Bacterial Pathogenesis

Introduction and definitions

Disease is caused by certain microorganisms known as ***pathogens*.** Microorganisms vary in their ability to cause disease in humans.
Pathogens: A microorganism capable of causing disease, especially if it causes disease in immunocompetent people, is called as a *pathogen*. These pathogens, however, represent a very small proportion of the microbial species.
Opportunistic pathogens: A microbe that is capable of causing disease only in immunocompromised people is known as *opportunistic pathogen*. These organisms can cause disease only if one or more of the usual defense mechanisms of humans are reduced or altered by accident, by intent (e.g., surgery), or by an underlying metabolic disorder or an infectious disease (e.g., AIDS).

infection

This is a process when an organism enters the body, increases in number, and causes damage to the host. The term *infection* has more than one meaning: (*a*) the presence of microbes in the body and (*b*) the symptoms of the disease

The presence of microbes in the body does not always result in symptoms of the disease. Bacteria cause symptoms of disease by two main mechanisms: (*a*) production of toxins, both endotoxin and exotoxin and (*b*) production of inflammation.

Virulence :The words “virulence” and “virulent” are derived from the Latin word *virulentus*, meaning “full of poison.”
Virulence is a measure of a microbe’s ability to cause disease. It is a quantitative measure of pathogenicity and is measured by the number of organisms required to cause disease. It means that a highly virulent microbe requires fewer organisms to cause disease than a less virulent one; hence it is directly dependent on the infectious dose of the organism.

The 50% lethal dose (LD50) is the number of organisms required to kill half of the hosts, whereas 50% infectious dose (ID50) is the number of microbes needed to cause infection in half of the hosts. The infectious dose of an organism required to cause disease varies among the pathogenic bacteria. For example, the infectious dose of *Shigella* to cause dysentery is less than 100 organisms, whereas that of *Salmonella* to cause diarrhea is more than 100,000 organisms. The virulence of a microbe is determined by virulence factors, such as capsules, exotoxins, or endotoxins.....
Pathogenicity is the capacity of a pathogen species to cause disease, while virulence is used to describe the sum of disease causing properties of a population (*strain*) within the species. Pathogens can be distinguished from their

avirulent counterparts by the presence of specific genes or gene clusters in the genome known as ***pathogenicity islands***.

The diseases that can be spread from one person to another are called ***communicable diseases***. Most microbial infections are communicable diseases. Three epidemiological terms are often used to describe infection: endemic, epidemic, and pandemic:

- **Endemic:** The infection that occurs at a persistent, usually low level in a certain geographical area is called endemic.
- **Epidemic:** The infection that occurs at a much higher rate than usual is known as epidemic.
- **Pandemic:** Infection that spreads rapidly over large areas of the world is known as a pandemic.

Types of Infections

Infections may be of the following types:

- **Primary infection:** infection with an organism in a host.
- **Reinfection:** subsequent infection with the same organism in the same host.
- **Secondary infection:** infection with a new organism in a host whose body resistance is already lowered by a pre-existing infectious disease.
- **Cross-infection:** infection with a new organism from another host or another external source in a patient who is already suffering from a disease.
- **Nosocomial infection:** Cross-infections acquired in hospitals are called hospital-acquired, hospital-associated, or nosocomial infections.
- **Iatrogenic infection:** physician induced infection as a result of therapy with drugs or investigation procedures.
- **Subclinical infection:** Inapparent clinical infections are called subclinical infections.
- **Latent infections:** a condition in which some organisms may remain in a latent or hidden stage in host and subsequently they multiply to produce clinical disease when host resistance is lowered.

Infection process:

Infectious diseases are complex. The outcome of infection depends on a variety of factors of the microbe and host as follows:

1. The ability of the organism to break host barriers and to evade destruction by innate local and tissue host defenses.
2. The ability of the organism to replicate, to spread, to establish infection, and to cause disease.
3. The ability of the organism to transmit to a new susceptible host.
4. The innate and adaptive immunologic ability of the host to control and eliminate the invading microorganism.

The infection process involves the following stages: (*a*) transmission of infection, (*b*) entry of the organisms and evasion of the local defenses, (*c*) adherence to cell surfaces, (*d*) growth and multiplication of the bacteria at the site of adherence, (*e*) manifestations of disease, and (*f*) termination of disease.

Transmission of Infection

There are three important components that play an important role in successful transmission of microbial diseases. These are (a) reservoir, (b) mode of transmission, and (c) susceptible host.

Reservoir

Reservoirs of microbial infections are human, animal, plant, soil, or inanimate matter in which organisms usually live, multiply, and cause the infections with or without overt clinical manifestations. Humans are usually the common reservoirs of many of the microbial infections. Animals are reservoirs of zoonotic infections, such as plague (e.g., rats), rabies (e.g., dogs), cysticercosis (e.g., pigs), etc.

Sources of infections: The sources of infections may be endogenous and exogenous:

■ ***Endogenous sources:*** The source of infection is the normal bacterial flora present in the human body. These bacteria are usually nonpathogenic but in certain situations become pathogenic and cause infections at different sites in the same host. For example, *Escherichia coli* present as normal flora of the intestine may cause urinary tract infection in the same host. Similarly, viridans streptococci present as a part of the normal flora of the mouth may cause infective endocarditis.

■ ***Exogenous sources:*** The source of infection is from outside the host's body. Most of the microbial infections are exogenous in nature. The exogenous sources include the following:

1. Humans: Humans are the most common sources of infections caused by the microorganisms. They may be either **patients** or **carriers**. The patient suffering from an active infection is an important source of infection to others. A carrier is a person who harbors pathogenic microorganisms without showing any signs and symptoms of disease.

Carriers are also important sources of infections. A carrier may be (*a*) healthy carrier, (*b*) convalescent carrier, (*c*) temporary carrier, and (*d*) chronic carrier.

■ **Healthy carrier** is the host who harbors the microorganism without ever suffering from the disease caused by that microorganism.

■ **Convalescent carrier** is the host who continues to harbor the microorganism even after recovering from the clinical disease caused by the same pathogen.

■ **Temporary carrier** is the host who harbors the microorganism up to 6 months after recovering from the disease caused by the same pathogen.

■ **Chronic carrier** is the host who harbors the microorganism for many years after recovering from the clinical disease caused by the same pathogen.

2. Animals: Animals are also important sources of infection for humans. The symptomatic as well as asymptomatic animals can transmit infections to humans. Asymptomatic animals act as a reservoir of human infections. These are called as ***reservoir hosts***. Infections transmitted from animals to humans are called ***zoonotic infections***.

The examples of zoonotic infections include bacterial (e.g., plague, anthrax, bovine tuberculosis, etc.).

3. Insects: Insects, such as mosquitoes, ticks, mites, flies, fleas, and lice may transmit a wide variety of microorganisms to the humans. The diseases transmitted by the insects are collectively referred to as ***arthropod-borne diseases*** and the insects transmitting these pathogens are called vectors.

4. Food: Food items contaminated with pathogens also act as source of infection and cause diarrhea, dysentery, food poisoning, and gastroenteritis.

5. Water: Water contaminated with microorganisms also acts as a source of infection and transmits water-borne diseases, such as leptospirosis, cholera, dysentery, hepatitis A infection, etc.

Modes of transmission

Microbial pathogens causing various infectious diseases are transmitted from one host to another by many ways: (*a*) contact, (*b*) inoculation, (*c*) ingestion, (*d*) inhalation, and (*e*) vectors.

1. Contact: Transmission of microorganisms from person to person occurs by direct or indirect contact.

2. Inoculation: Infections can be transmitted by inoculation of microorganisms directly into tissues of the host. ***Iatrogenic infection*** occurs following the use of unsterile syringes and equipment in a hospital.

3. Ingestion: Ingestion of water and food contaminated with microorganisms can transmit a wide variety of microbial infections. For example, food poisoning caused by *Bacillus cereus* . Cholera, typhoid, food poisoning, are the other examples of diseases transmitted by ingestion of contaminated food and water.

4. Inhalation: Infections are transmitted by inhalation of droplet nuclei that are discharged into the air by coughing, sneezing, or talking. Respiratory pathogens are shed into the environment by patients in secretions from the nose or throat during coughing, sneezing, or talking. whooping cough, tuberculosis, etc. are few examples of infectious diseases acquired by inhalation.

5. Vectors: Mosquitoes, flies, fleas, ticks, mite, and lice are the vectors that transmit many diseases as mentioned earlier.

Susceptible host

The infective agent enters the body by four main routes:
(*a*) genital tract, (*b*) respiratory tract, (*c*) gastrointestinal tract, and (*d*) skin.
The pathogens can be transmitted either as vertical or horizontal transmission.

