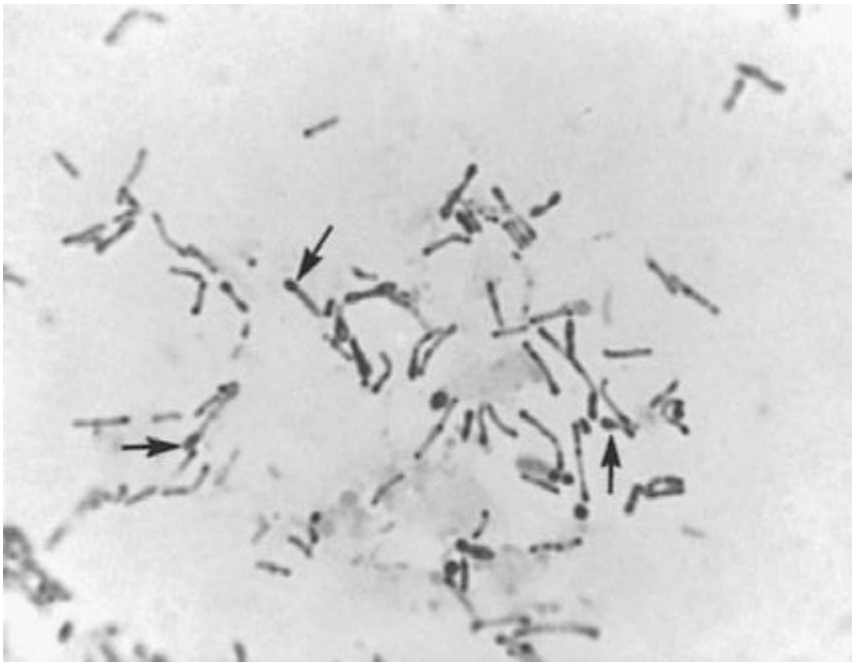
# Non-Spore-Forming Gram-Positive Bacilli

## *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 damag**e,

**( 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***

**Escherichi**a—*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) i**s 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 i***s 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 tes**t, 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 T**he 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

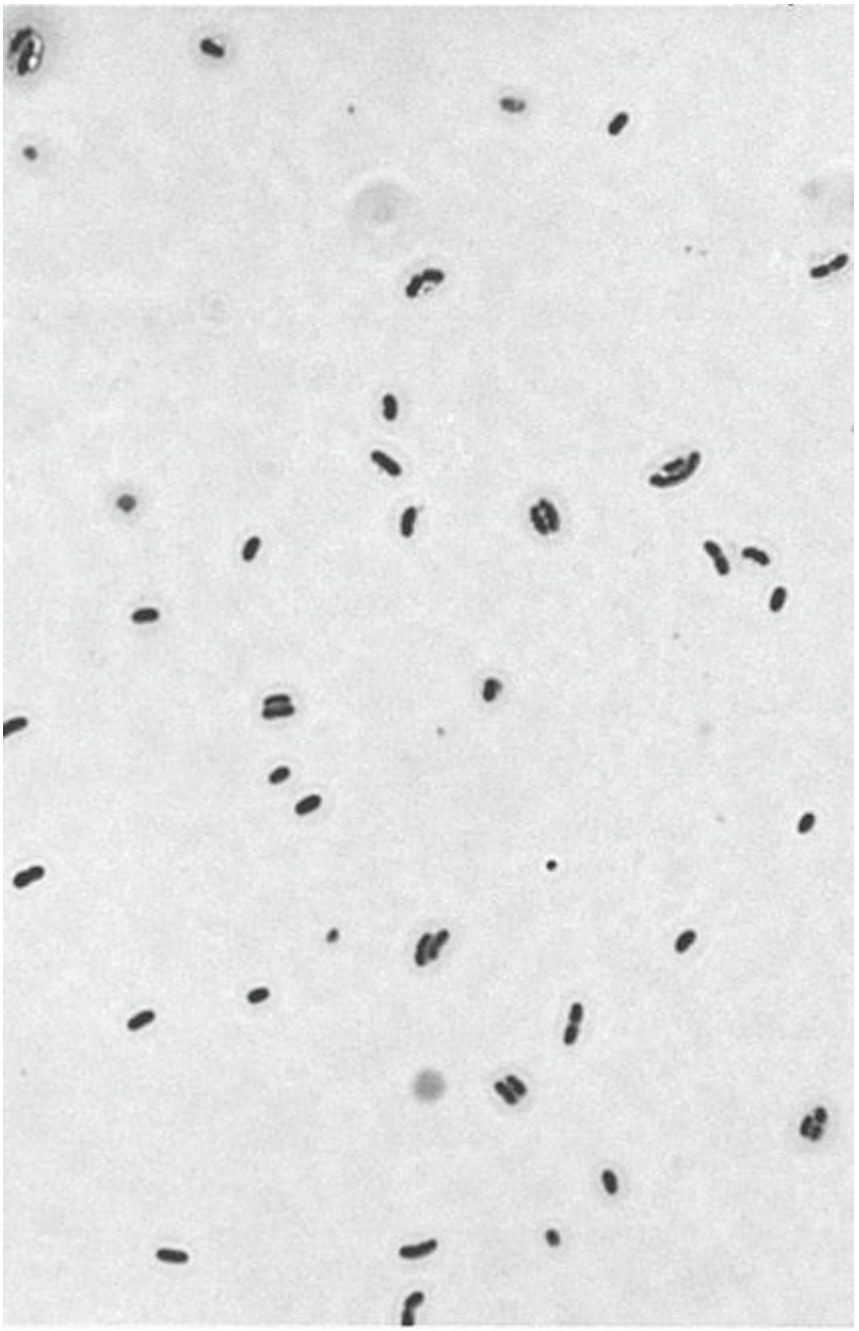
are gram-negative rods that are all widely distributed in nature. The vibrios are found in marine and surface water. *Vibrio cholerae* produces an enterotoxin that causes cholera, a profuse watery diarrhea that can rapidly lead to dehydration and death.

