Weak	Subject
1	*Staphylococcus aureus*
2	*STREPTOCOCCUS PYOGENES*
3	*STREPTOCOCCUS PNEUMONIAE*
4	*Neisseria gonorrhoeae (gonococci)*
5	*Neisseria meningitidis (meningococci*)
6	*Clostridium Species*
7	*BACILLUS ANTHRACIS , BACILLUS CEREU ,CORYNEBACTERIUM DIPHTHERIAE S*
8	*Examination*
9	Enteric Gram-Negative Rods (Enterobacteriaceae) *Escherichia coli, Proteus species ,klebsiella pneumoniaei*
10	*Shigellae species , Salmonellae Species*
11	*THE PSEUDOMONAD GROUP*
12	*Vibrio cholerae , H pylori*
13	*Haemophilus influenzae*
14	*THE BRUCELLAE Species*
15	*MYCOBACTERIUM TUBERCULOSIS*

References

1 -Jawetz, Melnick, & Adelberg's Medical Microbiology twenty-fourth edition Geo. F. Brooks, et al

2 -Medical Microbiology and Infection at a Glance Stephen H. et al

3 - SHERRIS MEDICAL MICROBIOLOGY AN INTRODUCTION TO INFECTIOUS DISEASES EDITORS KENNETH J. RYAN, M ,et al 4TH EDITION

The Staphylococci

The staphylococci are gram-positive spherical cells, usually arranged in grape-like irregular clusters. They grow readily on many types of media and are active metabolically, Some are members of the normal flora 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 :

1 - hemolyze blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins.

2 -The most common type of food poisoning is caused by a heat-stable staphylococcal enterotoxin.

3 -Staphylococci rapidly develop resistance to many antimicrobial agents and present difficult therapeutic problems.

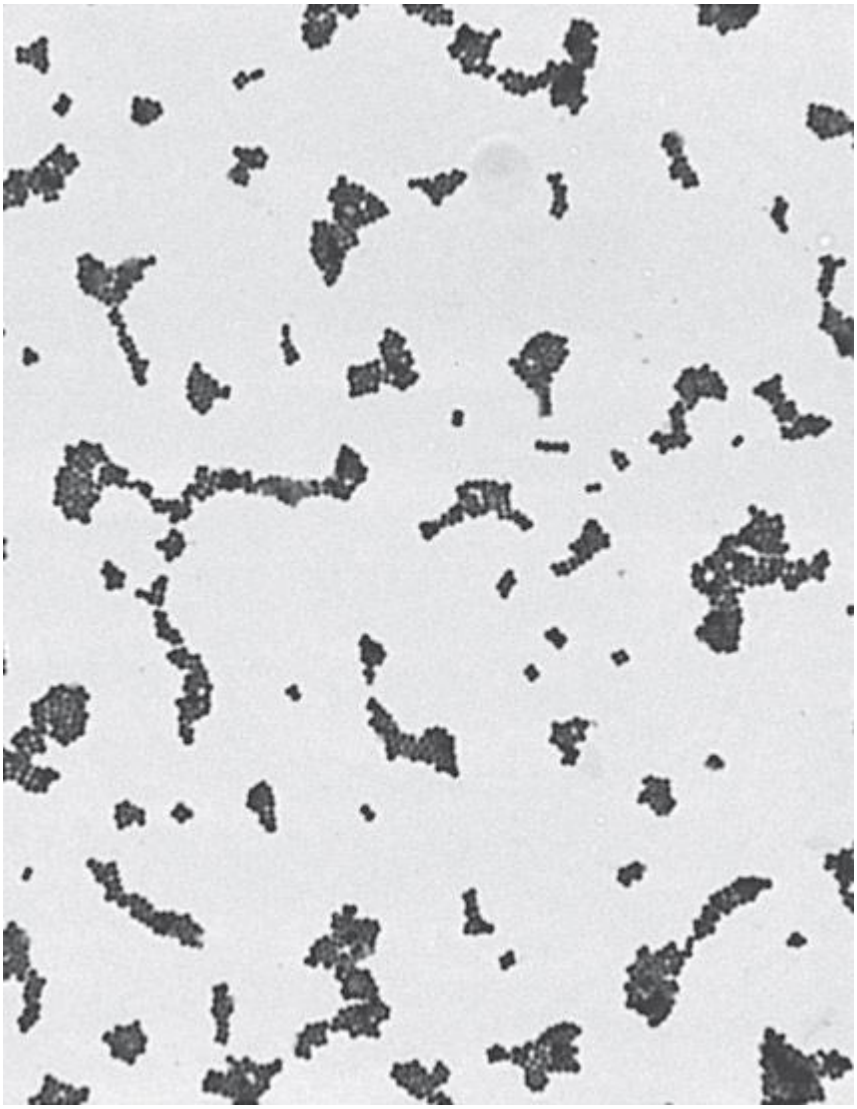
4 -The genus Staphylococcus has at least 35 species. The three main species of clinical importance *are Staphylococcus aureus, Staphylococcus epidermidis, and Staphylococcus saprophyticus. Staphylococcus aureus* is coagulase-positive, which differentiates it from the other species. S aureus is a major pathogen for humans.

5 -The coagulase-negative staphylococci are normal human flora and sometimes cause infection

Morphology & Identification

A. TYPICAL ORGANISMS

Staphylococci are spherical cells about 1 µm in diameter arranged in irregular clusters . 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 non motile and do not form spores.



B. CULTURE

Staphylococci grow readily on most bacteriologic media under aerobic or micro aerophilic 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. *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 an aerobically or in broth. Various degrees of hemolysis are produced by *S aureus* and occasionally by other species.

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 9% sodium chloride but are readily inhibited by certain chemicals, eg, 3% hexachlorophene .**Staphylococci are variably sensitive to many antimicrobial drugs**

D. VARIATION

A culture of staphylococci contains some bacteria that differ from the bulk of the population in

1- expression of colony characteristics (colony size, pigment, hemolysis) 2 - in enzyme elaboration . 3-in drug resistance, and in pathogenic .

4 - 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 .

Antigenic Structure :

Staphylococci contain antigenic polysaccharides and proteins as well as other substances important in cell wall structure:

1- Peptidoglycan, a polysaccharide polymer containing linked subunits, provides the rigid exoskeleton of the cell wall. 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 chemo attractant for polymorphonuclear leukocytes, have endotoxin-like activity, and activate complement.

2 - Teichoic acids, which are polymers of glycerol or ribitol phosphate, are linked to the peptidoglycan and can be antigenic. Antiteichoic acid

antibodies detectable by gel diffusion may be found in patients with active endocarditis due *to S aureus.*

3 - Protein A is a cell wall component of many *S aureus* strains that binds to the Fc portion of IgG molecules except IgG3. The Fab portion of 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 ,

4 - Some *S aureus* strains have capsules, which inhibit phagocytosis by polymorphonuclear leukocytes unless specific antibodies are present.

5 - Most strains of S aureus have coagulase, or clumping factor, on the cell wall surface; coagulase binds non enzymatically to fibrinogen. yielding aggregation of the bacteria.

Enzymes & Toxins

Staphylococci can produce disease both through :

1 - their ability to multiply and spread widely in tissues.

2 - and through their production of many extracellular substances. Some of these substances are enzymes; others are considered to be toxins, though 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**

1 - coagulase, an enzyme-like 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.

2 - Clumping factor is a *surface S aureus* compound 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.

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. EXOTOXINS

The α-toxin is a heterogeneous protein that acts on a broad spectrum of eukaryotic cell membranes. The α- toxin is a potent hemolysin**. 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 refers to three proteins that interact with the two proteins comprising the Panton-Valentine leukocidin (see below) 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.**

E. LEUKOCIDIN

This toxin of *S aureus* has two components. It can kill white blood cells of humans and rabbits. The two components act synergistically on the white blood cell membrane as described above for γ toxin. This toxin is an important virulence factor in community associated methicillin resistant *S aureus* infections.

F. EXFOLIATIVE TOXINS

These epidermolytic toxins of *S aureus* are two distinct proteins of the same molecular weight. Epidermolytic toxin A is a chromosomal gene product and is heat-stable (resists boiling for 20 minutes). Epidermolytic toxin B is plasmid-mediated and heat-labile. The 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 .

H. ENTEROTOXINS

There are multiple (A–E, G–I, K–M) enterotoxins. Approximately 50% *of S aureus* strains can produce one or more of them. Like TSST-1, the enterotoxins are superantigens. The enterotoxins are heat-stable and resistant to the action of gut enzymes. An important cause 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 flora 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.

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), **suppuration (abscess**) is typical of staphylococcal infections . 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 suppurat**ion.

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**). Staphylococci also cause disease through the elaboration of toxins, without apparent invasive infection. the scalded skin syndrome, is caused by the production of exfoliative toxins .

Diagnostic Laboratory Tests

A. SPECIMENS

Surface swab pus, blood, tracheal aspirate, or spinal fluid for culture, depending upon the localization of the process.

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 saprophytic *(S epidermidis*) from 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 flora can be cultured on media containing 7.5% NaCl; the salt inhibits most other normal flora but *not S aureus*. Mannitol salt agar or commercially available chromogenic media are used to screen for nasal carriers *of S aureus* and 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.

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 is positive. Coagulase-positive staphylococci are considered pathogenic .

F. SUSCEPTIBILITY TESTING

Broth micro dilution or disk diffusion susceptibility testing should be done routinely on staphylococcal isolates from clinically significant infections.

1- Resistance to penicillin G can be predicted by a positive test for β-lactamase; approximately 90% of S aureus produce β- lactamase

2 -.Resistance to nafcillin (and oxacillin and methicillin) occurs in about 35% *of S aureus* and approximately 75% of S epidermidis isolates. Nafcillin resistance correlates with the presence of mecA, the gene that codes for a penicillin-binding protein (PBP 2a) not affected by these drugs. The gene can be detected using the polymerase chain reaction.

Most clinical laboratories use a phenotypic method such as an oxacillin screening agar plate. Staphylococci that grow on Mueller-Hinton agar +9containing 4% NaCl and 6 µg/mL of oxacillin typically are mecA-po9+sitive and nafcillin-resistant. Alternatively, an assay for the mecA gene product, PBP 2a, is commercially available and is much more rapid than PCR for mecA or than testing for resistance using growth on oxacillin-containing salt agar.

Epidemiology & Control

Staphylococci are ubiquitous human parasites**. The chief sources** of infection are :

1- shedding human lesions, fomites contaminated from such lesions, and the human respiratory tract and skin.

2 - Contact spread of infection has assumed added importance in hospitals, where a large proportion of the staff and patients 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 , In hospitals, the areas at highest risk for severe staphylococcal infections are the newborn nursery, 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.so that Personnel with active *S aureus* lesions and carriers must be :

1- have to be excluded from these areas.

2 - In such individuals, the application of topical antiseptics to nasal or perineal carriage sites may diminish shedding of dangerous organisms. Rifampin coupled with a second oral anti staphylococcal 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.

3 - Patients who test positive by culture or PCR are placed upon contact precautions so as to minimize spread on the hands of health care workers ,by wearing gloves and washing hands before and after patient contact .

The Streptococci

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 and other cell wall or capsular antigens;

(3) biochemical reactions and resistance to physical and chemical factors; and

(4) ecologic features. Molecular genetics have also been used to study the streptococci. Combinations of the above methods have permitted the classification of streptococci for purposes of clinical and epidemiologic convenience .

A. HEMOLYSIS

1 - Complete disruption of erythrocytes with clearing of the blood around the bacterial growth is called β hemolysis.

2- Incomplete lysis of erythrocytes with reduction of hemoglobin and the formation of green pigment is called α hemolysis.

3 - Other streptococci are non-hemolytic (sometimes called gamma hemolysis). The hemolysis patterns of the streptococci of medical importance .

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 group-specific carbohydrate is determined by an amino sugar.

C - CAPSULAR POLYSACCHARIDES

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

D. BIOCHEMICAL REACTIONS

Biochemical tests include sugar fermentation reactions, tests for the presence of enzymesand tests for susceptibility or resistance to certain chemical agent

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 .