Vertical transmission: Certain bacteria (*Treponema pallidum*), can be transferred from mother to fetus by a process called vertical transmission. The organisms can be transmitted vertically by three ways:
(a) Across the placenta,
(b) Within birth canal during birth, and
(c) Through breast milk.

Horizontal transmission: Unlike vertical transmission, horizontal transmission occurs from person to person and is not from mother to off spring.

Entry of Organisms and Evasion of Local Defenses

Skin, mucus, ciliated epithelium, and secretions containing antibacterial substances (e.g., lysozyme) are the natural barriers of the human and animal hosts that prevent microbial entry. However, these barriers are sometimes broken (e.g., a break in the skin, an ulcer in the intestine, or a tumor, etc.), thereby allowing the entry of microbes into the host. On entry, the microbes spread through blood circulation to other sites in the body .

Skin: The skin with its superficial cornified anucleate layers is a simple and efficient mechanical barrier to prevent microbial invasion. Organisms gain access to the underlying tissues only by breaks or by way of hair follicles, sebaceous glands, and sweat glands that traverse the stratified layers The surface of the skin continuously desquamates and thereby tends to shed contaminating organisms. The skin also inhibits the growth of most extraneous microorganisms due to its low moisture, low pH, and the presence of substances with an antibacterial activity.

Mucus: Viscous mucus secreted by goblet cells protects the epithelium lining the respiratory and gastrointestinal tracts and urogenital system. Microorganisms become trapped in the mucus layer and may be swept away before they reach the epithelial cell surface. Secretory IgA, secreted into the mucus, and other secreted antimicrobials (such as lysozyme and lactoferrin) facilitate this cleansing process.

Ciliated epithelial cells: These cells constantly move the mucus away from the epithelial surfaces. For example, mucus in the respiratory tract—particles larger than 5 *M*m are washed and trapped in the mucus. Similarly, the multilayered transitional epithelium of the urinary tract uses the flushing effect of urine, and its relatively low pH acts as an additional defense mechanism to limit microbial entry and growth.

Secretions: The high level of hydrochloric acid and gastric enzymes in the normal stomach kills many ingested bacteria. Others are susceptible to pancreatic digestive enzymes or to the detergent effect of bile salts.

Adherence to Cell Surfaces

Adherence of bacteria to body surface is the most important event in the pathogenesis of disease. Once bacteria enter the body of the host, they must adhere to the cells of a tissue surface. If they do not adhere, they will be swept away by mucus and other fluids that bathe the tissue surface. Most pathogenic microorganisms have more than a single mechanism of host cell attachment. Adherence is important not only during the initial encounter between the pathogen and its host but also throughout the infection cycle. Adherence requires participation of two factors: bacterial adhesins and a receptor on the host cell.

- **Bacterial adhesins:**

Bacterial adherence to the cell surface is mediated by specialized molecules. The various molecules that mediate adherence to the cell surface are called ***adhesins***. These adhesins allow the bacteria to adhere to the surface of human cell, thereby promoting their ability to cause disease. Microorganisms that lack this mechanism are nonpathogenic. Bacterial adhesins can be divided into two major groups: pili (fimbriae) and nonpilus adhesins (afimbrial adhesins).
Pili: These are the main mechanisms by which bacteria adhere to human cells.
■ **Nonpilus adhesins:** These include glycocalyx and other adhesins present on the bacterial surfaces. The matrix formed by these adhesins forming proteins is called a ***biofilm***.
The biofilms are important in pathogenesis because they protect the bacteria by host defense and antibiotics .
■ *Streptococcus pyogenes* makes use of nonpilus adhesins (such as lipoteichoic acid, protein F, and M protein) to bind to epithelial cells. The lipoteichoic acid and protein F cause adherence of the streptococci to buccal epithelial cells. M protein acts as an antiphagocytic molecule.
■ Recently, it has been shown that certain strains of *E. coli* and *Shigella* spp. have surface proteins called ***curli,*** which help in the binding of bacteria to the host endothelium as well as to extracellular proteins.

- **Receptor on the host cell**

Certain receptors are present on the host cells to which pathogens adhere and initiate infections. For example, many a dhesion proteins are present at the tip of the pili of *E. coli.* These bind specific receptors on the surface of the urinary bladder to initiate urinary tract infections. Similarly, the gonococci adhere to the microvilli of nonciliated cells and start disease process.

Growth and Multiplication of Bacteria at the Site of Adherence

Bacteria cause diseases by three main mechanisms: (*a*) invasion of tissues followed by inflammation, (*b*) toxin production, and (*c*) immunopathogenesis.

- **Invasion of tissues followed by inflammation**

Invasiveness refers to the ability of an organism to invade the host cells after establishing infection. “Invasion” is the term commonly used to describe the entry of bacteria into host cells, implying an active role for the organisms and a passive role for the host cells.

Invasion of tissues followed by inflammation is enhanced by many factors, which include: (*a*) enzymes, (*b*) antiphagocytic factors, (*c*) biofilms, (*d*) inflammation, and (*e*) intracellular survival.

1. Enzymes: Invasion of bacteria is enhanced by many enzymes. Many species of bacteria produce enzymes that are not intrinsically toxic but do play important roles in the infectious process. Some of these enzymes are discussed below:

■ ***Hyaluronidases and collagenase:*** Hyaluronidases and collagenase are the enzymes that hydrolyze hyaluronic acid and degrade collagen, respectively; thereby allowing the bacteria to spread through subcutaneous tissues.

■ ***Coagulase:*** *Staphylococcus aureus* produces the enzyme coagulase, which in association with blood factors coagulates the plasma. Coagulase contributes to the formation of fibrin walls around staphylococcal lesions, which protects bacteria from phagocytosis by walling off the infected area.

■ ***Streptokinase (fibrinolysin):*** Many hemolytic streptococci produce enzyme streptokinase, which activates a proteolytic enzyme of plasma. This enzyme is then able to dissolve coagulated plasma and thereby possibly aids in the rapid spread of streptococci through tissues.

■ ***IgA1 proteases:*** Certain pathogenic bacteria produce enzymes IgA1 proteases that split IgA1 at specific proline– threonine or proline–serine bonds in the hinge region and inactivate its antibody activity.

2. Antiphagocytic factors: Many bacterial pathogens are rapidly killed once they are ingested by polymorphonuclear cells or macrophages. Some pathogens evade phagocytosis or leukocyte microbicidal mechanisms by several antiphagocytic factors; the most important being (*a*) capsule, (*b*) cell wall proteins, (*c*) cytotoxins, and (*d*) surface antigens.

■ ***Capsule:*** The capsule surrounding bacteria, such as *S. pneumoniae* .

■ ***Cell wall proteins:*** The cell wall proteins, such as the protein A and protein M, of *S. aureus* and *S. pyogenes* especially are antiphagocytic. For example, protein A of *S. aureus* binds to IgG and prevents the activation of complement. M protein of *S. pyogenes* is antiphagocytic.

■ ***Cytotoxins:*** Certain bacteria produce cytotoxins that interfere with chemotaxis or killing of phagocytes. For example, *S. aureus* produces hemolysins and leukocidins that lyse and damage RBCs and WBCs.

■ ***Surface antigens:*** Surface antigens of bacteria, such as Vi antigen of *S. typhi* and K antigen of *E. coli* make the bacteria resistant to phagocytosis and lytic activity of complement.

3. Biofilms: The biofilm is an aggregate of interactive bacteria attached to a solid surface or to each other and encased in an exopolysaccharide matrix.

Biofilms consist of single cells and microcolonies of bacteria, all found together in a highly hydrated, predominantly anionic exopolymer matrix.

4. Inflammation: Inflammation is an important host defense induced by the presence of bacteria in the body. It is of two types: pyogenic and granulomatous.

Pyogenic inflammation : is the host defense seen primarily against pyogenic or pusproducing bacteria, such as *S. pyogenes*. It typically consists of neutrophils and the production of specific antibodies and elevated level of complement. ***Granulomatous inflammation*** : is the host defense seen primarily against intracellular granuloma-producing bacteria, such as *Mycobacterium tuberculosis*, *Mycobacterium leprae*, etc. The response consists of production of macrophages and CD4+ T cells.

5. Intracellular survival: A few mechanisms that are suggested for intracellular survival of bacteria include (*a*) inhibition of phagolysosome fusion, (*b*) resistance to action of lysosomal enzymes, and (*c*) adaptation to cytoplasmic replication

Toxin production

Toxins produced by bacteria are generally classified into two groups: exotoxins and endotoxins.

1. Exotoxins: Exotoxins are heat-labile proteins that are produced by several Gram-positive and Gram-negative bacteria.

These are bacterial products, which are secreted into tissues and directly harm tissues or trigger destructive biological activities.