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 us**ing 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 T**he 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 aga**r**. 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

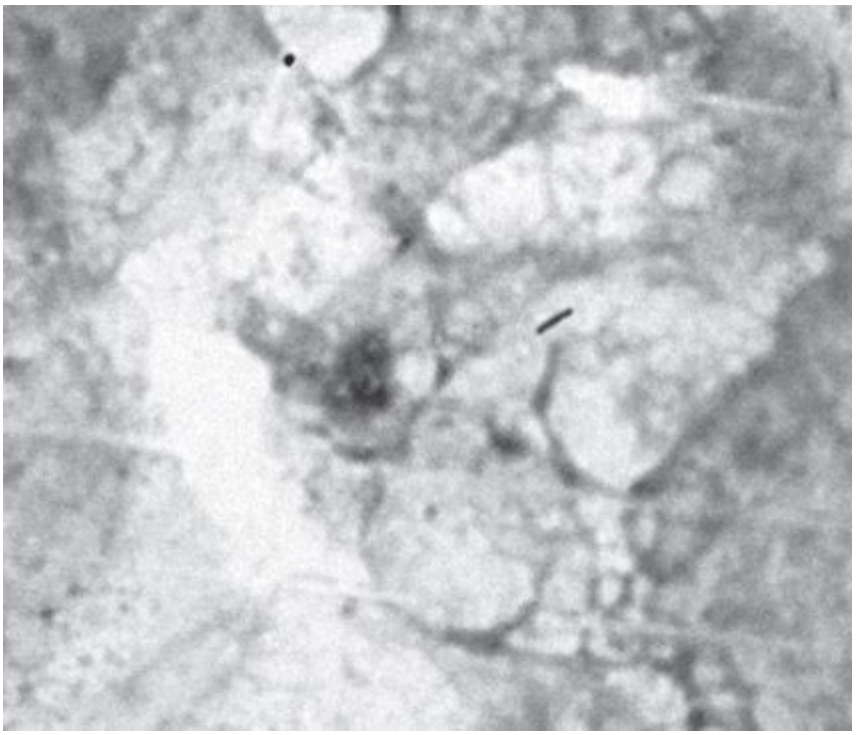
is a genus of Gram-positive bacilli that demonstrate the staining characteristic of acid-fastness. Its most important species, *Mycobacterium tuberculosis*, is the etiologic agent of tuberculosis .

## *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 inoc**ula. 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*