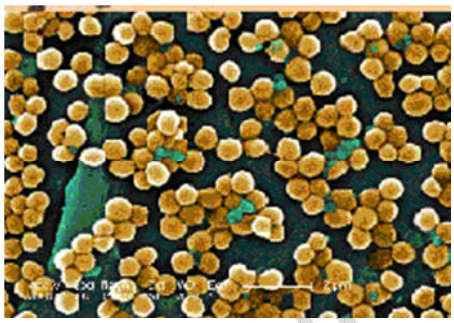
Staphylococci



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, **fermenting carbohydrates** and **producing pigments** that vary from **white to deep yellow**.

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 **hemolyze blood**, **coagulate plasma**, and **produce** a variety of **extracellular enzymes and toxins**. The most common type of food poisoning is caused by **a heat-stable staphylococcal enterotoxin.** Staphylococci **rapidly develop resistance to many antimicrobial agents** and present difficult therapeutic problems.

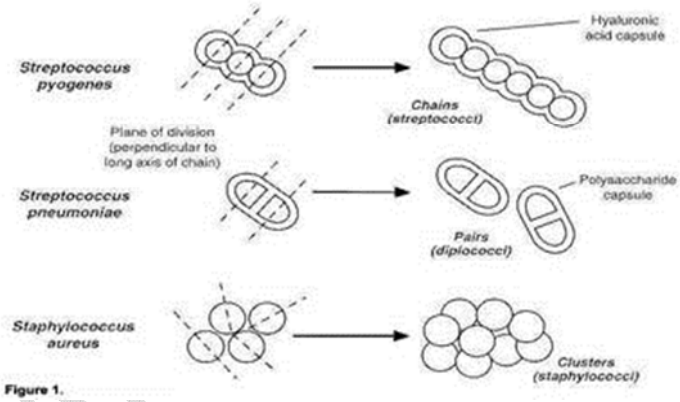


Figure 1.

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. Almost every person will have some type of *S. aureus* infection during a lifetime, ranging in severity from food poisoning or minor skin infections to severe life-threatening infections.

The **coagulase-negative staphylococci** are **normal human flora** and sometimes **cause infection**, often associated with implanted appliances and devices, especially in very young, old, and immunocompromised patients. Approximately 75% of these infections caused by **coagulase-negative** staphylococci are due to ***S epidermidis;*** infections due to ***Staphylococcus lugdunensis, Staphylococcus warneri, Staphylococcus hominis,*** and other species are less common. ***S saprophyticus*** is a relatively common cause of urinary tract infections in young women. Other species are important in veterinary medicine.

Streptococci

The streptococci are **gram-positive spherical** bacteria that characteristically **form pairs or chains during growth**. They are widely distributed in nature. Some of members are **the normal human flora**; others are **associated with important human**

diseases attributable in part **to infection by streptococci**, in part to sensitization to them. Streptococci elaborate a **variety of extracellular substances and enzymes**. The streptococci are a large and heterogeneous group of bacteria and no one system suffices to classify them. Yet, understanding the classification is key to understanding their medical importance.

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
(4) Ecologic features.

Hemolysis:

Many streptococci are able to hemolyze red blood cells in vitro in varying degrees. **Complete Lysis** of erythrocytes - clearing of the blood around the bacterial growth **(β hemolysis)**. **Incomplete Lysis** - reduction of hemoglobin and the formation of green pigment **(α-hemolysis).** Other streptococci are **non-hemolytic (**sometimes called **gamma hemolysis)**.

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*).

Biochemical Reactions:

Biochemical tests include **Sugar fermentation reactions, Tests for the presence of enzymes, and Tests for susceptibility or resistance to certain chemical agents.** Biochemical tests are most often used to classify streptococci **after the colony growth and hemolytic characteristics have been observed**. Biochemical tests are used for species that typically do not react with the commonly used antibody preparations for the group-specific substances, groups A, B, C, F, and G (**Lancefield classification groups A,B,C,D, F,G)**. For example, the **Viridans streptococci** are α-hemolytic or nonhemolytic and do not react with the antibodies commonly used for the **Lancefield classification**. Speciation of the viridans streptococci requires a battery of biochemical tests.

