157 serotypes may be sorbitol positive when grown in culture. Specific antisera are used to identify the O157:H7 strains. Tests for the detection of both Shiga toxins using commercially available enzyme immunoassays (EIAs) are done in many laboratories. Other sensitive test methods include cell culture cytotoxin testing using Vero cells and polymerase chain reaction for the direct detection of toxin genes directly from stool samples. Many cases of hemorrhagic colitis and its associated complications can be prevented by thoroughly cooking ground beef and by avoiding unpasteurized products such as apple cider. In 2011, the largest outbreak of hemorrhagic colitis attributed to a non-O157 serotype—namely, E coli O104:H4—was related to consumption of contaminated sprouts in Germany. This organism had increased virulence characterized by enhanced adherence as well as the production of shiga-like toxins.

Enteroinvasive E coli (EIEC) produce a disease very similar to shigellosis. The disease occurs most commonly in children in developing countries and in travelers to these countries. Similar to Shigella, EIEC strains are nonlactose or late lactose fermenters and are nonmotile. EIEC produce disease by invading intestinal mucosal epithelial cells. Enteroaggregative E coli (EAEC) causes acute and chronic diarrhea (>14 days in duration) in persons in developing countries. These

organisms also are the cause of foodborne illnesses in industrialized countries and have been associated with traveler's diarrhea and persistent diarrhea in patients with HIV. They are characterized by their specific patterns of adherence to human cells. This group of diarrheagenic E coli is quite heterogeneous, and the exact pathogenic mechanisms are still not completely elucidated. Some strains of EAEC produce STlike toxin; others a plasmid-encoded enterotoxin that produces cellular damage; and still others, a hemolysin. Diagnosis can be suspected clinically but requires confirmation by tissue culture adhesion assays not readily available in most clinical laboratories.

3. **Sepsis**—When normal host defenses are inadequate,
E coli may reach the bloodstream and cause sepsis. Newborns may be highly susceptible to E coli sepsis because they lack IgM antibodies. Sepsis may occur secondary to urinary tract infection and often the major clone associated with invasion is E coli O25b/ST131.

4. **Meningitis**—E coli and group B streptococci are the leading causes of meningitis in infants. Approximately 80% of E coli from meningitis cases have the K1 antigen. This antigen cross-reacts with the group B capsular polysaccharide of N meningitidis. The mechanisms of virulence associated with the K1 antigen are reviewed in the reference by Kim et al (2005).

KLEBSIELLA

The pathogenesis of disease caused by these groups of enteric gram-negative rods is similar to that of the nonspecific factors in disease caused by E coli.

1. **Klebsiella**—Klebsiella pneumoniae is present in the respiratory tract and feces of about 5% of normal individuals. It causes a small proportion (~1%) of bacterial pneumonias. K pneumoniae can produce extensive hemorrhagic necrotizing consolidation of the lung. It produces urinary tract infection and bacteremia with focal lesions in debilitated patients. Other enterics also may produce pneumonia. Recently a particular clone of K pneumoniae has emerged as a cause of community acquired pyogenic liver abscess that is seen mostly among Asian males worldwide. This particular K1 encapsulated strain phenotypically appears hypermucoviscous when grown in culture. Klebsiella species rank among the top 10 bacterial pathogens responsible for hospital-acquired infections. Multilocus sequencing typing has identified global emergence of two particularly important clones. Sequence type 16 has elaborated extended spectrum β-lactamases resulting in resistance to a broad range of penicillins and cephalosporins (but not carbapenem antibiotics). ST 258 is a multidrug resistant strain called a “carbapenamase producer” because it is resistant to all β-lactam antibiotics including the broad spectrum carbapenem agents. Typically it is resistant to other antimicrobial agents as a result of acquisition of plasmids that carry multiple resistance genes. Two other Klebsielleae are associated with inflammatory conditions of the upper respiratory tract: K pneumoniae subspecies ozaenae has been isolated from the nasal mucosa in ozena, a fetid, progressive atrophy of mucous membranes; and K pneumoniae subspecies rhinoscleromatis form rhinoscleroma, a destructive granuloma of the nose and pharynx. Klebsiella granulomatis (formerly Calymmatobacterium granulomatis) causes a chronic genital ulcerative disease, granuloma inguinale, an uncommon sexually transmitted disease. The organism grows with difficulty on media containing egg yolk. Ampicillin or tetracycline is effective treatment.

PROTEUS

Proteus species produce infections in humans only when the bacteria leave the intestinal tract. They are found in urinary tract infections and produce bacteremia, pneumonia, and focal lesions in debilitated patients or those receiving contaminated intravenous infusions. P mirabilis causes urinary tract infections and occasionally other infections. Proteus vulgaris and M morganii are also important nosocomial pathogens.

Proteus species produce urease, resulting in rapid hydrolysis of urea with liberation of ammonia. Thus, in urinary tract infections with Proteus species, the urine becomes alkaline, promoting stone formation and making acidification virtually impossible. The rapid motility of Proteus may contribute to its invasion of the urinary tract. Strains of Proteus vary greatly in antibiotic susceptibility. P mirabilis is often inhibited by penicillins; the most active antibiotics for other members of the group are aminoglycosides and cephalosporins.

Diagnostic Laboratory Tests

A. Specimens

Specimens include urine, blood, pus, spinal fluid, sputum, or other material, as indicated by the localization of the disease process.

B. Smears

The Enterobacteriaceae resemble each other morphologically. The presence of large capsules is suggestive of Klebsiella species.

C. Culture

Specimens are plated on both blood agar and differential media. With differential media, rapid preliminary identification of gram-negative enteric bacteria is often possible.

D. Nucleic Acid Amplification Tests (NAATs)

A variety of multiplex NAATs designed to detect the most common pathogens responsible for particular syndromes, are currently available and many more are entering clinical trials. These panel tests detect members of the Enterobacteriaceae in specimens such as positive blood cultures, cerebrospinal fluid, respiratory specimens, and stool. In some of these assays resistance markers are also detected. The reader should consult the literature for the most up to date information on these assays.

Immunity

Specific antibodies develop in systemic infections, but it is uncertain whether significant immunity to the organisms follows.

Treatment

No single specific therapy is available. The sulfonamides, ampicillin, cephalosporins, fluoroquinolones, and aminoglycosides have marked antibacterial effects against the enterics, but variation in susceptibility is great, and laboratory tests for antibiotic susceptibility are essential. Multiple drug resistance is common and is under the control of transmissible plasmids. Certain

conditions predisposing to infection by these organisms require surgical correction, such as relief of urinary tract obstruction, closure of a perforation in an abdominal organ, or resection of a bronchiectatic portion of lung. Treatment of gram-negative bacteremia and impending septic shock requires rapid institution of antimicrobial therapy, restoration of fluid and electrolyte balance, and treatment of disseminated intravascular coagulation.

Various means have been proposed for the prevention of traveler's diarrhea, including daily ingestion of bismuth subsalicylate suspension (bismuth subsalicylate can inactivate E coli enterotoxin in vitro) and regular doses of tetracyclines or other antimicrobial drugs for limited periods. Because none of these methods are entirely successful or lacking in adverse effects, it is widely recommended that caution be observed in regard to food and drink in areas where environmental sanitation is poor and that early and brief treatment (eg, with ciprofloxacin or trimethoprim–sulfamethoxazole) be substituted for prophylaxis.

Epidemiology, Prevention, and Control

The enteric bacteria establish themselves in the normal intestinal tract within a few days after birth and from then on constitute a main portion of the normal aerobic (facultative anaerobic) microbial flora. E coli is the prototype. Enterics found in water or milk are accepted as proof of fecal contamination from sewage or other sources. Control measures are not feasible as far as the normal endogenous microbiota is concerned. Enteropathogenic E coli serotypes should be controlled like salmonellae. Some of the enterics constitute a major problem in hospital infection. It is particularly important to recognize that many enteric bacteria are "opportunists" that cause illness when they are introduced into debilitated patients. Within hospitals or other institutions, these bacteria commonly are transmitted by personnel, instruments, or parenteral medications.

Their control depends on handwashing, rigorous asepsis, sterilization of equipment, disinfection, restraint in intravenous therapy, and strict precautions in keeping the urinary tract sterile (ie, closed drainage). For control of the multidrug-resistant pathogens, especially carbapenamase producers, surveillance of hospitalized patients with prompt implementation of contact precautions for colonized patients is often employed.

2.3 THE SHIGELLAE

The natural habitat of shigellae is limited to the intestinal tracts of humans and other primates, where they produce bacillary dysentery.

2.3.1 Morphology and Identification

A. Typical Organisms

Shigellae are slender gram-negative rods; coccobacillary forms occur in young cultures.

B. Culture

Shigellae are facultative anaerobes but grow best aerobically. Convex, circular, transparent colonies with intact edges reach a diameter of about 2 mm in 24 hours.

C. Growth Characteristics

All shigellae ferment glucose. With the exception of Shigella sonnei, they do not ferment lactose. The inability to ferment lactose distinguishes shigellae on differential media. Shigellae form acid from carbohydrates but rarely produce gas. They may also be divided into those organisms that ferment mannitol and those that do not.

2.3.2 Antigenic Structure

Shigellae have a complex antigenic pattern. There is great overlapping in the serologic behavior of different species, and most of them share O antigens with other enteric bacilli. The somatic O antigens of shigellae are lipopolysaccharides. Their serologic specificity depends on the polysaccharide. There are more than 40 serotypes. The classification of shigellae relies on biochemical and antigenic characteristics. The pathogenic species are *S sonnei, Shigella flexneri, S dysenteriae,* and *Shigella boydii*.

2.3.3 Pathogenesis and Pathology

Shigella infections are almost always limited to the gastrointestinal tract; bloodstream invasion is quite rare. Shigellae are highly communicable; the infective dose is on the order of 103 organisms (it usually is 105–108 for salmonellae and vibrios). The essential pathologic process is invasion of the mucosal epithelial cells (eg, M cells) by induced phagocytosis, escape from the phagocytic vacuole, multiplication and spread within the epithelial cell cytoplasm, and passage to adjacent cells. Microabscesses in the wall of the large intestine and terminal ileum lead to necrosis of the mucous membrane, superficial ulceration, bleeding, and formation of a “pseudomembrane” on the ulcerated area. This consists of fibrin, leukocytes, cell debris, a necrotic mucous membrane, and bacteria. As the process subsides, granulation tissue fills the ulcers, and scar tissue forms.

2.3.4 Toxins

A. Endotoxin

Upon autolysis, all shigellae release their toxic lipopolysaccharide. This endotoxin probably contributes to the irritation of the bowel wall.

B. Shigella Dysenteriae Exotoxin

S dysenteriae type 1 (Shiga bacillus) produces a heat-labile exotoxin that affects both the gut and the central nervous system. The exotoxin is a protein that is antigenic (stimulating production of antitoxin) and lethal for experimental animals. Acting as an enterotoxin, it produces diarrhea as does the E coli Shiga-like toxin, perhaps by the same mechanism. In humans, the exotoxin also inhibits sugar and amino acid absorption in the small intestine. Acting as a "neurotoxin," this material may contribute to the extreme severity and fatal nature of S dysenteriae infections and to the central nervous system reactions observed in them (ie, meningismus, coma). Patients with S flexneri or S sonnei infections develop antitoxin that neutralizes S dysenteriae exotoxin in vitro. The toxic activity is distinct from the invasive property of shigellae in dysentery. The two may act in sequence, the toxin producing an early nonbloody, voluminous diarrhea and the invasion of the large intestine, resulting in later dysentery with blood and pus in stools.

2.3.5 Clinical Findings

After a short incubation period (1–2 days), there is a sudden onset of abdominal pain, fever, and watery diarrhea. A day or so later, as the infection involves the ileum and colon, the number of stools increases; they are less liquid but often contain mucus and blood. Each bowel movement is accompanied by straining and tenesmus (rectal spasms), with resulting lower abdominal pain. In more than half of adult cases, fever and diarrhea subside spontaneously in 2–5 days. However, in children and elderly adults, loss of water and electrolytes may lead to dehydration, acidosis, and even death. The illness caused by S dysenteriae may be particularly severe.

On recovery, most persons shed dysentery bacilli for only a short period. Upon recovery from the infection, most persons develop circulating antibodies to shigellae, but these do not protect against reinfection.

2.3.6 Diagnostic Laboratory Tests

A. Specimens

Specimens include fresh stool, mucus flecks, and rectal swabs for culture. Large numbers of fecal leukocytes and some red blood cells often are seen microscopically.

B. Culture

The materials are streaked on differential media (eg, MacConkey or EMB agar) and on selective media (Hektoen enteric agar or xylose-lysine-deoxycholate agar), which suppress other

Enterobacteriaceae and gram-positive organisms. Colorless (lactose-negative) colonies are inoculated into TSI agar. Organisms that fail to produce H2S, that produce acid but not gas in the butt and an alkaline slant in TSI agar medium, and that are nonmotile, should be subjected to slide agglutination by specific Shigella antisera. Shigella and E coli cannot be differentiated by MALDI-ToF MS.

C. Serology

Healthy persons often have agglutinins against several Shigella species. However, serial determinations of antibody titers may show a rise in specific antibody. Serology is not used to diagnose Shigella infections. D. Nucleic Acid Amplification Tests There are several commercial NAATs that directly detect shigellae in fecal samples along with some of the other major enteric pathogens.

Immunity Infection is followed by a type-specific antibody response. Injection of killed shigellae stimulates production of antibodies in serum but fails to protect humans against infection. IgA antibodies in the gut may be important in limiting reinfection; these may be stimulated by live attenuated strains given orally as experimental vaccines. Serum antibodies to somatic Shigella antigens are IgM.

Treatment

Ciprofloxacin, ampicillin, doxycycline, and trimethoprim– sulfamethoxazole are most commonly inhibitory for Shigella isolates and can suppress acute clinical attacks of dysentery and shorten the duration of symptoms. Azithromycin is often used to treat children with shigellosis. Some antibiotics may fail to eradicate the organisms from the intestinal tract. Multiple drug resistance can be transmitted by plasmids, and resistant infections are widespread. Many cases are selflimited. Opioids should be avoided in Shigella dysentery.

2.3.7 Epidemiology, Prevention, and Control

Shigellae are transmitted by “food, fingers, feces, and flies” from person to person. A low infectious dose (1–100 organisms) is capable of causing disease. Most cases of Shigella infection occur in children younger than 10 years of age. Shigellosis, caused primarily by S sonnei, has become an important problem in daycare centers in the United States. S dysenteriae can spread widely. Because humans are the main recognized host of pathogenic shigellae, control efforts must be directed at eliminating the organisms from this reservoir by (1) sanitary control of water, food, and milk; sewage disposal and fly control; (2) isolation of patients and disinfection of excreta; (3) detection of subclinical cases and carriers, particularly food handlers; and (4) antibiotic treatment of infected individuals.

2.4 THE SALMONELLAE

Salmonellae are often pathogenic for humans or animals when acquired by the oral route. They are transmitted from animals and animal products to humans, where they cause enteritis, systemic infection, and enteric fever.

Morphology and Identification

Salmonellae vary in length. Most isolates are motile with peritrichous flagella. Salmonellae grow readily on simple media, but they almost never ferment lactose or sucrose. They form acid and sometimes gas from glucose and mannose. They usually produce H2S. They survive freezing in water for long periods. Salmonellae are resistant to certain chemicals (eg, brilliant green, sodium tetrathionate, sodium deoxycholate) that inhibit other enteric bacteria; such compounds are therefore useful for inclusion in media to isolate salmonellae from feces.

Classification

The classification of salmonellae is complex because the organisms are a continuum rather than a defined species. The members of the genus Salmonella were originally classified on the basis of epidemiology; host range; biochemical reactions; and structures of the O, H, and Vi (when present) antigens. The names (eg, S typhi, Salmonella typhimurium) were written as if they were genus and species; this form of the nomenclature remains in widespread but incorrect use. DNA–DNA hybridization studies have demonstrated that there are seven evolutionary groups. Currently, the genus Salmonella is divided into two species each with multiple subspecies and serotypes. The two species are Salmonella enterica and Salmonella bongori (formerly subspecies V). S enterica contains five subspecies, which are subspecies enterica (subspecies I), subspecies salamae (subspecies II), subspecies arizonae (subspecies IIIa), subspecies diarizonae (subspecies IIIb), subspecies houtenae (subspecies IV), and subspecies indica (subspecies VI).

Most human illness is caused by the subspecies I strains, written as S enterica subspecies enterica. Rarely human infections may be caused by subspecies IIIa and IIIb or the other subspecies frequently found in cold-blooded animals. Frequently, these infections are associated with exotic pets such as reptiles. It seems probable that the widely accepted nomenclature for classification will be as follows: S enterica subspecies enterica serotype Typhimurium, which can be shortened to S Typhimurium with the genus name in italics and the serotype name in roman type. National and international reference laboratories may use the antigenic formulas following the subspecies name because they impart more precise information about the isolates. There are more than 2500 serotypes of salmonellae, including more than 1400 in DNA hybridization group I that can infect humans. Four serotypes of salmonellae that cause enteric fever can be identified in the clinical

laboratory by biochemical and serologic tests. These serotypes should be routinely identified because of their clinical significance. They are as follows: Salmonella paratyphi A (serogroup A), S paratyphi B (serogroup B), Salmonella choleraesuis (serogroup C1), and S typhi (serogroup D). Salmonella serotypes Enteritidis and Typhimurium are the two most common serotypes reported in the United States. The more than 1400 other salmonellae that are isolated in clinical laboratories are serogrouped by their O antigens as A, B, C1, C2, D, and E; some are nontypeable with this set of antisera. The isolates are then sent to reference laboratories for definitive serologic identification. This allows public health officials to monitor and assess the epidemiology of Salmonella infections on a statewide and nationwide basis.