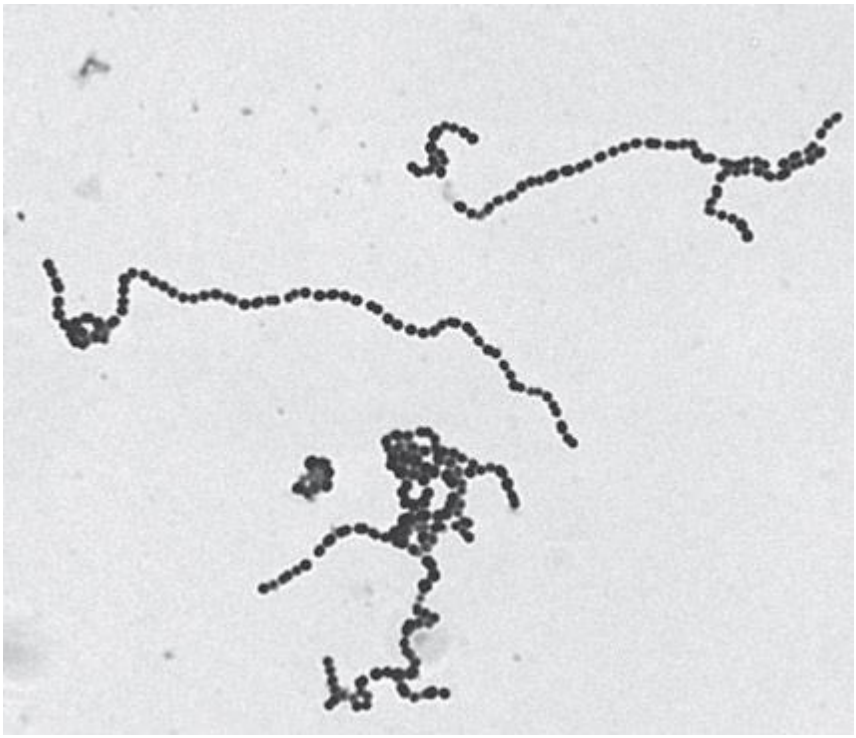
STREPTOCOCCUS PYOGENES

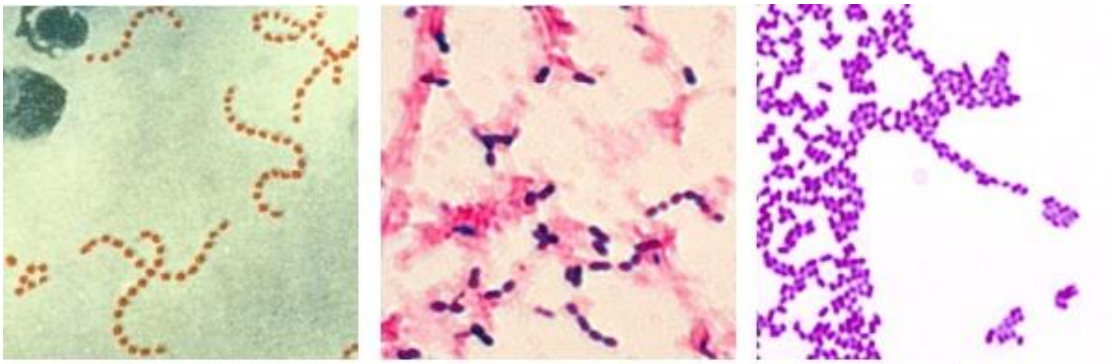
Most streptococci that contain the group A antigen *are S pyogenes*. It is a prototypical human pathogen*, S pyogenes* is the main human pathogen associated with local or systemic invasion and poststreptococcal immunologic disorders.

Morphology & Identification

A. TYPICAL ORGANISMS

Individual cocci are spherical or ovoid and are arranged in chains 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.** Capsules of other streptococci (eg*, S agalactiae and S pneumonia*e) 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.

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 , Streptococci grown in broth showing gram-positive cocci in chains. colonies**. Matte colonies** consist of organisms that produce much M protein and generally are virulent. The *S pyogenes* **in glossy colonie**s tend to produce little M protein and are often not virulent.

Antigenic Structure

A. M PROTEIN This substance is a major virulence factor of group A

S pyogenes. M protein appears as hair-like projections of 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*. S pyogenes* that lack M protein are not virulent.

B. T SUBSTANCE This antigen has no relationship to virulence of streptococci, T substance is acid-labile and heat-labile. It T substance permits differentiation of certain types of streptococci by agglutination with specific antisera, while other types share the same T substance. Yet another surface antigen has been called R protein.

C. NUCLEOPROTEINS Extraction of streptococci with weak alkali yields mixtures of proteins and other substances of little serologic specificity, called P substances, which probably make up most of the streptococcal cell body.

Toxins & 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.

B. STREPTODORNASE (streptococcal deoxyribonuclease) depolymerizes DNA. Mixtures of streptodornase and streptokinase 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 splits hyaluronic acid, an important component of the ground substance of connective tissue. Thus, hyaluronidase aids in spreading infecting microorganisms (spreading factor).

D. PYROGENIC EXOTOXINS (ERYTHROGENIC TOXIN) Pyrogenic exotoxins are elaborated by S pyogenes. There are three antigenically distinct streptococcal pyrogenic exotoxins: A, B, and C. The streptococcal pyrogenic exotoxins have been associated with streptococcal toxic shock syndrome and scarlet fever.

E. DIPHOSPHOPYRIDINE NUCLEOTIDASE This enzyme is may be related to the organism's ability to kill leukocytes**. Proteinases and amylase** are produced by some strains.

F. HEMOLYSINS The β-hemolytic group A *S pyogenes* elaborates two hemolysins (streptolysins).

A - Streptolysin O is a protein (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 is in cuts deep into the medium in blood**

agar plates. It combines quantitatively with antistreptolysin O, an antibody that appears in humans following 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 antistreptolysin O (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 due to an exaggerated immune response to an earlier exposure in a hypersensitive person.**

B - 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 .

STREPTOCOCCUS PYOGENES infections :

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

1. Erysipelas—If the portal of entry is the skin, erysipelas results, with massive brawny edema and a rapidly advancing margin of infection

2. Cellulitis—Streptococcal cellulitis is an acute, rapidly spreading infection of the skin and subcutaneous tissues. 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)— This is infection of the subcutaneous tissues and fascia. There is extensive and very rapidly spreading necrosis of the skin and subcutaneous tissues .

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/Sepsis—Infection of traumatic or surgical wounds with streptococci results in bacteremia, which rapidly can be fatal. S pyogenes bacteremia can also follow skin infections, such as cellulitis and rarely pharyngitis.

B. DISEASES ATTRIBUTABLE TO LOCAL INFECTION WITH S PYOGENES AND THEIR BY-PRODUCTS

1. Streptococcal Sore Throat—The most common infection due to β-hemolytic *S pyogenes* is streptococcal sore throat or pharyngitis

2. Streptococcal Pyoderma—Local infection of superficial layers of skin, especially in children, is called impetigo

C. INVASIVE GROUP A STREPTOCOCCAL INFECTIONS, STREPTOCOCCAL TOXIC SHOCK SYNDROME, AND SCARLET FEVER Fulminant, invasive *S pyogenes i*nfections with streptococcal toxic shock syndrome are characterized by shock, bacteremia, respiratory failure, and multiorgan failure. Death occurs in about 30% of patients.

D. POSTSTREPTOCOCCAL DISEASES (RHEUMATIC FEVER, GLOMERULONEPHRITIS) .

1. Acute Glomerulonephritis—This sometimes develops 3 weeks after S *pyogenes* skin infection (pyoderma, impetigo). Glomerulonephritis may be initiated by antigen-antibody complexes on the glomerular basement membrane. The most important antigen is probably in the streptococcal protoplast membranee majority recover 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 .

Diagnostic Laboratory Tests

A. SPECIMENS to be obtained depend upon the nature of the streptococcal infection. A throat swab, pus, or blood is obtained for culture. Serum is obtained for antibody determinations.

B. 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 occasionally do 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. ANT IGEN DETECTION TESTS Several commercial kits are available for rapid detection of group A streptococcal antigen from throat swabs.

E. SEROLOGIC TESTS A rise in the titer of antibodies to many group A streptococcal antigens can be estimated. Such antibodies include antistreptolysin O (ASO), particularly in respiratory disease; anti-DNase and antihyaluronidase, particularly in skin infections; antistreptokinase;

anti-M type-specific antibodies; and others. Of these, the antiASO titer is most widely used.

Control procedures are directed mainly at the human source:

(1) Detection and early antimicrobial therapy of respiratory and skin infections with group A streptococci.

(2) Antistreptococcal chemoprophylaxis in persons who have suffered an attack of rheumatic fever .

(3) Eradication of *S pyogenes* from carriers. This is especially important when carriers are in areas such as obstetric delivery rooms, operatin

VIRIDANS STREPTOCOCCI

The viridans streptococci include *S mitis, S mutans, S salivarius, S sangui*s, and others. Typically they are α- hemolytic, but they may be nonhemolytic.

Their growth is not inhibited **by Optochin,** and colonies are not soluble in bile (deoxycholate).

The viridans streptococci are the most prevalent members of the normal flora of the upper respiratory tract and are important for the healthy state of the mucous membranes there. They may reach the bloodstream as a result of trauma and are a principal cause of endocarditis on abnormal heart valves.

Some viridans streptococci (eg*, S mutans*) synthesize large polysaccharides such as dextrans or levans from sucrose and contribute importantly to the genesis of dental caries.

STREPTOCOCCUS PNEUMONIAE

The pneumococci (*S pneumoniae*) are gram-positive diplococci, often lancet-shaped or arranged in chains, possessing a capsule of polysaccharide that permits typing with specific antisera. Pneumococci are normal inhabitants of the upper respiratory tract of 5–40% of humans and can cause pneumonia, sinusitis, otitis, bronchitis, bacteremia, meningitis, and other infectious processes.

Morphology & Identification

A. TYPICAL ORGANISMS

The typical gram-positive, lancet-shaped diplococci , are often seen in specimens of young cultures. In sputum or pus, single cocci or chains are also seen. With age, the organisms rapidly become gram-negative and tend to lyse spontaneously. Autolysis of pneumococci is greatly enhanced by surface-active agents**. Lysis of pneumococci occurs in a few minutes when ox bile (10%) or sodium deoxycholate (2%) is added to a broth culture or suspension of** organisms at neutral pH.

On solid media, the growth of pneumococci is inhibited around a disk of Optochin; viridans streptococci are not inhibited by Optochin. Other identifying points include almost uniform virulence for mice when injected intraperitoneally and **the "capsule swelling test," or quellung reaction .**

B. CULTURE Pneumococci form small round colonies, at first domeshaped and later developing a central plateau with an elevated rim. Pneumococci are **α-hemolytic on blood agar**. Growth is enhanced by 5–10% CO2 .

c -VARIATION Pneumococcal isolates that produce large amounts of capsules produce large mucoid colonies.

Antigenic Structure

A. COMPONENT STRUCTURES The pneumococcal cell wall has peptidoglycan and teichoic acid, like other streptococci. The capsular polysaccharide is covalently bound to the peptidoglycan and to the cell wall polysaccharide. The capsular polysaccharide is immunologically distinct for each of the more than 90 types.

B. QUELLUNG REACTION When pneumococci of a certain type are mixed with specific anti polysaccharide serum of the same type—or with polyvalent antiserum—on a microscope slide, the capsule swells markedly, and the organisms agglutinate by cross linking of the antibodies**. This reaction is useful for rapid identification and for typing**

of the organisms, either in sputum or in cultures. The polyvalent antiserum, which contains antibody to all of the types (“omniserum”), is a good reagent for rapid microscopic determination of whether or not pneumococci are present in fresh sputum.

A. TYPES OF PNEUMOCOCCI

In adults, types 1–8 are responsible for about 75% of cases of pneumococcal pneumonia and for more than half of all fatalities in pneumococcal bacteremia; in children, types 6, 14, 19, and 23 are frequent causes.

B. PRODUCTION OF DISEASE

Pneumococci produce disease through their ability to multiply in the tissues. They produce no toxins of significance. The virulence of the organism is a function of its capsule, which prevents or delays ingestion by phagocytosis .

C. LOSS OF NATURAL RESISTANCE

Since 40–70% of humans are at some time carriers of virulent pneumococci, the normal respiratory mucosa must possess great natural resistance to the pneumococcus

(1) Viral and other respiratory tract infections that damage surface cells; abnormal accumulations of mucus (eg, allergy), which protect pneumococci from phagocytosis; bronchial obstruction (eg, atelectasis); and respiratory tract injury due to irritants disturbing its mucociliary function.

(2) Alcohol or drug intoxication, which depresses phagocytic activity, depresses the cough reflex, and facilitates aspiration of foreign material.
(3) Abnormal circulatory dynamics (eg, pulmonary congestion, heart failure).

(4) Other mechanisms, eg, malnutrition, general debility, sickle cell anemia, hyposplenism, nephrosis, or complement deficiency.

Diagnostic Laboratory Tests

Blood is drawn for culture; CSF and sputum are collected for demonstration of pneumococci by smear and culture. Serum antibody tests are impractical. Sputum may be examined in several ways

A. STAINED SMEARS A Gram-stained film of rusty-red sputum shows typical organisms, many polymorphonuclear neutrophils, and many red cells.

B. CAPSULE SWELLING TESTS Fresh emulsified sputum mixed with antiserum causes capsule swelling (the quellung reaction) for identification of pneumococci .

C. CULTURE The culture is created by sputum cultured on blood agar and incubated in CO2 or a candle jar. A blood culture is also taken. Immunity Immunity to infection with pneumococci is type-specific and depends both on antibodies to capsular polysaccharide and on intact phagocytic function.

ENTEROCOCCI

The enterococci have the group D group-specific substance and were previously classified as group D streptococci. Because the group D cell wall specific antigen is a teichoic acid, it is not an antigenically good marker; enterococci are usually identified by characteristics other than immunologic reaction with group-specific antisera.

They are part of the normal enteric flora. They are usually nonhemolytic, but occasionally α-hemolytic. Enterococci are PYR-positive. They grow in the presence of bile and hydrolyze esculin (bile esculin-positive). They grow in 6.5% NaCl. They grow well at between 10 °C and 45 °C whereas streptococci generally grow at a much narrower temperature range. They are more resistant to penicillin G than the streptococci, and rare isolates have plasmids that encode for β-lactamase.

Enterococci are transmitted from one patient to another primarily on the hands of hospital personnel, some of whom may carry the enterococci in their gastrointestinal tracts. Enterococci occasionally are

transmitted on medical devices. In patients, the most common sites of infection are the urinary tract, wounds, biliary tract, and blood.

Enterococci may cause meningitis and bacteremia in neonates. In adults, enterococci can cause endocarditis. However, in intra-abdominal, wound, urine, and other infections .