Many species of streptococci, including ***S pyogenes* (group A)**, ***S agalactiae* (group B)**, and the **enterococci (group D),** are characterized by combinations of features: **colony growth characteristics**, **hemolysis patterns on blood agar** (α -hemolysis, β-hemolysis, or no hemolysis), **antigenic composition of group-specific cell wall substances**, and **biochemical reactions**. *S pneumoniae* (pneumococcus) types are further classified by the antigenic composition of the capsular polysaccharides. The viridans streptococci can be α -hemolytic or nonhemolytic and are generally speciated by biochemical reactions

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, and enteric bacteria are the terms used in this chapter, 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. In this lecture, taxonomic refinements will be minimized, and the names commonly employed in the medical literature will generally be used.

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; and have a **39–59% G + C** DNA content.

Morphology and Identification:

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.

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 **Shigella** produce colonies similar to ***E. coli*** but **do not ferment lactose**. Some strains of ***E. coli*** produce **hemolysis on blood agar**.

Growth Characteristics:

Carbohydrate fermentation patterns and the activity of **amino acid decarboxylases** and **other enzymes** are used in **biochemical differentiation**. Some 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 non-lactose-fermenting colonies (nonpigmented) and may allow rapid presumptive identification of enteric bacteria .

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 H_2S 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. 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 CO_2 and H_2O and released from the slant and as oxidative decarboxylation of proteins continues with formation of amines, the slant turns alkaline (red). 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.

Escherichia

E. coli typically produces positive tests for indole, lysine decarboxylase, and mannitol fermentation and produces gas from glucose. An isolate from urine can be quickly identified as *E. coli* by its hemolysis on blood agar, 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.

Klebsiella-Enterobacter-Serratia Group

Klebsiella species exhibit mucoid growth, large polysaccharide capsules, and lack of motility, and they usually give positive tests for lysine decarboxylase and citrate. Most *Enterobacter* species give positive tests for motility, citrate, and ornithine decarboxylase and produce gas from glucose. *Enterobacter aerogenes* has small capsules. Serratia produces DNase, lipase, and gelatinase. Klebsiella, enterobacter, and serratia usually give positive Voges-Proskauer reactions.

Proteus-Morganella-Providencia Group

The members of this group deaminate phenylalanine, are motile, grow on potassium cyanide medium (KCN), and ferment xylose. *Proteus* species move very actively by means of peritrichous flagella, resulting in "swarming" on solid media unless the swarming is inhibited by chemicals, eg, phenylethyl alcohol or CLED (cystine-lactose-electrolyte-deficient) medium. *Proteus* species and *Morganella morganii* are urease-positive, while *Providencia* species usually are urease-negative. The proteus-providencia group ferments lactose very slowly or not at all. *Proteus mirabilis* is more susceptible to antimicrobial drugs, including penicillins, than other members of the group.

Citrobacter

These bacteria typically are citrate-positive and differ from the salmonellae in that they do not decarboxylate lysine. They ferment lactose very slowly if at all.

Shigella

Shigellae are nonmotile and usually do not ferment lactose but do ferment other carbohydrates, producing acid but not gas. They do not produce H_2S. The four *Shigella* species are closely related to *E coli.* Many share common antigens with one another and with other enteric bacteria (eg, *Hafnia alvei* and *Plesiomonas shigelloides*).

Salmonella

Salmonellae are motile rods that characteristically ferment glucose and mannose without producing gas but do not ferment lactose or sucrose. Most salmonellae produce H_2S. They are often pathogenic for humans or animals when ingested. Arizona is included in the salmonella group.