Variation

Organisms may lose H antigens and become nonmotile. Loss of O antigen is associated with a change from smooth to rough colony form. Vi antigen may be lost partially or completely. Antigens may be acquired (or lost) in the process of transduction.

Pathogenesis and Clinical Findings

S serotype Typhi, S serotype, and perhaps S serotype Paratyphi and S serotype Paratyphi B are primarily infective for humans, and infection with these organisms implies acquisition from a human source. The vast majority of salmonellae, however, are chiefly pathogenic in animals that constitute the reservoir for human infection; these include poultry, pigs, rodents, cattle, pets (from turtles to parrots), and many others. The organisms almost always enter via the oral route, usually with contaminated food or drink. The mean infective dose to produce clinical or subclinical infection in humans is 105–108 salmonellae (but perhaps as few as 103 S serotype Typhi organisms). Among the host factors that contribute to resistance to salmonella infection are gastric acidity, normal intestinal microbiota, and local intestinal immunity. Salmonellae produce three main types of disease in humans, but mixed forms are frequent.

A. The “Enteric Fevers” (Typhoid Fever)

This syndrome is produced by only a few of the salmonellae, of which S serotype Typhi (typhoid fever) is the most important. The ingested salmonellae reach the small intestine, from which they enter the lymphatics and then the bloodstream. They are carried by the blood to many organs, including the intestine. The organisms multiply in intestinal lymphoid tissue and are excreted in stools.

After an incubation period of 10–14 days, fever, malaise, headache, constipation, bradycardia, and myalgia occur. The fever rises to a high plateau, and the spleen and liver become enlarged. Rose spots, usually on the skin of the abdomen or chest, are seen briefly in rare cases. The white blood

cell count is normal or low. In the preantibiotic era, the chief complications of enteric fever were intestinal hemorrhage and perforation, and the mortality rate was 10–15%. Treatment with antibiotics has reduced the mortality rate to less than 1%. The principal lesions are hyperplasia and necrosis of lymphoid tissue (eg, Peyer's patches); hepatitis; focal necrosis of the liver; and inflammation of the gallbladder, periosteum, lungs, and other organs.

B. Bacteremia With Focal Lesions

This is associated commonly with S serotype choleraesuis but may be caused by any salmonella serotype. After oral infection, there is early invasion of the bloodstream (with possible focal lesions in lungs, bones, meninges, etc), but intestinal manifestations are often absent. Blood culture results are positive.

C. Enterocolitis

This is the most common manifestation of salmonella infection. In the United States, S Typhimurium and Salmonella Enteritidis are prominent, but enterocolitis can be caused by any of the more than 1400 group I serotypes of salmonellae. Eight to 48 hours after ingestion of salmonellae, there is nausea, headache, vomiting, and profuse diarrhea, with few leukocytes in the stools. Low-grade fever is common, but the episode usually resolves in 2–3 days. Inflammatory lesions of the small and large intestine are present. Bacteremia is rare (2–4%) except in immunodeficient persons. Blood culture results are usually negative, but stool culture results are positive for salmonellae and may remain positive for several weeks after clinical recovery.

Diagnostic Laboratory Tests

A. Specimens

Blood for culture must be taken repeatedly. In enteric fevers and septicemias, blood culture results are often positive in the first week of the disease. Bone marrow cultures may be useful. Urine culture results may be positive after the second week. Stool specimens also must be taken repeatedly. In enteric fevers, the stools yield positive results from the second or third week on; in enterocolitis, the stools yield positive results during the first week. A positive culture of duodenal drainage establishes the presence of salmonellae in the biliary tract in carriers. B. Bacteriologic Methods for Isolation of Salmonellae 1. Differential medium cultures—EMB, MacConkey, or desoxycholate medium permits rapid detection of lactose nonfermenters (not only salmonellae and shigellae but also Proteus, Serratia, Pseudomonas, etc). Gram-positive organisms are somewhat inhibited. Bismuth sulfite medium permits rapid detection of salmonellae, which form black colonies because of H2S production. Many salmonellae produce H2S. 2. Selective medium cultures—The specimen is plated on salmonella-shigella (SS) agar, Hektoen enteric agar, xyloselysine desoxycholate (XLD) agar, or desoxycholate-citrate agar, which favor growth of

salmonellae and shigellae over other Enterobacteriaceae. Chromogenic agars specifically for salmonella recovery are also available. 3. Enrichment cultures—The specimen (usually stool) also is put into selenite F or tetrathionate broth, both of which inhibit replication of normal intestinal bacteria and permit multiplication of salmonellae. After incubation for 1–2 days, this is plated on differential and selective media. 4. Final identification—Suspect colonies from solid media are identified by biochemical reaction patterns and slide agglutination tests with specific sera. C. Serologic Methods Serologic techniques are used to identify unknown cultures with known sera and may also be used to determine antibody titers in patients with unknown illness, although the latter is not very useful in the diagnosis of Salmonella infections.

1. **Agglutination test**

In this test, known sera and unknown culture are mixed on a slide. Clumping, when it occurs, can be observed within a few minutes. This test is particularly useful for rapid preliminary identification of cultures. There are commercial kits available to agglutinate and serogroup salmonellae by their O antigens: A, B, C1, C2, D, and E.

2. Tube dilution agglutination test (Widal test)—

Serum agglutinins rise sharply during the second and third weeks of S serotype Typhi infection. The Widal test to detect these antibodies against the O and H antigens has been in use for decades. At least two serum specimens, obtained at intervals of 7–10 days, are needed to prove a rise in antibody titer. Serial dilutions of unknown sera are tested against antigens from representative salmonellae. False-positive and falsenegative results occur. The interpretive criteria when single serum specimens are tested vary, but a titer against the O antigen of greater than 1:320 and against the H antigen of greater than 1:640 is considered positive. High titer of antibody to the Vi antigen occurs in some carriers. Alternatives to the Widal test include rapid colorimetric and EIA methods. There are conflicting reports in the literature regarding superiority of these methods to the Widal test. Results of serologic tests for Salmonella infection cannot be relied upon to establish a definitive diagnosis of typhoid fever and are most often used in resource poor areas of the world where blood cultures are not readily available.

D. Nucleic Acid Amplification Tests As mentioned above for the shigellae, several commercial NAATs are available for direct detection of salmonellae in fecal samples of patients with acute diarrhea. Since these assays are new, performance characteristics of the assays and their impact on public health surveillance are still under investigation.

Immunity

Infections with S serotype Typhi or S paratyphi usually confer a certain degree of immunity. Reinfection may occur but is often milder than the first infection. Circulating antibodies to O and

Vi are related to resistance to infection and disease. However, relapses may occur in 2–3 weeks after recovery despite antibodies. Secretory IgA antibodies may prevent attachment of salmonellae to intestinal epithelium. Persons with S/S hemoglobin (sickle cell disease) are exceedingly susceptible to Salmonella infections, particularly osteomyelitis. Persons with A/S hemoglobin (sickle cell trait) may be more susceptible than normal individuals (those with A/A hemoglobin).

Treatment

Although enteric fevers and bacteremias with focal lesions require antimicrobial treatment, the vast majority of cases of enterocolitis do not. Antimicrobial treatment of Salmonella enteritis in neonates is important. In enterocolitis, clinical symptoms and excretion of the salmonellae may be prolonged by antimicrobial therapy. In severe diarrhea, replacement of fluids and electrolytes is essential. Antimicrobial therapy of invasive Salmonella infections is with ampicillin, trimethoprim–sulfamethoxazole, or a third-generation cephalosporin. Multiple drug resistance transmitted genetically by plasmids among enteric bacteria is a problem in Salmonella infections. Susceptibility testing is an important adjunct to selecting a proper antibiotic. In most carriers, the organisms persist in the gallbladder (particularly if gallstones are present) and in the biliary tract. Some chronic carriers have been cured by ampicillin alone, but in most cases cholecystectomy must be combined with drug treatment.

Epidemiology

The feces of persons who have unsuspected subclinical disease or are carriers are a more important source of contamination than frank clinical cases that are promptly isolated, such as when carriers working as food handlers are “shedding” organisms. Many animals, including cattle, rodents, and fowl, are naturally infected with a variety of salmonellae and have the bacteria in their tissues (meat), excreta, or eggs. The high incidence of salmonellae in commercially prepared chickens has been widely publicized. The incidence of typhoid fever has decreased, but the incidence of other Salmonella infections has increased markedly in the United States. The problem probably is aggravated by the widespread use of animal feeds containing antimicrobial drugs that favor the proliferation of drug-resistant salmonellae and their potential transmission to humans.

A. Carriers

After manifest or subclinical infection, some individuals continue to harbor salmonellae in their tissues for variable lengths of time (ie, convalescent carriers or healthy permanent carriers). Three percent of survivors of typhoid become permanent carriers, harboring the organisms in the gallbladder, biliary tract, or, rarely, the intestine or urinary tract.

B. Sources of Infection

The sources of infection are food and drink that have been contaminated with salmonellae. The following sources are important: 1. Water—Contamination with feces often results in explosive epidemics 2. Milk and other dairy products (ice cream, cheese, custard)—Contamination with feces and inadequate pasteurization or improper handling; some outbreaks are traceable to the source of supply 3. Shellfish—From contaminated water 4. Dried or frozen eggs—From infected fowl or contaminated during processing 5. Meats and meat products—From infected animals (poultry) or contamination with feces by rodents or humans 6. “Recreational” drugs—Marijuana and other drugs 7. Animal dyes—Dyes (eg, carmine) used in drugs, foods, and cosmetics 8. Household pets—Turtles, dogs, cats, exotic pets such as reptiles, and so on

Prevention and Control

Sanitary measures must be taken to prevent contamination of food and water by rodents or other animals that excrete salmonellae. Infected poultry, meats, and eggs must be thoroughly cooked. Carriers must not be allowed to work as food handlers and should observe strict hygienic precautions. Two typhoid vaccines are currently available in the United States: an oral live, attenuated vaccine (Ty 21a) and a Vi capsular polysaccharide vaccine (Vi CPS) for intramuscular use. Vaccination is recommended for travelers to endemic regions, especially if the traveler visits rural areas or small villages where food choices are limited. Both vaccines have an efficacy of 50–80%. The time required for primary vaccination and age limits for each vaccine varies, and individuals should consult the Centers for Disease Control and Prevention’s Web site or obtain advice from a travel clinic regarding the latest vaccine information.

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UNIT – III – Medical Bacteriology – SMB3101

3.MYCOBACTERIA

The mycobacteria are rod-shaped, aerobic bacteria that do not form spores. Although they do not stain readily, after being stained, they resist decolorization by acid or alcohol and are therefore called “acid-fast” bacilli. Mycobacterium tuberculosis causes tuberculosis and is a very important pathogen of humans. Mycobacterium leprae causes leprosy. Mycobacterium avium-intracellulare (M avium complex, or MAC) and other nontuberculous mycobacteria (NTM) frequently infect patients with AIDS, are opportunistic pathogens in other immunocompromised persons, and occasionally cause disease in patients with normal immune systems. There are more than 200 Mycobacterium species, including many that are saprophytes. The mycobacteria that infect humans are listed in Table 23-1.

MYCOBACTERIUM TUBERCULOSIS

Morphology and Identification

A. Typical Organisms

In tissue, tubercle bacilli are thin, straight rods measuring about 0.4 × 3 μm (Figure 23-1). On artificial media, coccoid and filamentous forms are seen with variable morphology from one species to another. Mycobacteria cannot be classified as either gram positive or gram negative. When stained by basic dyes, they cannot be decolorized by alcohol, regardless of treatment with iodine. True tubercle bacilli are characterized by “acid fastness”—that is, 95% ethyl alcohol containing 3% hydrochloric acid (acid-alcohol) quickly decolorizes all bacteria except the mycobacteria. Acid fastness depends on the integrity of the waxy envelope. The Ziehl-Neelsen technique of staining is used for identification of acid-fast bacteria. The method is detailed in Chapter 47. In smears of sputum or sections of tissue, mycobacteria can be demonstrated by yellow-orange fluorescence after staining with fluorochrome stains (eg, auramine, rhodamine). The ease with which acid-fast bacteria can be visualized with fluorochrome stains makes them the preferred stains for clinical specimens (Figure 23-1B). The availability of ultrabright light-emitting diode microscopes, some of which do not require electricity, has advanced fluorescence microscopy in resource-limited countries.

B. Culture

The media for primary culture of mycobacteria should include a nonselective medium and a selective medium. Selective media contain antibiotics to prevent the overgrowth of contaminating bacteria and fungi. There are three general formulations that can be used for both the nonselective and selective media. Agar-based (solid) media are useful for observing colony morphology, for detection of mixed cultures, for antimicrobial susceptibility testing, and can also provide some indication of the quantity of organisms in a particular specimen.

1. Semisynthetic agar media—These media (eg, Middlebrook 7H10 and 7H11) contain defined salts, vitamins, cofactors, oleic acid, albumin, catalase, and glycerol; the 7H11 medium also contains casein hydrolysate. The albumin neutralizes the toxic and inhibitory effects of fatty acids in the specimen or medium. Large inocula yield growth on these media in several weeks. Because large inocula may be necessary, these media may be less sensitive than other media for primary isolation of mycobacteria.
2. Inspissated egg media—These media (eg, Löwenstein- Jensen) contain defined salts, glycerol, and complex organic substances (eg, fresh eggs or egg yolks, potato flour, and other ingredients in various combinations). Malachite green is included to inhibit other bacteria. Small inocula in specimens from patients will grow on these media in 3–6 weeks. These media with added antibiotics (Gruft and Mycobactosel) are used as selective media.
3. Broth media—Broth media (eg, Middlebrook 7H9 and 7H12) support the proliferation of small inocula. Ordinarily, mycobacteria grow in clumps or masses because of the hydrophobic character of the cell surface. If tweens (water-soluble esters of fatty acids) are added, they wet the surface and thus permit dispersed growth in liquid media. Growth is often more rapid than on complex media. There are several commercial sources of these media that are used in many clinical and reference laboratories. These include the MGIT system (Becton Dickinson, Sparks, MD), VersaTREK® Culture System (ThermoFisher Scientific, Houston, TX), and MB Redox (Heipha Diagnostica Biotest, Eppelheim, Germany).

C. Growth Characteristics

Mycobacteria are obligate aerobes and derive energy from the oxidation of many simple carbon compounds. Increased CO2 tension enhances growth. Biochemical activities are not characteristic, and the growth rate is much slower than that of most bacteria. The doubling time of tubercle bacilli is about 18 hours. Saprophytic forms tend to grow more rapidly, to proliferate well at 22–33°C, to produce more pigment, and to be less acid fast than pathogenic forms.

D. Reaction to Physical and Chemical Agents

Mycobacteria tend to be more resistant to chemical agents than other bacteria because of the hydrophobic nature of the cell surface and their clumped growth. Dyes (eg, malachite green) or antibacterial agents (eg, penicillin) that are bacteriostatic to other bacteria can be incorporated into media without inhibiting the growth of tubercle bacilli. Acids and alkalies permit the survival of some exposed tubercle bacilli and are used to help eliminate contaminating organisms and for "concentration" of clinical specimens. Tubercle bacilli are resistant to drying and survive for long periods in dried sputum.

E. Variation

Variation can occur in colony appearance, pigmentation, virulence, optimal growth temperature, and many other cellular or growth characteristics. F. Pathogenicity of Mycobacteria There are marked differences in the ability of different mycobacteria to cause lesions in various host species. Humans and guinea pigs are highly susceptible to M tuberculosis infection, but fowl and cattle are resistant. M tuberculosis and Mycobacterium bovis are equally pathogenic for humans. The route of infection (respiratory vs intestinal) determines the pattern of lesions. In developed countries, M bovis has become very rare. Some "atypical" mycobacteria, now designated as NTM (eg, Mycobacterium kansasii), produce human disease indistinguishable from tuberculosis; others (eg, Mycobacterium fortuitum) cause only surface lesions or act as opportunists.

Constituents of Tubercle Bacilli

The constituents listed as follows are found mainly in cell walls. Mycobacterial cell walls can induce delayed hypersensitivity and some resistance to infection and can replace whole mycobacterial cells in Freund's adjuvant. Mycobacterial cell contents only elicit delayed hypersensitivity reactions in previously sensitized animals.

A. Lipids

Mycobacteria are rich in lipids. These include mycolic acids (long-chain fatty acids C78–C90), waxes, and phosphatides. In the cell, the lipids are largely bound to proteins and polysaccharides. Muramyl dipeptide (from peptidoglycan) complexed with mycolic acids can cause granuloma formation; phospholipids induce caseous necrosis. Lipids are to some extent responsible for acid fastness. Their removal with hot acid destroys acid fastness, which depends on both the integrity of the cell wall and the presence of certain lipids. Acid fastness is also lost after sonication of mycobacterial cells.

Analysis of lipids by gas chromatography reveals patterns that aid in classification of different species. Virulent strains of tubercle bacilli form microscopic "serpentine cords" in which acid-fast bacilli are arranged in parallel chains. Cord formation is correlated with virulence. A "cord factor" (trehalose-6,6′-dimycolate) has been extracted from virulent bacilli with petroleum ether. It inhibits migration of leukocytes, causes chronic granulomas, and can serve as an immunologic "adjuvant."

B. Proteins

Each type of MYCOBACTERIUM contains several proteins that elicit the tuberculin reaction. Proteins bound to a wax fraction can, upon injection, induce tuberculin sensitivity. They can also elicit the formation of a variety of antibodies.