The Neisseriae

The family Neisseriaceae includes the genera Neisseria, Kingella, Eikenella, Simonsiella, Alysiella, and several unnamed species **The neisseriae are gram-negative cocci that usually occur in pairs. *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

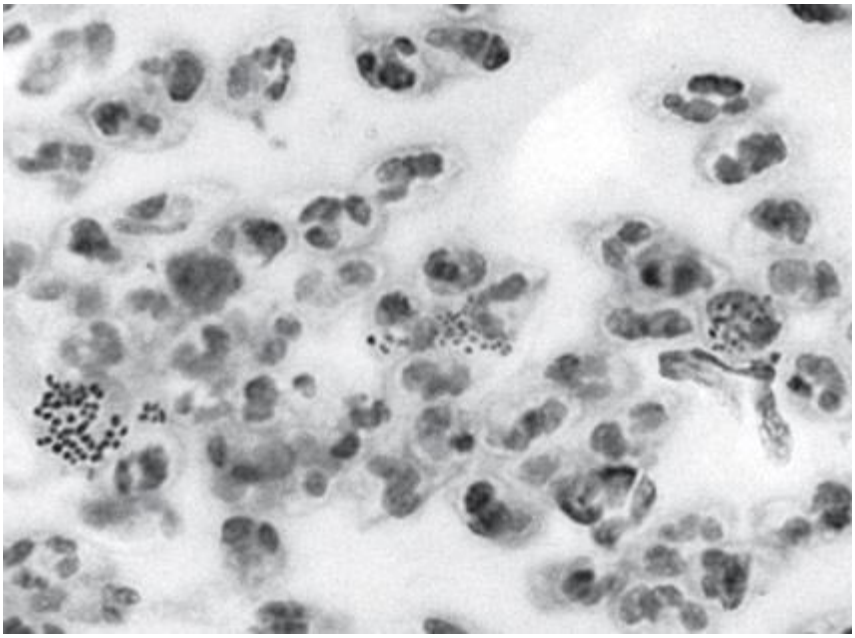
of the human respiratory tract, rarely if ever cause disease, and occur extracellularly .

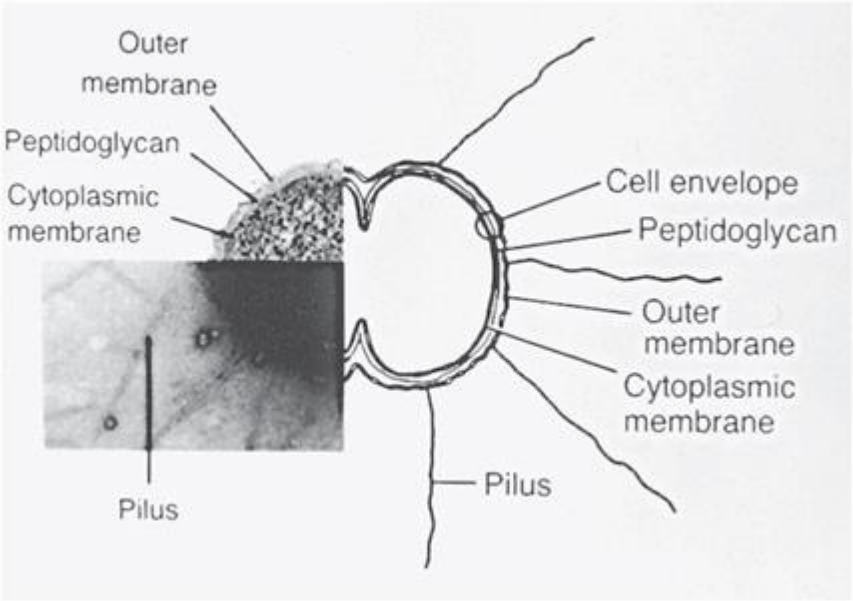
(comparison) : Gonococci and meningococci are closely related, with 70% DNA homology, and are differentiated by a few laboratory tests and specific characteristics:

1 - Meningococci have polysaccharide capsules, whereas gonococci do not.

2 - and meningococci rarely have plasmids whereas most gonococci do.

3 - 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, while gonococci cause genital infections.





Morphology & Identification

A. TYPICAL ORGANISMS

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

B. CULTURE

In 48 hours on enriched media (eg, Mueller-Hinton, modified Thayer-Martin), gonococci and meningococci form convex, glistening, elevated, mucoid colonies 1–5 mm in diameter. Colonies are transparent or opaque, non pigmented, and non hemolytic. *Neisseria flavescens, Neisseria subflava, and Neisseria lactamica* have yellow pigmentation. *Neisseria sicca* produces opaque, brittle, wrinkled colonies. *M catarrhalis* produces non pigmented or pinkish-gray opaque colonies.

C. GROWTH CHARACTERISTICS

The neisseriae grow best under aerobic conditions, but some will grow in an anaerobic environment. They have complex growth requirements. Most neisseriae ferment carbohydrates, producing acid but not gas, and their carbohydrate fermentation 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 tetramethyl paraphenylenediamine 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% CO2 (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 .

NEISSERIA GONORRHOEAE

Gonococci ferment 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– , 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 non piliated 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 - (FIBRIAE) Pili are the hair-like 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 (MW 17,000–21,000).

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 occurs in **trimers to form 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**. The molecular weight of Por varies from 34,000 to 37,000. Each strain of gonococcus expresses only one of two types of Por, but the Por of different strains is antigenically different .

C. OPA PROTEINS

These proteins function in adhesion of gonococci within colonies and in attachment of gonococci to host cells .

D. RMP (PROTEIN III)

This protein (MW about 33,000) is antigenically conserved in all gonococci. It is a reduction-modifiable protein (Rmp) and changes its apparent molecular weight when in a reduced state**. It associates with Por in the formation of pores in the cell surface.**

E. LIPOOLIGOSACCHARIDE (LOS) In contrast to the enteric gram-negative rods , gonococcal LPS does not have long Oantigen side chains and is called a lipooligosaccharide. Its molecular weight is 3000–7000. Gonococci can express more than one antigenically different LOS chain simultaneously.

Toxicity in gonococcal infections is largely due to the endotoxic effects of LOS. In a form of molecular mimicry, gonococci make LOS molecules that structurally resemble human cell membrane glycosphingolipids. A structure 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.

F. OTHER PROTEINS Several antigenically constant proteins of gonococci have poorly defined roles in pathogenesis.

Pathogenesis

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 mid cycle**. Gonococci that form transparent** colonies are frequently isolated from men with asymptomatic urethral infection, from menstruating women, and from 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.

In males, there is usually 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 females, 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 or proctitis is often asymptomatic

Gonococcal **bacteremia leads to skin lesion**s (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. Complement deficiency is frequently found in patients with **gonoccal bacteremia. Gonococcal ophthalmi**a **neonatorum**, an infection of the eye of the newborn, is acquired during passage through an infected birth canal.

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

B. SMEARS

Gram-stained smears of urethral or endocervical exudate reveal many diplococci within pus cells. Stained smears of endocervical exudates have a sensitivity of about 50% and a specificity of about 95% when examined by an experienced microscopist. Cultures of urethral exudate from men are not necessary when the stain is positive, but 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) and incubated in an atmosphere containing 5% CO2 (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 CO2-containing transport-culture system. Forty-eight hours after culture, the organisms can be quickly identified by their appearance on a Gram-stained smear, by oxidase positivity, and by co agglutination, immuno fluorescence staining, or other laboratory tests. The species of sub cultured bacteria may be determined by fermentation reactions .

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.

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 ELISA (enzyme-

linked immunosorbent assay) tests. However, these tests are not useful as diagnostic aids for several reasons: gonococcal antigenic heterogeneity; the delay in development of antibodies in acute infection; and a high background level of antibodies in the sexually active population.

NEISSERIA MENINGITIDIS

Antigenic Structure At least 13 serogroups of meningococci have been **identified by immunologic specificity of capsular polysaccharides**. The most important sero groups associated with disease in humans are A, B, C, Y, and W-135. Meningococcal antigens are found in blood and cerebrospinal fluid of patients with active disease.

The outer membrane proteins of meningococci have been divided into classes on the basis of molecular weight. All strains have either class 1, class 2, or class 3 proteins; these are analogous to the Por proteins of gonococci and are responsible for the serotype specificity of meningococci.

Meningococcal LPS is responsible for many of the toxic effects found in meningococcal disease.

Pathogenesis

Humans are the only natural hosts for whom meningococci are pathogenic**. The nasopharynx is the portal of entr**y. There, the organisms attach to epithelial cells with the aid of pili; they may form part of the transient flora without producing symptoms. From the nasopharynx, organisms may reach the bloodstream, producing bacteremia**; Meningitis** is the most common complication of meningococcemia. It usually begins suddenly, with 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 petechia**l hemorrhages. There may be **interstitial myocarditis, arthritis, and skin lesions.**

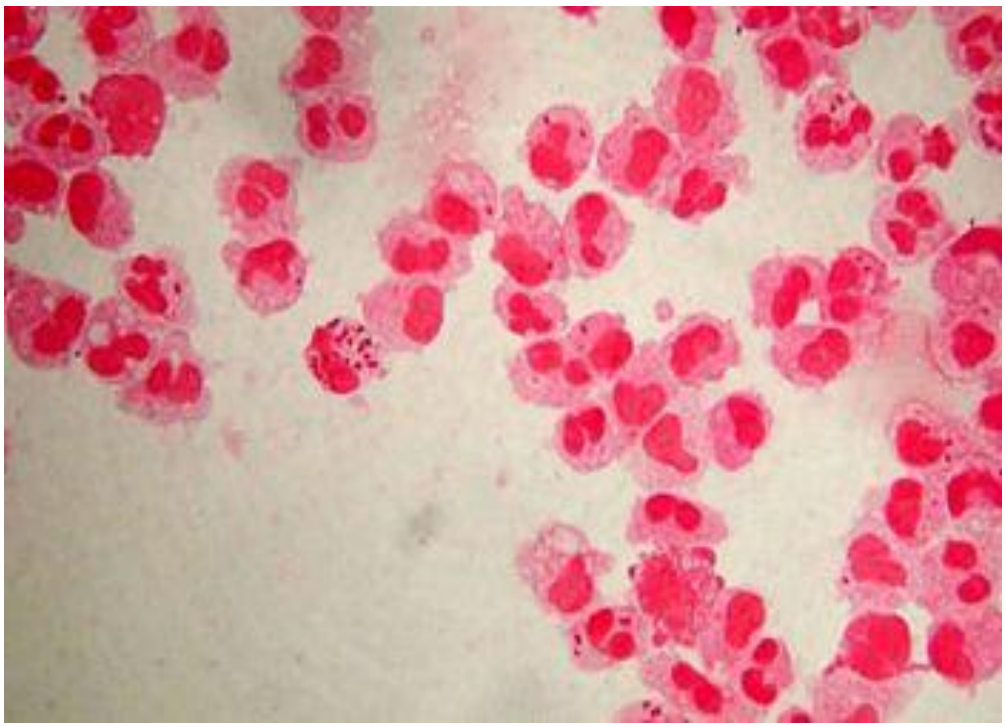
Neisseria bacteremia is favored by the absence of bactericidal antibody (IgM and IgG), inhibition of serum bactericidal action by a blocking IgA antibody, or a complement component deficiency (C5, C6, C7, or C8). Meningococci are readily phagocytosed in the presence of a specific opsonin.

Diag**nostic Laboratory Tests**

A. SPECIMENS

Specimens of blood are taken for culture, and specimens **of spinal fluid** are taken for smear, culture, and chemical determinations. **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 fluidpetechial aspirate often show typical neisseriae wpolymorphonucleukocytes or extracellularly.polymorpor extracellularl



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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**

(candle jar). Freshly drawn spinal fluid can be directly incubated at 37 °C if agar culture media are not immediately available**. A modified Thayer-Martin medium 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 fermentation 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.

Epidemiology, Prevention, & Control

Meningococcal meningitis occurs in epidemic waves , Five to 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.

More important is the reduction of personal contacts in a population with a high carrier rate.

Spore-Forming Gram-Positive Bacilli: Bacillus & Clostridium Species

The gram-positive spore-forming bacilli are the Bacillus and Clostridium species. These bacilli are ubiquitous, and because they form spores they can survive in the environment for many years**. Bacillus species are aerobes, whereas clostridia are anaerobes. is caused by *Bacillus anthracis.*** Anthrax remains an important disease of animals and occasionally of humans, *and B anthracis* is a major agent of bioterrorism and biologic warfare. **Bacillus cereus causes food poisoning** and occasionally eye or other localized infections.