Superantigens: Superantigens are special group of toxins.

2. Endotoxins: The term endotoxin was coined in 1893 by Pfeiffer to distinguish the class of toxic substances released after lysis of bacteria from the toxic substances (exotoxins) secreted by bacteria.

Biological activity of endotoxin: Gram-negative bacteria produce endotoxin during infection. The toxicity of endotoxin is low in comparison with exotoxins. All endotoxins usually produce the same generalized effect of fever and shock. The lipid A protein of LPS is responsible for endotoxin activities.

- **Immunopathogenesis**

In certain diseases, the symptoms are caused not by the organism itself, but due to immune response to the presence of organisms. For example, immune complexes deposited in the glomerulus of the kidney cause poststreptococcal glomerulonephritis. Antibodies that are produced against the M proteins of *S. pyogenes* cross-react with joint, heart, and brain tissues producing disease manifestations of rheumatic fever. Similarly, the host immune response is an important cause of disease symptoms in patients suffering from syphilis caused by *T. pallidum*, Lyme disease caused by *Borrelia*, and other diseases.

The genus *Staphylococcus*

Family Micrococcaceae consists of Gram-positive cocci, which are aerobic and anaerobic, and are arranged in tetrads or clusters. Micrococcaceae consists of four genera, *Staphylococcus, Micrococcus, Planococcus,* and *Stomatococcus*. Among these, *Staphylococcus* is the only genus of medical importance. Differences between these genera are summarized in Table 1.

Table 1 **Features distinguishing *Staphylococcus, Micrococcus,* and *Planococcus***

Characters	*Staphylococcus*	*Micrococcus*	*Planococcus*
Arrangement of bacteria	Clusters	Clusters/ Tetrads	Tetrads
Presence of teichoic acid	+	−	−
Production of brown pigment	−	−	+
Glucose fermentation	+	+	−

The genus *Staphylococcus* consists of 32 species, most of which are animal pathogens or commensals. The bacteria belonging to this genus are aerobic and facultative anaerobic, catalase positive, oxidase negative, and are arranged in clusters, pairs, or tetrads.

- *Staphylococcus aureus* is the most important human pathogen.
- The other important human pathogens are coagulase negative staphylococci (CONS), which include *Staphylococcus epidermidis, Staphylococcus saprophyticus, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus warneri, Staphylococcus saccharolyticus, Staphylococcus schleiferi*, and *Staphylococcus lugdunensis*. Staphylococci are capable of acquiring resistance to many antibiotics and therefore can cause major clinical and epidemiological problems in hospitals.

Staphylococcus aureus

- **Morphology**

Staphylococci show following features:

- They are Gram-positive cocci, measuring around 1 *M*m in diameter.
- They are nonmotile, nonsporing.

■ They are noncapsulated. They, however, contain a microcapsule, which can be visualized by electron microscope only, but not by a light microscope.

The cocci are typically arranged in irregular grape-like clusters. This appearance is due to incomplete separation of daughter cells during successive divisions of bacteria, which takes place in perpendicular planes. The grape-like clustering is seen when the bacteria are grown in solid media, but usually short chains are seen when grown in liquid media. In smears taken from pus, the cocci are present either singly or in pairs, in clusters, or in short chains of three or four cells.

- **Culture**

Staphylococci are aerobes and facultative anaerobes but can grow in the absence of oxygen also. They grow at a temperature range of 10–42°C (optimum temperature 37°C) and a pH range of 7.4–7.6 (optimum pH 7).

Culture on solid media: Staphylococci can grow on a wide range of media including Mueller–Hinton agar, nutrient agar, blood agar, and MacConkey agar. Primary isolation can be made on nutrient agar and blood agar.

at Nutrient agar: *S. aureus* produces round, convex, well defined colonies measuring 2–4 mm in diameter. The colonies show a consistency with a smooth surface.

S. aureus produces characteristic golden-yellow colonies due to production of a nondiffusible golden-yellow pigment. The production of the pigment is enhanced by incubation at 22°C in the presence of oxygen. Milk agar and 1% glycerol monoacetate agar are other media that facilitate the production of pigment. On nutrient agar slopes, the growth gives a characteristic “oil paint” appearance.

at Blood agar: *S. aureus* produces a clear zone of hemolysis (beta-hemolysis) surrounding the colonies. Hemolysis is well marked on sheep or rabbit blood agar, especially when incubated in an atmosphere of 20–25% CO2. Sheep blood agar is used for primary isolation. Hemolysis is weak on horse blood agar. Human blood is not used, as it may contain antibiotics or other inhibitors. Other species of *Staphylococcus* do not produce hemolysis.

at MacConkey agar: *S. aureus* produces small pink colonies due to fermentation of lactose.

Selective media: Mannitol salt agar, milk agar, and glycerol monoacetate agar are the commonly used selective media for isolation of *S. aureus* from clinical specimens containing normal bacterial flora (e.g., stools). Mannitol salt agar contains 1% mannitol, 7.5% sodium chloride, and 0.0025% phenol red indicator. Most strains of *S. aureus* ferment mannitol with acid production, which gives rise to yellow zone formation around the colonies.

Culture in liquid media: *S. aureus* produces turbidity in liquid media and there is no production of pigment.

- **Biochemical reactions**

S. aureus shows following reactions:

■ It is coagulase positive. The production of coagulase is used as a test to differentiate *S. aureus* from *S. epidermidis* and other CONS.

■ It is phosphatase positive. Phosphatase production can also be used to differentiate *S. aureus* from *S. epidermidis*, as the latter either does not produce or has very weak phosphatase activity.

■ It is catalase positive. It produces enzyme catalase (unlike *Streptococcus*), which degrades H2O2 into nascent oxygen and water.

■ It is oxidase negative.

■ *S. aureus* ferments mannitol, sucrose, maltose, and trehalose under aerobic conditions, with the production of acid but no gas. Fermentation of mannitol is of diagnostic importance, because most strains of *S. aureus* ferment mannitol while those of *S. epidermidis* and *S. saprophyticus* do not ferment mannitol.

■ It liquefies gelatin, hydrolyzes urea, reduces nitrate to nitrite, and is "Voges-Proskauer (VP)" and "methyl red (MR)" positive but indole negative.

The cocci withstand moist heat at 60°C for 30 minutes but are killed after 30 minutes. They are also killed rapidly by disinfectants, such as phenol, chlorhexidine, and hexachlorophene. The cocci are very sensitive to aniline dyes, such as crystal violet.

Cell Wall Components and Antigenic Structure

Cell wall associated proteins and polymers include the following:

- **Cell wall peptidoglycan :**Peptidoglycan is a polymer of the polysaccharide, which provides rigidity to the cell wall of the bacteria.
- **Teichoic acid :** the major antigenic determinant of the cell wall of *S. aureus*. Antibodies to teichoic acids develop in some staphylococcal infections.
- **Protein A**

It is the major protein in the cell wall and has a molecular weight of 13,000 Da. The antigen is present in more than 90% strains of *S. aureus*. Protein A is absent in both the coagulase-negative staphylococci (CONS) and micrococci.

Pathogenesis and Immunity

S. aureus causes disease by multiplying in tissues and causing inflammation, and also by liberating toxin.

- **Virulence factors**

  S. aureus produces several virulence factors (Table 2), which include the following:

Table 2 **Virulence factors of *Staphylococcus aureus***

Virulence factors	Biological functions
Cell wall associated polymers and proteins	
Peptidoglycan	Inhibits chemotaxis of inflammatory cells
Capsular polysaccharide	Inhibits phagocytosis and chemotaxis
Teichoic acid	Mediates attachment of staphylococci to mucosal cell
Protein A	Chemotactic, anticomplementary, and antiphagocytic; causes platelet injury; and elicits hypersensitivity reactions
Enzymes	
Coagulase	The enzyme coats the bacterial cells with fibrin, rendering them resistant to opsonization and phagocytosis
Catalase	Produces nascent oxygen which causes oxidative damage to host tissue
Hyaluronidase	Hydrolyzes hyaluronic acids present in the matrix of the connective tissues, thereby facilitating the spread of bacteria in the tissues
Penicillinase	Inactivates penicillins
Nuclease	Hydrolyzes DNA
Lipases	Hydrolyzes lipids
Toxins	
Toxic shock syndrome toxin	Superantigen, stimulates the release of large amount of interleukins (IL-1 and IL-2)
Enterotoxin	Superantigen, acts by producing large amounts of interleukins (IL-1 and IL-2)
Exfoliative toxin	Splits intercellular bridges in the stratum granulosum of epidermis of the skin
Leukocidin toxin	Leukolysin is thermostable and causes lysis of leukocytes
Hemolysin	Causes lysis of erythrocytes

(a) Cell wall associated proteins and polymers
(b) Extracellular enzymes
(c) Toxins

Cell wall associated proteins and polymers

1- **Capsular polysaccharide:** Few strains of *S. aureus* are capsulated. These strains are more virulent than the noncapsulated ones.