C. Polysaccharides

Mycobacteria contain a variety of polysaccharides. Their role in the pathogenesis of disease is uncertain. They can induce the immediate type of hypersensitivity and can serve as antigens in reactions with sera of infected persons.

Pathogenesis

Mycobacteria are emitted in droplets smaller than 25 μm in diameter when infected persons cough, sneeze, or speak. The droplets evaporate, leaving organisms that are small enough, when inhaled, to be deposited in alveoli. Inside the alveoli, the host's immune system responds by release of cytokines and lymphokines that stimulate monocytes and macrophages. Mycobacteria begin to multiply within macrophages. Some of the macrophages develop an enhanced ability to kill the organism, but others may be killed by the bacilli. Pathogenic lesions associated with infection appear in the lung 1–2 months after exposure. Two types of lesions as described later under Pathology may develop. Resistance and hypersensitivity of the host greatly influence development of disease and the type of lesions that are seen.

Pathology

The production and development of lesions and their healing or progression are determined chiefly by (1) the number of mycobacteria in the inoculum and their subsequent multiplication and (2) the type of host and immune response.

A. Two Principal Lesions

1. Exudative type—This consists of an acute inflammatory reaction with edema fluid; polymorphonuclear leukocytes; and, later, monocytes around the tubercle bacilli. This type is seen particularly in lung tissue, where it resembles bacterial pneumonia. It may heal by resolution so that the entire exudate becomes absorbed; it may lead to massive necrosis of tissue or may develop into the second (productive) type of lesion. During the exudative phase, the tuberculin test result becomes positive.
2. Productive (proliferative) type—When fully developed, this lesion, a chronic granuloma, consists of three zones:
   (1) a central area of large, multinucleated giant cells containing tubercle bacilli;
   (2) a mid zone of pale epithelioid cells, often arranged radially; and
   (3) a peripheral zone of fibroblasts, lymphocytes, and monocytes. Later, peripheral fibrous tissue develops, and the central area undergoes caseation necrosis. Such a lesion is called a tubercle. A caseous tubercle may break into a bronchus, empty its contents there, and form a cavity. It may subsequently heal by fibrosis or calcification.

B. Spread of Organisms in the Host

Tubercle bacilli spread in the host by direct extension, through the lymphatic channels and bloodstream, and via the bronchi and gastrointestinal tract. In the first infection, tubercle bacilli always spread from the initial site via the lymphatics to the regional lymph nodes. The bacilli may spread farther and reach the bloodstream, which in turn distributes bacilli to all organs (miliary distribution). The bloodstream can be invaded also by erosion of a vein by a caseating tubercle or lymph node. If a caseating lesion discharges its contents into a bronchus, they are aspirated and distributed to other parts of the lungs or are swallowed and passed into the stomach and intestines. C. Intracellular Site of Growth When mycobacteria establish themselves in tissue, they reside principally intracellularly in monocytes, reticuloendothelial cells, and giant cells. The intracellular location is one of the features that makes chemotherapy difficult and favors microbial persistence. Within the cells of immune animals, multiplication of tubercle bacilli is greatly inhibited.

Primary Infection and Reactivation

Types of Tuberculosis

When a host has first contact with tubercle bacilli, the following features are usually observed: (1) An acute exudative lesion develops and rapidly spreads to the lymphatics and regional lymph nodes. The exudative lesion in tissue often heals rapidly. (2) The lymph node undergoes massive caseation, which usually calcifies (Ghon lesion). (3) The tuberculin test result becomes positive.
As described in the early 20th century, primary infection type occurred usually in childhood, and involved any part of the lung but most often the mid-lung fields or the base. Enlarged hilar and mediastinal lymph nodes are frequently observed.

The reactivation type is usually caused by tubercle bacilli that have survived in the primary lesion. Reactivation tuberculosis is characterized by chronic tissue lesions, the formation of tubercles, caseation, and fibrosis. Regional lymph nodes are only slightly involved, and they do not caseate. The reactivation type almost always begins at the apex of the lung, where the oxygen tension (PO2) is highest. These differences between primary infection and reinfection or reactivation are attributed to (1) resistance and (2) hypersensitivity induced by the first infection. It is not clear to what extent each of these components participates in the modified response in reactivation tuberculosis.

Immunity and Hypersensitivity During the first infection with tubercle bacilli, a certain resistance is acquired, and there is an increased capacity to localize tubercle bacilli, retard their multiplication, limit their spread, and reduce lymphatic dissemination. This can be attributed to the development of cellular immunity, with evident ability of mononuclear phagocytes to limit the multiplication of ingested organisms and even to destroy them. In the course of primary infection,

the host also acquires hypersensitivity to the tubercle bacilli. This is made evident by the development of a positive tuberculin reaction. Tuberculin sensitivity can be induced by whole tubercle bacilli or by tuberculoprotein in combination with the chloroform-soluble wax D of the tubercle bacillus but not by tuberculoprotein alone. Hypersensitivity and resistance appear to be distinct aspects of related cell-mediated reactions.

Tuberculin Test

A. Material

Old tuberculin is a concentrated filtrate of broth in which tubercle bacilli have grown for 6 weeks. In addition to the reactive tuberculoproteins, this material contains a variety of other constituents of tubercle bacilli and of growth medium. A purified protein derivative (PPD) is obtained by chemical fractionation of old tuberculin. PPD is standardized in terms of its biologic reactivity as tuberculin units (TU). By international agreement, the TU is defined as the activity contained in a specified weight of Siebert's PPD Lot No. 49608 in a specified buffer. This is PPD-S, the standard for tuberculin against which the potency of all products must be established by biologic assay (ie, by reaction size in humans). First-strength tuberculin has 1 TU, intermediate-strength has 5 TU, and second-strength has 250 TU. Bioequivalency of PPD products is not based on the weight of the material but on comparative activity.

B. Dose of Tuberculin

A large amount of tuberculin injected into a hypersensitive host may give rise to severe local reactions and a flare-up of inflammation and necrosis at the main sites of infection (focal reactions). For this reason, tuberculin tests in surveys use 5 TU in 0.1 mL solution; in persons suspected of extreme hypersensitivity, skin testing is begun with 1 TU. The volume is usually 0.1 mL injected intracutaneously, usually on the volar aspect of the forearm. The PPD preparation must be stabilized with polysorbate 80 to prevent adsorption to glass.

C. Reactions to Tuberculin

After the tuberculin skin test is placed, the area is examined for the presence of induration no later than 72 hours after placement. It is imperative that a person trained in the accurate reading of these tests examine the area in question. Erythema alone should not be interpreted as a reactive test result. The Centers for Disease Control and Prevention (CDC) has established three different cut points defining a positive test result, considering both the sensitivity and specificity of the test and the prevalence of tuberculosis in various populations. For patients at the highest risk of developing active disease (eg, HIV-infected persons, people who have had exposure to persons with active tuberculosis) 5 mm or larger of induration is considered positive; larger than 10 mm is considered positive for persons with increased probability of recent infection. This category might include

individuals such as recent immigrants from high-prevalence countries, injection drug users, and health care workers with exposure to tuberculosis. For persons at low risk for tuberculosis, 15 mm or larger of induration is considered a positive test result. In an individual who has not had contact with mycobacteria, there is generally no reaction to PPD-S. Positive test results tend to persist for several days. Weak reactions may disappear more rapidly. The tuberculin test result becomes positive within 4–6 weeks after infection (or injection of avirulent bacilli). It may be negative in the presence of tuberculous infection when "anergy" develops because of overwhelming tuberculosis, measles, Hodgkin disease, sarcoidosis, AIDS, or immunosuppression.

A positive tuberculin test result may occasionally revert to negative upon isoniazid (INH) treatment of a recent converter. After bacillus Calmette-Guérin (BCG) vaccination, people convert to a positive test result, but this may last for only 3–7 years. Only the elimination of viable tubercle bacilli results in reversion of the tuberculin test result to negative. However, persons who were PPD positive years ago and are healthy may fail to give a positive skin test result. When such persons are retested 2 weeks later, their PPD skin test result—"boosted" by the recent antigen injection—will give a positive size of induration again. A positive tuberculin test result indicates that an individual has been infected in the past. It does not imply that active disease or immunity to disease is present. Tuberculin-positive persons are at risk of developing disease from reactivation of the primary infection, but tuberculin-negative persons who have never been infected are not subject to that risk, although they may become infected from an external source.

D. Interferon-Gamma Release Assays for Detection of Tuberculosis

Sometimes the results of the tuberculin skin test are equivocal, particularly in persons who have been vaccinated with BCG or who live in areas where NTM are highly prevalent in the environment. In an effort to improve diagnostic accuracy, whole-blood interferon-γ release assays (IGRAs) have been commercially developed. These assays are based on the host's immune responses to specific M tuberculosis antigens ESAT-6 (early secretory antigenic target-6), CFP-10 (culture filtrate protein-10), and TB7.7, which are absent from most NTM and BCG. The tests detect interferon-γ that is released by sensitized CD4 T cells in response to these antigens. Currently, two commercial assays are available in the United States.

The Quantiferon-Gold In-Tube test (QFT-GIT) (Cellestis Limited, Carnegie, Victoria, Australia) is an enzyme-linked immunosorbent assay (ELISA) that detects interferon-γ in whole blood. The T-SPOT-TB (Oxford Immunotec, Oxford, UK) is an ELISA ImmunoSpot assay that uses purified peripheral blood mononuclear cells. Results for both tests are reported as positive, negative, or indeterminate. These assays are still undergoing extensive evaluation. They are susceptible to biological variation in the immune response. However, multiple studies have shown that these

assays are comparable to the tuberculin skin test in evaluating latent infection, particularly in persons who have received BCG. However, they should not be used in severely immunocompromised hosts or in very young children (< 5 years of age). The CDC has drafted updated guidelines summarizing recommendations on the use of the IGRAs. Patients that who have newly converted from having a negative to a positive result by skin test or IGRA as well as others who have had a positive test result and meet certain criteria for increased risk of active disease if infected are usually given prophylaxis with INH daily for 9 months. Recently, the CDC has published new recommendations for treatment of latent tuberculosis that significantly shorten the length of therapy to 12 weeks. The new regimen consists of once-weekly treatment with INH and rifapentine by directly observed therapy. The new regimen was shown to be equivalent to the old treatment in three randomized controlled trials.

Clinical Findings

Because the tubercle bacillus can involve every organ system, its clinical manifestations are protean. Fatigue, weakness, weight loss, fever, and night sweats may be signs of tuberculous disease. Pulmonary involvement giving rise to chronic cough and spitting of blood usually is associated with far-advanced lesions. Meningitis or urinary tract involvement can occur in the absence of other signs of tuberculosis. Bloodstream dissemination leads to miliary tuberculosis with lesions in many organs and a high mortality rate.

Diagnostic Laboratory Tests

A positive tuberculin test result does not prove the presence of active disease caused by tubercle bacilli. Isolation of tubercle bacilli provides such proof.

A. Specimens

Specimens consist of fresh sputum, gastric washings, urine, pleural fluid, cerebrospinal fluid, joint fluid, biopsy material, blood, or other suspected material.

B. Decontamination and Concentration of Specimens

Specimens from sputum and other nonsterile sites should be liquefied with N-acetyl-L-cysteine decontaminated with NaOH (kills many other bacteria and fungi), neutralized with buffer, and concentrated by centrifugation. Specimens processed in this way can be used for acid-fast stains and for culture. Specimens from sterile sites, such as cerebrospinal fluid, do not need the decontamination procedure but can be directly centrifuged, examined, and cultured.

C. Smears

Sputum, exudate, or other material is examined for acid-fast bacilli by staining. Stains of gastric washings and urine generally are not recommended because saprophytic mycobacteria may be present and yield a positive stain. Fluorescence microscopy with auramine-rhodamine stain is more sensitive than traditional acid-fast stains, such as Ziehl-Neelsen, and is the preferred method for clinical material. If acid-fast organisms are found in an appropriate specimen, this is presumptive evidence of mycobacterial infection.

D. Culture, Identification, and Susceptibility Testing

Processed specimens from nonsterile sites and centrifuged specimens from sterile sites can be cultured directly onto selective and nonselective media. The selective broth culture often is the most sensitive method and provides results most rapidly. A selective agar media (eg, Löwenstein-Jensen or Middlebrook 7H10/7H11 biplate with antibiotics) should be inoculated in parallel with broth media cultures. Incubation is at 35–37°C in 5–10% CO2 for up to 8 weeks. If culture results are negative in the setting of a positive acid-fast stain or if slowly growing NTM are suspected, then a set of inoculated media should be incubated at a lower temperature (eg, 24–33°C) and both sets incubated for 12 weeks.

Blood for culture of mycobacteria (usually MAC) should be anticoagulated and processed by one of two methods: (1) commercially available lysis centrifugation system or (2) inoculation into commercially available broth media specifically designed for blood cultures. It is medically important to characterize and separate M tuberculosis complex from all the other species of mycobacteria. Isolated mycobacteria should be identified as to species. Conventional methods for identification of mycobacteria include observation of rate of growth, colony morphology, pigmentation, and biochemical profiles. The conventional methods often require 6–8 weeks for identification and are rapidly becoming of historical interest because they are inadequate to identify the expanding numbers of clinically relevant species. Most laboratories have abandoned reliance on these biochemical tests. Growth rate separates the rapid growers (growth in ≤7 days) from other mycobacteria.

Photochromogens produce pigment in light but not in darkness, scotochromogens develop pigment when growing in the dark, and nonchromogens (nonphotochromogens) are nonpigmented or have light tan or buff-colored colonies. Molecular probe methods are available for four species and are much faster than the conventional methods. The probes can be used on mycobacterial growth from solid media or from broth cultures. DNA probes specific for ribosomal RNA (rRNA) sequences of the test organism are used in a hybridization procedure. There are approximately 10,000 copies of the rRNA per mycobacterial cell, providing a natural amplification system, enhancing detection. Double-stranded hybrids are separated from unhybridized single-stranded probes. The DNA

probes are linked with chemicals that are activated in the hybrids and detected by chemiluminescence. Probes for the M tuberculosis complex (M tuberculosis, M bovis, Mycobacterium africanum, Mycobacterium caprae, Mycobacterium microti, Mycobacterium canetti, and Mycobacterium pinnipedii), MAC (M avium, M intracellulare, and closely related mycobacteria), M kansasii, and Mycobacterium gordonae are in use. The use of these probes has shortened the time to identification of clinically important mycobacteria from several weeks to as little as 1 day.

In the United States, these four groups (M tuberculosis complex, M avium complex, M kansasii, and M gordonae) make up 95% or more of clinical isolates of mycobacteria. For species that cannot be identified by DNA probes, many laboratories with molecular capabilities have implemented 16S rRNA gene sequencing to rapidly identify probe-negative species or send such organisms to a reference laboratory with sequencing capability. High-performance liquid chromatography (HPLC) has been applied to identification of mycobacteria. The method is based on development of profiles of mycolic acids, which vary from one species to another. HPLC to speciate mycobacteria is available in reference laboratories. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is not yet FDA cleared for identification of

Mycobacterium species recovered in culture although progress is being made. It is anticipated that this method will be available in the near future. Susceptibility testing of mycobacteria is an important adjunct in selecting drugs for effective therapy. A standardized broth culture technique can be used to test for susceptibility to first-line drugs. The complex and more arduous conventional agarbased technique usually is performed in reference laboratories; first- and second-line drugs can be tested by this method. A modification of liquid broth cultures involves inoculating mycobacteria on a multi-well plate with and without addition of antibiotics (Microscopic Observation Drug Susceptibility, MODS assay) and examining for cording that is characteristic of M tuberculosis complex. This method is largely used outside the United States.

E. Nucleic Acid Amplification Tests (NAATs)

NAATs are available for the rapid and direct detection of M tuberculosis in clinical specimens. An advance over the inlab– developed PCR tests and the existing FDA-cleared commercial assays is the GeneXpert MTB/RIF test (Cepheid, Sunnyvale, CA), a real-time multiplex PCR method that both identifies the Mtb complex and also detects genes that encode rifampin resistance. One of the earlier publications on this method reported a sensitivity for smear positive respiratory specimens of 98.2% and for smear negative samples, 72.5%. Overall specificity was 99.2%. In terms of the detection of rifampin resistance, the assay does detect the common mutations, but discrepancies between phenotypic test results and genotypic results still challenge complete reliance on this

component of the test. This assay is not yet widely available in the United States but is available in other countries.

The characterization of specific strains of M tuberculosis can be important for clinical and epidemiologic purposes. It facilitates tracking transmission, analysis of outbreaks of tuberculosis, and demonstration of reactivation versus reinfection in individual patients. DNA fingerprinting is done using a standardized protocol based on restriction fragment length polymorphism. Many copies of the insertion sequence 6110 (IS6110) are present in the chromosome of most strains of M tuberculosis, and these are located at variable positions. DNA fragments are generated by restriction endonuclease digestion and separated by electrophoresis. A probe against IS6110 is used to determine the genotypes. Other useful methods for strain characterization include spoligotyping, a PCR-based technique that targets the direct repeat locus of M tuberculosis and mycobacterial interspersed repetitive unitsvariable number of tandem repeats (MIRU-VNTR) analysis. The latter method is slowing replacing IS6110 typing. Genotyping is done at the CDC, at some state health department laboratories, and in research laboratories.