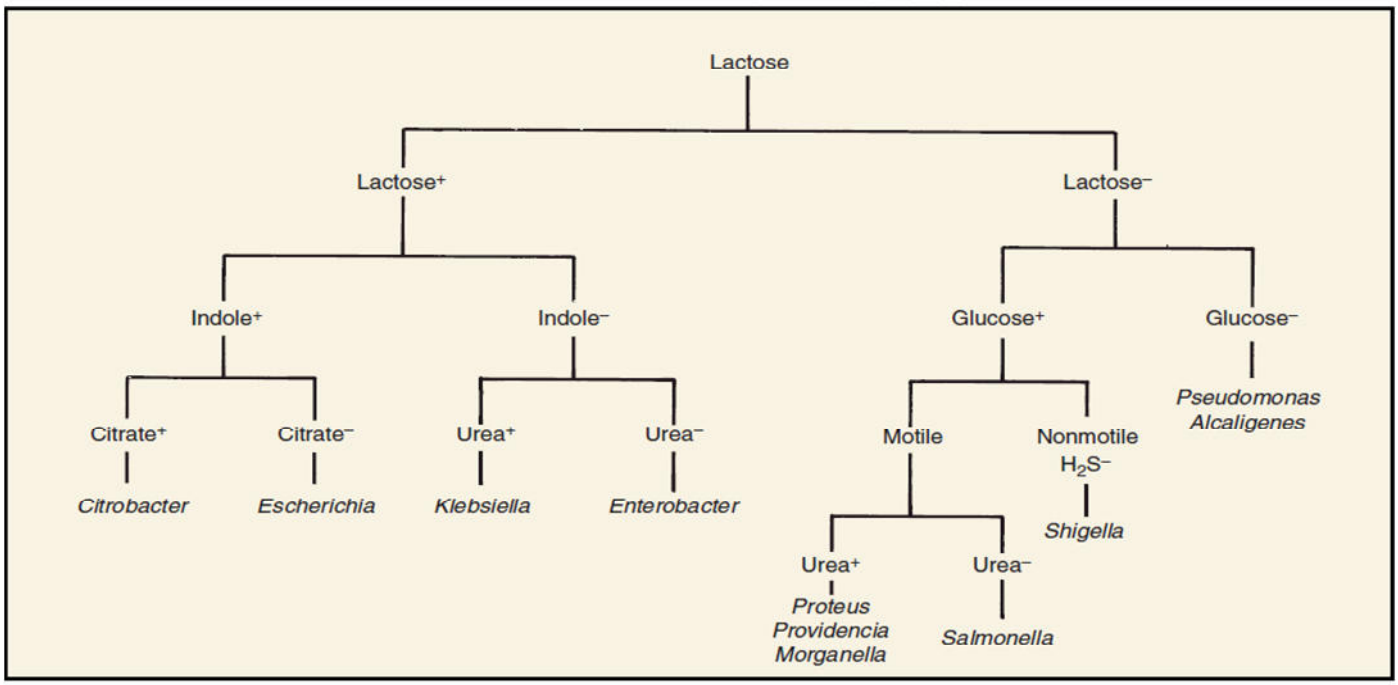
Antigenic Structure

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

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).

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. Such H antigens agglutinate with anti-H antibodies, mainly IgG. The determinants in H antigens are a function of the amino acid sequence in flagellar protein (flagellin).



Separation outline of Enterobacteriaceae

Figure 3 is a separation outline that is the basis for the series of tests that are used to demonstrate the presence of salmonella or Shigella in a patient's blood, urine, or feces. Note that lactose fermentation separates the salmonella and Shigella from most of the other Enterobacteriaceae. Final differentiation of the two enteric pathogens from *Proteus* relies on motility, hydrogen sulfide production, and urea hydrolysis. The differentiation information of the positive lactose fermenters on the left side of the separation outline is provided here mainly for comparative references that can be used for the identification of other unknown enterics.

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.

Morphology and Identification

Typical Organisms

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

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.

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.

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; when intravenous or urinary catheters are used; or when neutropenia is present, as in cancer chemotherapy. 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, leukocytosis and leukopenia, disseminated intravascular coagulation (DIC), and adult respiratory distress syndrome. *P aeruginosa* and other pseudomonads 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:** when introduced by lumbar puncture; 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**; this occurs commonly in patients with leukemia or lymphoma who have received antineoplastic drugs or radiation therapy and in patients with severe burns.

In most *P. aeruginosa* infections, the symptoms and signs are nonspecific and are related to the organ involved. Occasionally, **verdoglobin** (a breakdown product of hemoglobin) or **fluorescent pigment** can be detected in wounds, burns, or urine by ultraviolet fluorescence.