- The capsule protects the bacteria from phagocytosis.
- The capsule also facilitates adherence of the cocci to host cells and to prosthetic implants.

2- Protein A: Protein A is an important virulence factor since it has :

- It is chemotactic, anticomplementary, and antiphagocytic.
- It causes platelet injury and elicits hypersensitivity reactions.

3- Peptidoglycan: It activates the complement, stimulates production of the antibodies, and inhibits chemotaxis by inflammatory cells.

4- Teichoic acid: It mediates attachment of staphycocci to mucosal cell.

Extracellular enzymes

1- **Coagulase:** *S. aureus* has a unique ability to clot a variety of mammalian plasma. Clotting of plasma is brought about by the action of the enzyme coagulase secreted by the pathogenic strains of *S. aureus*. The enzyme coagulase is of two types:

(*a*) free coagulase and (*b*) bound coagulase.

A. Free coagulase: Free coagulase is a heat-labile and filterable enzyme. It has eight antigenic types (A, B, C, D, E, F, G, and H). Antigenic type A is produced by most human *S. aureus* strains. The enzyme coagulase in association with coagulasereacting factor (CRF) present in plasma converts fibrinogen to fibrin. In the absence of CRF, coagulase cannot bring about clotting like in case of the guinea pig plasma. This fibrin coats the bacterial cells, rendering them resistant to opsonization and phagocytosis and hence making bacteria more virulent.

B. Bound coagulase: Bound coagulase is otherwise known as clumping factor. It is a heat-stable protein and is present in the cell wall. This enzyme brings about clumping of the staphylococci when mixed with plasma by directly acting on fibrinogen. Lysis of the cell releases the enzyme. Unlike free coagulase, clumping factor does not need CRF for its action; till date only one type has been identified. Bound coagulase is not a virulence factor.

2- Catalase: The enzyme catalase reduces H2O2 to nascent oxygen and water. This nascent oxygen causes oxidative damage of host tissue. This enzyme is produced after phagocytosis or during metabolism of the bacteria.

3- Hyaluronidase: The enzyme hyaluronidase hydrolyzes the acidic mucopolysaccharides present in the matrix of the connective tissues, thereby facilitating the spread of bacteria in tissues.

4- Penicillinase: More than 90% of *S. aureus* produce enzyme penicillinase. The enzyme inactivates penicillin group of antibiotics, hence is responsible for widespread occurrence of penicillin-resistant staphylococci. The gene for this enzyme is acquired through plasmids.

5- Other enzymes: These include phosphatase, deoxyribonucleases, nucleases, proteases, phospholipase, and lipases.

Toxins

1- **Toxic shock syndrome toxin:** Toxic shock syndrome toxin (TSST) is a protein with a molecular weight of 22,000 Da. It is antigenic. Production of toxin is pH dependent and occurs at pH 7–8. The toxin causes toxic shock syndrome (TSS).

2- **Enterotoxin:** Enterotoxin is a heat-stable protein, capable of resisting boiling for about 30 minutes. The toxin is produced by nearly one-third of all the strains.

Nine antigenic types (A, B, C1,2,&3, D, E, G, H, I, and J) of enterotoxins have been described, out of which type A and B are most important. These proteins are of molecular weights ranging from 26,000 to 30,000 Da. The enterotoxins are responsible for clinical conditions like staphylococcal food poisoning and pseudomembranous enterocolitis postantibiotic therapy.

3- Exfoliative toxin: Exfoliative toxin is of two types: (*a*) toxin A (molecular weight of 30,000 Da) and (*b*) toxin B (molecular weight of 29,500 Da). Toxin A is heat stable, while toxin B is heat labile. The toxin is antigenic, and specific antibodies against the toxin are protective.

The toxin breaks intercellular bridges in the epidermis and resulting in exfoliating disease of the skin. Toxin causes impetigo and causes staphylococcal scalded skin syndrome (SSSS) in children below 4 years of age.

4- Leukocidins: Leukocidins include :

- The alpha-lysin is the most important leukocidin. It causes marked necrosis of the skin and hemolysis by damaging the cell membrane.
- PV–leukocidins are six in number, each consisting of two components. The molecular weight is around 32 kDa. These toxins cause death of human leukocytes and macrophages without causing any lysis.
- Leukolysin is thermostable and causes lysis of leukocytes and necrosis of tissues *in vivo*.

5- Hemolysins: *S. aureus* produces four hemolysins: alpha (α), beta (β), gamma (ϒ), and delta (δ) hemolysins.

- Alpha-hemolysin is a protein with a molecular weight of 33 kDa. It has lethal effects on a wide variety of cell types and lyses erythrocytes of several animal species.
- Beta-hemolysin is a sphingomyelinase that is active on a variety of cells. It is a protein with a molecular weight of 35 kDa. It is a hot–cold hemolysin; i.e., its hemolytic properties are increased by exposure of the RBCs to cold temperature.
- Delta-hemolysin is a protein with a molecular weight of 8 kDa. It acts primarily as a surfactant.
- Gamma-hemolysin actually consists of three proteins.

Pathogenesis of staphylococcal infections

S. aureus are pyogenic bacteria that cause localized lesions in contrast to streptococci that are spreading in nature. Staphylococci adhere to the damaged skin, mucosa, or tissue surfaces. At these sites, they evade defense mechanisms of the host, colonize, and cause tissue damage. They produce disease by:

- Multiplying in tissues,
- Liberating toxins, and
- Stimulating inflammation.

S. aureus infection does not cause any life-long immunity. It causes repeated infections in a susceptible host.

The diseases caused by *S. aureus* can be divided into two groups: (*a*) inflammatory and (*b*) toxin-mediated staphylococcal diseases.

❖ Inflammatory staphylococcal diseases

These include the followings:

- Staphylococcal skin infections include impetigo, folliculitis, furuncles, carbuncles, paronychia, surgical wound infection, blepharitis, and postpartum breast infection.
- *S. aureus* is the most common cause of boils. The infection is acquired either by self-inoculation from a carrier site, such as the nose or through contact with another person harboring the bacteria.
- Bacteremia and septicemia may occur from any localized lesion, especially wound infection or as a result of intravenous drug abuse.
- *S. aureus* is an important cause of acute bacterial endocarditis, of normal or prosthetic heart valves, which is associated with high mortality.
- *S. aureus* is the most common cause of osteomyelitis in children. The bacteria reach bone through blood stream or by direct implantation following trauma.
- *S. aureus* causes pneumonia in postoperative patients following viral respiratory infection, it also leads to chronic sinusitis.
- *S. aureus* causes deep-seated abscesses in any organ after bacteremia.

❖ Toxin-mediated staphylococcal diseases

These include (*a*) staphylococcal food poisoning, (*b*) staphylococcal toxic shock syndrome, and (*c*) staphylococcal scalded skin syndrome.

- **Staphylococcal food poisoning:** Staphylococcal food poisoning is caused by enterotoxin. The enterotoxin is already present in the contaminated food before consumption.

Milk and milk products and animal products like fish and meat kept at room temperature after cooking are mainly incriminated. When kept at room temperature, the contaminating staphylococci multiply and produce toxin adequate enough (as little as 25 µg of toxin B can lead to illness) to cause food poisoning.

Often a food handler, who either is a carrier of *S. aureus* (nose, skin) or is suffering from staphylococcal skin infection, is the source of infection. The onset of symptoms is sudden, appearing within 2–6 hours of ingestion of food. It is a self limiting condition characterized by nausea, vomiting, abdominal cramps, and watery, nonbloody diarrhea.

- **Staphylococcal toxic shock syndrome:** Staphylococcal toxic shock syndrome (STSS) is caused by TSST. The toxin is a superantigen, which causes STSS by stimulating the release of large amounts of interleukins IL-1 and IL-2 in the body.

The STSS is an acute and potentially life-threatening condition similar to Gram-negative sepsis and septic shock. STSS is a multisystem disease characterized by fever, hypotension, myalgia, vomiting, diarrhea, mucosal hyperemia, and an erythematous rash followed by desquamation of the skin

- **Staphylococcal scalded skin syndrome:** Staphylococcal scalded skin syndrome (SSSS) is caused by the exfoliative toxin, exfoliatin. The condition is seen commonly in infants and children. It is associated with extensive exfoliation of the skin, in which outer layer of the epidermis is separated from the underlying tissue and is characterized by the appearance of extensive bullae. These bullae when ruptured may leave behind scalded, red, tender skin.