Treatment

The primary treatment for mycobacterial infection is specific chemotherapy. The drugs for treatment of mycobacterial infection are discussed in Chapter 28. Two cases of tuberculosis are presented in Chapter 48. Between one in 106 and one in 108 tubercle bacilli are spontaneous mutants resistant to first-line antituberculosis drugs. When the drugs are used singly, the resistant tubercle bacilli emerge rapidly and multiply. Therefore, treatment regimens use drugs in combination to yield cure rates of greater than 95%. The two major drugs used to treat tuberculosis are INH and RMP. The other first-line drugs are pyrazinamide (PZA) and ethambutol (EMB). Second-line drugs are more toxic or less effective (or both), and they should be used in therapy only under extenuating circumstances (eg, treatment failure, multiple drug resistance).

Second-line drugs include kanamycin, capreomycin, ethionamide, cycloserine, ofloxacin, and ciprofloxacin. A four-drug regimen of INH, RMP, PZA, and EMB is recommended for persons in the United States who have a slight to moderate risk for being infected with drug-resistant tubercle bacilli. The risk factors include recent emigration from Latin America or Asia, persons with HIV infections or who are at risk for HIV infection and live in an area with a low prevalence of multidrug-resistant tubercle bacilli, and persons who were previously treated with a regimen that did not include RMP. These four drugs are continued for 2 months. If the isolate is susceptible to INH and RMP, PZA and EMB can be discontinued, and the remaining treatment with INH and RMP is continued to complete a 6-month course. In patients with cavitary disease or in whom the sputum culture results are still positive after 2 months of treatment, an additional 3 months of therapy (total course duration of 9 months) should be given to prevent relapse.

In noncompliant patients, directly observed therapy is important. Drug resistance in M tuberculosis is a worldwide problem. Mechanisms explaining the resistance phenomenon for many, but not all, of the resistant strains have been defined. INH resistance has been associated with deletions or mutations in the catalase-peroxidase gene (katG); these isolates become catalase negative or have decreased catalase activity. INH resistance has also been associated with alterations in the inhA gene, which encodes an enzyme that functions in mycolic acid synthesis. Streptomycin resistance has been associated with mutations in genes encoding the ribosomal S12 protein and 16S rRNA, rpsL and rrs, respectively. RMP resistance has been associated with alterations in the B subunit of RNA polymerase, the rpoB gene. Mutations in the DNA gyrase gene gyrA have been associated with resistance to fluoroquinolones. The possibility that drug resistance is present in a patient's M tuberculosis isolate must be taken into account when selecting therapy.

Multidrug-resistant M tuberculosis (resistant to both INH and RMP) is a major problem in tuberculosis treatment and control. Such strains are prevalent in certain geographic areas and certain populations (eg, hospitals, prisons). There have been many outbreaks of tuberculosis with multidrugresistant strains. They are particularly important in persons with HIV infections in resource-poor countries. Persons infected with multidrug-resistant organisms or who are at high risk for such infections, including exposure to another person with such an infection, should be treated according to susceptibility test results for the infecting strain. If susceptibility results are not available, the drugs should be selected according to the known pattern of susceptibility in the community and modified when the susceptibility test results are available. Therapy should include a minimum of three and preferably more than three drugs to which the organisms have demonstrated susceptibility.

Extensively drug-resistant (XDR) strains are now globally recognized. These are defined by the World Health Organization (WHO) as isolates of M tuberculosis with resistance to INH and RMP; any fluoroquinolone; and at least one of three injectable second-line drugs such as amikacin, capreomycin, or kanamycin. The true prevalence of XDR tuberculosis is underestimated in resource-limited countries because of the lack of available diagnostic and susceptibility tests. Factors that have contributed to the global epidemic include ineffective tuberculosis treatment; lack of proper diagnostic testing; and most importantly, poor infection control practices. Persons infected with XDR tuberculosis have a poorer clinical outcome and are 64% more likely to die during treatment than persons infected with susceptible strains. In 2006, the WHO Global Task Force on XDR-TB issued multifaceted and comprehensive recommendations to address the XDR-TB epidemic (available at http://www.who.int/tb/ features_archive/global_taskforce_report/en/).

Epidemiology

The most frequent source of infection is humans who excrete, particularly from the respiratory tract, large numbers of tubercle bacilli. Close contact (eg, in the family) and massive exposure (eg, in medical personnel) make transmission by droplet nuclei most likely. Susceptibility to tuberculosis is a function of the risk of acquiring the infection and the risk of clinical disease after infection has occurred. For tuberculin-negative people, the risk of acquiring tubercle bacilli depends on exposure to sources of infectious bacilli, principally sputum-positive patients. This risk is proportionate to the rate of active infection in the population, crowding, socioeconomic disadvantage, and inadequacy of medical care. The development of clinical disease after infection may have a genetic component (proven in animals and suggested in humans by a higher incidence of disease in those with HLA-Bw15 histocompatibility antigen). It is influenced by age (high risk in infancy and in elderly adults); by undernutrition; and by immunologic status, coexisting diseases (eg, silicosis, diabetes), and other individual host resistance factors. Infection occurs at an earlier age in urban than in rural populations. Disease occurs only in a small proportion of infected individuals. In the United States at present, active disease has several epidemiologic patterns in which individuals are at increased risk, including minorities, predominantly African Americans and Hispanics; immigrants from countries of high endemicity; HIV-infected patients; homeless persons; and very young and very old individuals. The incidence of tuberculosis is especially high in minority persons with HIV infections. Primary infection can occur in any person exposed to an infectious source. Patients who have had tuberculosis can be infected exogenously a second time. Endogenous reactivation tuberculosis occurs most commonly among persons with AIDS immunosuppression and elderly malnourished or alcoholic destitute men.

Prevention and Control

1. Prompt and effective treatment of patients with active tuberculosis and careful follow-up of their contacts with tuberculin tests, radiographs, and appropriate treatment are the mainstays of public health tuberculosis control.
2. Drug treatment of asymptomatic tuberculin-positive persons in the age groups most prone to develop complications (eg, children) and in tuberculin-positive persons who must receive immunosuppressive drugs greatly reduces reactivation of infection.
3. Nonspecific factors may reduce host resistance, thus favoring the conversion of asymptomatic infection into disease. Such factors include starvation, gastrectomy, and suppression of cellular immunity by drugs (eg, corticosteroids) or infection. HIV infection is a major risk factor for tuberculosis.
4. Various living avirulent tubercle bacilli, particularly BCG (an attenuated bovine organism), have been used to induce a certain amount of resistance in those heavily exposed to infection. Vaccination with these organisms is a substitute for primary infection with

virulent tubercle bacilli without the danger inherent in the latter. The available vaccines are inadequate from many technical and biologic standpoints. Nevertheless, BCG is given to children in many countries. Statistical evidence indicates that an increased resistance for a limited period follows BCG vaccination.

5. Eradication of tuberculosis in cattle and pasteurization

MYCOBACTERIUM LEPRAE

Although this organism was described by Hansen in 1873 (9 years before Koch's discovery of the tubercle bacillus), it has not been cultivated on nonliving bacteriologic media. It causes leprosy. Globally the majority of cases occur in Brazil and the Indian subcontinent. Typical acid-fast bacilli—singly, in parallel bundles, or in globular masses—are regularly found in scrapings from skin or mucous membranes (particularly the nasal septum) in patients with lepromatous leprosy. The bacilli are often found within the endothelial cells of blood vessels or in mononuclear cells. When bacilli from human leprosy (ground tissue nasal scrapings) are inoculated into the footpads of mice, local granulomatous lesions develop with limited multiplication of bacilli. Inoculated armadillos develop extensive lepromatous leprosy, and armadillos naturally infected with leprosy have been found in Texas and Mexico. M leprae from armadillo or human tissue contains a unique o-diphenoloxidase, perhaps an enzyme characteristic of leprosy bacilli.

Clinical Findings

The onset of leprosy is insidious. The lesions involve the cooler tissue of the body, including the skin, superficial nerves, nose, pharynx, larynx, eyes, and testicles. The skin lesions may occur as pale, anesthetic macular lesions 1–10 cm in diameter; diffuse or discrete erythematous, infiltrated nodules 1–5 cm in diameter; or a diffuse skin infiltration. Neurologic disturbances are manifested by nerve infiltration and thickening, with resultant anesthesia, neuritis, paresthesia, trophic ulcers, and bone resorption and shortening of the digits. The disfigurement caused by the skin infiltration and nerve involvement in untreated cases may be extreme.

The disease is divided into two major types, lepromatous and tuberculoid, with several intermediate stages. In the lepromatous type, the course is progressive and malignant, with nodular skin lesions; slow, symmetric nerve involvement; abundant acid-fast bacilli in the skin lesions; continuous bacteremia; and a negative lepromin (extract of lepromatous tissue) skin test result. In lepromatous leprosy, cell-mediated immunity is markedly deficient, and the skin is infiltrated with suppressor T cells. In the tuberculoid type, the course is benign and nonprogressive, with a small number of macular skin lesions containing few bacilli, severe asymmetric nerve involvement of sudden onset, and a positive lepromin skin test result. In tuberculoid leprosy, cell-mediated immunity is intact, and the skin is infiltrated with helper T cells. Systemic manifestations of anemia and lymphadenopathy may also occur. Eye involvement is common. Amyloidosis may develop.

Diagnosis

Scrapings with a scalpel blade from skin or nasal mucosa or from a biopsy of earlobe skin are smeared on a slide and stained by the Ziehl-Neelsen technique. Biopsy of skin or of a thickened

nerve gives a typical histologic picture. No serologic tests are of value. Non-treponemal serologic tests for syphilis frequently yield false-positive results in patients with leprosy.

Treatment

Sulfones such as dapsone are first-line therapy for both tuberculoid and lepromatous leprosy. RMP and/ or clofazimine generally are included in the initial treatment regimens. Other drugs active against M leprae include minocycline, clarithromycin, and some fluoroquinolones. Regimens recommended by the WHO are practical. Several years of therapy may be necessary to adequately treat leprosy.

Epidemiology

Transmission of leprosy is most likely to occur when small children are exposed for prolonged periods to heavy shedders of bacilli. Nasal secretions are the most likely infectious material for family contacts. The incubation period is probably 2–10 years. Without prophylaxis, about 10% of exposed children may acquire the disease. Treatment tends to reduce and abolish the infectivity of patients. The naturally infected armadillos found in Texas and Mexico probably play no role in transmission of leprosy to humans.

Prevention and Control

In the United States, the current recommendations for prevention of leprosy include a thorough examination of household contacts and close relatives. This should include a complete skin examination and an examination of the peripheral nervous system. The U.S. Public Health Service National Hansen's Disease Program does not recommend routine dapsone prophylaxis. A therapeutic trial may be indicated for patients whose signs and symptoms are suggestive of leprosy but who do not have a definitive diagnosis. BCG does provide some protection against leprosy especially among household contacts of cases.

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – IV – Medical Bacteriology – SMB3101

4. SPIROCHETES

The spirochetes are a large, heterogeneous group of spiral, motile bacteria. One family (Spirochaetaceae) of the order Spirochaetales consists of two genera whose members are human pathogens, Borrelia and Treponema. The other family (Leptospiraceae) includes one genus of medical importance: Leptospira. The spirochetes have many structural characteristics in common, as typified by Treponema pallidum (Figure 24-1). They are long, slender, helically coiled, spiral, or corkscrewshaped bacilli. *T pallidum* has an outer sheath or glycosaminoglycan coating. Inside the sheath is the outer membrane, which contains peptidoglycan and maintains the structural integrity of the organisms. Endoflagella (axial filaments) are the flagella-like organelles in the periplasmic space encased by the outer membrane. The endoflagella begin at each end of the organism and wind around it, extending to and overlapping at the midpoint. Inside the endoflagella is the inner membrane (cytoplasmic membrane) that provides osmotic stability and covers the protoplasmic cylinder. A series of cytoplasmic tubules (body fibrils) are inside the cell near the inner membrane. Treponemes reproduce by transverse fission.

4.1 TREPONEMA PALLIDUM AND SYPHILIS

Morphology and Identification

A. Typical Organisms

T pallidum are slender spirals measuring about 0.2 μm in width and 5–15 μm in length. The spiral coils are regularly spaced at a distance of 1 μm from one another. The organisms are actively motile, rotating steadily around their endoflagella even after attaching to cells by their tapered ends. The long axis of the spiral is ordinarily straight but may sometimes bend so that the organism forms a complete circle for moments at a time, returning then to its normal straight position. The spirals are so thin that they are not readily seen unless immunofluorescent stain or dark-field illumination is used. They do not stain well with aniline dyes, but they can be seen in tissues when stained by a silver impregnation method.

B. Culture

Pathogenic *T pallidum* has never been cultured continuously on artificial media, in fertile eggs, or in tissue culture. In proper suspending fluids and in the presence of reducing substances, *T pallidum* may remain motile for 3–6 days at 25°C. In whole blood or plasma stored at 4°C, organisms remain viable for at least 24 hours, which is of potential importance in blood transfusions.

D. Reactions to Physical and Chemical Agents

Drying kills the spirochete rapidly, as does elevation of the temperature to 42°C. Treponemes are rapidly immobilized and killed by trivalent arsenical, mercury, and bismuth (contained in drugs of historical interest in the treatment of syphilis). Penicillin is treponemicidal in minute concentrations, but the rate of killing is slow, presumably because of the metabolic inactivity and slow multiplication rate of *T pallidum* (estimated division time is 30 hours). Resistance to penicillin has not been demonstrated in syphilis.

E. Genome

The *T pallidum* genome is a circular chromosome of approximately 1,138,000 base pairs, which is small for bacteria. Most pathogenic bacteria have transposable elements, but *T pallidum* does not, which suggests that the genome is highly conserved and may explain its continued susceptibility to penicillin. There are few genes involved in energy production and synthesis of nutrients, indicating that *T pallidum* obtains these from the host.

Antigenic Structure

The fact that *T pallidum* cannot be cultured in vitro has markedly limited the characterization of its antigens. The outer membrane surrounds the periplasmic space and the peptidoglycan–cytoplasmic membrane complex. Membrane proteins are present that contain covalently bound lipids at their amino terminals. The lipids appear to anchor the proteins to the cytoplasmic or outer membranes and keep the proteins inaccessible to antibodies.

The endoflagella are in the periplasmic space. *T pallidum* has hyaluronidase that breaks down the hyaluronic acid in the ground substance of tissue and presumably enhances the invasiveness of the organism. The endoflagella are composed of three core proteins that are homologous to other bacterial flagellin proteins plus an unrelated sheath protein. Cardiolipin is an important component of the treponemal antigens.

Humans with syphilis develop antibodies capable of staining *T pallidum* by indirect immunofluorescence, immobilizing and killing live motile *T pallidum* and fixing complement in the presence of a suspension of *T pallidum* or related spirochetes. The spirochetes also cause the development of a distinct antibody-like substance, reagin, which gives positive complement fixation (CF) and flocculation test results with aqueous suspensions of cardiolipin extracted from normal mammalian tissues. Both reagin and antitreponemal antibody can be used for the serologic diagnosis of syphilis.

Pathogenesis, Pathology, and Clinical Findings

A. Acquired Syphilis

Natural infection with *T pallidum* is limited to the human host. Human infection is usually transmitted by sexual contact, and the infectious lesion is on the skin or mucous membranes of genitalia. In 10–20% of cases, however, the primary lesion is intrarectal, perianal, or oral. It may be anywhere on the body. *T pallidum* can probably penetrate intact mucous membranes, or the organisms may enter through a break in the epidermis. Based on experiments in rabbits, as few as four to eight spirochetes may cause infection. Spirochetes multiply locally at the site of entry, and some spread to nearby lymph nodes and then reach the bloodstream. Within 2–10 weeks after infection, a papule develops at the site of infection and breaks down to form an ulcer with a clean, hard base ("hard chancre"). The inflammation is characterized by a predominance of lymphocytes and plasma cells. This "primary lesion" always heals spontaneously, but 2–10 weeks later, the "secondary" lesions appear. These consist of a red maculopapular rash anywhere on the body, including the hands and feet, and moist, pale papules (condylomas) in the anogenital region, axillae, and mouth. The patient may also have syphilitic meningitis, chorioretinitis, hepatitis, nephritis (immune complex type), or periostitis. The secondary lesions also subside spontaneously. Both primary and secondary lesions are rich in spirochetes and are highly infectious. Contagious lesions may recur within 3–5 years after infection, but thereafter the individual is not infectious. Syphilitic infection may remain subclinical, and the patient may pass through the primary or secondary stage (or both) without symptoms or signs yet develop tertiary lesions. In about 30% of cases, early syphilitic infection progresses spontaneously to complete cure without treatment. In another 30%, the untreated infection remains latent (principally evident by positive serologic test results). In the remainder, the disease progresses to the "tertiary stage" characterized by the development of granulomatous lesions (gummas) in the skin, bones, and liver; degenerative changes in the central nervous system (meningovascular syphilis, paresis, tabes); or cardiovascular lesions (aortitis, aortic aneurysm, aortic valve insufficiency). In all tertiary lesions, treponemes are very rare, and the exaggerated tissue response must be attributed to hypersensitivity to the organisms. However, treponemes can occasionally be found in the eye or central nervous system in late syphilis.

B. Congenital Syphilis

A pregnant woman with syphilis can transmit *T pallidum* to the fetus through the placenta beginning in the 10th–15th weeks of gestation. Some of the infected fetuses die, and miscarriages result; others are stillborn at term. Others are born live but develop the signs of congenital syphilis in childhood, including interstitial keratitis, Hutchinson's teeth, saddlenose, periostitis, and a variety of central nervous system anomalies. Adequate treatment of the mother during pregnancy prevents congenital syphilis. The reagin titer in the blood of the child rises with active infection

but falls with time if antibody was passively transmitted from the mother. In congenital infection, the child makes IgM antitreponemal antibody.