Clostridia cause several important toxin-mediated diseases: *Clostridium tetani, te**tanus**; Clostridium botulinum, **botulism;** Clostridium perfringens, **gas gangrene**; and Clostridium difficile, pseudomembranous **colitis***

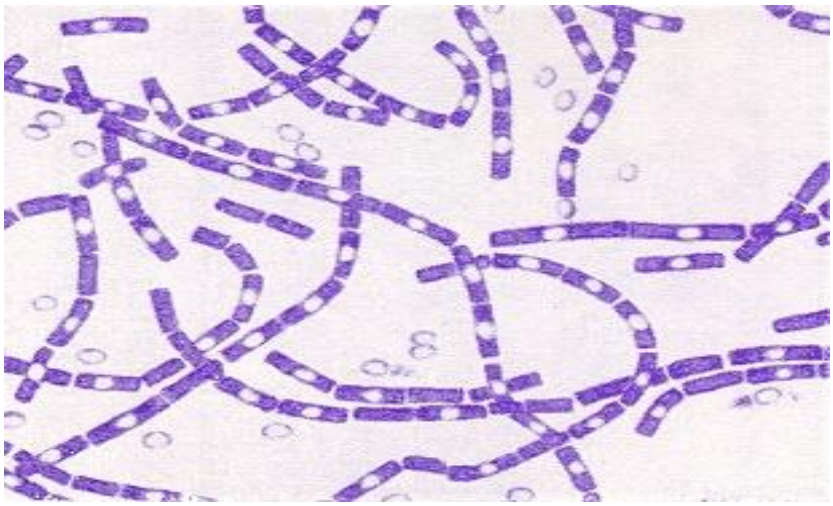
■ ***BACILLUS SPECIES***

The genus bacillus **includes large aerobic, gram-positive rod**s occurring in chains.

Morphology & Identification

A. TYPICAL ORGANISMS

The typical cells, measuring 1 × 3–4 µm, have square ends and are arranged in long chains; **spores are located in the center** of **the non motile bacilli.**



B. CULTURE

Colonies *of B anthracis* are round and have a “cut glass” appearance in transmitted light. Hemolysis is uncommon with B anthracis but common with the saprophytic bacilli. Gelatin is liquefied, and growth in gelatin stabs resembles an inverted fir tree.

C. GROWTH CHARACTERISTICS

The spores are resistant to environmental changes, withstand dry heat and certain chemical disinfectants for moderate periods, and persist for years in dry earth. Animal products contaminated with anthrax spores (eg, hides, bristles, hair, wool, bone) can be sterilized by autoclaving

BACILLUS ANTHRACIS

Pathogenesis Anthrax is primarily a disease of herbivores—goats, sheep, cattle, horses, etc; other animals (eg, rats) are relatively resistant to the infection. **Humans become infected incidentally by contact with infected animals or their products. In animals, the portal of entry is the mouth and the gastrointestinal tract. Spores from contaminated soil** find easy access when ingested with spiny or irritating vegetation.

In humans, the infection is usually acquired by the entry of spores through injured skin (cutaneous anthrax) or rarely the mucous membranes (gastrointestinal anthrax), or by inhalation of spores into the lung (inhalation anthrax). The spores germinate in the tissue at the site of entry, and growth of the vegetative organisms results in formation of a gelatinous edema and congestion. Bacilli spread via lymphatics to the bloodstream, and they multiply freely in the blood and tissues shortly before and after the animal's death.

B anthracis that **does not produce a capsule is not virulent and does not induce anthrax in test animals.** The poly-D-glutamic acid capsule is antiphagocytic. The capsule gene is on a plasmid.

Anthrax toxin is made up of three proteins: protective antigen (PA), edema factor (EF), and lethal factor (LF). PA binds to specific cell receptors, and following proteolytic activation it forms a membrane channel that mediates entry of EF and LF into the cell. EF is an adenylyl cyclase; with PA it forms a toxin known as edema toxin. **LF plus PA form lethal toxin, which is a major virulence factor and cause of death in infected animals .**

In inhalation anthrax ("woolsorter's disease"), the spores from the dust of wool, hair, or hides are inhaled, phagocytosed in the lungs, and

transported by the lymphatic drainage to the mediastinal lymph nodes, where germination occurs. This is followed by toxin production and the development of hemorrhagic mediastinitis and sepsis, which are usually rapidly fatal.

Clinical Findings In humans, approximately 95% of cases are cutaneous anthrax and 5% are inhalation. Gastrointestinal anthrax is very rare .Cutaneous anthrax generally occurs on exposed surfaces of the arms or hands, followed in frequency by the face and neck.

Diagnostic Laboratory Tests

Specimens to be examined are fluid or pus from a local lesion, blood, and sputum. Stained smears from the local lesion or of blood from dead animals often show chains of large gram-positive rods. Anthrax can be identified in dried smears by immunofluorescence staining techniques

When grown on blood agar plates, the organisms produce non hemolytic gray to white colonies with a rough texture and a ground-glass appearance. Comma-shaped outgrowths (Medusa head) may project from the colony. Gram stain shows large gram-positive rods. Carbohydrate fermentation is not useful. In semisolid medium,

anthrax bacilli are always non motile, whereas related nonpathogenic organisms (eg, B cereus) exhibit motility by “swarming.

. Contact with infected animals or with their hides, hair, and bristles is the source of infection in human

Control measures include (1) disposal of animal carcasses by burning or by deep burial in lime pits, (2) decontamination (usually by autoclaving) of animal products, (3) protective clothing and gloves for handling potentially infected materials, and (4) active immunization of domestic animals with live attenuated vaccines. Persons with high occupational risk should be immunized.

BACILLUS CEREUS

Food poisoning caused by Bacillus cereus has two distinct forms: the emetic type, associated with fried rice, and the diarrheal type, associated with meat dishes and sauces.

. ■ **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 or the intestinal tract of animals and humans, where they live as saprophytes.

Morphology & 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, sub terminally, or terminally. Most species of clostridia are** motile and possess peritrichous flagella. A gram stain of a Clostridium species with terminal spor.

B. CULTURE

Clostridia are **anaerobes and grow under anaerobic conditions**; a few species are aero tolerant and will also grow in ambient air. . In general, the clostridia grow well on the blood-enriched media used to grow anaerobes and on other media used to culture anaerobes as well.

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 on the agar , Clostridium Gram stain. Individual gram positive bacilli are present (short arrow). Some bacilli have terminal spores (long arrow**). Many clostridia produce a zone of hemolysis on blood agar,*C perfringens* typically produces multiple zones of hemolysis around colonies.**

D. GROWTH CHARACTERISTICS

Clostridia can ferment a variety of sugars; many can digest proteins. 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 .

E. ANTIGENIC CHARACTERISTICS

Clostridia share some antigens but also possess specific soluble antigens that permit grouping by precipitin tests.

CLOSTRIDIUM BOTULINUM

***Clostridium botulinum*, which causes botulism,** is worldwide in distribution; **it is found in soil and occasionally in animal feces.** 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. Toxin During the growth *of C botulinum* and during autolysis of the bacteria, toxin is liberated into the environment**. Seven antigenic varieties of toxin (A–G) are known. Types A, B, and E (and occasionally F) are the principal causes of human illness**. The toxin is a 150,000-MW protein that is cleaved into 100,000-MW and 50,000-MW proteins linked by a disulfide bond.

Botulinum toxin action is absorbed from the gut and binds to receptors of presynaptic membranes of motor neurons of the peripheral nervous system and cranial nerves. Proteolysis—by the light chain of botulinum toxin—of the target **SNARE proteins in the neurons inhibits the release of acetylcholine at the synapse, resulting in lack of muscle contraction and paralysis.** *C botulinum t*oxins are among the most toxic substances known: The lethal dose for a human is probably about 1–2 µg. The toxins are destroyed by heating for 20 minutes at 100 °C.

Pathogenesis

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; under anaerobic conditions, vegetative forms grow and produce toxin. The toxin acts by blocking release of acetylcholine at synapses and neuromuscular junctions .**

Epidemiology, Prevention, & Control

Since spores *of C botulinum* are widely distributed in soil, they often contaminate vegetables, fruits, and other materials. . 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. The risk from home-canned foods can be reduced if the food is boiled for more than 20 minutes before consumption. **Toxoids are used for active immunization of cattle .**

CLOSTRIDIUM TETANI

Clostridium 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 c*an be distinguished by specific flagellar antigens. All share a common O (somatic) antigen**, which may be masked, and all produce the same antigenic type of neurotoxin, tetanospasmin. Toxin The vegetative cells *of C tetani* produce the toxin tetanospasmin (MW 150,000) that is cleaved by a bacterial protease into two peptides (MW 50,000 and 100,000) linked by a disulfide bond.

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 brain stem and and

cause Hyperreflexia, muscle spasms, and spastic paralysis result. **Extremely small amounts of toxin can be lethal for humans.**

Clinical Findings

The incubation period may range from 4–5 days to as many 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**. Death usually results from interference with the mechanics of respiration. The mortality rate in generalized tetanus is very high.**

Diagnosis

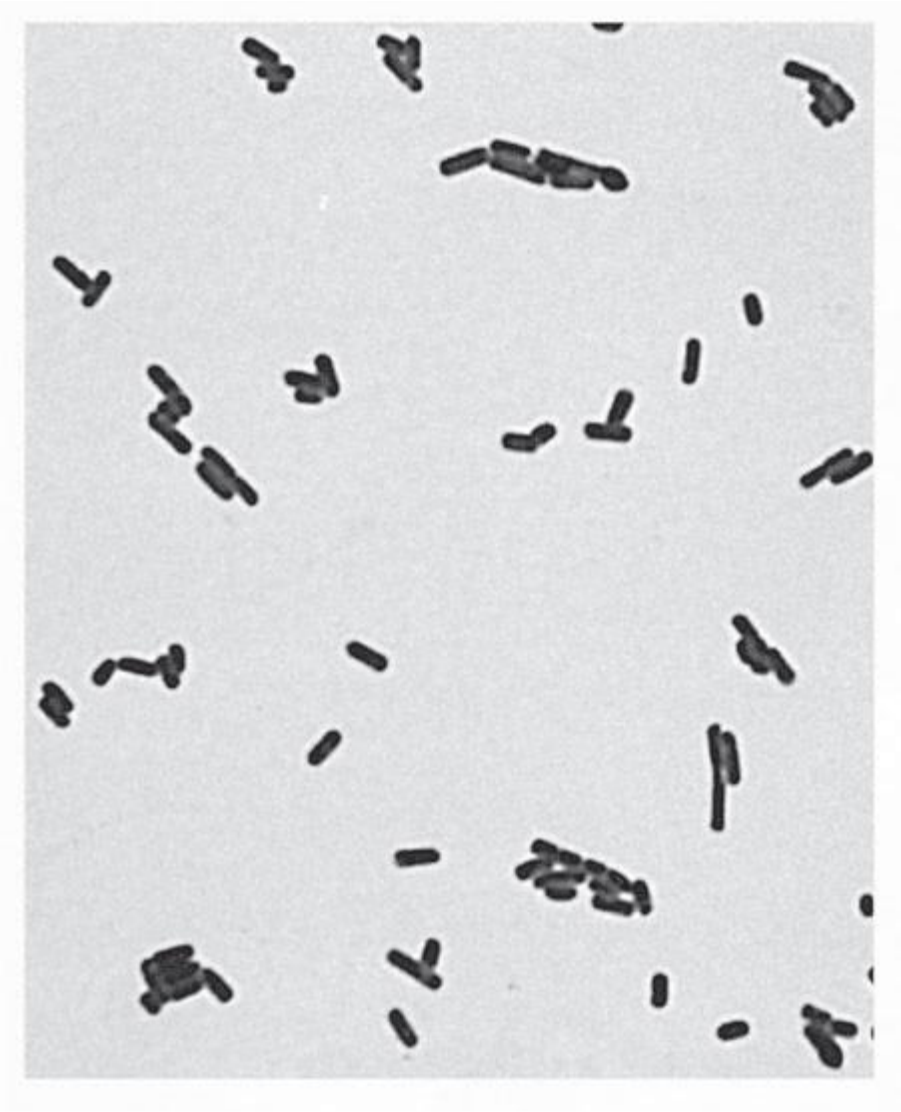
The diagnosis rests on the clinical picture and a history of injury, although only 50% of patients with tetanus have an injury for which they seek medical attention. The primary differential diagnosis of tetanus is strychnine poisoning. Anaerobic culture of tissues from contaminated wounds may yield *C tetani,* Proof of isolation *of C tetani* must rest on production of toxin and its neutralization by specific antitoxin.

Prevention & Treatment

The results of treatment of tetanus are not satisfactory. Therefore, prevention is all-important**. Prevention of tetanus depends upon** (1) active immunization with toxoids; (2) proper care of wounds contaminated with soil, etc; (3) prophylactic use of antitoxin; and (4) administration of penicillin.

CLOSTRIDIA THAT PRODUCE INVASIVE INFECTIONS

Clostridium perfringens



Clostridium perfringens can produce invasive infection (**including myonecrosis and gas gangrene) if introduced into damaged tissue**. 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.

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. **The theta toxin** has similar hemolytic and necrotizing effects but is not a lecithinase**. DNase and hyaluronidase, a collagenas**e that **digests collagen of subcutaneous tissue and muscle**, are also produced.

Some strains of C perfringens produce a **powerful enterotoxin, especially when grown in meat dishes**.

The action *o**f 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.

Pathogenesis &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.

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

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

CULTURE

Material is inoculated into chopped meat-glucose medium and thioglycolate medium and onto blood agar plates incubated anaerobically. The growth from one of the media is transferred into milk. A clot torn by gas in 24 hours is suggestive *of C perfringens*. Once 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 form.

Lecithinase activity is evaluated by the precipitate formed around colonies on egg yolk media.

Final identification rests on toxin production and neutralization by specific antitoxin. *C perfringens* rarely produces spores when cultured on agar in the laboratory.