Laboratory Diagnosis

Laboratory diagnosis of staphylococcal infections is based on the demonstration of staphylococci, in appropriate clinical specimens, by microscopy and culture

- **Specimens**

Specimens to be collected for demonstration of staphylococci depend on the nature of lesion (Table 3).

Table 3 **Various specimens collected in staphylococcal infections**

Specimen	Condition
Pus	Suppurative lesions and osteomyelitis
Sputum	Respiratory infections
Blood	Bacteremia
Feces and vomitus	Food poisoning
Urine	Urinary tract infections
Nasal and perineal swab	Suspected carriers

Microscopy

Demonstration of Gram-positive cocci arranged in clusters and pus cells in the Gram-stained smears of pus, wound exudate, etc. are the characteristic features of pyogenic infection caused by *S. aureus.* It is noteworthy that microscopy: (Alone is not adequate to differentiate various species of staphylococci or micrococci from one another and also of no value for sputum and other specimens where mixed bacterial flora is present).

- **Culture**

The identification of staphylococci is confirmed by culture and other identification tests comprising a range of biochemical and enzymatic tests followed by antibiotic sensitivity. The specimens are inoculated onto nutrient agar and blood agar and incubated at 37°C for 24 hours. On nutrient agar, large, circular, smooth, convex, and glistening colonies showing goldenyellow pigments can be observed. On blood agar, the colonies show a zone of beta-hemolysis, which is not shown by any other species of staphylococci. Specimens from heavily contaminated sources, such as vomitus and feces, are inoculated on selective media (e.g., mannitol salt agar or salt milk agar).

These media inhibit growth of Gram-negative bacteria but allow the growth of staphylococci and certain other Gram-positive cocci.

- **Identification of bacteria**

The identifying features of *S. aureus* are summarized in Box 23-2.

Coagulase test

Coagulase test is an important test carried out to detect *S. aureus.* The test is done in two ways: tube coagulase test (carried out to detect free coagulase) and slide coagulase test (detects the bound coagulase or the clumping factor).

Phosphatase test

The production of phosphatase can be demonstrated by culturing a mixed specimen on phenolphthalein phosphate agar and exposing the colonies to ammonium vapors. *S. aureus* colonies turn bright pink due to the release of phenolphthalein.

Novobiocin sensitivity

Novobiocin sensitivity is a simple disk diffusion test to differentiate *S. aureus* from other staphylococci. *S. aureus* is novobiocin sensitive, while *S. saprophyticus* is novobiocin resistant.

Polymyxin B resistance

Polymyxin B sensitivity is again a simple disk diffusion test to differentiate *S. aureus* from other staphylococci. *S. aureus* is usually polymyxin resistant.

Bacteriophage typing

Strains of staphylococci can be typed by bacteriophage typing, which is useful in epidemiological studies. Bacteriophage typing is based on the susceptibility of cocci to bacteriophages. This is carried out by pattern method, where a set of 23 standard typing phages of *S. aureus* is used to type staphylococcal isolates and distinguish them from one another by their patterns of susceptibility to lysis. In this strain of *S. aureus* to be typed is inoculated on a nutrient agar plate to produce a lawn culture. After drying the plate, various phages at their routine test dilution (RTD) are applied over marked squares on plate. Such plates are then incubated overnight at 30°C and observed for the presence or absence of lysis of the colonies by the phages.

The phage type of a strain is known by designation of the phages that lyse it. Thus, if a strain is lysed by phages 83A, 84, and 85, it is called phage type 83A/84/85. By this method, most of the strains of staphylococci can be classified and are divided into five lytic groups, while there are a few which cannot be classified and constitute the unclassified group (Table 23-5). The national reference centre for staphylococcal phage typing in India is located in the Department of Microbiology, Maulana Azad Medical College, New Delhi.

Other typing methods

S. aureus has been classified into six biotypes (A, B, C, D, E, and F). Most human pathogenic strains belong to biotype A. Other typing methods include , plasmid profile, and serotyping....

Coagulase-negative staphylococci (CONS)

Coagulase-negative staphylococci (CONS) are the normal flora of the skin. CONS are opportunistic bacteria. They cause infections in immunocompromised patients and in patients fitted with urinary catheters, cardiac valves, and artificial joints.

■ They form white nonpigmented colonies, morphologically similar to those of *S. aureus*.

■ They are differentiated from *S. aureus* by their failure to coagulate the plasma due to the absence of the enzyme coagulase. CONS of medical importance include (*a*) *S. epidermidis,* (*b*) *S. saprophyticus,* (*c*) *S. haemolyticus,* (*d*) *S. saccharolyticus,* (*e*) *S. hominis,* (*f*) *S. schleiferi,* (*g*) *S. lugdunensis,* and (*h*) *Staphylococcus simulans*.

Staphylococcus epidermidis

S. epidermidis forms white colonies on blood agar. It is catalase positive, coagulase negative, and does not ferment mannitol. It tolerates salt, survives drying, and is highly antibiotic resistant. It is a normal skin commensal. Carriage rate is as high as 100%. This bacterium is transmitted by self-inoculation or by contact with infected patients and hospital personnel.

Ability to produce slime is an important virulence factor of the bacterium. *S. epidermidis* causes infection by adhering itself to the surface of the intravenous plastic catheters and prosthetic devices. The adherence is believed to be facilitated by polysaccharide glycocalyx known as slime, produced in large quantities by the bacteria. Slime also inhibits the action of lymphocytes and neutrophils. *S. epidermidis* is an important agent of hospital-acquired infection. It causes:

■ infection in compromised hosts, such as neutropenic patients, particularly in association with intravenous catheters and other prosthetic devices, such as heart valves.

■ endocarditis in patients with prosthetic valves, intravenous catheter infections, CSF shunt infections, catheter-associated peritonitis and endocarditis.

■ sepsis in neonates, osteomyelitis, wound infections, vascular graft infections, and mediastinitis. Vancomycin is the drug of choice for treatment of infection caused by *S. epidermidis*.

Staphylococcus saprophyticus

S. saprophyticus forms white colonies on blood agar. It is catalase positive, coagulase negative, and does not ferment mannitol. It normally inhabits the skin and genital mucosa. The bacterium causes:

■ Urinary tract infection by endogenous spread in colonized women. It adheres to the epithelial cells lining the urogenital tract. It causes dysuria, pyuria, and hematuria.

■ Urethritis, catheter-associated urinary tract infections, prostatitis in elderly men, and rarely, sepsis and endocarditis. Urinary tract infection caused by *S. saprophyticus* can be treated with quinolones (such as norfloxacin) or with trimethoprim–sulfamethoxazole. *S. epidermidis* and *S. saprophyticus* are distinguished from each other by their reaction to antibiotic novobiocin—*S. epidermidis* is sensitive, while *S. saprophyticus* is resistant. The differences between *S. aureus, S. epidermidis*, and *S. saprophyticus* are summarized in Table 4.

Table 4

Differences between *Staphylococcus aureus, Staphylococcus epidermidis,* and *Staphylococcus saprophyticus*

Test	*S. aureus*	*S. epidermidis*	*S. saprophyticus*
Coagulase	+	—	—
Clumping factor	+	—	—
Heat-stable nuclease	+	—	—
Urease	Variable	—	+
β-galactosidase	—	—	+
Alkaline phosphatase	—	+	—
Polymyxin B	Resistant	Resistant	Sensitive
Novobiocin	Sensitive	Sensitive	Resistant
Acid from mannitol	—	—	—
Acid from trehalose	+	—	
Acid from mannose	—	+	—
PYR test	—	—	+

Other Coagulase-Negative Staphylococci

There are many other coagulase-negative staphylococci that have been reported recently to cause human infections. These include the following:

1. *S. haemolyticus* causes bacteremia, endocarditis, urinary tract infection, and wound infection.

2. *S. saccharolyticus* causes endocarditis.

3. *S. hominis* causes bacteremia in cancer patients.

4. *S. schleiferi* causes wound infections, bacteremia, and indwelling catheter infections.

5. *S. lugdunensis* causes endocarditis, peritonitis, osteomyelitis, and breast abscesses.

6. *S. simulans* causes septicemia, osteomyelitis, and septic arthritis.

Streptococci

Bacteria of the genus *Streptococcus* are Gram-positive cocci arranged in chains that form a significant portion of the indigenous microflora of the oropharynx. In addition to relatively harmless species, the genus includes three of the most important pathogens of humans. One is *S. pyogenes,* the cause of "strep throat Second is *S. agalactiae,* the most frequent cause of sepsis in newborns. Third is *S. pneumoniae,* a leading cause of pneumonia and meningitis in persons of all ages.