Diagnostic Laboratory Tests

A. Specimens

Specimens include tissue fluid expressed from early surface lesions for demonstration of spirochetes by either dark-field microscopy or immunofluorescence; such specimens can also for serologic tests; cerebrospinal fluid (CSF) is useful for Venereal Disease Research Laboratory (VDRL) testing (see later discussion).

B. Dark-Field Examination

A drop of tissue fluid or exudate is placed on a slide, and a coverslip is pressed over it to make a thin layer. The preparation is then examined under oil immersion within 20 minutes of collection with dark-field illumination for typical motile spirochetes. Dark-field microscopy should not be performed on lesions within the oral cavity because it is not possible to differentiate pathogenic from commensal spirochetes. Treponemes disappear from lesions within a few hours after the beginning of antibiotic treatment.

C. Immunofluorescence

Tissue fluid or exudate is spread on a glass slide, air dried, and sent to the laboratory. It is fixed, stained with a fluoresceinlabeled antitreponeme antibody, and examined by means of immunofluorescence microscopy for typical fluorescent spirochetes.

D. Serologic Tests for Syphilis

These tests use either nontreponemal or treponemal antigens.

1. Nontreponemal tests

The nontreponemal tests are universally used as screening tests for syphilis. The tests are widely available, lend themselves to automation with ease of performance in large numbers, and have a low cost. In addition to their function as screening tests, they can be used to follow the efficacy of therapy. The drawbacks to the nontreponemal tests are that they are not very sensitive in early syphilis, and the results may not turn positive until a few weeks after initial infection; false-positive results can occur with many other diseases; and there may be a prozone phenomenon, particularly in secondary syphilis (antibody excess produces a negative result at low serum dilutions but positive results at higher dilutions). The antigens in these tests contain measured amounts of cardiolipin, cholesterol, and purified lecithin in quantities sufficient to yield a standardized amount of reactivity. Historically, the cardiolipin was extracted from beef heart or liver with added lecithin and cholesterol to enhance reaction with syphilitic “reagin” antibodies. Reagin is a mixture of IgM

and IgG antibodies reactive with the cardiolipin–cholesterol– lecithin complex. All of the tests are based on the fact that the particles of the lipid antigen remain dispersed in normal serum but flocculate when combining with reagin. The VDRL and unheated serum reagin (USR) tests require microscopic examination to detect flocculation. The rapid plasma reagin (RPR) test and toluidine red unheated serum test (TRUST) have colored particles that become caught in the mesh of the antigen–antibody complex, allowing the tests to be read without microscopic magnification. Results develop within a few minutes, particularly if the suspension is agitated.

The nontreponemal tests can give quantitative results using serial twofold dilutions. An estimate of the amount of reagin present in serum can be expressed as the titer or as the highest dilution giving a positive result. Quantitative results are valuable in establishing a diagnosis and in evaluating the effect of treatment. Positive nontreponemal test results develop after 2–3 weeks of untreated syphilis and are positive in high titer in secondary syphilis. Positive nontreponemal test results typically revert to negative, often in 6–18 months and generally by 3 years after effective treatment of syphilis. A positive nontreponemal test result late after treatment for syphilis suggests ineffective treatment or reinfection. The VDRL test is standardized for use on CSF, and the result becomes positive in patients with neurosyphilis. Reagin antibodies generally do not reach the CSF from the bloodstream but are probably formed in the central nervous system in response to syphilitic infection. The serologic diagnosis of neurosyphilis is complex.

2. Treponemal antibody tests

The treponemal tests measure antibodies against *T pallidum* antigens. The tests are used to determine if a positive result from a nontreponemal test is truly positive or falsely positive. A positive result of a treponemal test on a serum specimen that is also positive on a nontreponemal test is a strong indication of *T pallidum* infection. The traditional treponemal tests are less useful as screening tests because once positive after initial syphilitic infection the tests remain positive for life independent of therapy for syphilis. Serial dilutions of serum are not done in the treponemal tests, and results are reported as reactive or nonreactive (or occasionally inconclusive). The treponemal antibody tests tend to be more costly than the nontreponemal test, which is important when large groups of people (eg, blood donors) are being screened. The T pallidum–particle agglutination (TP-PA) test is perhaps the most widely used treponemal test in the United States. Gelatin particles sensitized with *T pallidum* subspecies pallidum antigens are added to a standard dilution of serum. When anti-*T pallidum* antibodies (IgG, IgM, or both) react with the sensitized particles, a mat of agglutinated particles forms in the well of the microdilution tray. Gelatin particles that are not sensitized are tested with diluted serum to exclude nonspecific agglutination.

The *T pallidum* hemagglutination (TPHA) and the microhemagglutination *T pallidum* (MHA-TP) are based on the same principles as the TP-PA but use sheep erythrocytes rather than gelatin particles and may be more prone to nonspecific agglutination. The fluorescent treponemal antibody absorbed (FTAABS) test is the treponemal antibody test used for many years. Because it is difficult to perform, the test is used only in selected circumstances. The test uses indirect immunofluorescence to detect reactive antibodies, including killed *T pallidum* and the patient's serum absorbed with sonicated saprophytic Reiter spirochetes plus antihuman γ-globulin labeled with a fluorescent compound. The presence of IgM
FTA in the blood of newborns is a good evidence of in utero infection (ie, congenital syphilis). A negative FTA-ABS result on CSF tends to exclude neurosyphilis, but a positive FTAABS result on CSF can occur by transfer of antibodies from serum and is not helpful in the diagnosis of neurosyphilis.

Multiple relatively similar treponemal antibody tests using enzyme immunoassay (EIA) or chemiluminescence (CIA) formats for *T pallidum* are available. These tests use antigens obtained by sonication of *T pallidum* or recombinant antigens. An aliquot of serum at a standard dilution is added to a sensitized well of a microdilution plate. After washing, addition of an enzyme-labeled conjugate, and further washing, a precursor substrate is added. A color change or CIA indicates a reactive serum. Because some of these assays are available as high-throughput automated tests, many laboratories have now reversed the traditional algorithm for screening. Instead of screening with the nontreponemal test and verifying with a treponemal assay, the high throughput allows screening with a more sensitive treponemal test. The advantage to this approach is that patients with early disease or untreated latent disease are more likely to be detected (see earlier discussion).
There are some concerns about variability in assay performance among these tests that result in more false positives when testing low-prevalence populations. Because of this, the Centers for Disease Control and Prevention (CDC) has recommended an algorithm for confirming a positive EIA or CIA test result with a quantitative RPR or other nontreponemal test. If the RPR result is positive, a current or past infection with syphilis is likely. If the RPR result is negative, then additional testing with a traditional treponemal test such as the TP-PA is recommended. If the TP-PA result is positive, syphilis is likely; if it is negative, syphilis is unlikely.

Immunity

A person with active or latent syphilis appears to be resistant to superinfection with T pallidum. However, if early syphilis is treated adequately and the infection is eradicated, the individual again becomes fully susceptible. The various immune responses usually fail to eradicate the infection or arrest its progression.

Treatment

Penicillin in concentrations of 0.003 U/mL has definite treponemicidal activity, and penicillin is the treatment of choice. Syphilis of less than 1 year's duration is treated by a single injection of benzathine penicillin G 2.4 million units intramuscularly. In older or latent syphilis, benzathine penicillin G intramuscularly is given three times at weekly intervals. In neurosyphilis, the same therapy is acceptable, but larger amounts of intravenous penicillin are sometimes recommended. Other antibiotics (eg, tetracyclines or erythromycin) can occasionally be substituted. Treatment of gonorrhea is thought to cure incubating syphilis. Prolonged follow-up is essential. In neurosyphilis, treponemes occasionally survive such treatment. Severe neurologic relapses of treated syphilis have occurred in patients with AIDS who are infected with both HIV and T pallidum. A typical Jarisch-Herxheimer reaction may occur within hours after treatment is begun. It is caused by the release of toxic products from dying or killed spirochetes.

Epidemiology, Prevention, and Control

With the exceptions of congenital syphilis and the rare occupational exposure of medical personnel, syphilis is acquired through sexual exposure. Reinfection in treated persons is common. An infected person may remain contagious for 3–5 years during "early" syphilis. "Late" syphilis, of more than 5 years' duration, is usually not contagious. Consequently, control measures depend on (1) prompt and adequate treatment of all discovered cases, (2) follow-up on sources of infection and contacts so they can be treated, and (3) safe sex with condoms. Several sexually transmitted diseases can be transmitted simultaneously. Therefore, it is important to consider the possibility of syphilis when any one sexually transmitted disease has been found.

LEPTOSPIRA AND LEPTOSPIROSIS

Leptospirosis is a zoonosis of worldwide distribution. It is caused by spirochetes of the genus Leptospira. There is one pathogenic species, Leptospira interrogans, but more than 200 serovars of L interrogans. These serovars are further organized into over two dozen serogroups. The serogroups are based on shared antigenicity and are primarily for laboratory use. Morphology and Identification

A. Typical Organisms

Leptospirae are tightly coiled, thin, flexible spirochetes 5–15 μm long, with very fine spirals 0.1–0.2 μm wide; one end is often bent, forming a hook. They are actively motile, which is best seen using a dark-field microscope. Electron micrographs show a thin axial filament and a delicate membrane. The spirochete is so delicate that in the dark-field view, it may appear only as a chain of minute cocci. It does not stain readily but can be impregnated with silver.

B. Culture

Leptospires grow best under aerobic conditions at 28–30°C in semisolid medium (eg, Ellinghausen-McCullough-Johnson- Harris, EMJH) in 10 mL test tubes with 0.1% agar and 5-fluorouracil (see also Diagnostic Laboratory Tests). After 1–2 weeks, the leptospires produce a diffuse zone of growth near the top of the tube and later a ring of growth at a level in the tube corresponding to the level of the optimal oxygen tension for the organisms.

C. Growth Requirements

Leptospirae derive energy from oxidation of long-chain fatty acids and cannot use amino acids or carbohydrates as major energy sources. Ammonium salts are a main source of nitrogen. Leptospirae can survive for weeks in water, particularly at alkaline pH.

Antigenic Structure

The main strains (“serovars”) of L interrogans are all serologically related and exhibit cross-reactivity in serologic tests.

This indicates considerable overlapping in antigenic structure, and quantitative tests and antibody absorption studies are necessary for a specific serologic diagnosis. The outer envelope contains large amounts of lipopolysaccharide of antigenic structure that is variable from one strain to another. This variation forms the basis for the serologic classification of the Leptospira species. It also determines the specificity of the human immune response to leptospirae.

Pathogenesis and Clinical Findings

Human infection usually results from leptospires, often in bodies of water, entering the body through breaks in the skin (cuts and abrasions) and mucous membranes (mouth, nose, conjunctivae). Ingestion is considered to be less important. After an incubation period of 1–2 weeks, there is a variable febrile onset during which spirochetes are present in the bloodstream. They then establish themselves in the parenchymatous organs (particularly liver and kidneys), producing hemorrhage and necrosis of tissue and resulting in dysfunction of those organs (jaundice, hemorrhage, nitrogen retention). The illness is often biphasic. After initial improvement, the second phase develops when the IgM antibody titer rises. It manifests itself often as "aseptic meningitis" with an intense headache, stiff neck, and pleocytosis of the CSF. Nephritis and hepatitis may also recur, and there may be skin, muscle, and eye lesions. The degree and distribution of organ involvement vary in the different diseases produced by different leptospirae in various parts of the world. Many infections are mild or subclinical. Hepatitis is frequent in patients with leptospirosis.

Kidney involvement in many animal species is chronic and results in the shedding of large numbers of leptospirae in the urine; this is probably the main source of environmental contamination resulting in infection of humans. Human urine also may contain spirochetes in the second and third weeks of disease. Agglutinating, complement-fixing, and lytic antibodies develop during the infection. Serum from convalescent patients protects experimental animals against an otherwise fatal infection. The immunity resulting from infection in humans and animals appears to be serovar specific.

Diagnostic Laboratory Tests

A. Specimens

Specimens consist of aseptically collected blood in a heparin tube, CSF, or tissues for microscopic examination and culture. Urine should be collected using great care to avoid contamination. Serum is collected for agglutination tests.

B. Microscopic Examination

Dark-field examination or thick smears stained by the Giemsa technique occasionally show leptospirae in fresh blood from early infections. Results of dark-field examination of centrifuged urine may also be positive. Fluorescein-conjugated antibodies or other immunohistochemical techniques can be used also.

C. Culture

Whole fresh blood or urine can be cultured in a semisolid medium. Because of inhibitory substances in blood, only one or two drops should be placed in each of five tubes containing 5 or

10 mL of medium. Up to 0.5 mL of CSF can be used. One drop of undiluted urine can be used followed by one drop each of 10-fold serially diluted urine for a total of four tubes. Tissue approximately 5 mm in diameter should be crushed and used as the inoculum. Growth is slow, and cultures should be kept for at least 8 weeks.

D. Serology

The diagnosis of leptospirosis in most cases is confirmed serologically. Agglutinating antibodies first appear 5–7 days after infection and develop slowly, reaching a peak at 5–8 weeks. Very high titers may be attained (>1:10,000). The reference laboratory standard for detection of leptospiral antibody uses microscopic agglutination of live organisms, which can be hazardous. The test is highly sensitive, but it is difficult to standardize; the end point is 50% agglutination, which is difficult to determine. Agglutination of the live suspensions is most specific for the serovar of the infecting leptospires. Agglutination tests are generally performed only in reference laboratories. Paired sera that show a significant change in titer or a single serum with high-titer agglutinins plus a compatible clinical illness can be diagnostic. Because of the difficulty in performing the definitive agglutination tests, a variety of other tests have been developed for use primarily as screening tests.

Immunity

Serovar-specific immunity follows infection, but reinfection with different serovars may occur.

Treatment

Treatment of mild leptospirosis should be with oral doxycycline, ampicillin, or amoxicillin. Treatment of moderate or severe disease should be with intravenous penicillin or ampicillin.

Epidemiology, Prevention, and Control

The leptospiroses are essentially animal infections; human infection is only accidental, occurring after contact with water or other materials contaminated with the excreta of animal hosts. Rats, mice, wild rodents, dogs, swine, and cattle are the principal sources of human infection. They excrete leptospirae in urine both during the active illness and during the asymptomatic carrier state. Leptospirae remain viable in stagnant water for several weeks; drinking, swimming, bathing, or food contamination may lead to human infection. Persons most likely to come in contact with water contaminated by rats (eg, miners, sewer workers, farmers, and fishermen) run the greatest risk of infection. Children acquire the infection from dogs more frequently than adults do. Control consists of preventing exposure to potentially contaminated water and reducing contamination by rodent control. Doxycycline, 200 mg orally once weekly during heavy exposure, is effective prophylaxis. Dogs can receive distemper–hepatitis–leptospirosis vaccinations.

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DEPARTMENT OF BIOTECHNOLOGY

UNIT – V – Medical Bacteriology – SMB3101

5. MYCOPLASMAS

There are more than 200 known species in the class of Mollicutes (cell wall–free bacteria). At least 16 of these species are thought to be of human origin; others have been isolated from animals and plants. In humans, four species are of primary importance: Mycoplasma pneumoniae causes pneumonia and has been associated with joint and other infections. Mycoplasma hominis sometimes causes postpartum fever and has been found with other bacteria in uterine tube infections. Ureaplasma urealyticum is a cause of nongonococcal urethritis in men and is associated with lung disease in premature infants of low birth weight. Mycoplasma genitalium is closely related to M pneumoniae and has been associated with urethral and other urogenital infections. Other members of the genus Mycoplasma are pathogens of the respiratory and urogenital tracts and joints of humans and animals. The smallest genome of known mycoplasmas, M genitalium, is little more than twice the genome size of certain large viruses. Mycoplasmas are the smallest organisms that can be free living in nature and self-replicating on laboratory media.

They have the following characteristics:

(1) the smallest mycoplasmas are 125–250 nm in size;
(2) they are highly pleomorphic because they lack a rigid cell wall and instead are bounded by a triple-layered "unit membrane" that contains a sterol (mycoplasmas require the addition of serum or cholesterol to the medium to produce sterols for growth);
(3) mycoplasmas are completely resistant to penicillin because they lack the cell wall structures at which penicillin acts, but they are inhibited by tetracycline or erythromycin;
(4) mycoplasmas can reproduce in cell-free media; on agar, the center of the whole colony is characteristically embedded beneath the surface;
(5) growth of mycoplasmas is inhibited by specific antibody; and (6) mycoplasmas have an affinity for mammalian cell membranes.

Morphology and Identification

A. Typical Organisms

Mycoplasmas cannot be studied by the usual bacteriologic methods because of the small size of their colonies and the plasticity and delicacy of their individual cells. Growth in fluid media gives rise to many different forms. Growth on solid media consists principally of protoplasmic masses of indefinite shape that are easily distorted. These structures vary greatly in size, ranging from 50 to 300 nm in diameter. The morphology appears different according to the method of examination (eg, dark field, immunofluorescence, Giemsastained films from solid or liquid media, and agar fixation).

B. Culture

Culture of mycoplasmas that cause disease in humans requires media with serum, a metabolic substrate such as glucose or urea, and growth factors such as yeast extract. There is no one medium that is optimal for all the species because of different properties and substrate requirements. After incubation at 37°C for 48–96 hours, there may be no turbidity in broth cultures; however, Giemsa stains of the centrifuged sediment show the characteristic pleomorphic structures, and subculture on appropriate solid media yields minute colonies. After 2–6 days on biphasic (broth over agar) and agar medium incubated in a Petri dish that has been sealed to prevent evaporation, isolated colonies of the more rapidly growing mycoplasmas measuring 20–500 μm can be detected with a hand lens. These colonies are round, with a granular surface and a dark center typically buried in the agar. They can be subcultured by cutting out a small square of agar containing one or more colonies and streaking this material on a fresh plate or dropping it into liquid medium.