Hemorrhagic necrosis of skin occurs often in sepsis due to *P aeruginosa;* the lesions, called **ecthyma gangrenosum,** are surrounded by erythema and often do not contain pus. *P aeruginosa* can be seen on Gram-stained specimens from ecthyma lesions, and cultures are positive. **Ecthyma gangrenosum is uncommon in bacteremia due to organisms other than *P aeruginosa.***

Vibrios:

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**. 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.

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, including defined media containing mineral salts and asparagine as sources of carbon and nitrogen. *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.** Cultures containing fermentable carbohydrates therefore quickly become sterile. Under natural conditions, *V. cholerae* is pathogenic only for humans. A person with normal gastric acidity may have to ingest as many as 10^{10} 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 10^2–10^4 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*.

In areas where cholera is **endemic**, direct cultures of stool on selective media such as TCBS, and enrichment cultures in alkaline peptone water are appropriate. However, routine stool cultures on special media such as TCBS generally are not necessary or cost-effective in areas where **cholera is rare**.

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 microvilli** of the **brush border** of **epithelial cells**. There they multiply and **liberate cholera toxin** and perhaps **mucinases** and **endotoxin**.

Neisseriae:

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. 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**.

***Haemophilus influenzae* :**

Haemophilus influenzae Gram-negative, coccobacillary, facultatively anaerobic pathogenic bacterium belonging to the *Pasteurellaceae* family. 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. The organisms are short (1.5 mu) coccoid bacilli . On enriched medium have a definite capsule. The capsule is the antigen used for "typing" *H. influenzae* .

Mycobacteria:

The mycobacteria are **rod-shaped**, aerobic bacteria that do **not form spores**. Although they do not stain readily, once stained they resist decolorization by acid or alcohol and are **therefore called "acid-fast" bacilli.** ***Mycobacterium tuberculosis*** causes **tuberculosis** and is a very important pathogen of humans.

Mycobacterium leprae causes l**eprosy**. ***Mycobacterium avium****-intracellulare* (***M.avium*** **complex, or MAC**) and other **atypical mycobacteria** frequently infect patients with AIDS, are opportunistic pathogens in other immunocompromised persons, and occasionally cause disease in patients with normal immune systems.

Mycoplasmas:

There are over **150 species** in the **class of cell wall-free bacteria**. At least 15 of these species are thought to be of human origin, while others have been isolated from animals and plants. In humans, four species are of primary importance: ***Mycoplasma pneumoniae*** causes **pneumonia** and has been associated with **joint and other infections**. ***Mycoplasma hominis*** sometimes causes **postpartum fever** and has been found with other bacteria **in uterine tube** infections. ***Ureaplasma urealyticum*** is a cause of nongonococcal **urethritis** in men and is associated with **lung disease in** premature infants of low birth weight. ***Mycoplasma genitalium*** is closely related to *M. pneumoniae* and has been associated with urethral and other infections. Other members of the genus *Mycoplasma* are pathogens of the respiratory and urogenital tracts and joints of animals. They have the following characteristics:

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

Rickettsia

They are **obligate intracellular parasites** and, except for **Q fever**, are transmitted **to humans by arthropods**. Many rickettsiae are transmitted transovarially in the arthropod, which serves as both vector and reservoir. Rickettsial infections, typically are manifested by fever, rashes, and vasculitis.

Chlamydiae

Chlamydiae that infect humans are divided into **three species**—***Chlamydia trachomatis*** (is a major infectious cause of human genital and eye disease.), ***Chlamydia pneumoniae*** (is a major cause of pneumonia), and ***Chlamydia psittaci*** (may cause respiratory psittacosis in humans often starts with flu-like symptoms and becomes a life-threatening pneumonia)—on the basis of antigenic composition, intracellular inclusions, sulfonamide susceptibility, and disease production. A fourth species, ***Chlamydia pecorum,*** infects a variety of animals but is not known to infect humans. All Chlamydiae exhibit similar morphologic features, share a common group antigen, and multiply in the cytoplasm of their host cells by a distinctive developmental cycle. The Chlamydiae can be viewed as gram-negative bacteria that lack mechanisms for the production of metabolic energy and cannot synthesize ATP. This defect restricts them to an intracellular existence, where the host cell furnishes energy-rich intermediates. Thus, Chlamydiae are **obligate intracellular parasites.**