MORPHOLOGY

Streptococci stain readily with common dyes, demonstrating coccal cells that are generally smaller and more ovoid in shape than staphylococci. They are usually arranged in chains with oval cells touching end to end, because they divide in one plane and tend to remain attached. Length may vary from a single pair to continuous chains of over 30 cells, depending on the species and growth conditions. do not form spores, and are nonmotile. Some members form capsules composed of polysaccharide complexes or hyaluronic acid.

CULTURAL AND BIOCHEMICAL CHARACTERISTICS

Streptococci grow best in enriched media under aerobic or anaerobic conditions (facultative). Growth of many strains is enhanced by the presence of carbon dioxide. Blood agar is preferred because it satisfies the growth requirements and also serves as an indicator for patterns of hemolysis. The colonies are small, ranging from pinpoint size to 2 mm in diameter, and they may be surrounded by a zone where the erythrocytes suspended in the agar have been hemolyzed. When this zone is clear, this state is called β-**hemolysis.**

When the result is hazy (incomplete hemolysis), with a green discoloration of the agar, it is called α-**hemolysis.** Streptococci are metabolically active, attacking a variety of carbohydrates, proteins, and amino acids. Glucose fermentation yields mostly lactic acid. In contrast to staphylococci, streptococci are catalase negative.

CLASSIFICATION

At the turn of the 20th century, a classification based on hemolysis and biochemical tests was sufficient to associate some streptococcal species with infections in humans and animals. Rebecca Lancefield, who demonstrated

carbohydrate antigens in cell-wall extracts of the β-hemolytic streptococci, put this taxonomy on a sounder basis. Her studies formed a classification by serogroups (eg, A, B, C), each of which is generally correlated with an established species. Later it was discovered that some nonhemolytic streptococci had the same cell wall antigens.

For practical purposes, the type of hemolysis and certain biochemical reactions remain valuable for the initial recognition and presumptive classification of streptococci,. The streptococci will be considered as follows: (1) **pyogenic streptococci** (Lancefield groups); (2) **pneumococci**; (3) **viridans and other streptococci**. Table (1) explanes different classification methods for streptococci .

Table (1)

Classification of Streptococci and Enterococci

			MAJOR ANTIGENS/STRUCTURES				
GROUP/SPECIES	**COMMON TERM**	**HEMOLYSIS**	**LANCEFIELD CELL WALL**	**SURFACE PROTEIN**	**CAPSULE**	**VIRULENCE FACTORS**	**DISEASE**
Streptococci							
Pyogenic							
Streptococcus pyogenes	Group A strep (GAS)	β	A	M protein (100+)	Hyaluronic acid	M protein, lipoteichoic acid, StrepSAgs, streptolysin O, streptokinase	Strep throat, impetigo, pyogenic infections, toxic shock, rheumatic fever, glomerulonephritis
S agalactiae	Group B strep (GBS)	β, –	B	–	Sialic acid (9)	Capsule	Neonatal sepsis, meningitis, pyogenic infections
S equi		β	C	–	–	StrepSAg genes	Pyogenic infections
S bovis		–, α	D	–	–	–	Pyogenic infections
Other species		β, α, –	E-W	–	–	–	Pyogenic infections
Pneumococcus							
S pneumoniae	Pneumococcus	α	–	Choline-binding protein	Polysaccharide (90+)	Capsule, pneumolysin, neuraminidase	Pneumonia, meningitis, otitis media, pyogenic infections
Viridans and Nonhemolytic							
S sanguis		α	–	–	–	–	Low virulence, endocarditis
S salivarius		α					Low virulence, endocarditis
S mutans		α	–	–			Dental caries
Other species		α, –	–	–	–	–	Low virulence, endocarditis
Enterococci							
Enterococcus faecalis	Enterococcus	–, α	D	–	–	–	Urinary tract, pyogenic infections
E faecium	Enterococcus	–, α	D	–	–	–	Urinary tract, pyogenic infections
Other species		–, α	D, –	–	–	–	Urinary tract, pyogenic infections

Streptococcus pyogenes

(Group A streptococci)

MORPHOLOGY AND GROWTH

Group A streptococci typically appear in purulent lesions or broth cultures as spherical or ovoid cells in chains of short to medium length (4 to 10 cells). On blood agar plates, colonies are usually compact, small, and surrounded by a 2- to 3-mm zone of β hemolysis that is easily seen and sharply demarcated. β-hemolysis is caused by either of two hemolysins, **streptolysin S** and the oxygen-labile **streptolysin O,** both of which are produced by most group A strains. Strains that lack streptolysin S are β-hemolytic only under anaerobic conditions, because the remaining streptolysin O is not active in the presence of oxygen. This feature is of practical importance, because such strains would be missed if cultures were incubated only aerobically.

STRUCTURE

The structure of group A streptococci is illustrated in Figure (1). The cell wall is built on a peptidoglycan matrix that provides rigidity, as in other Gram-positive bacteria. Within this matrix lies the group carbohydrate antigen, which by definition is present in all group A streptococci. A number of other molecules such as M protein and lipoteichoic acid (LTA) are attached to the cell wall but extend beyond often in association with the hair-like pili. Group A streptococci are divided into more than 80 serotypes based on antigenic differences in the M protein. Some strains have an overlying nonantigenic hyaluronic acid capsule.

The **M protein** itself is a fibrillar coiled-coil molecule (Fig 1) with structural homology to myosin. Its carboxy terminus is rooted in the peptidoglycan of the cell wall, and the amino-terminal regions extend out from the surface. The specificity of the more than 80 serotypes of M protein is determined by variations in the amino sequence of the aminoterminal portion of the molecule. **Other Surface Molecules** have been described on the basis of their similarity with M protein or some unique binding capacity. Of these, a fibronectin binding **protein F** and **LTA** are both exposed on the streptococcal surface and may have a role in pathogenesis. Group A streptococci may have a **hyaluronic acid capsule,** which is a polymer containing repeating units of glucuronic acid and N-acetylglucosamine.

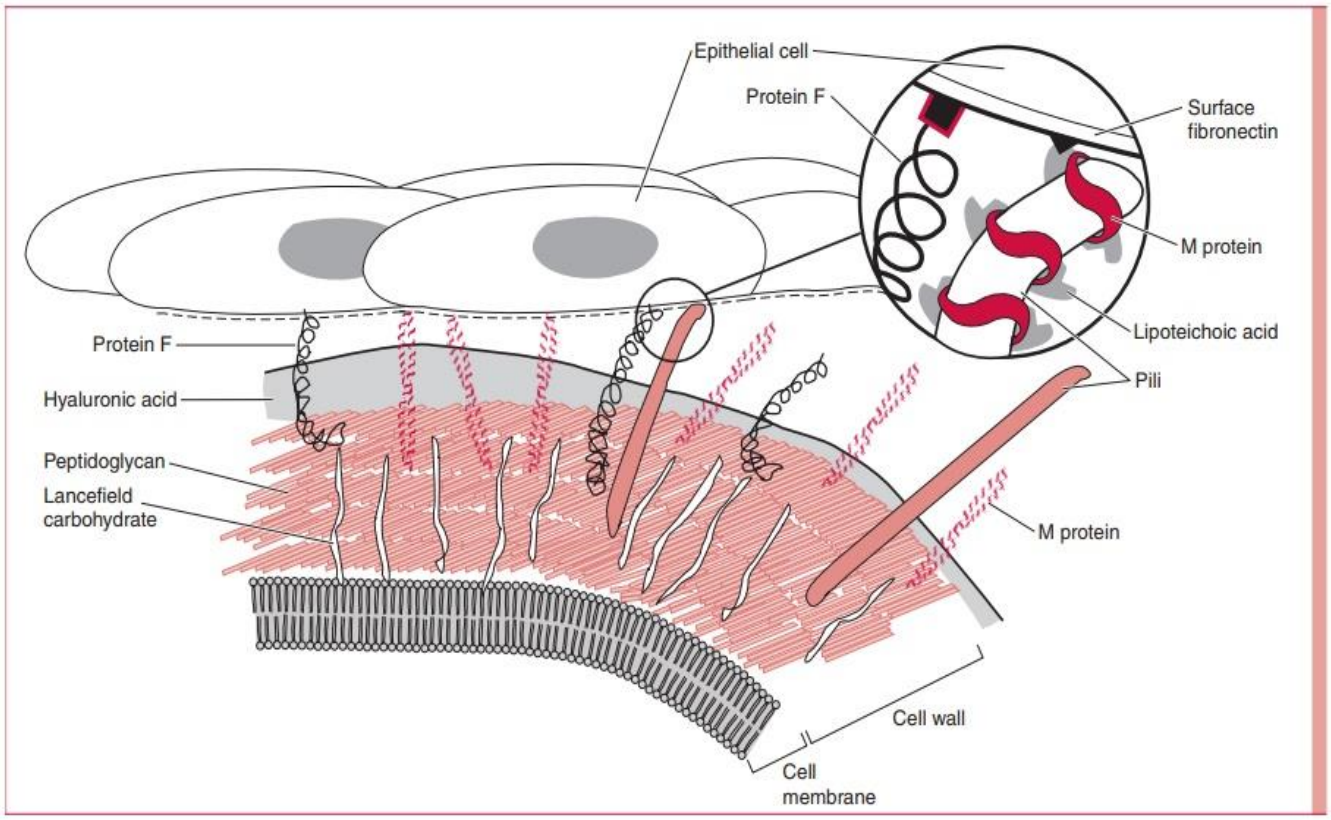


Figure (1)

Antigenic structure of *S. pyogenes* and adhesion to an epithelial cell. The location of peptidoglycan and Lancefield carbohydrate antigen in the cell wall is shown in the diagram. M protein and lipoteichoic acid are associated with the cell surface and the pili. Lipoteichoic acid and protein F mediate binding to fibronectin on the host surface.