C. Growth Characteristics

Mycoplasmas are unique in microbiology because of (1) their lack of a call wall; (2) their extremely small size; and (3) their growth on complex but cell-free media. Mycoplasmas pass through filters with 450-nm pore size and thus are comparable to chlamydiae or large viruses. However, parasitic mycoplasmas grow on cell-free media that contain lipoprotein and sterol. This sterol requirement for growth and membrane synthesis is unique. Many mycoplasmas use glucose as a source of energy; ureaplasmas require urea.

Some human mycoplasmas produce peroxides and hemolyze red blood cells. In cell cultures and in vivo, mycoplasmas are observed predominantly at cell surfaces. Many established animal and human cell culture lines carry mycoplasmas as contaminants; often the mycoplasmas are intracellular as well.

D. Variation

The extreme pleomorphism of mycoplasmas is one of their principal characteristics.

Antigenic Structure At least 16 antigenically distinct species can be identified from humans, including M hominis, M pneumoniae, M genitalium, and U urealyticum. Most Mycoplasma species have high evolved systems for variation of outer membrane antigens presumably for evading the host immune response during infection. The species are classified by biochemical and serologic features. The complement fixation (CF) antigens of mycoplasmas are glycolipids. Antigens for enzyme-linked immunoassay (ELISA) tests are proteins. Some species have more than one serotype.

Pathogenesis

Many pathogenic mycoplasmas have flasklike or filamentous shapes and have specialized polar tip structures that mediate adherence to host cells. These structures are a complex group of interactive proteins, adhesins (eg, the P1 adhesin of M pneumoniae and the MgPa adhesin of M genitalium), and adherenceaccessory proteins. The proteins are proline rich, which influence the protein folding and binding and are important in the adherence to cells. The mycoplasmas attach to the surfaces of ciliated and nonciliated cells, probably through the mucosal cell sialoglycoconjugates and sulfated glycolipids. Some mycoplasmas lack the distinct tip structures but use adhesin proteins or have alternative mechanisms to adhere to host cells. The subsequent events in infection are less well understood but may include several factors as follows: direct cytotoxicity through generation of hydrogen peroxide and superoxide radicals, cytolysis mediated by antigen–antibody reactions or by chemotaxis and action of mononuclear cells, and competition for and depletion of nutrients.

Mycoplasmal Infection

Mycoplasmas have been cultivated from human mucous membranes and tissues, particularly from the genital, urinary, and respiratory tracts. Mycoplasmas are part of the normal microbiota of the mouth and can be grown from normal saliva, oral mucous membranes, sputum, or tonsillar tissue. M hominis is found in the oropharynx of fewer than 5% of adults. M pneumoniae in the oropharynx is generally associated with disease.

Some mycoplasmas are inhabitants of the genitourinary tract, particularly in women. In both men and women, genital carriage of mycoplasmas is directly related to the number of lifetime sex partners. M hominis can be cultured from 1–5% of asymptomatic men and 30–70% of asymptomatic women; the rates increase to 20% and more than 90% positive for men and women, respectively, in sexually transmitted disease clinics. U urealyticum is found in the genital tracts of 5–20% of sexually active men and 40–80% of sexually active women. Approximately 10% of women attending sexually transmitted disease clinics have M genitalium in their lower genital tracts. The presence of M genitalium in the male urethra is typically associated with disease, a syndrome termed nongonococcal urethritis. Other mycoplasmas also occur in the lower genital tract.

Diagnostic Laboratory Tests

A. Specimens

Specimens consist of throat swabs; sputum; inflammatory exudates; and respiratory, urethral, or genital secretions.

B. Microscopic Examination

Direct examination of a specimen for mycoplasmas is useless. Cultures are examined as described earlier.

C. Cultures

The material is inoculated into broth and onto special solid media depending on the organism sought. Agar media is best incubated at 37°C with 5–10% CO_2 (under microaerophilic conditions or even anaerobic conditions). Broths require incubation at 37°C under atmospheric (aerobic) conditions. The duration of incubation varies from 2–4 days for organisms such as M hominis and U urealyticum to up to 4 weeks for M pneumoniae.

One or two transfers of media may be necessary before growth appears that is suitable for microscopic examination by staining or immunofluorescence. Colonies of M hominis may have a typical "fried egg" appearance on agar, but those of M pneumoniae and M genitalium are smaller and may lack the typical appearance. Specimens submitted for diagnosis of Ureaplasma species are usually inoculated to broth or agar media (eg, A8 agar) containing urea. Growth is signaled by a color change indicating hydrolysis of urea.

D. Serology

Antibodies develop in humans infected with mycoplasmas and can be demonstrated by several methods. CF tests can be performed with glycolipid antigens extracted with chloroform– methanol from cultured mycoplasmas. M pneumoniae and M genitalium are serologically cross-reactive using CF tests. HI tests can be applied to tanned red blood cells with adsorbed Mycoplasma antigens. Indirect immunofluorescence may be used. The test that measures growth inhibition by antibody is quite specific. Enzyme immunoassays (EIAs) are available in most laboratories, but sensitivity and specificity are quite variable depending on the assay. In general, EIAs are considered better than CF. With all of these serologic techniques there is adequate specificity for different human Mycoplasma species, but a rising antibody titer is required for diagnostic significance because of the high incidence of positive serologic test results in normal individuals.

E. Nucleic Acid Amplification Tests

Molecular methods for the detection of the human mycoplasmas and ureaplasma are available in many reference laboratories, and a variety of primers and probes have been published. Very few assays are cleared by the U.S. Food and Drug Administration, although many platforms are in development and this situation will likely improve. Nucleic acid amplification tests (NAATs) are particularly useful for those organisms that are difficult to cultivate such as M pneumoniae and M genitalium and less useful for the more rapidly growing organisms. The difficulty arises when these test results are positive in the absence of corroborating clinical or other positive diagnostic

test results. At this time, these assays are best used in combination with other traditional diagnostic methods such as serology until more clinical data become available.

Treatment

Many strains of mycoplasmas are inhibited by a variety of antimicrobial drugs, but most strains are resistant to penicillins, cephalosporins, and vancomycin. Tetracyclines and erythromycins are effective both in vitro and in vivo and are, at present, the drugs of choice in mycoplasmal pneumonia. Some ureaplasmas are resistant to tetracycline. Treatment of M genitalium urethritis in men is typically through a single dose of azithromycin administered in the clinic. This ensures compliance and reduces the likelihood of sexual transmission to other partners.

Epidemiology, Prevention, and Control

M pneumoniae behaves like a communicable viral respiratory pathogen (see later discussion) and is capable of causing both endemic and epidemic infections. The genital mycoplasmas and ureaplasma are spread by genital or oral–genital contact and may be transmitted along with other sexually acquired pathogens. Safe sexual practices should reduce spread. No vaccines are available to protect against any of these organisms.

CHLAMYDIA spp.

Chlamydiae that infect humans are divided into three species, Chlamydia trachomatis, Chlamydia pneumoniae, and Chlamydia psittaci, on the basis of antigenic composition, intracellular inclusions, sulfadiazine susceptibility, and disease production. The separation of the genus Chlamydia into the genera Chlamydia and Chlamydophila was controversial; in this chapter, the three chlamydiae that are pathogens of humans are considered to be in the genus Chlamydia in keeping with publications that do not support the new taxonomy. Other chlamydiae infect animals but rarely if ever infect humans. All chlamydiae exhibit similar morphologic features, share a common group antigen, and multiply in the cytoplasm of their host cells by a distinctive developmental cycle. The chlamydiae can be viewed as gram-negative bacteria that lack mechanisms for the production of metabolic energy and cannot synthesize adenosine triphosphate (ATP). This restricts them to an intracellular existence, where the host cell furnishes energy-rich intermediates. Thus, chlamydiae are obligate intracellular pathogens.

Developmental Cycle

All chlamydiae share a common and unique biphasic developmental cycle. The environmentally stable infectious particle (transmissible form) is a small cell called the elementary body (EB). These are about 0.3 μm in diameter (Figure 27-1) with an electron-dense nucleoid. The EB membrane proteins have highly cross-linked membrane proteins. The EBs have a high affinity for host epithelial cells and rapidly enter them. The first step in entry involves interaction between outer membrane proteins of the EB and heparin sulfate proteoglycan of the host cells. The second step involves additional and irreversible binding to a variety of other host cell receptors. There appear to be multiple adhesins, such as OmcB, the major outer membrane protein (MOMP), glycosylated MOMP, and other surface proteins. Following adherence, the mechanisms thought to mediate entry into the host cell also vary and involve cytoskeletal rearrangements and activation of type III secretion systems and other effectors. EBs are usually seen attached near the base of microvilli, where they are subsequently engulfed by the host cell. More than one mechanism appears to be functional: receptor-mediated endocytosis into clathrin-coated pits and pinocytosis via noncoated pits. Lysosomal fusion is inhibited, creating a protected membrane- bound environment around the chlamydiae. Shortly after entry into the host cell, the disulfide bonds of the EB membrane proteins are reduced (no longer cross-linked), and the EB is reorganized into a larger structure called a reticulate body (RB) [replicative form] measuring about 0.5–1 μm and devoid of an electron-dense nucleoid. Within the membrane-bound vacuole, the RB grows in size and divides repeatedly by binary fission. Eventually, the entire vacuole becomes filled with EBs derived from the RBs to form a cytoplasmic inclusion. The newly formed EBs may be liberated from the host cell to infect new cells. The developmental cycle takes 48–72 hours.

Structure and Chemical Composition

In chlamydiae, the outer cell wall resembles the cell wall of gram-negative bacteria. It has a relatively high lipid content including lipopolysaccharide of low endotoxic activity. It is rigid but does not contain a typical bacterial peptidoglycan. As mentioned above, another important structural component is the MOMP encoded by ompA. MOMP antigenic variants of C trachomatis are associated with different clinical syndromes. Penicillin-binding proteins occur in chlamydiae, and chlamydial cell wall formation is inhibited by penicillins and other drugs that inhibit transpeptidation of bacterial peptidoglycan. Lysozyme has no effect on chlamydial cell walls. N-acetylmuramic acid appears to be absent from chlamydial cell walls. Both DNA and RNA are present in EBs and RBs. The RBs contain about four times as much RNA as DNA, whereas the EBs contain about equal amounts of RNA and DNA. In EBs, most DNA is concentrated in the electron-dense central nucleoid. Most RNA exists in ribosomes. The circular genome of chlamydiae is 1.04 megabases in length, encodes 900 genes, and is one of the smallest bacterial genomes. Multiple chlamydial genomes have been sequenced, providing insight into the basic biology of the organisms. For example, chlamydiae have a type III secretion system, which may allow them to inject effector proteins into host cells as part of the infectious process (see discussion above under Developmental Cycle).

Staining Properties

Chlamydiae have distinctive staining properties (similar to those of rickettsiae). Elementary bodies stain purple with Giemsa stain—in contrast to the blue of host cell cytoplasm. The larger, noninfective RBs stain blue with Giemsa stain. The Gram reaction of chlamydiae is negative or variable and is not useful in identification of the agents. Chlamydial particles and inclusions stain brightly by immunofluorescence, with group specific, species-specific, or serovar-specific antibodies. Fully formed, mature intracellular inclusions of C trachomatis are compact masses near the nucleus that are dark purple when stained with Giemsa stain because of the densely packed mature particles. If stained with dilute Lugol's iodine solution, some of the inclusions of C trachomatis (but not C pneumoniae or C psittaci) appear brown because of the glycogen matrix that surrounds the particles. In contrast, inclusions of C psittaci appear as diffuse intracytoplasmic aggregates.

Antigens

Chlamydiae possess shared group (genus)–specific antigens. These are heat-stable lipopolysaccharides with 2-keto- 3-deoxyoctanoic acid as an immunodominant component. Antibody to these genus-specific antigens can be detected by complement fixation (CF) and immunofluorescence. Species-specific or serovar-specific antigens are mainly outer membrane

proteins. Specific antigens can best be detected by immunofluorescence, particularly using monoclonal antibodies. Specific antigens are shared by only a limited number of chlamydiae, but a given organism may contain several specific antigens. There are at least 15 serovars of C trachomatis that are separated into two biovariants that cause different clinical syndromes. The trachoma biovar includes serovars A, B, Ba, and C as well as the genital tract serovars D–K. The lymphogranuloma venereum (LGV) biovar includes serovars L1, L2, and L3. Several serovars of C psittaci can be demonstrated by CF and microimmunofluorescence (MIF) tests. Only one serovar of C pneumoniae has been described.

Growth and Metabolism

Chlamydiae require an intracellular habitat because of the small genome size, which make them dependent upon host cells for their development and for energy requirements. Chlamydiae grow in cultures of a variety of eukaryotic cells lines. McCoy cells treated with cycloheximide commonly are used to isolate chlamydiae; C pneumoniae grows better in HL or HEp-2 cells. All types of chlamydiae proliferate in embryonated eggs, particularly in the yolk sac.

Some chlamydiae have an endogenous metabolism similar to other bacteria. They can liberate CO_2 from glucose, pyruvate, and glutamate; they also contain dehydrogenases. Nevertheless, they require energy-rich intermediates from the host cell to carry out their biosynthetic activities. The replication of chlamydiae can be inhibited by many antibacterial drugs. Cell wall inhibitors such as penicillins and cephalosporins result in the production of morphologically defective forms but are not effective in treatment of clinical diseases. Inhibitors of protein synthesis (tetracyclines, erythromycins) are effective in most clinical infections. C trachomatis strains synthesize folates and are susceptible to inhibition by sulfonamides. Aminoglycosides are noninhibitory.

Characteristics of Host–Parasite Relationship

The outstanding biologic feature of infection by chlamydiae is the balance that is often reached between host and parasite, resulting in prolonged persistence of infection. Subclinical infection is the rule—and overt disease the exception—in the natural hosts of these agents. Spread from one species to another (eg, birds to humans, as in psittacosis) more frequently leads to disease. Antibodies to several antigens of chlamydiae are regularly produced by the infected host. These antibodies have little protective effect against reinfection. The infectious agent commonly persists in the presence of high antibody titers. Treatment with effective antimicrobial drugs (eg, tetracyclines) for prolonged periods may eliminate the chlamydiae from the infected host. Very early, intensive treatment may suppress antibody formation. Late treatment with antimicrobial drugs in moderate doses may suppress disease but permit persistence of the infecting agent in tissues. The immunization of humans has been singularly unsuccessful in protecting against

reinfection. Prior infection or immunization at most tends to result in milder disease upon reinfection, but at times, the accompanying hypersensitization aggravates inflammation and scarring (eg, in trachoma).

Classification

Chlamydiae are classified according to their pathogenic potential, host range, antigenic differences, and other methods. Three species that infect humans have been characterized.

A. Chlamydia trachomatis

This species produces compact intracytoplasmic inclusions that contain glycogen; it is usually inhibited by sulfonamides. It includes agents of human disorders such as trachoma, inclusion conjunctivitis, nongonococcal urethritis, salpingitis, cervicitis, pneumonitis of infants, and LGV.

B. **Chlamydia pneumoniae**

This species produces intracytoplasmic inclusions that lack glycogen; it is usually resistant to sulfonamides. It causes respiratory tract infections in humans.

C. **Chlamydia psittaci**

This species produces diffuse intracytoplasmic inclusions that lack glycogen; it is usually resistant to sulfonamides. It includes agents of psittacosis in humans, ornithosis in birds, feline pneumonitis, and other animal diseases.

CHLAMYDIA TRACHOMATIS OCULAR, GENITAL, AND RESPIRATORY INFECTIONS

Humans are the natural host for C trachomatis. Monkeys and chimpanzees can be infected in the eye and genital tract. C trachomatis also replicates in cells in tissue culture. C trachomatis of different serovars replicates differently. Isolates from trachoma do not grow as well as those from LGV or genital infections. Intracytoplasmic replication results in the formation of compact inclusions with a glycogen matrix in which EBs are embedded.

TRACHOMA

Trachoma is an ancient eye disease, well described in the Ebers Papyrus, which was written in Egypt 3800 years ago. It is a chronic keratoconjunctivitis that begins with acute inflammatory changes in the conjunctiva and cornea and progresses to scarring and blindness. The C trachomatis serovars A, B, Ba, and C are associated with clinical trachoma.

Clinical Findings

The incubation period for chlamydial conjunctival infection is 3–10 days. In endemic areas, initial infection occurs in early childhood, and the onset of the long-term consequence, trachoma, is

insidious. Chlamydial infection is often mixed with bacterial conjunctivitis in endemic areas, and the two together produce the clinical picture. The earliest symptoms of trachoma are lacrimation, mucopurulent discharge, conjunctival hyperemia, and follicular hypertrophy. Microscopic examination of the cornea reveals epithelial keratitis, subepithelial infiltrates, and extension of limbal vessels into the cornea (pannus). As the pannus extends downward across the cornea, there are scarring of the conjunctiva, eyelid deformities (entropion, trichiasis), and an added insult caused by eyelashes sweeping across the cornea (trichiasis). With secondary bacterial infection, loss of vision progresses over a period of years. There are, however, no systemic symptoms or signs of infection. The World Health Organization has a grading scheme for assessment of trachoma (see reference by Batteiger and Tan).