Prevention & Control

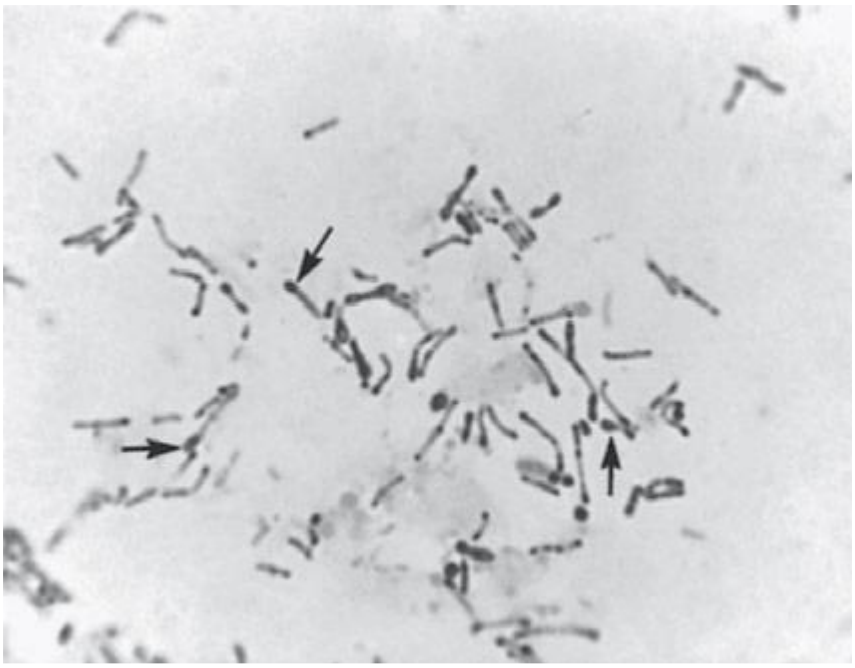
Early and adequate cleansing of contaminated wounds and surgical debridement, together with the administration of antimicrobial drugs directed against clostridia (eg, penicillin), are the best available preventive measures.

Non-Spore-Forming Gram-Positive Bacilli

CORYNEBACTERIUM DIPHTHERIAE

Morphology & Identification

Corynebacteria are 0.5–1 µm in diameter and several micrometers long. Characteristically, they possess irregular swellings at one end that give them the “club-shaped” appearance . Irregularly distributed within the rod (often near the poles) are granules staining deeply with aniline dyes (metachromatic granules) that give the rod a beaded appearance. Individual corynebacteria in stained smears tend to lie parallel or at acute angles to one another. True branching is rarely observed



cultures.

On blood agar, *the C diphtheriae* colonies are small, granular, and gray, with irregular edges, and may have small zones of hemolysis. On agar containing potassium tellurite, the colonies are brown to black with a brown-black halo because the tellurite is reduced intracellularly (staphylococci and streptococci can also produce black colonies).

Four biotypes *of C diphtheriae* have been widely recognized: gravis, mitis, intermedius, and belfanti. These variants have been classified on the basis of growth characteristics such as colony morphology, biochemical reactions, and, severity of disease produced by infection.

Pathogenesis

The principal human pathogen of the group *is C diphtheriae*. In nature*, C diphtheriae* occurs in the respiratory tract, in wounds, or on the skin of infected persons or normal carriers. It is spread by droplets or by contact to susceptible individuals; the bacilli then grow on mucous membranes or in skin abrasions, and those that are toxigenic start producing toxin.

All toxigenic C diphtheriae are capable of elaborating the same disease-producing exotoxIn vitro production of this toxin depends largely on the **concentration of iron.** Toxin production is optimal at 0.14 µg of iron per milliliter of medium but is virtually suppressed at 0.5 µg/ mL. Other factors influencing the yield of toxin in vitro are **osmotic pressure, amino acid concentration, pH, and availability of suitable carbon and nitrogen sources. .**

Diphtheria toxin is a heat-labile polypeptide (MW 62,000) that can be lethal in a dose of 0.1 µg/kg. If disulfide bonds are broken, the molecule can be split into two fragments. Fragment B (MW = 38,000) A into the cell. Fragment A inhibits polypeptide chain elongation—provided nicotinamide adenine dinucleotide (NAD) is present—by inactivating the elongation factor EF-2. This factor is required for translocation of polypeptidyl-transfer RNA from the acceptor to the donor site on the eukaryotic ribosome. Toxin fragment A inactivates EF-2 by catalyzing a reaction that yields free nicotinamide plus an inactive adenosine diphosphate-ribose-EF-2 complex. It is assumed that the abrupt arrest of protein synthesis is responsible for the necrotizing and neurotoxic effects of diphtheria toxin.

Diphtheria toxin is absorbed into the mucous membranes and causes destruction of epithelium and a superficial inflammatory response. The necrotic epithelium becomes embedded in exuding fibrin and red and white cells, so that a **grayish "pseudomembrane"** is formed—commonly over the tonsils, pharynx, or larynx. Any attempt to remove the pseudomembrane exposes and tears the capillaries and thus results in bleeding. The regional lymph nodes in the neck enlarge, and there may be marked edema of the entire neck.

The diphtheria bacilli within the membrane continue to produce toxin actively. This is absorbed and results in distant toxic damage,

(complications) particularly parenchymatous degeneration, fatty infiltration, and necrosis in heart muscle, liver, kidneys, and adrenals, sometimes accompanied by gross hemorrhage. The toxin also produces nerve damage, resulting often in paralysis of the soft palate, eye muscles, or extremities .

Clinical Findings

When diphtheritic inflammation begins in the respiratory tract, sore throat and fever usually develop. Prostration and dyspnea soon follow because of the obstruction caused by the membrane. This obstruction may even cause suffocation if not promptly relieved by intubation or tracheostomy. Irregularities of cardiac rhythm indicate damage to the heart. Later, there may be difficulties with vision, speech, swallowing, or movement of the arms or legs.

Diagnostic Laboratory Tests

Dacron swabs from the nose, throat, or other suspected lesions must be obtained before antimicrobial drugs are administered. Swabs should be collected beneath any visible membrane.

The swab should then be placed in **semisolid transport** media such as Amies. **Smears stained** with alkaline methylene blue or Gram stain show beaded rods in typical arrangement. **Inoculate** a blood agar plate (to rule out hemolytic streptococci), a Loeffler slant, and a tellurite plate (eg, cystine-tellurite agar or modified Tinsdale medium) and incubate all at 37 °C. In 12–18 hours, the Loeffler slant may yield organisms of typical “diphtheria-like” morphology. In 36–48 hours, the colonies on tellurite medium are sufficiently definite for recognition of C diphtheriae.

A presumptive *C diphtheriae* isolate should be subjected to testing for toxigenicity. Such tests are performed only in reference public health laboratories. There are several methods, as follows:

1 - A filter paper disk containing antitoxin is placed on an agar plate. The cultures to be tested for toxigenicity are spot innoculated 7 to 9 mm away from the disk. After 48 hours of incubation, the antitoxin diffusing from the paper disk has precipitated the toxin diffusing from toxigenic cultures and has resulted in precipitate bands between the disk and the bacterial growth. This is the modified Elek method described by the WHO Diphtheria Reference Unit.

2 - Polymerase chain reaction-based methods have been described for detection of the diphtheria toxin gene (tox). PCR assays for tox can also be used directly on patient specimens before culture results are available. A positive culture confirms a positive PCR assay. A negative culture following antibiotic therapy along with a positive PCR assay suggests that the patient probably has diphtheria.

3 - Enzyme-linked immunosorbent assays can be used to detect diphtheria toxin from clinical *C diphtheriae* isolates.

4 - An immunochromographic strip assay allows detection of diphtheria toxin in a matter of hours. This assay is highly sensitive. Historically, toxigenicity of a *C diphtheriae* isolate has been demonstrated by injecting two guinea pigs with the emulsified isolate. If the guinea pig protected with diphtheria antitoxin survives while the unprotected one dies, the isolate is considered to be toxigenic. This test has largely been replaced by more modern technology.

Epidemiology, Prevention, & Control

Before artificial immunization, diphtheria was mainly a disease of small children. Active immunization in childhood with diphtheria toxoid yields antitoxin levels that are generally adequate until adulthood.

A filtrate of broth culture of a toxigenic strain is treated with 0.3% formalin and incubated at 37 °C until toxicity has disappeared. This fluid toxoid is purified and standardized in flocculating units (Lf doses). Fluid toxoids prepared as above are adsorbed onto aluminum hydroxide or aluminum phosphate , Such toxoids are commonly combined with

tetanus toxoid (Td) and sometimes with pertussis vaccine (DPT or DaPT) as a single injection to be used in initial immunization of children.

Enteric Gram-Negative Rods (Enterobacteriaceae)

The Enterobacteriaceae are a large, heterogeneous group of gram-negative rods whose natural habitat is the intestinal tract of humans and animals. The family includes many genera (Escherichia, Shigella, Salmonella, Enterobacter, Klebsiella, Serratia, Proteus, and others). Some enteric organisms, eg*, Escherichia coli*, are part of the normal flora and incidentally cause disease, while others, the salmonellae and shigellae, are regularly pathogenic for humans. The Enterobacteriaceae are facultative anaerobes or aerobes, ferment a wide range of carbohydrates, possess a complex antigenic structure, and produce a variety of toxins and other virulence factors. Enterobacteriaceae, enteric gram-negative rods . but these bacteria may also be called coliforms.

CLASSIFICATION

The Enterobacteriaceae are the most common group of gram-negative rods cultured in the clinical laboratory and along with staphylococci and streptococci are among the most common bacteria that cause disease. The taxonomy of the Enterobacteriaceae is complex and rapidly changing since the introduction of techniques that measure evolutionary distance, such as nucleic acid hybridization and sequencing. More than 25 genera and 110 species or groups have been defined; however, the clinically significant Enterobacteriaceae comprise 20–25 species, and other species are encountered infrequently.

The family Enterobacteriaceae have the following characteristics: They are gram-negative rods, either motile with peritrichous flagella or nonmotile; they grow on peptone or meat extract media without the addition of sodium chloride or other supplements; grow well on MacConkey’s agar; grow aerobically and anaerobically (are facultative anaerobes); ferment rather than oxidize glucose, often with gas production; are catalase-positive, oxidase-negative, and reduce nitrate to nitrite .

Morphology & Identification

A. TYPICAL ORGANISMS

The Enterobacteriaceae are short gram-negative rods. Typical morphology is seen in growth on solid media in vitro, but morphology is highly variable in clinical specimens. Capsules are large and regular in klebsiella, less so in enterobacter, and uncommon in the other species.

B. CULTURE

E coli and most of the other enteric bacteria form circular, convex, smooth colonies with distinct edges. Enterobacter colonies are similar but somewhat more mucoid. Klebsiella colonies are large and very mucoid and tend to coalesce with prolonged incubation**. The salmonellae and shigellae produce colonies similar to E coli but do not ferment lactose.** Some strains of E coli produce hemolysis on blood agar.

C. GROWTH CHARACTERISTICS

Carbohydrate fermentation patterns and the activity of amino acid decarboxylases and other enzymes are used in biochemical differentiation . Examples of Biochemical Reactions of Selected Enteric Gram-Negative Rods.1 Indole Production Methyl Red Voges-Proskauer Simmons' Citrate Hydrogen Sulfide Urea Hydrolysis Phenylalanine Deaminase Lysine Decarboxylase Arginine Dihydrolase Ornithine Decarboxylase Motility (36 °C) Gelatin Hydrolysis (22 °C) D-Glucose, Acid D-Glucose, Gas Lactose Fermentation Sucrose Fermentation D-Mannitol Fermentation Dulcitol Fermentation .

tests, eg, the production of indole from tryptophan, are commonly used in rapid identification systems, while others, eg, the Voges-Proskauer reaction (production of acetylmethylcarbinol from dextrose), are used less often.

Culture on "differential" media that contain special dyes and carbohydrates (eg, eosin-methylene blue [EMB], MacConkey's, or deoxycholate medium) distinguishes lactose-fermenting (colored) from nonlactose-fermenting colonies (nonpigmented) .

.**Many complex media have been devised to help in identification of the enteric bacteria One such medium is triple sugar iron (TSI) agar**, which is often used to help differentiate salmonellae and shigellae from other enteric gram-negative rods in stool cultures. The medium contains 0.1% glucose, 1% sucrose, 1% lactose, ferrous sulfate (for detection of H2S production), tissue extracts (protein growth substrate), and a pH indicator (phenol red). It is poured into a test tube to produce a slant with a deep butt and is inoculated by stabbing bacterial growth into the butt.

A - If only glucose is fermented, the slant and the butt initially turn yellow from the small amount of acid produced; as the fermentation products are subsequently oxidized to CO2 and H2O and released from the slant and as oxidative decarboxylation of proteins continues with formation of amines, the slant turns alkaline (red).

B- If lactose or sucrose is fermented, so much acid is produced that the slant and butt remain yellow (acid).

Salmonellae and shigellae typically yield an alkaline slant and an acid butt. Although proteus, providencia, and morganella produce an alkaline slant and acid butt, they can be identified by their rapid formation of red color in Christensen's urea medium. Organisms producing acid on the slant and acid and gas (bubbles) in the butt are other enteric bacteria.

Antigenic Structure

Enterobacteriaceae have a complex antigenic structure. They are classified by more than 150 different heat-stable somatic O (lipopolysaccharide) antigens, more than 100 heat-labile K (capsular) antigens, and more than 50 H (flagellar) antigens . *In Salmonella typhi,* the capsular antigens are called Vi antigens.

O antigens are the most external part of the cell wall
lipopolysaccharide and consist of repeating units of polysaccharide. Some O-specific polysaccharides contain unique sugars. O antigens are resistant to heat and alcohol and usually are detected by bacterial agglutination. Antibodies to O antigens are predominantly IgM .