BIOLOGICALLY ACTIVE EXTRACELLULAR PRODUCTS

Streptolysin O

Streptolysin O is a general cytotoxin, lysing leukocytes, tissue cells, and platelets. The toxin inserts directly into the cell membrane of host cells, forming transmembrane pores. StreptolysinO is antigenic and the quantitation of antibodies against it is the basis of a standard serologic test called antistreptolysin O (ASO).

Pyrogenic Exotoxins

The manifestations of classical **scarlet fever** have long been associated with the action of an erythrogenic toxin. This toxin is now included in a family of nine proteins called **streptococcal pyrogenic exotoxins (SPEs),** They have multiple effects including fever, rash (scarlet fever), T-cell proliferation, B-lymphocyte suppression.

Other Extracellular Products

Most strains of group A streptococci produce a number of other extracellular products including streptokinase, hyaluronidase, nucleases, and a **C5a peptidase**. The C5a peptidase is an enzyme that degrades complement component C5a,. **Streptokinase** causes lysis of fibrin clots.

PATHOGENESIS

Acute Infections

As with other pathogens, adherence to mucosal surfaces is a crucial step in initiating disease. A dozen adhesins have been described that facilitate the ability of the group A streptococcus to adhere to epithelial cells of the nasopharynx and/or skin. Of these, the most important are M protein, LTA, and protein F. In the nasopharynx, all three appear to be involved in mediating attachment to the fatty acid – binding sites in the glycoprotein fibronectin covering the epithelial cell surface. The role of M protein is not direct but it appears to provide a scaffold for LTA, which is essential for it to reach its binding site .

On the other hand, M protein appears to be direct and dominant in binding to the skin through its ability to interact with subcorneal keratinocytes, the most numerous cell type in cutaneous tissue. Protein F is also involved primarily adherence. Clinical evidence makes it clear that group A streptococci have the capacity to be highly invasive.

After the initial events of attachment and invasion, it appears that the concerted activity of the M protein, immunoglobulin-binding proteins, and the C5a peptidase play the key roles in allowing the streptococcal infection to continue. Although the hyaluronic acid capsule contributes to resistance to phagocytosis,. The role of other bacterial factors in the pathogenesis of acute infection is uncertain, but the combined effect of streptokinase, DNAase, and hyaluronidase may prevent effective localization of the infection, while the streptolysins produce tissue injury and are toxic to phagocytic cells.

In **streptococcal toxic shock syndrome (STSS),** as with staphylococcal toxic shock syndrome, the findings of shock, renal impairment, and diarrhea.

Streptococcal Pharyngitis

Although it may occur at any age, streptococcal pharyngitis is most frequent between the ages of 5 and 15 years. The illness is characterized by acute sore throat, malaise, fever, and headache. Infection typically involves the tonsillar pillars, uvula, and soft palate, which become red, swollen, and covered with a yellow-white exudate. The cervical lymph nodes that drain this area may also become swollen and tender. Group A streptococcal pharyngitis is usually self-limiting. Typically, the fever is gone by the third to fifth day, and other manifestations subside within 1 week. Occasionally the infection may spread locally to produce peritonsillar or retropharyngeal abscesses, otitis media, suppurative cervical adenitis, and acute sinusitis.

Disease Associated with Streptococcal Pyrogenic Exotoxins

**** Scarlet Fever***

Infection with strains that elaborate any of the Streptococcal Pyrogenic Exotoxins (SPEs) may superimpose the signs of scarlet fever on a patient with streptococcal pharyngitis. In scarlet fever, the buccal mucosa, temples, and cheeks are deep red, except for a pale area around the mouth and nose. Punctate hemorrhages appear on the hard and soft palates, and the tongue becomes covered with a yellow-white exudate through which the red papillae are prominent (strawberry tongue). A diffuse red "sandpaper" rash appears on the second day of illness, spreading from the upper chest to the trunk and extremities.

**** Streptococcal Toxic Shock Syndrome (STSS)***

STSS may begin at the site of any group A streptococcal infection even at the site of seemingly minor trauma. The systemic illness starts with vague myalgia, chills, and severe pain at the infected site. Most commonly, this is in the skin and soft tissues and leads to necrotizing fasciitis and myonecrosis. STSS continues with nausea, vomiting, and diarrhea followed by hypotension, shock, and organ failure.

Poststreptococcal Sequelae

**** Acute Rheumatic Fever (ARF)***

Attacks typically begin 3 weeks (range, 1 to 5 weeks) after an attack of group A streptococcal pharyngitis, and in the absence of antiinflammatory therapy last 2 to 3 months. Of the many theories advanced to explain the role of group A streptococci in ARF, an autoimmune mechanism related to antigenic similarities between streptococci and human tissue antigens has the most experimental support. Streptococcal pharyngitis patients who develop ARF have higher levels of antistreptococcal and autoreactive antibodies and T cells than those who do not. Some of these have been shown to react with both heart tissue and streptococcal antigens.

The antigen stimulating these antibodies is most probably M protein, but the group A carbohydrate is also a possibility. The similarity between the structure of M protein and myosin is an obvious connection, and M protein fragments have been shown to stimulate antibodies that bind to human heart sarcolemma membranes.

**** Acute Glomerulonephritis***

Poststreptococcal glomerulonephritis is primarily a disease of childhood that begins 1 to 4 weeks after streptococcal pharyngitis and 3 to 6 weeks after skin infection. It is characterized clinically by edema, hypertension, hematuria, proteinuria,. The renal injury of acute glomerulonephritis is caused by deposition in the glomerulus of antigen–antibody complexes with complement activation and consequent inflammation. The M proteins of some nephritogenic strains have been shown to share antigenic determinants with glomeruli, which suggests an autoimmune mechanism similar to rheumatic fever.

Impetigo

The primary lesion of streptococcal impetigo is a small (up to 1 cm) vesicle surrounded by an area of erythema. The vesicle enlarges over a period of days, becomes pustular, and eventually breaks to form a yellow crust. The lesions usually appear in 2- to 5-year-old children on exposed body surfaces, typically the face and lower extremities. Multiple lesions may coalesce to form deeper ulcerated areas. Although *S. aureus* produces a clinically distinct bullous form of impetigo it can also cause vesicular lesions resembling streptococcal impetigo. Both pathogens are isolated from some cases.

DIAGNOSIS

Although these clinical features of streptococcal pharyngitis are fairly typical, there is enough overlap with viral pharyngitis that a culture of the posterior pharynx and tonsils is required for diagnosis.

A direct Gram-stained smear of the throat is unhelpful because of the other streptococci in the pharyngeal flora, but smears from normally sterile sites usually demonstrate streptococci.

Blood agar plates incubated anaerobically give the best yield because they favor the demonstration of β-hemolysis. β-hemolytic colonies are identified by Lancefield grouping using agglutination methods.

Several serologic tests have been developed to aid in the diagnosis of poststreptococcal sequelae by providing evidence of a previous group A streptococcal infection. They include the ASO, anti-DNAase B, and some tests that combine multiple antigens. High titers of ASO are usually found in sera of patients with rheumatic fever, so that test is used most widely Table (2)

.

Table (2)

Usual Hemolytic, Biochemical, and Cultural Reactions of Common Streptococci and Enterococci[a]

	Susceptibility to		Bile	Bile/Esculin	
	Bacitracin	Optochin	Solubility	Reaction[b]	PYR[c]
Streptococci					
β-Hemolytic					
Lancefield group A	+	−	−	−	+
Lancefield groups B, C, F, G	−	−	−	−	−
α-Hemolytic					
S. pneumoniae	−	+	+	−	−
Viridans group	−	−	−	−	−
Nonhemolytic	−	−	−	−	−
Enterococci	−	−	−	+	+

[a] All are tests commonly substituted for serologic identification in clinical laboratories.
[b] Tests for the ability to grow in bile and reduce esculin.
[c] PYR = pyrrolidonyl arylamidase test.