Laboratory Diagnosis

A. Culture

Typical cytoplasmic inclusions are found in epithelial cells of conjunctival scrapings stained with fluorescent antibody or by the Giemsa method. These occur most frequently in the early stages of the disease and on the upper tarsal conjunctiva. Inoculation of conjunctival scrapings into cycloheximide treated McCoy cell cultures permits growth of C trachomatis if the number of viable infectious particles is sufficiently large. Centrifugation of the inoculum into the cells increases the sensitivity of the method. The diagnosis can sometimes be made in the first passage after 2–3 days of incubation by looking for inclusions by immunofluorescence or staining with iodine or Giemsa stain.

B. Serology

Infected individuals often develop both group antibodies and serovar-specific antibodies in serum and in eye secretions. Immunofluorescence is the most sensitive method for their detection. Neither ocular nor serum antibodies confer significant resistance to reinfection.

C. Molecular Methods

Developing countries, where trachoma is endemic, generally do not have the resources to apply polymerase chain reaction (PCR) or other molecular methods to the diagnosis of C trachomatis infections of the eye. Developed countries have relatively little trachoma and little need for such tests. Thus, the molecular methods have been developed for the diagnosis of genital infections. Only research projects have used PCR in studies of trachoma.

Treatment

Clinical trials in villages with endemic trachoma using mass azithromycin treatment show that infection and clinical disease are greatly decreased at 6 and 12 months after therapy; this is true

even with single-dose therapy. Thus, azithromycin has replaced erythromycin and doxycycline in the mass treatment of endemic trachoma. Topical therapy is of little value.

Epidemiology and Control

It is believed that more than 400 million people throughout the world have trachoma and that 20 million are blinded by it. The disease is most prevalent in sub-Saharan Africa, Asia, and the Mediterranean basin, where hygienic conditions are poor and water is scarce. In such hyperendemic areas, childhood infection may be universal, and severe blinding disease (resulting from frequent bacterial superinfection) is common. In the United States, trachoma occurs sporadically in some areas, and endemic foci persist. The World Health Organization has initiated the S-A-F-E program to eliminate blinding trachoma and at least markedly reduce clinically active disease. The S-A-F-E program is as follows: surgery for deformed eyelids, periodic azithromycin therapy, face washing and hygiene, and environmental improvement such as building latrines and decreasing the number of flies that feed on conjunctival exudates. It is clear that improved socioeconomic conditions enhance the disappearance of endemic trachoma.

CHLAMYDIA TRACHOMATIS GENITAL INFECTIONS AND INCLUSION CONJUNCTIVITIS

C trachomatis serovars D–K cause sexually transmitted diseases, especially in developed countries, and may also produce infection of the eye (inclusion conjunctivitis). In sexually active men, C trachomatis causes nongonococcal urethritis and, occasionally, epididymitis. In women, C trachomatis causes urethritis, cervicitis, and pelvic inflammatory disease, which can lead to sterility and predispose to ectopic pregnancy. Proctitis and proctocolitis may occur in men and women, although these infections appear to be most common in men who have sex with men. Any of these anatomic sites of infection may give rise to symptoms and signs, or the infection may remain asymptomatic but communicable to sex partners. Up to 50% of nongonococcal urethritis (men) or the urethral syndrome (women) is attributed to chlamydiae and produces dysuria, nonpurulent discharge, and frequency of urination. Genital secretions of infected adults can be selfinoculated into the conjunctiva, resulting in inclusion conjunctivitis, an ocular infection that closely resembles acute trachoma.

The newborn acquires the infection during passage through an infected birth canal. Probably 30–50% of infants of infected mothers acquire the infection, with 15–20% of infected infants manifesting eye symptoms and 10–40% manifesting respiratory tract involvement. Inclusion conjunctivitis of the newborn begins as a mucopurulent conjunctivitis 5–12 days after delivery. It tends to subside with erythromycin or tetracycline treatment or spontaneously after weeks or months. Occasionally, inclusion conjunctivitis persists as a chronic chlamydial infection with a

clinical picture indistinguishable from that of subacute or chronic childhood trachoma in nonendemic areas and usually not associated with bacterial conjunctivitis.

Laboratory Diagnosis

A. Specimen Collection

Proper specimen collection is the key to the laboratory diagnosis of chlamydia infection. Because the chlamydiae are obligate intracellular bacteria, it is important that the specimens contain infected human cells as well as the extracellular material where they might also be present. Endocervical specimens should be collected after removal of discharge and secretions from the cervix. A swab or cytology brush is used to scrape epithelial cells from 1–2 cm deep into the endocervix. Dacron, cotton, or rayon on a plastic shaft should be used to collect the specimen; some other swab materials (calcium alginate) and wooden shafts are toxic to chlamydiae. A similar method is used to collect specimens from the vagina, urethra, or conjunctiva. The commercial diagnostic nonculture tests for chlamydia do not require viable organisms. In general, these proprietary tests include the specimen collection swabs and transport tubes that have been demonstrated to be suitable for the specific tests. For culture, the swab specimens should be placed in a chlamydiae transport medium, such as 2-sucrose phosphate supplemented with bovine serum and antibiotics that inhibit normal microbiota, and kept at refrigerator temperature before transport to the laboratory. Urine can be tested for the presence of chlamydial nucleic acid. Only the first 20 mL of the void should be collected because a larger volume of bladder urine would dilute the initial urine that passes through the urethra; this could result in a negative test result because of the dilution.

B. Nucleic Acid Detection

Nonamplified probe assays—In one nucleic acid hybridization test, a DNA probe hybridizes to a specific sequence of C trachomatis 16S rRNA; chlamydiae have up to 104 copies of the 16S rRNA. After the hybrids are formed, they are absorbed onto beads, and the amount of hybrid is detected by chemiluminescence. This assay is no longer commercially available in the United States. Another hybridization assay used RNA probes to detect chlamydiae DNA sequences. The overall sensitivity and specificity of these tests are good but are not as good as the nucleic acid amplification tests (NAATs). The hybridization assays, however, are less expensive than the NAATs. Nucleic acid amplification tests—NAATs are the tests of choice for the diagnosis of genital C trachomatis infections. There are at least five U.S. Food and Drug Administration (FDA)-cleared assays in the United States. They use a variety of molecular methods that target the C trachomatis cryptic plasmid or 23SrRNA, including PCR, strand displacement, and transcription-mediated amplification. These tests have become widely used and have replaced most of the

nonamplification methods. Although they are highly sensitive and specific, they are not perfect. New assays to diagnose chlamydiae infection can be compared to combined results from two NAATs as the reference standard. Specimen types that are appropriate for testing by NAATs include first void urine from males and females and vaginal, cervical, and urethral swabs. Some of the commercial companies that market these platforms are in the process of validating or have validated extragenital sources such as conjunctival, oropharyngeal, and rectal samples. The nucleic acid detection tests have been adapted to simultaneously detect Neisseria gonorrhoeae.

C. Direct Cytologic Examination (Direct Fluorescent Antibody) and Enzyme-Linked Immunoassay

Commercially available direct fluorescent antibody (DFA) and enzyme-linked immunoassay (EIA) assays to detect C trachomatis continue to be marketed. The DFA uses monoclonal antibodies directed against a species-specific antigen on the chlamydial MOMP. The EIA detects the presence of genus-specific antigens extracted from EBs in the specimen. DFA remains useful for detection of chlamydiae in extragenital samples, such as conjunctival swabs. Because of their very low sensitivity and the widespread availability of the more sensitive NAATs, EIAs are being phased out as acceptable methods for screening for both chlamydia and gonorrhea.

D. Culture

Culture of C trachomatis has historically been used to diagnose chlamydia infections. Culture, however, is costly and arduous. Results are delayed compared with the timeliness of NAATs and other tests. Culture is generally much less sensitive than NAATs; the degree of lower sensitivity is largely dependent on the culture method used. Culture is now done in a limited number of reference laboratories. A number of susceptible cell lines can be used, most often McCoy, HeLa 229, or HEp-2. The cells are grown in monolayers on coverslips in dram or shell vials. Some laboratories use flat-bottomed microdilution trays, but cultures by this method are not as sensitive as those achieved with the shell vial method. The cells are treated with cycloheximide to inhibit metabolism and increase the sensitivity of isolation of the chlamydiae. The inoculum from the swab specimen is centrifuged onto the monolayer and incubated at 35–37°C for 48–72 hours. A second monolayer can be inoculated, and after incubation, it can be sonicated and passaged to another monolayer to enhance sensitivity. The monolayers are examined by direct immunofluorescence to visualize the cytoplasmic inclusions. Chlamydial cultures by this method are about 80% sensitive but 100% specific.

E. Serology

Because of the relatively great antigenic mass of chlamydiae in genital tract infections, serum antibodies occur much more commonly than in trachoma and are of higher titer. A titer rise occurs

during and after acute chlamydial infection. Because of the high prevalence of chlamydial genital tract infections in some societies, there is a high background of antichlamydial antibodies in the population; serologic tests to diagnose genital tract chlamydial infections generally are not useful. In genital secretions (eg, cervical), antibody can be detected during active infection and is directed against the infecting immunotype (serovar).

Treatment

It is essential that chlamydial infections be treated simultaneously in both sex partners and in offspring to prevent reinfection. Tetracyclines (eg, doxycycline) are commonly used in nongonococcal urethritis and in nonpregnant infected women. Azithromycin is effective and can be given to pregnant women. Topical tetracycline or erythromycin is used for neonatal N gonorrhoeae infections but may not effectively prevent neonatal C trachomatis infection. Systemic therapy should be used for inclusion conjunctivitis because topical therapy may not cure the eye infections or prevent respiratory disease.

Epidemiology and Control

Genital chlamydial infection and inclusion conjunctivitis are sexually transmitted diseases that are spread by contact with infected sex partners. Neonatal inclusion conjunctivitis originates in the mother's infected genital tract. Prevention of neonatal eye disease depends on diagnosis and treatment of the pregnant woman and her sex partner. As in all sexually transmitted diseases, the presence of multiple etiologic agents (eg, gonococci, treponemes, trichomonads, herpes) must be considered. Instillation of erythromycin or tetracycline into the newborn's eyes does not prevent development of chlamydial conjunctivitis. The ultimate control of this— and all—sexually transmitted disease depends on safe sex practices and on early diagnosis and treatment of infected persons. To accomplish the latter, the Centers for Disease Control and Prevention recommends annual screening of all sexually active women ages 25 years and younger.

CHLAMYDIA TRACHOMATIS AND NEONATAL PNEUMONIA

Of newborns infected by the mother, 10–20% may develop respiratory tract involvement 2–12 weeks after birth, culminating in pneumonia. Affected newborns have nasal obstruction or discharge, striking tachypnea, a characteristic paroxysmal staccato cough, an absence of fever, and eosinophilia. Interstitial infiltrates and hyperinflation can be seen on radiographs. The diagnosis should be suspected if pneumonitis develops in a newborn who has inclusion conjunctivitis and can be established by isolation of C trachomatis from respiratory secretions. In such neonatal pneumonia, an immunoglobulin M (IgM) antibody titer to C trachomatis of 1:32 or more is considered diagnostic. Oral erythromycin for 14 days is recommended; systemic erythromycin is effective treatment in severe cases.

LYMPHOGRANULOMA VENEREUM

LGV is a sexually transmitted disease caused by C trachomatis and is characterized by suppurative inguinal adenitis; it is most common in tropical climates.

Properties of the Agent

The particles contain CF heat-stable chlamydial group antigens that are shared with all other chlamydiae. They also contain one of three serovar antigens (L1–L3), which can be defined by immunofluorescence.

Clinical Findings

Several days to several weeks after exposure, a small, evanescent papule or vesicle develops on any part of the external genitalia, anus, rectum, or elsewhere. The lesion may ulcerate, but usually it remains unnoticed and heals in a few days. Days to weeks later, the regional lymph nodes enlarge and tend to become matted and painful. In men, inguinal nodes are most commonly involved both above and below Poupart's ligament and the overlying skin often turns purplish as the nodes suppurate (bubo formation) and eventually discharge pus through multiple sinus tracts. In women and in homosexual men, the perirectal nodes are prominently involved, with proctitis and a bloody mucopurulent anal discharge. During the stage of active lymphadenitis, there are often marked systemic symptoms, including fever, headaches, meningismus, conjunctivitis, skin rashes, nausea and vomiting, and arthralgias. Meningitis, arthritis, and pericarditis occur rarely. Unless effective antimicrobial drug treatment is given at that stage, the chronic inflammatory process progresses to fibrosis, lymphatic obstruction, and rectal strictures. The lymphatic obstruction may lead to elephantiasis of the penis, scrotum, or vulva. The chronic proctitis of women or homosexual men may lead to progressive rectal strictures, rectosigmoid obstruction, and fistula formation.

Laboratory Diagnosis

A. Smears

Pus, buboes, or biopsy material may be stained, but particles are rarely recognized.

B. Nucleic Acid Amplification Tests

All of the commercial NAATs detect all of the LGV serovars but cannot differentiate them from other C trachomatis serovars.

C. Culture

Suspected material is inoculated into McCoy cell cultures. The inoculum can be treated with an aminoglycoside (but not with penicillin) to lessen bacterial contamination. The agent is identified by morphology and serologic tests.

D. Serology

Antibodies are commonly demonstrated by the CF reaction. The test becomes positive 2–4 weeks after onset of illness. In a clinically compatible case, a rising antibody level or a single titer of more than 1:64 is good evidence of active infection. If treatment has eradicated the LGV infection, the CF titer falls. Serologic diagnosis of LGV can use immunofluorescence, but the antibody is broadly reactive with many chlamydial antigens.

Immunity

Untreated infections tend to be chronic, with persistence of the agent for many years. Little is known about active immunity. The coexistence of latent infection, antibodies, and cell-mediated reactions is typical of many chlamydial infections.

Treatment The sulfonamides and tetracyclines have been used with good results, especially in the early stages. Some drug-treated persons have a marked decline in complement-fixing antibodies, which may indicate that the infective agent has been eliminated from the body. Late stages require surgery.

Epidemiology and Control

Although the highest incidence of LGV has been reported from subtropical and tropical areas, the infection occurs all over the world. The disease is most often spread by sexual contact but not exclusively so. The portal of entry may sometimes be the eye (conjunctivitis with an oculoglandular syndrome). The genital tracts and rectums of chronically infected (but at times asymptomatic) persons serve as reservoirs of infection. Laboratory personnel exposed to aerosols of C trachomatis serovars L1–L3 can develop a chlamydial pneumonitis with mediastinal and hilar adenopathy. If the infection is recognized, treatment with tetracycline or erythromycin is effective. The measures used for the control of other sexually transmitted diseases also apply to the control of LGV. Case finding and early treatment and control of infected persons are essential.

CHLAMYDIA PNEUMONIAE AND RESPIRATORY INFECTIONS

The first C pneumoniae strain was obtained in the 1960s in chick embryo yolk sac culture. After the development of cell culture methods, this initial strain was thought to be a member of the species C psittaci. Subsequently, C pneumoniae was firmly established as a new species that causes respiratory disease in humans and nonhuman species.

Properties of the Agent

C pneumoniae produces round, dense, glycogen-negative inclusions that are sulfonamide resistant, similar to C psittaci (see Table 27-1). The EBs sometimes have a pear-shaped appearance. The genetic relatedness of C pneumoniae isolates is greater than 95%. Only one serovar has been demonstrated.

Clinical Findings

Most infections with C pneumoniae are asymptomatic or associated with mild illness, but severe disease has been reported. There are no signs or symptoms that specifically differentiate C pneumoniae infections from those caused by many other agents. Both upper and lower airway diseases occur. Pharyngitis is common. Sinusitis and otitis media may occur and be accompanied by lower airway disease. An

Laboratory Diagnosis

A. Smears Direct detection of EBs in clinical specimens using fluorescent antibody techniques is insensitive. Other stains do not effectively demonstrate the organism.

B. Culture

Swab specimens of the pharynx should be put into a chlamydiae transport medium and placed at 4°C; C pneumoniae is rapidly inactivated at room temperature. It grows poorly in cell culture, forming inclusions smaller than those formed by the other chlamydiae. C pneumoniae grows better in HL and HEp-2 cells than in HeLa 229 or McCoy cells; the McCoy cells are widely used to culture C trachomatis. The sensitivity of the culture is increased by incorporation of cycloheximide into the cell culture medium to inhibit the eukaryotic cell metabolism and by centrifugation of the inoculum onto the cell layer. Growth is better at 35°C than 37°C. After 3 days' incubation, the cells are fixed and inclusions detected by fluorescent antibody staining with genus- or species-specific antibody or, preferably, with a C pneumoniae–specific monoclonal antibody conjugated with fluorescein. Giemsa staining is insensitive, and the glycogen-negative inclusions do not stain with iodine. It is moderately difficult to grow C pneumoniae— as evidenced by the number of isolates described compared with the incidence of infection.

C. Serology

Serology using the MIF test is the most sensitive method for diagnosis of C pneumoniae infection. The test is species specific and can detect IgG or IgM antibodies by using the appropriate reagents. Primary infection yields IgM antibody after about 3 weeks followed by IgG antibody at 6–8 weeks. In reinfection, the IgM response may be absent or minimal, and the IgG response occurs in 1–2 weeks. The following criteria have been suggested for the serologic diagnosis of C pneumoniae infection: a single IgM titer of 1:16 or greater, a single IgG titer of 1:512 or greater, and a fourfold rise in either the IgM or IgG titers. The CF test can be used, but it is group reacting, does not differentiate C pneumoniae infection from psittacosis or LGV, and is less sensitive than the MIF test.