K antigens are external to O antigens on some but not all Enterobacteriaceae. Some are polysaccharides, including the K antigens of E coli; others are proteins. K antigens may interfere with agglutination by O antisera, and they may be associated with virulence (eg, *E coli* strains producing K1 antigen are prominent in neonatal meningitis, and K antigens *of E coli* cause attachment of the bacteria to epithelial cells prior to gastrointestinal or urinary tract invasion). Klebsiellae form large capsules consisting of polysaccharides (K antigens) covering the somatic (O or H) antigens and can be identified by capsular swelling tests with specific antisera. Human infections of the respiratory tract are caused particularly by capsular types 1 and 2; those of the urinary tract, by types 8, 9, 10, and 24.

H antigens are located on flagella and are denatured or removed by heat or alcohol. They are preserved by treating motile bacterial variants with formalin.

There are many examples of overlapping antigenic structures between Enterobacteriaceae and other bacteria. Most Enterobacteriaceae share the O14 antigen of E coli. The type 2 capsular polysaccharide of klebsiellae is very similar to the polysaccharide of type 2 pneumococci. Some K antigens cross-react with capsular polysaccharides of *Haemophilus influenzae or Neisseria meningitidis.*

Colicins (Bacteriocins) Many gram-negative organisms produce bacteriocins. These virus-like bactericidal substances are produced by certain strains of bacteria active against some other strains of the same or closely related species. Their production is controlled by plasmids. Colicins are produced by *E coli, marcescens* by serratia, and pyocins by pseudomonas. Bacteriocin-producing strains are resistant to their own bacteriocin; thus, bacteriocins can be used for “typing” of organisms.

Toxins & Enzymes

Most gram-negative bacteria possess complex Antigenic structure of Enterobacteriaceae. polysaccharides in their cell walls. These substances, Lipopolysaccharide O side chains (O) Capsule (K) Flagella (H) Cell envelope (cytoplasmic membrane, peptidoglycan, outer membrane)

, endotoxins . Many gram-negative enteric bacteria also produce exotoxins of clinical importance.

The bacteria become pathogenic only when they reach tissues outside of their normal intestinal or other less common normal flora sites. The most frequent sites of clinically important infection are the urinary tract, biliary tract, and other sites in the abdominal cavity, but any anatomic site (eg, bacteremia, prostate gland, lung, bone, meninges) can be the site of disease.

Pathogenesis & Clinical Findings

The clinical manifestations of infections with E coli and the other enteric bacteria depend on the site of the infection : ***I***

Escherichia—*E coli* typically produces positive tests for indole, lysine decarboxylase, and mannitol fermentation and produces gas from glucose. , typical colonial morphology with an iridescent “sheen” on differential media such as EMB agar, and a positive spot indole test. Over 90% of E coli isolates are positive for β-glucuronidase using the substrate 4-methylumbelliferyl-β-glucuronide (MUG). Isolates from anatomic sites other than urine, with characteristic properties (above plus negative oxidase tests) often can be confirmed as *E coli* with a positive MUG test

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 .

2. E coli-Associated Diarrheal Diseases—*E coli* that cause diarrhea are extremely common worldwide.

A - Enteropathogenic E coli (EPEC) is an important cause of diarrhea in infants, especially in developing countries.

EPEC previously was associated with outbreaks of diarrhea in nurseries in developed countries. EPEC adhere to the mucosal cells of the small bowel. The result of EPEC infection is watery diarrhea , EPEC diarrhea has been associated with multiple specific serotypes *of E coli.*

B - Enterotoxigenic E coli (ETEC) is a common cause of “traveler’s diarrhea” and a very important cause of diarrhea in infants in developing countries

ETEC colonization factors specific for humans promote adherence of ETEC to epithelial cells of the small bowel. Some strains of ETEC produce a heat-labile exotoxin (LT) (MW 80,000) that is under the genetic control of a plasmid. I

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 colonization factors that facilitate the attachment of *E coli* strains to intestinal epithelium

C - Enterohemorrhagic E coli (EHEC) produces v**erotoxin**. EHEC has been associated with hemorrhagic colitis, a severe form of diarrhea, and with hemolytic uremic syndrome, a disease resulting in acute renal failure, micro angiopathic hemolytic anemia, and thrombocytopenia..

D - Enteroinvasive E coli (EIEC) produces a disease very similar to shigellosis. The disease occurs most commonly in children in developing countries , EIEC produce disease by invading intestinal mucosal epithelial cells.

E - Enteroaggregative E coli (EAEC) causes acute and chronic diarrhea in persons in developing countries.

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.

4. Meningitis—*E coli* and group B streptococci are the leading causes of meningitis in infants. Approximately 75% *of E coli* from meningitis cases have the K1 antigen.

Klebsiella pneumoniae is present in the respiratory tract and feces of about 5% of normal individuals. It causes a small proportion (about 1%) of bacterial pneumonias*. K pneumoniae* can produce extensive hemorrhagic necrotizing consolidation of the lung. It occasionally produces urinary tract infection and bacteremia with focal lesions in debilitated patients.

Enterobacter aerogenes—This organism has small capsules, may be found free-living as well as in the intestinal tract, and causes urinary tract infections and sepsis.

Serratia marcescens is a common opportunistic pathogen in hospitalized patients. Serratia (usually nonpigmented) causes pneumonia, bacteremia, and endocarditis—especially in narcotics addicts and hospitalized patients.

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 intravenous infusions*. P mirabilis* causes urinary tract infections and occasionally other infections*. Proteus vulgaris and Morganella morganii* are important nosocomial pathogens. Proteus species produce urease, resulting in rapid hydrolysis of urea with liberation of ammonia. Thus, in urinary tract infections with proteus, 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.

Providencia—Providencia species (Providencia rettgeri, Providencia alcalifaciens, and Providencia stuartii) are members of the normal intestinal flora. All cause urinary tract infections and occasionally other infections and are often resistant to antimicrobial therapy

Citrobacter—Citrobacter can cause urinary tract infections and sepsis.

Diagnostic Laboratory Tests

A. SPECIMENS included 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.

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 .

THE SHIGELLAE

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

Morphology & Identification

A. TYPICAL ORGANISMS

Shigellae are slender gram-negative rods; cocco bacillary 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 that ferment mannitol and those that do not.

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.

Pathogenesis & 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 (whereas 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. Micro abscesses 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.

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.

In humans, the exotoxin also inhibits sugar and amino acid absorption in the small intestine. 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.

Clinical findings after a short incubation period (1–2 days), there is a sudden onset of abdominal pain, fever, and watery diarrhea. The diarrhea has been attributed to an exotoxin acting in the small intestine (see above). 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 .

Diagnostic Laboratory Tests

A. 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. Serum specimens, if desired, must be taken 10 days apart to demonstrate a rise in titer of agglutinating antibodies.

B. CULTURE The materials are streaked on differential media (eg, MacConkey's or EMB agar) and on selective media (Hektoen enteric agar or **salmonella-shigella agar), which suppress other Enterobacteriaceae and gram-positive organisms**. Colorless (lactose-negative) colonies are inoculated into triple sugar iron agar. Organisms that fail to produce H2S, that produce acid but not gas in the butt and an alkaline slant in triple sugar iron agar medium, and that are non motile should be subjected to slide agglutination by specific shigella antisera.

Epidemiology, Prevention, & Control

Shigellae are transmitted by "food, fingers, feces, and flies" from person to person. Most cases of shigella infection occur in children under 10 years of age. *S dysenteriae* can spread widely**. Since 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.

THE SALMONELLA-ARIZONA GROUP

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 & 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.

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), Salmonella Paratyphi B (serogroup B), Salmonella Choleraesuis (serogroup C1), and Salmonella Typhi (serogroup D).*

Pathogenesis & Clinical Findings

Salmonella Typhi, Salmonella Choleraesuis, and *perhaps Salmonella Paratyphi A* and Salmonella 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: 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 Salmonella Typhi organisms). **Among the host factors that contribute to resistance to salmonella infection are gastric acidity, normal intestinal microbial flora, and local intestinal immunity .**

Salmonellae produce three main types of disease in humans, but mixed forms are frequency :

A. THE "ENTERIC FEVERS" (TYPHOID FEVER) This syndrome is produced by only a few of the salmonellae, of *which Salmonella 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.

B. BACTEREMIA WITH FOCAL LESIONS

This is associated commonly with *S choleraesuis* but may be caused by any salmonella serotype. Following oral infection, there is early invasion of the bloodstream (with possible focal lesions in lungs, bones, meninges,

C. ENTEROCOLITIS

This is the most common manifestation of salmonella infection. In the United States, *Salmonella Typhimurium* and *Salmonella Enteritidis* are prominent, but enterocolitis can be cause 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 cultures are usually negative, but stool cultures 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 cultures are often positive in the first week of the disease. Bone marrow cultures may be useful. Urine cultures 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, 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's, or deoxycholate medium permits rapid detection of lactose non fermenters , 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 o**n salmonella-shigella (SS) agar**, Hektoen enteric agar, XLD, or deoxycholate-citrate agar, which favor growth of salmonellae and shigellae over other Enterobacteriaceae

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 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

2. Tube Dilution Agglutination Test (Widal Test)

Serum agglutinins rise sharply during **the second and third weeks of Salmonella 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. a titer against the O antigen of > 1:320 and against the H antigen of > 1:640 is considered positive. Results of serologic tests for salmonella infection must be interpreted cautiously because the possible presence of cross-reactive antibodies limits the use of serology.

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 (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, etc.

Prevention & 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.

THE PSEUDOMONAD GROUP

The pseudomonads are gram-negative, motile, aerobic rods some of which produce water-soluble pigments. Pseudomonads occur widely in soil, water, plants, and animals. *Pseudomonas aeruginosa* is frequently present in small numbers in the normal intestinal flora and on the skin of humans and is the major pathogen of the group. Other pseudomonads

infrequently cause disease. The classification of pseudomonads is based on rRNA/DNA homology and common culture characteristics.

Pseudomonas aeruginosa is widely distributed in nature and is commonly present in moist environments in hospitals. It can colonize normal humans, in whom it is a saprophyte. It causes disease in humans with abnormal host defenses.

Morphology & Identification

A. TYPICAL ORGANISMS

P aeruginosa is motile and rod-shaped, measuring about 0.6 × 2 µm. It is gram-negative and occurs as single bacteria, in pairs, and occasionally in short chains.

B. CULTURE

P aeruginosa is an obligate aerobe that grows readily on many types of culture media, sometimes producing a sweet or grape-like or corn taco-like odor. Some strains hemolyze blood*. P aeruginosa* forms smooth round colonies with a fluorescent greenish color. It often produces the nonfluorescent bluish pigment pyocyanin, which diffuses into the agar. Other Pseudomonas species do not produce pyocyanin.

Many strains *of P aeruginosa* also produce the fluorescent pigment pyoverdin, which gives a greenish color to the agar. Some strains produce the dark red pigment pyorubin or the black pigment pyomelanin. *P aeruginosa* in a culture can produce multiple colony types*. P aeruginosa* from different colony types may also have different biochemical and enzymatic activities and different antimicrobial susceptibility patterns. Sometimes it is not clear if the colony types represent different strains *of P aeruginosa* or are variants of the same strain. Cultures from patients with cystic fibrosis often yield *P aeruginosa* organisms that form mucoid colonies as a result of overproduction of alginate, an exopolysaccharide. In cystic fibrosis patients, the exopolysaccharide appears to provide the matrix for the organisms to live in a biofilm .

C. GROWTH CHARACTERISTICS *P aeruginosa* grows well at 37–42 °C; its growth at 42 °C helps differentiate it from other Pseudomonas species in the fluorescent group. **It is oxidase-positive**. It does not ferment carbohydrates, but many strains oxidize glucose. Identification is usually based on colonial morphology, oxidase positivity, the presence of characteristic pigments, and growth at 42 °C. Differentiation of *P aeruginosa* from other pseudomonads on the basis of biochemical activity requires testing with a large battery of substrates.

Antigenic Structure & Toxins

Pili (fimbriae) extend from the cell surface and promote attachment to host epithelial cells. **The exopolysaccharide** is responsible for the mucoid colonies seen in cultures from patients with cystic fibrosis. The **lipopolysaccharide,** which exists in multiple immunotypes, is responsible for many of the endotoxic properties of the organism. *P aeruginosa* can be typed by lipopolysaccharide immunotype and by pyocin (bacteriocin) susceptibility. Most *P aeruginosa* isolates from clinical infections **produce extracellular enzymes, including elastases, proteases, and two hemolysins: a heat-labile phospholipase C and a heatstable glycolipid.** Many strains of *P aeruginosa* **produce exotoxin** A, which causes tissue necrosis and is lethal for animals when injected in purified form.

Pathogenesis

P aeruginosa is pathogenic only when introduced into areas devoid of normal defenses, eg, when mucous membranes and skin are disrupted by direct tissue damage; The bacterium attaches to and colonizes the mucous membranes or skin, invades locally, and produces systemic disease. These processes are promoted by the pili, enzymes, and toxins described above. Lipopolysaccharide plays a direct role in causing fever, shock, oliguria.

P aeruginosa a are resistant to many antimicrobial agents and therefore become dominant and important when more susceptible bacteria of the normal flora are suppressed.