D. Nucleic Acid Amplification Methods

Although many research and reference laboratories have attempted to develop molecular assays targeting genes such as the 16SrRNA gene and the ompA gene, among others, progress has been

hampered by the lack of a reliable gold standard. However, recently BioFire Diagnostics, Inc. (Salt Lake City, UT) received FDA approval for the addition of C pneumoniae to its FilmArray Respiratory panel. Such tests are needed so that the true contribution of C pneumoniae to clinical disease can be fully determined.

Immunity

Little is known about active or potentially protective immunity. Prolonged infections can occur with C pneumoniae, and asymptomatic carriage may be common.

Treatment

C pneumoniae is susceptible to the macrolides and tetracyclines and to some fluoroquinolones. Treatment with doxycycline, azithromycin, or clarithromycin, levofloxacin or moxifloxacin, appears to significantly benefit patients with C pneumoniae infection, but there are only limited data on the efficacy of antibiotic treatment. Reports indicate that the symptoms may continue or recur after routine courses of therapy with erythromycin, doxycycline, or tetracycline, and these drugs should be given for 10- to 14-day courses.

Epidemiology

Infection with C pneumoniae is common. Worldwide, 30–50% of people have antibody to C pneumoniae. Few young children have antibody, but after the age of 6–8 years, the prevalence of antibody increases through young adulthood. Infection is both endemic and epidemic, with multiple outbreaks attributed to C pneumoniae. There is no known animal reservoir, and transmission is presumed to be from person to person, predominantly by the airborne route. Lines of evidence suggesting that C pneumoniae is associated with atherosclerotic coronary artery and cerebrovascular disease consist of seroepidemiologic studies, detection of C pneumoniae in atherosclerotic tissues, cell culture studies, animal models, and trials of prevention using antibiotic agents. However, other studies have shown no association. The possible link between C pneumoniae infection and coronary artery disease remains controversial.

CHLAMYDIA PSITTACI AND PSITTACOSIS

The term psittacosis is applied to the human C psittaci disease acquired from contact with birds and also the infection of psittacine birds (eg, parrots, parakeets, cockatoos). The term ornithosis is applied to infection with similar agents in all types of domestic birds (eg, pigeons, chickens, ducks, geese, turkeys) and free-living birds (eg, gulls, egrets, petrels). In humans, C psittaci produces a spectrum of clinical manifestations ranging from severe pneumonia and sepsis with a high mortality rate to a mild inapparent infection.

Properties of the Agent

C psittaci can be propagated in embryonated eggs, in mice and other animals, and in some cell cultures. The heat-stable group-reactive CF antigen resists proteolytic enzymes and appears to be a lipopolysaccharide. Treatment of C psittaci infection with deoxycholate and trypsin yields extracts that contain group-reactive CF antigens, but the cell walls retain the species-specific antigen. Antibodies to the species-specific antigen are able to neutralize toxicity and infectivity. Specific serovars characteristic for certain mammalian and avian species may be demonstrated by immunofluorescence typing. Neutralization of infectivity of the agent by specific antibody or cross-protection of immunized animals can also be used for serotyping, and the results parallel those of immunofluorescence typing.

Pathogenesis and Pathology

The agent enters through the respiratory tract, is found in the blood during the first 2 weeks of the disease, and may be found in the sputum at the time the lung is involved. Psittacosis causes a patchy inflammation of the lungs in which consolidated areas are sharply demarcated. The exudates are predominantly mononuclear. Only minor changes occur in the large bronchioles and bronchi. The lesions are similar to those found in pneumonitis caused by some viruses and mycoplasmas. The liver, spleen, heart, and kidney are often enlarged and congested.

Clinical Findings

A sudden onset of illness taking the form of influenza or nonbacterial pneumonia in a person exposed to birds is suggestive of psittacosis. The incubation period averages 10 days. The onset is usually sudden, but can be insidious, with malaise, fever, anorexia, sore throat, photophobia, and severe headache. The disease may progress no further, and the patient may improve in a few days. In severe cases, the signs and symptoms of bronchial pneumonia appear at the end of the first week of the disease. The clinical picture often resembles that of influenza, nonbacterial pneumonia, or typhoid fever. The mortality rate may be as high as 20% in untreated cases, especially in elderly adults.

Laboratory Diagnosis

A. Culture

Culture of C psittaci can be dangerous, and detection of the organism using immunoassays or PCR is preferred. If necessary, C psittaci can be cultured from blood or sputum or from lung tissue by culture in tissue culture cells, embryonated eggs, or mice in an appropriate biosafety level-3 laboratory. Isolation of C psittaci is confirmed by the serial transmission, its microscopic demonstration, and serologic identification.

B. Antigen Detection of Chlamydia psittaci

Antigen detection by DFA staining or by immunoassay or molecular diagnosis by PCR is done in reference or research laboratories.

C. Serology

A diagnosis of psittacosis is usually confirmed by demonstrating complement-fixing or microimmunofluorescent antibodies in serum specimens. A confirmed case is one with a positive culture result or associated with a compatible clinical illness plus a fourfold or greater change in antibody titer to at least 1:32 or a single MIF IgM titer of at least 1:16. A probable case is one associated with a compatible illness linked epidemiologically with a confirmed case or a titer of at least 1:32 in a single specimen. The CF test is cross-reactive with C trachomatis and C pneumoniae. The MIF test is more sensitive and specific than the CF test, but cross-reactions do occur. MIF allows detection of IgM and IgG. Although antibodies usually develop within 10 days, the use of antibiotics may delay their development for 20–40 days or suppress it altogether.

In live birds, infection is suggested by a positive CF test result and an enlarged spleen or liver. This can be confirmed by demonstration of particles in smears or sections of organs and by passage of the agent in mice and eggs.

D. Molecular Methods

Multiple PCR assays have been developed to detect C psittaci in respiratory tract specimens, vascular tissues, serum, and mononuclear cells from peripheral blood. These tests are done in reference or research laboratories.

Immunity

Immunity in animals and humans is incomplete. A carrier state in humans can persist for 10 years after recovery. During this period, the agent may continue to be excreted in the sputum. Live or inactivated vaccines induce only partial resistance in animals. They have not been used in humans.

Treatment

Because of the difficulty in obtaining laboratory confirmation of C psittaci infection, most infections are treated based only on the clinical diagnosis. Information on therapeutic efficacy comes from several clinical trials. Doxycycline and tetracycline are the preferred agents for treatment; macrolides and fluoroquinolones may be alternatives.

Epidemiology and Control

Outbreaks of human disease can occur whenever there is close and continued contact between humans and infected birds that excrete or shed large amounts of infectious agent. Birds often acquire infection as fledglings in the nest, may develop diarrheal illness or no illness, and often carry the infectious agent for their normal lifespan. When subjected to stress (eg, malnutrition, shipping), birds may become sick and die. The agent is present in tissues (eg, the spleen) and is often excreted in feces by healthy birds. The inhalation of infected dried bird feces is a common method of human infection. Another source of infection is the handling of infected tissues (eg, in poultry rendering plants) and inhalation of an infected aerosol. Birds kept as pets have been an important source of human infection. Foremost among these were the many imported psittacine birds. Latent infections often flared up in these birds during transport and crowding, and sick birds excreted exceedingly large quantities of infectious agent. Control of bird shipment, quarantine, testing of imported birds for psittacosis infection, and prophylactic tetracyclines in bird feed have helped to control this source. Pigeons kept for racing or as pets or raised for squab meat have been important sources of infection. Pigeons populating buildings and thoroughfares in many cities, if infected, shed relatively small quantities of agent.

HELICOBACTER PYLORI

H pylori is a spiral-shaped, gram-negative rod. *H pylori* is associated with antral gastritis, duodenal (peptic) ulcer disease, gastric ulcers, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphomas.

Morphology and Identification

A. Typical Organisms

H pylori has many characteristics in common with campylobacters. It has multiple flagella at one pole and is actively motile.

B. Culture

Culture sensitivity can be limited by prior therapy, contamination with other mucosal bacteria, and other factors. *H pylori* grows in 3–6 days when incubated at 37°C in a microaerophilic environment, as for C jejuni. The media for primary isolation include Skirrow's medium with vancomycin, polymyxin B, and trimethoprim, chocolate medium, and other selective media with antibiotics (eg, vancomycin, nalidixic acid, amphotericin). The colonies are translucent and 1–2 mm in diameter.

C. Growth Characteristics

H pylori is oxidase positive and catalase positive, has a characteristic morphology, is motile, and is a strong producer of urease.

Pathogenesis and Pathology

H pylori grows optimally at a pH of 6.0–7.0 and would be killed or not grow at the pH within the gastric lumen. Gastric mucus is relatively impermeable to acid and has a strong buffering capacity. On the lumen side of the mucus, the pH is low (1.0–2.0); on the epithelial side, the pH is about 7.4. *H pylori* is found deep in the mucous layer near the epithelial surface where physiologic pH is present. *H pylori* also produces a protease that modifies the gastric mucus and further reduces the ability of acid to diffuse through the mucus. *H pylori* produces potent urease activity, which yields production of ammonia and further buffering of acid. *H pylori* is quite motile, even in mucus, and is able to find its way to the epithelial surface. *H pylori* overlies gastric-type but not intestinal-type epithelial cells. In human volunteers, ingestion of *H pylori* resulted in development of gastritis and hypochlorhydria. There is a strong association between the presence of *H pylori* infection and duodenal ulceration. Antimicrobial therapy results in clearing of *H pylori* and improvement of gastritis and duodenal ulcer disease.

The mechanisms by which *H pylori* causes mucosal inflammation and damage are not well defined but probably involve both bacterial and host factors. The bacteria invade the epithelial cell surface to a limited degree. Toxins and lipopolysaccharide may damage the mucosal cells, and the ammonia produced by the urease activity may also directly damage the cells. Histologically, gastritis is characterized by acute and chronic inflammation. Polymorphonuclear and mononuclear cell infiltrates are seen within the epithelium and lamina propria. Vacuoles within cells are often pronounced. Destruction of the epithelium is common, and glandular atrophy may occur. *H pylori* thus is a major risk factor for gastric cancer.

Clinical Findings

Acute infection can yield an upper gastrointestinal illness with nausea and pain; vomiting and fever may also be present. The acute symptoms may last for less than 1 week or as long as 2 weeks. After colonization, the *H pylori* infection persists for years and perhaps decades or even a lifetime. About 90% of patients with duodenal ulcers and 50–80% of those with gastric ulcers have *H pylori* infection. Recent studies confirm that *H pylori* also is a risk factor for gastric carcinoma and lymphoma.

Diagnostic Laboratory Tests

A. Specimens

Gastric biopsy specimens can be used for histologic examination or minced in saline and used for culture. Blood is collected for determination of serum antibodies. Stool samples may be collected for *H pylori* antigen detection.

B. Smears

The diagnosis of gastritis and *H pylori* infection can be made histologically. A gastroscopy procedure with biopsy is required. Routine stains demonstrate gastritis, and Giemsa or special silver stains can show the curved or spiral-shaped organisms.

C. Culture

As above, culture is performed when patients are not responding to treatment, and there is a need to assess susceptibility patterns.

D. Antibodies

Several assays have been developed to detect serum antibodies specific for H pylori. The serum antibodies persist even if the *H pylori* infection is eradicated, and the role of antibody tests in diagnosing active infection or after therapy is therefore limited.

E. Special Tests

Rapid tests to detect urease activity are widely used for presumptive identification of *H pylori* in specimens. Gastric biopsy material can be placed onto a urea-containing medium with a color indicator. If *H pylori* is present, the urease rapidly splits the urea (1–2 hours), and the resulting shift in pH yields a color change in the medium. In vivo tests for urease activity can be done also. In urea breath tests, 13C- or 14C-labeled urea is ingested by the patient. If *H pylori* is present, the urease activity generates labeled CO_2 that can be detected in the patient's exhaled breath. Detection of *H pylori* antigen in stool specimens is appropriate as a test of cure for patients with known *H pylori* infection who have been treated.

Immunity

Patients infected with *H pylori* develop an IgM antibody response to the infection. Subsequently, IgG and IgA are produced, and these persist, both systemically and at the mucosa, in high titer in chronically infected persons. Early antimicrobial treatment of *H pylori* infection blunts the antibody response; such patients are thought to be subject to repeat infection.

Treatment

Triple therapy with metronidazole and either bismuth subsalicylate or bismuth subcitrate plus either amoxicillin or tetracycline for 14 days eradicates *H pylori* infection in 70–95% of patients. An acid-suppressing agent given for 4–6 weeks enhances ulcer healing. Proton pump inhibitors (PPIs) directly inhibit *H pylori* and appear to be potent urease inhibitors. The preferred initial therapy is 7–10 days of a PPI plus amoxicillin and clarithromycin or a quadruple regimen of a PPI metronidazole, tetracycline, and bismuth for 10 days.

Epidemiology and Control

H pylori is present on the gastric mucosa of fewer than 20% of persons younger than years 30 but increases in prevalence to 40–60% of persons age 60 years, including persons who are asymptomatic. In developing countries, the prevalence of infection may be 80% or higher in adults. Person-to-person transmission of *H pylori* is likely because intrafamilial clustering of infection occurs. Acute epidemics of gastritis suggest a common source for H pylori.

CAMPYLOBACTER

Campylobacters cause both diarrheal and systemic diseases, and are among the most widespread causes of infection in the world. Campylobacter infection of domesticated animals also is widespread. C jejuni is the prototype organism in the group and is a very common cause of diarrhea in humans.

CAMPYLOBACTER JEJUNI

C jejuni has emerged as common human pathogen, causing mainly enteritis and occasionally systemic infection. These bacteria are at least as common as salmonellae and shigellae as a cause of diarrhea; an estimated 2 million cases occur in the United States each year.

Morphology and Identification

A. Typical Organisms

C jejuni are Gram-negative rods with comma, S, or "gull wing" shapes (Figure 17-3). They are motile, with a single polar flagellum, and do not form spores.

B. Culture

The culture characteristics are most important in the isolation and identification of C jejuni. Selective media are needed, and incubation must be in an atmosphere with reduced O2 (5% O2) with added CO2 (10% CO2). A relatively simple way to produce the incubation atmosphere is to place the plates in an anaerobe incubation jar without the catalyst and to produce the gas with a commercially available gas-generating pack or by gas exchange. Incubation of primary plates for isolation of C jejuni should be at 42°C. Although C jejuni grows well at 36–37°C, incubation at 42°C prevents growth of most of the other bacteria present in feces, thus simplifying the identification of C jejuni. Several selective media are in widespread use. Skirrow's medium contains vancomycin, polymyxin B, and trimethoprim to inhibit growth of other bacteria, but this medium may be less sensitive than other commercial products that contain charcoal, other inhibitory compounds, and cephalosporin antibiotics. The selective media are suitable for isolation of C jejuni at 42°C. The colonies tend to be colorless or gray. They may be watery and spreading or round and convex, and both colony types may appear on one agar plate.

C. Growth Characteristics

Because of the selective media and incubation conditions for growth, an abbreviated set of tests is usually all that is necessary for identification. C jejuni are positive for both oxidase and catalase. Campylobacters do not oxidize or ferment carbohydrates. Gram-stained smears show typical morphology. Nitrate reduction, hydrogen sulfide production, hippurate tests, and antimicrobial susceptibilities can be used for further identification of species.

Antigenic Structure and Toxins

The campylobacters have lipopolysaccharides with endotoxic activity. Cytopathic extracellular toxins and enterotoxins have been found, but the significance of the toxins in human disease is not well defined.

Pathogenesis and Pathology

The infection is acquired by the oral route from food, drink, or contact with infected animals or animal products, especially poultry. C jejuni is susceptible to gastric acid, and ingestion of about 104 organisms is usually necessary to produce infection. This inoculum is similar to that required for Salmonella and Shigella infection but less than that for Vibrio infection. The organisms multiply in the small intestine, invade the epithelium, and produce inflammation that results in the appearance of red and white blood cells in the stools. Occasionally, the bloodstream is invaded, and a clinical picture of enteric fever develops. Localized tissue invasion coupled with the toxic activity appears to be responsible for the enteritis.

Clinical Findings

Clinical manifestations are acute onset of crampy abdominal pain, profuse diarrhea that may be grossly bloody, headache, malaise, and fever. Usually the illness is self-limited to a period of 5–8 days, but occasionally it continues longer. C jejuni isolates are usually susceptible to erythromycin, and therapy shortens the duration of fecal shedding of bacteria. Most cases resolve without antimicrobial therapy; however, in about 5–10% of patients, symptoms may recur. Certain serotypes of C jejuni have been associated with postdiarrheal Guillain-Barré syndrome, a form of ascending paralytic disease. Reactive arthritis and Reiter's syndrome may also follow acute Campylobacter diarrhea.

Diagnostic Laboratory Tests

A. Specimens

Diarrheal stool is the usual specimen. C jejuni may occasionally be recovered from blood cultures usually from immunocompromised or elderly patients. Other extraintestinal infections are uncommon.

B. Smears

Gram-stained smears of stool may show the typical "gull wing"–shaped rods. Dark-field or phase contrast microscopy may show the typical darting motility of the organisms.

C. Culture

Culture on the selective media described earlier is the definitive test to diagnose C jejuni enteritis. If another species of Campylobacter is suspected, medium without a cephalosporin should be used and incubated at 36–37°C.

Epidemiology and Control

Campylobacter enteritis resembles other acute bacterial diarrheas, particularly shigella dysentery. The source of infection may be food (eg, milk, undercooked fowl) or contact with infected animals or humans and their excreta. Outbreaks arising from a common source, such as unpasteurized milk, may require public health control measures.