Clinical Findings

P aeruginosa produces **infection of wounds and burns**, giving rise to blue-green pus; meningitis; and **urinary tract infection**, when introduced by catheters and instruments or in irrigating solutions. Involvement of the respiratory tract, especially from contaminated respirators, results in necrotizing **pneumonia**. The bacterium is often found in mild **otitis externa** in swimmers. It may cause invasive (malignant) otitis externa in diabetic patients. **Infection of the eye**, which may lead to rapid destruction of the eye, occurs most commonly after injury or surgical procedures. In infants or debilitated persons, *P aeruginosa* **may invade the bloodstream and result in fatal sepsis .**

Diagnostic Laboratory Tests

A. SPECIMENS

Specimens from skin lesions, pus, urine, blood, spinal fluid, sputum, and other material should be obtained as indicated by the type of infection.

B. SMEARS

Gram-negative rods are often seen in smears. There are no specific morphologic characteristics that differentiate pseudomonads in specimens from enteric or other gram-negative rods.

C. CULTURE

Specimens are plated on blood agar and the differential media commonly used to grow the enteric gram-negative .

Vibrios

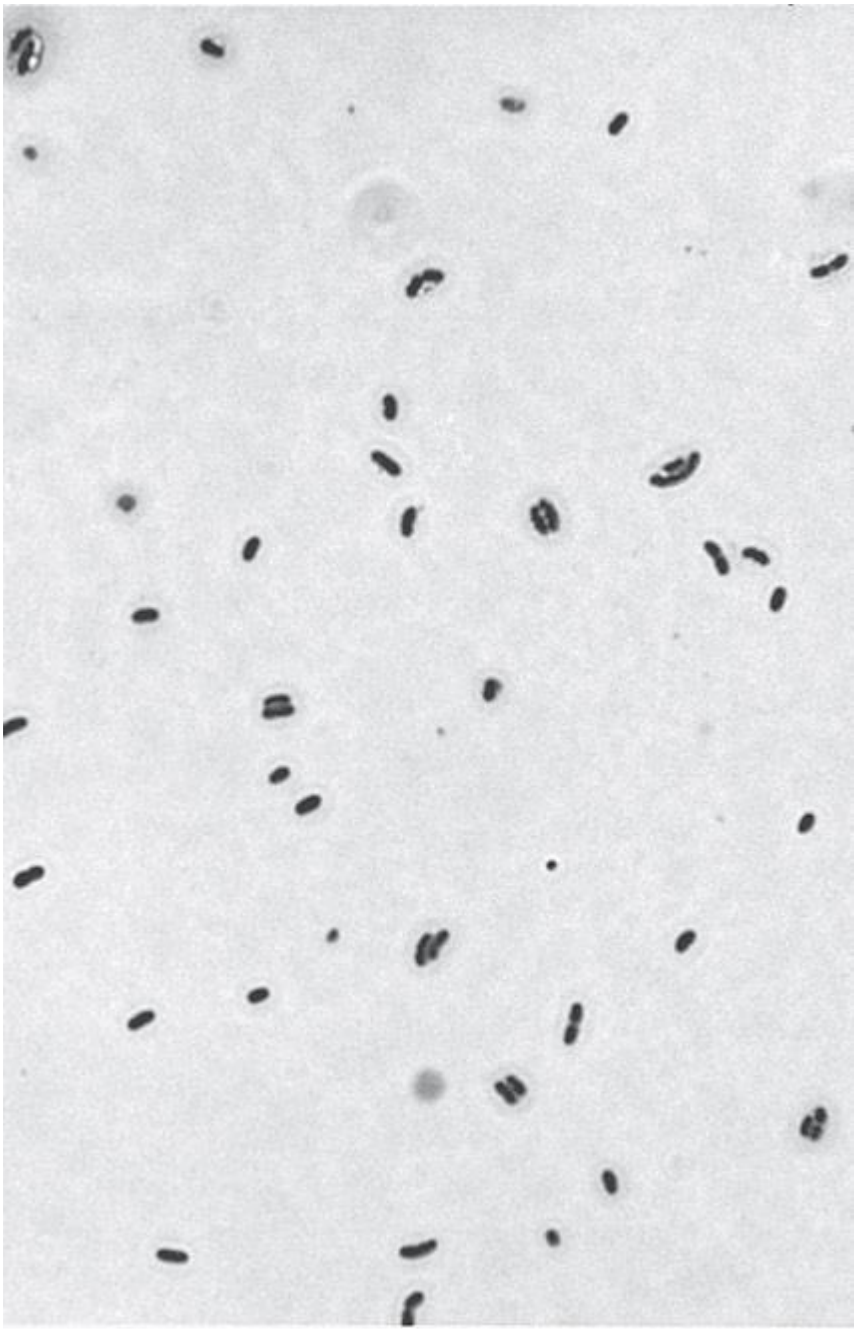
are gram-negative rods that are all widely distributed in nature. The vibrios are found in marine and surface water. *Vibrio cholerae* produces an enterotoxin that causes cholera, a profuse watery diarrhea that can rapidly lead to dehydration and death.

Vibrios are among the most common bacteria in surface waters worldwide. They are curved aerobic rods and are motile, possessing a polar flagellum. *V cholerae* serogroups O1 and O139 cause cholera in humans, while other vibrios may cause sepsis or enteritis.

Morphology & Identification

A. TYPICAL ORGANISMS

Upon first isolation, *V cholerae* is a comma-shaped, curved rod 2–4 µm long , It is actively motile by means of a polar flagellum. On prolonged cultivation, vibrios may become straight rods that resemble the gram negative enteric bacteria.



B. CULTURE

V cholerae produces convex, smooth, round colonies that are opaque and granular in transmitted light. *V cholerae* and most other vibrios grow well at 37 °C on many kinds of media. ***V cholerae*** **grows well on thiosulfate-citrate-bile-sucrose (TCBS) agar, on which it produces yellow colonies that are readily visible against the dark-green background of the agar. Vibrios are oxidase-positive, which**

differentiates them from enteric gram-negative bacteria. Characteristically, vibrios grow at a very high pH (8.5–9.5) and are rapidly killed by acid.

C. GROWTH CHARACTERISTICS

V cholerae regularly ferments sucrose and mannose but not arabinose. A positive oxidase test is a key step in the preliminary identification *of V cholerae* and other vibrios. Vibrio species are susceptible to the compound O/129 (2,4-diamino-6,7-diisopropylpteridine phosphate), which differentiates them from Aeromonas species, which are resistant to O/129. Most Vibrio species are halotolerant, and NaCl often stimulates their growth. Some vibrios are halophilic, requiring the presence of NaCl to grow. Another difference between vibrios and aeromonas is that vibrios grow on media containing 6% NaCl, whereas aeromonas does not.

Antigenic Structure & Biologic Classification

Many vibrios share a single heat-labile flagellar H antigen. Antibodies to the H antigen are probably not involved in the protection of susceptible hosts*. V cholerae* has O lipopolysaccharides that confer serologic specificity. There are at least 139 O antigen groups*. V cholerae* strains of O group 1 and O group 139 cause classic cholera. *The V cholerae* serogroup O1 antigen has determinants that make possible further typing; the serotypes are Ogawa, Inaba, and Hikojima. Two biotypes of *epidemic V cholerae* have been defined, classic and El Tor. The El Tor biotype produces a hemolysin, gives positive results on the Voges-Proskauer test, and is resistant to polymyxin B. Molecular techniques can also be used to *type V cholerae .*

Vibrio cholerae Enterotoxin

V cholerae produce a heat-labile enterotoxin with a molecular weight of about 84,000, consisting of subunits A (MW 28,000) and B (see Chapter 10). Ganglioside GM1 serves as the mucosal receptor for subunit B, which promotes entry of subunit A into the cell. Activation of subunit A1 yields increased levels of intracellular cAMP and results in

prolonged hyper secretion of water and electrolytes. There is increased sodium-dependent chloride secretion, and absorption of sodium and chloride is inhibited. Diarrhea occurs—as much as 20–30 L/d—with resulting dehydration, shock, acidosis, and death. The genes for *V cholerae* enterotoxin are on the bacterial chromosome.

Pathogenesis & Pathology

Under natural conditions, *V cholerae* is pathogenic only for humans. A person with normal gastric acidity may have to ingest as many as 1010 or *more V cholerae* to become infected when the vehicle is water, because the organisms are susceptible to acid. When the vehicle is food, as few as 102 –104 organisms are necessary because of the buffering capacity of food. Any medication or condition that decreases stomach acidity makes a person more susceptible to infection *with V cholerae.*

Cholera is not an invasive infection. The organisms do not reach the bloodstream but remain within the intestinal tract. *Virulent V cholerae* organisms attach to the micro villi of the brush border of epithelial cells. There they multiply and liberate cholera toxin .

Clinical Findings

About 60% of infections with classic *V cholerae* are asymptomatic, as are about 75% of infections with the El Tor biotype. The incubation period is 1–4 days for persons who develop symptoms, Stools, which resemble “rice water,” contain mucus, epithelial cells, and large numbers of vibrios. There is rapid loss of fluid and electrolytes, The El Tor biotype tends to cause milder disease than the classic biotype.

Diagnostic Laboratory Tests

A. SPECIMENS for culture consist of mucus flecks from stools.

B. SMEARS

The microscopic appearance of smears made from stool samples is not distinctive. Dark-field or phase contrast microscopy may show the rapidly motile vibrios.

C. CULTURE

Growth is rapid in peptone agar, on blood agar with a pH near 9.0, **or on TCBS agar**, and typical colonies can be picked in 18 hours. For enrichment, a few drops of stool can be incubated for 6–8 hours in taurocholate peptone broth (pH 8.0–9.0); organisms from this culture can be stained or subcultured .

D. SPECIFIC TESTS *V cholerae* organisms are further identified by slide agglutination tests using anti-O group 1 or group 139 antisera and by biochemical reaction patterns.

Morphology & Identification

A. TYPICAL ORGANISMS

H pylori has many characteristics, 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, **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 & 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) while 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. **. There is a strong association between the presence of H pylori infection and duodenal ulceration.**

Toxins and lipopolysaccharide may damage the mucosal cells, and the ammonia produced **by the urease** activity may directly damage the cells also. Histologically, gastritis is characterized by chronic and active inflammation. Destruction of the epithelium is common, and glandular atrophy may occur. *H pylori* thus may be a major risk factor for gastric cancer.

Clinical Finding

About 90% of patients **with duodenal ulcers** and 50–80% of those with **gastric ulcers** have *H pylori* infection. *H pylori* also may have a role **in 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.

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 spiraled organisms.

C. CULTURE As above.

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 following 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 pylor*i is present, the urease rapidly splits the urea (1–2 days) and the resulting shift in pH yields a color change in the medium. In vivo tests **for urease** activity can be done also.

E**pidemiology & Control H pylori**

present on the gastric mucosa of less than 20% of persons under age 30 but increases in prevalence to 40–60% of persons age 60, including persons who are asymptomatic. In developing countries, the prevalence of infection may be 80% or higher in adult

Haemophilus influenzae

Haemophilus influenzae is found on the mucous membranes of the upper respiratory tract in humans. It is an important cause of meningitis in children and occasionally causes respiratory tract infections in children and adults.

Morphology & Identification

A. TYPICAL ORGANISMS , the organisms are short (1.5 µm) coccoid bacilli, Gram negative, sometimes occurring in pairs or short chains. In cultures, the morphology depends both on age and on the medium. At 6–8 hours in rich medium, the small coccobacillary forms predominate. Later there are longer rods, lysed bacteria, and very pleomorphic forms.

Organisms in young cultures (6–18 hours) on enriched medium have a definite capsule.

B. CULTURE

On chocolate agar, flat, grayish-brown colonies with diameters of 1–2 mm are present after 24 hours of incubation. IsoVitaleX in media enhances growth.

C. GROWTH CHARACTERISTICS

Identification of organisms of the haemophilus group depends in part upon demonstrating **the need for certain growth factors called X and V. Factor X acts physiologically as hemin; factor V can be replaced by nicotinamide adenine nucleotide (NAD) or other coenzymes**. Colonies of staphylococci on sheep blood agar cause the release of NAD, yielding the satellite growth phenomenon.

D. VARIATION In addition to morphologic variation*, H influenzae* has a marked tendency to lose its capsule and the associated type specificity.

E. TRANSFORMATION Under proper experimental circumstances, the DNA extracted from a given type of *H influenzae* is capable of transferring that type specificity to other cells (transformation). Resistance to ampicillin and chloramphenicol is controlled by genes on transmissible plasmids.

Antigenic Structure Encapsulated *H influenzae* contains capsular polysaccharides (MW > 150,000) of one of six types (a–f). The capsular antigen of type b is a polyribose-ribitol phosphate (PRP). Encapsulated H influenzae can be typed by slide agglutination, A capsule swelling test with specific antiserum is analogous to the quellung test for pneumococci. Typing can also be done by immunofluorescence

Pathogenesis

H influenzae **produces no exotoxin**. The nonencapsulated organism is a regular member of the normal respiratory flora of humans. The capsule is antiphagocytic in the absence of specific anticapsular antibodies. The polyribose phosphate capsule of type *b H influenzae* is

the major virulence factor. Type b H influenzae causes meningitis, pneumonia and empyema, epiglottitis, cellulitis, septic arthritis, and occasionally other forms of invasive infection.

***H influenzae* type b and pneumococci are two of the most common etiologic agents of bacterial otitis media and acute sinusitis.**

Diagnostic Laboratory Tests

A. SPECIMENS consist of nasopharyngeal swabs, pus, blood, and spinal fluid for smears and cultures.

B. DIRECT IDENTIFICATION

Commercial kits are available for immunologic detection of *H influenzae* antigens in spinal fluid. A positive test indicates that the fluid contains high concentrations of specific polysaccharide from H *influenzae type b.*

C. CULTURE Specimens are grown on IsoVitaleX-enriched chocolate agar until typical colonies appear*. H influenzae* is differentiated from related gram-negative bacilli by its requirements for X and V factors and by its lack of hemolysis on blood agar . Tests for X (heme) and V (nicotinamide-adenine dinucleotide) factor requirements can be done in several ways. The Haemophilus species that require V factor grow around paper strips or disks containing V factor placed on the surface of agar that has been autoclaved before the blood was added (V factor is heat-labile). Alternatively, a strip containing X factor can be placed in parallel with one containing V factor on agar deficient in these nutrients. Growth of Haemophilus in the area between the strips indicates requirement for both factors.

Epidemiology, Prevention, & Control

Encapsulated *H influenzae* type b is **transmitted from person to person by the respiratory route.** *H influenza*e type b disease can be prevented by administration of Haemophilus b conjugate vaccine to children.

THE BRUCELLAE

The brucellae are obligate parasites of animals and humans and are characteristically located intracellularly. They are relatively inactive metabolically. *Brucella melitensis* typically infects goats*; Brucella suis, swine; Brucella abortus, cattle; and Brucella canis,* dogs. Other species are found only in animals. **. The disease in humans, brucellosis (undulant fever, Malta fever),**

Morphology & Identification

A. TYPICAL ORGANISMS The appearance in young cultures varies from cocci to rods 1.2 µm in length, with short coccobacillary forms predominating. They are gram-negative but often stain irregularly, and they are aerobic, nonmotile, and non-spore-forming.

B. CULTURE Small, convex, smooth colonies appear on enriched media in 2–5 days

C. GROWTH CHARACTERISTICS Brucellae are adapted to an intracellular habitat, and their nutritional requirements are complex. Some strains have been cultivated on defined media containing amino acids, vitamins, salts, and glucose. Fresh specimens from animal or **human sources are usually inoculated on trypticase-soy agar or blood culture media.** *B abortus* requires 5–10% CO2 for growth, whereas the other three species grow in air. Brucellae utilize carbohydrates but produce neither acid nor gas in amounts sufficient for classification. **Catalase and oxidase are produced by the four species that infect humans.** Hydrogen sulfide is produced by many strains, and nitrates are reduced to nitrites**. Brucellae are moderately sensitive to heat and acidity. They are killed in milk by pasteurization**.

D. VARIATION The typical virulent organism forms a smooth, transparent colony; upon culture, it tends to change to the rough form, which is a virulent**. The serum of susceptible animal**s contains a globulin and a lipoprotein that suppress growth of non smooth, a virulent types and favor the growth of virulent types. **Resistant animal species lack** these factors. Antigenic Structure Differentiation among Brucellae species or biovars is made possible by their characteristic sensitivity to **dyes and their production of H2S.**

Pathogenesis & Pathology

The common routes of infection in humans are the intestinal tract (ingestion of infected milk), mucous membranes (droplets), and skin (contact with infected tissues of animals**). Cheese made from unpasteurized goats' milk is a particularly common vehicle**. The organisms progress from the portal of entry, via lymphatic channels and regional lymph nodes, to the thoracic duct and the bloodstream, which distributes them to the parenchymatous organs. Granulomatous nodules that may develop into abscesses form in lymphatic tissue, liver, spleen, bone marrow, and other parts of the reticuloendothelial system. In such lesions, the brucellae are principally intracellular**. Osteomyelitis, meningitis, or cholecystitis also occasionally occurs .**

Clinical Findings

incubation period is 1–6 weeks. The onset is insidious, with malaise, fever, weakness, aches, and sweats. The fever usually rises in the afternoon; its fall during the night is accompanied by drenching sweat.

Diagnostic Laboratory Tests

A. SPECIMENS Blood should be taken for culture, biopsy material for culture (lymph nodes, bone, etc), and serum for serologic tests.

B. CULTURE

Brucella agar was specifically designed to culture Brucella species bacteria. The medium is highly enriched and—in reduced form—is used primarily in cultures for anaerobic bacteria. In oxygenated form, the medium grows Brucella species bacteria very well.

The Brucella species bacteria will grow on commonly used media, including **trypticase soy medium** with or without 5% sheep blood**, brain heart infusion medium, and chocolate agar. Blood culture media** readily grow Brucella species bacteria , All cultures should be **incubated in 8–10% CO2 at 35–37 °**C and should be observed for 3 weeks before being discarded as negative;

The observation of tiny gram-negative cocco bacilli that **are catalase-positive and oxidase-positive ,urease positive suggests Brucella species.**

C. SEROLOGY IgM antibody levels rise during the first week of acute illness, peak at 3 months, and may persist during chronic disease. Even with appropriate antibiotic therapy, high IgM levels may persist for up to 2 years in a small percentage of patients. IgG antibody levels rise about 3 weeks after onset of acute disease, peak at 6–8 weeks, and remain high during chronic disease. IgA levels parallel the IgG levels.

1. Agglutination Test—To be reliable, serum agglutination tests must be performed with standardized heat killed, phenolized, **smooth** brucella antigens. IgG agglutinin titers above 1:80 indicate active infection.

2. Blocking Antibodies—These are IgA antibodies that interfere with agglutination by IgG and IgM and cause a serologic test to be negative in low serum **dilutions (prozone)** although positive in higher dilutions. These antibodies appear during the subacute stage of infection, tend to persist for many years independently of activity of infection, and are detected by the Coombs antiglobulin method.

3. ELISA Assays—IgG, IgA, and IgM antibodies may be detected using ELISA assays, which use cytoplasmic proteins as antigens. These assays tend to be more sensitive and specific than the agglutination test.

d. Epidemiology, Prevention, & Control

Brucellae are animal pathogens transmitted to humans by accidental contact with infected animal feces, urine, milk, and tissues.

The common sources of infection for humans are unpasteurized milk, milk products, and cheese, and occupational contact (eg, farmers, veterinarians, slaughterhouse workers) with infected animals. Cheese made from unpasteurized goat's milk is a particularly common vehicle for transmission of brucellosis. Occasionally the airborne route may be important. Because of occupational contact, brucellae infection is much more frequent men**. Control rests on limitation of spread and possible**

eradication of animal infection, pasteurization of milk and milk products, and reduction of occupational hazards wherever possible.

Mycobacterium

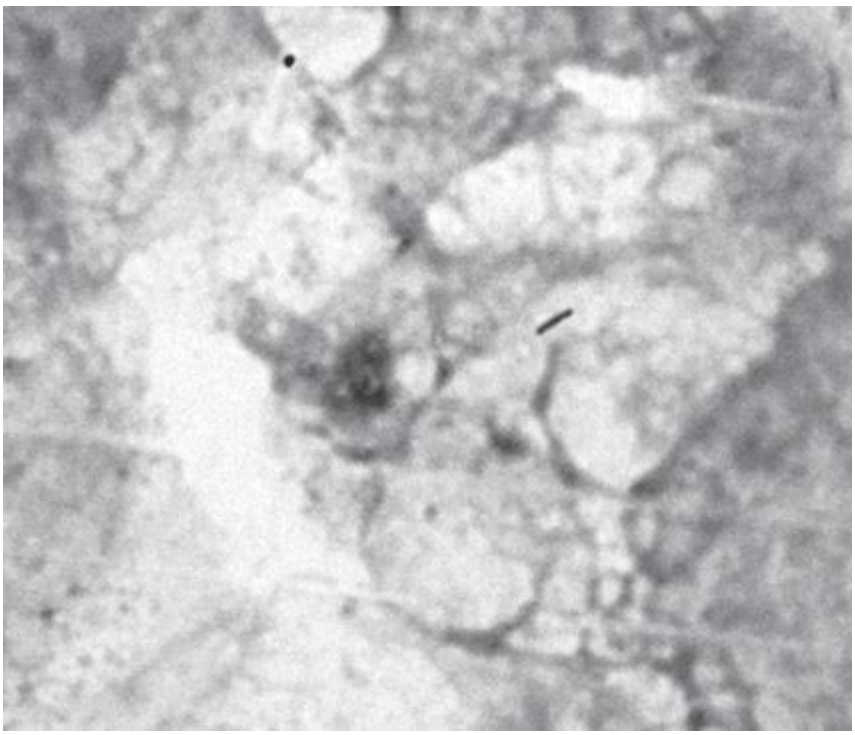
is a genus of Gram-positive bacilli that demonstrate the staining characteristic of acid-fastness. Its most important species, *Mycobacterium tuberculosis,* is the etiologic agent of tuberculosis .

MYCOBACTERIUM TUBERCULOSIS

Morphology & Identification

A. TYPICAL ORGANISMS

In tissue, tubercle bacilli are thin straight rods measuring about 0.4 × 3 µm . **On artificial media**, coccoid and filamentous forms are seen with variable morphology from one species to another. Once stained by basic dyes they cannot be decolorized by alcohol, regardless of treatment with iodine , The Ziehl-Neelsen technique of staining is employed for identification of acid-fast bacteria.



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.

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 contains casein hydrolysate also. The albumin neutralizes the toxic and inhibitory effects of fatty acids in the specimen or medium.

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 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.

C. GROWTH CHARACTERISTICS

Mycobacteria are obligate aerobes and derive energy from the oxidation of many simple carbon compounds. Increased CO2 tension enhances growth, the growth rate is much slower than that of most bacteria. **The doubling time of tubercle bacilli is about 18 hours**.

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 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*. M tuberculosis and Mycobacterium bovis* are equally pathogenic for humans. **The route of infection (respiratory versus intestinal) determines the pattern of lesions.**

Constituents of Tubercle Bacilli The constituents listed below are found mainly in cell walls.

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. Lipids are to some extent responsible for acid fastness. Analysis of lipids by gas chromatography reveals patterns that aid in classification of different species.

B. PROTEINS Each type of mycobacterium contains several proteins that elicit the tuberculin reaction.

C. POLYSACCHARIDES Mycobacteria contain a variety of polysaccharides. They can induce the immediate type of hypersensitivity and can serve as antigens in reactions with sera of infected persons.

Pathogenesis Mycobacteria in droplets 1–5 µm in diameter are inhaled and reach alveoli. **The disease results from establishment and proliferation of virulent organisms and interactions with the host**

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.

(2) the resistance and hypersensitivity of the host.

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, the tuberculin test becomes positive.

2. Productive 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.

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) .

C. INTRACELLULAR SITE OF GROWTH

Once 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 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

(2) The lymph node undergoes massive caseation, which usually calcifies.

(3) The tuberculin test becomes positive.

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.**

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 .

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 inflam) The volume is usually 0.1 mL injected intracutaneously. The

PPD preparation must be stabilized with polysorbate 80 to prevent adsorption to glass.

C. REACTIONS TO TUBERCULIN In an individual who has not had contact with mycobacteria, there is no reaction to PPD-S. An individual who has had a primary infection with tubercle bacilli develops induration, edema, erythema in 24–48 hours, and, with very intense reactions, even central necrosis. The skin test should be read in 48 or 72 hours. It is considered positive if the injection of 5 TU is followed by induration 10 mm or more in diameter.

D. INTERPRETATION OF TUBERCULIN TEST A positive tuberculin test indicates that an individual has been infected in the past

Clinical Findings Since the tubercle bacillus can involve every organ system, its clinical manifestations are protean. Fatigue, weakness, weight loss, and fever may be signs of tuberculous disease. Pulmonary involvement giving rise to chronic cough and spitting of blood usually is associated with far-advanced lesions.

Diagnostic Laboratory Tests

A positive tuberculin test does not prove the presence of active disease due to tubercle bacilli. Isolation of tubercle bacilli provides such proof.

A. 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 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, exudates, or other material is examined for acid-fast bacilli **by Ziehl-Neelsen staining , Fluorescence microscop**y with auramine-rhodamine stain is more sensitive than acid fast stain.

D. CULTURE, IDENTIFICATION, AND SUSCEPTIBILITY TESTING

Processed specimens from non sterile 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.

Conventional methods for identification of mycobacteria include observation of rate of growth, colony morphology, pigmentation, and biochemical profiles.

The conventional methods for classifying mycobacteria are rapidly becoming of historical interest because molecular probe methods are much faster and easier. Molecular probes provide a rapid, sensitive, and specific method to identify mycobacteria.

The use of these probes has shortened the time to identification of clinically important mycobacteria from several weeks to as little as 1 day.

High-performance liquid chromatography (HPLC) has been applied to speciation of mycobacteria. The method is based on development of profiles of mycolic acids, which vary from one species to anothers .

E. DNA DETECTION (PCR)

The polymerase chain reaction holds great promise for the rapid and direct detection of M tuberculosis in clinical specimens .

Epidemiology

The most frequent source of infection is the human who excretes, 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.

Prevention & Control

(1) Prompt and effective treatment of patients with active tuberculosis and careful follow-up of their contacts with tuberculin tests, x-rays, and appropriate treatment are the mainstays of public health tuberculosis control.

(2) Drug treatment of asymptomatic tuberculin-positive persons /

(3) Individual host resistance: 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) Immunization: Various living avirulent tubercle bacilli, particularly **BCG (bacillus Calmette-Guérin, an attenuated bovine organism**), have been used to induce a certain amount of resistance in those heavily exposed to infection.**(5)** The eradication of tuberculosis in cattle and the pasteurization of milk have greatly reduced *M bovis infection*