TREATMENT

Group A streptococci are highly susceptible to penicillin G, the antimicrobic of choice. Concentrations as low as 0.01 _g/mL have a bactericidal effect, and penicillin resistance is so far unknown. Numerous other antimicrobics are also active, including other penicillins, cephalosporins, tetracyclines, and macrolides, but not aminoglycosides. Patients allergic to penicillin are usually treated with erythromycin if the organisms are susceptible. Impetigo is often treated with erythromycin to cover the prospect of *S. aureus* involvement. Adequate treatment of streptococcal pharyngitis within 10 days of onset prevents rheumatic fever by removing the antigenic stimulus; its effect on the duration of the pharyngitis is less, because of the short course of the natural infection. Treatment does not prevent the development of acute glomerulonephritis.

Streptococcus agalactiae

(Group B Streptococci)

MORPHOLOGY

Group B streptococci (GBS) produce short chains and diplococcal pairs of spherical or ovoid Gram-positive cells. Colonies are larger and β-hemolysis is less distinct than with group A streptococci and may even be absent. In addition to the Lancefield B antigen, GBS produce polysaccharide capsules of nine antigenic types (Ia, Ib, II through VIII).

PATHOGENESIS and MANIFESTATIONS

GBS disease requires the proper combination of organism and host factors. The GBS capsule is the major organism factor.. Antibody is protective against GBS disease, but as with group A streptococcal M protein, the antibody must be specific to the infecting type of GBS Newborns will have this antibody only if they receive it from their mother as transplacental IgG.

GBS infections in adults are uncommon and may be bacteremia, the mother's side of the neonatal syndrome. Other infections include pneumonia and a variety of skin and soft tissue infections similar to those produced by other pyogenic streptococci. Although adult GBS infections may be serious, they are usually not fatal unless patients are immunocompromised. GBS are not associated with rheumatic fever or acute glomerulonephritis.

The clinical findings are nonspecific and similar to those found in other serious infections in the neonatal period. Respiratory distress, fever, lethargy, irritability, apnea, and hypotension are common. Fever is sometimes absent, and infants may even be hypothermic. Pneumonia is common, and meningitis is present in 5 to 10% of cases, but most infections have GBS circulating in the bloodstream without localizing findings. The onset is typically in the first few days of life, and signs of infection are present at birth in almost 50% of cases. The late-onset (1 to 3 month) cases have similar findings but are more likely to have meningitis and focal infections in the bones and joints. Even with appropriate and prompt treatment, the mortality rate for early onset GBS infection approaches 20%.

DIAGNOSIS

The laboratory diagnosis of GBS infection is by culture of blood, cerebrospinal fluid, or other appropriate specimen. Definitive identification

involves serologic determination of the Lancefield group by the same methods used for group A streptococci.

TREATMENT

GBS are susceptible to the same antimicrobics as group A organisms. Although penicillin is the treatment of choice, GBS are slightly less susceptible to β-lactams than other streptococci. For this reason neonatal infections are often initially treated with combinations of penicillin (or ampicillin) and an aminoglycoside. These combinations have been shown to accelerate killing of GBS in vitro.

Streptococcus pneumoniae

MORPHOLOGY AND STRUCTURE

S. pneumoniae (pneumococci) are Gram-positive, oval cocci typically arranged end to end in pairs (diplococcus). The distinguishing structural feature of the pneumococcus is its capsule. All virulent strains have surface capsules composed of high molecular-weight polysaccharide polymers that are complex mixtures of monosaccharides, oligosaccharides, and sometimes other components.

Pneumococcal cell wall structure is similar to other streptococci. Teichoic acid, LPA, and phosphocholine are rooted in the peptidoglycan extending outward into the capsule where they provide binding domains for a variety of surface proteins. At least one of these, **binding protein,** is able to bind to both pneumococcal cell wall and surface of epithelial cells.

GROWTH

On blood agar, pneumococci produce round, glistening 0.5- to 2.0-mm colonies surrounded by a zone of α-hemolysis. Both colonies and broth cultures have a tendency to undergo autolysis due to their susceptibility to peroxides produced during growth and the action of **autolysins,** a family of pneumococcal enzymes that degrade peptidoglycan. Accelerating the autolytic process with bile salts is the basis of the bile solubility test that separates pneumococci from other α-hemolytic streptococci.

EXTRACELLULAR PRODUCTS

All pneumococci produce **pneumolysin,**. The pneumococcus does not secrete pneumolysin but it is released on lysis of the organisms by autolysins. Pneumococci also produce a **neuraminidase,** which cleaves sialic acid present in host.

PATHOGENESIS

Pneumococcal Pneumonia

Clinically, pneumococcal pneumonia begins abruptly with a shaking chill and high fever. Cough with production of sputum pink to rusty in color (indicating the presence of RBCs) and chest pain are common.

Although infection may occur at any age, the incidence and mortality of pneumococcal pneumonia increase sharply after 50 years.

Pneumococcal Meningitis

S. pneumoniae is one of the three leading causes of bacterial meningitis. The signs and symptoms are similar to those produced by other bacteria. Acute meningitis may follow pneumococcal pneumonia,. The mortality and frequency of sequelae are slightly higher with pneumococcal meningitis than with other forms of pyogenic meningitis.

Other Infections

Pneumococci are common causes of sinusitis and otitis media. The latter frequently occurs in children in association with viral infection. Pneumococci do not cause pharyngitis or tonsillitis.

DIAGNOSIS

Gram smears of material from sputum and other sites of pneumococcal infection typically show Gram-positive, lancet-shaped diplococci. Sputum collection may be difficult, however, and specimens contaminated with respiratory flora are useless for diagnosis.

Other types of lower respiratory specimens may be needed for diagnosis. *S. pneumoniae* grows well overnight on blood agar medium and is usually distinguished from viridans streptococci by susceptibility to the synthetic chemical (Optochin) or by a bile solubility. Bacteremia is common in pneumococcal pneumonia and meningitis, and blood cultures are valuable supplements to cultures of local fluids or exudates. Detection of pneumococcal capsular antigen in body fluids is possible but valuable primarily when cultures are negative.

TREATMENT

For decades pneumococci were uniformly susceptible to penicillin

Penicillin is still the antimicrobic of choice for susceptible strains but resistance rates now exceed 10% in most locales and may be greater than 30% in some areas. Penicillin resistant strains may be treated with erythromycin, vancomycin, or quinolones, if susceptible.

Viridans and Nonhemolytic Streptococci

The viridans group comprises all α-hemolytic streptococci. Characteristically members of the normal flora of the oral and nasopharyngeal cavities, they have the basic bacteriologic features of streptococci but lack the specific antigens, toxins, and virulence of the other groups. Although the viridans group includes many species

Although their virulence is very low, viridans strains can cause disease when they are protected from host defenses. The prime example is subacute bacterial endocarditis. In this disease, viridans streptococci reach previously damaged heart valves as a result of transient bacteremia associated with manipulations, such as tooth extraction, that disturb their usual habitat. Protected by fibrin and platelets, they multiply on the valve, causing local and systemic disease that is fatal if untreated. Extracellular production of glucans, complex polysaccharide polymers, may enhance their attachment to cardiac valves. The clinical course of viridans streptococcal endocarditis is subacute, with slow progression over weeks or months. It is effectively treated with penicillin, but uniformly fatal if untreated. The disease is particularly associated with valves damaged by recurrent rheumatic fever.

ENTEROCOCCI

Until DNA homology studies dictated their separation into the genus *Enterococcus,* the enterococci were classified as streptococci. Indeed, the most common enterococcal species share the bacteriologic characteristics described above for pyogenic streptococci, including presence of the Lancefield group D antigen. The term enterococcus derives from their presence in the intestinal tract and the many biochemical and cultural features that reflect that habitat. These include the ability to grow in the presence of high concentrations of bile salts and sodium chloride. Most enterococci produce nonhemolytic or α-hemolytic colonies that are larger than those of most streptococci. *E. faecalis, E. faecium,* and several other species are recognized based on biochemical and cultural reactions,.

PATHOGENESIS

Enterococci are a significant cause of disease in specialized hospital settings, but they are not highly virulent. On their own, they do not produce fulminant disease and in wound and soft tissue infections are usually mixed with other members of the intestinal flora. Some have even doubted their

significance when isolated with more virulent members of the Enterobacteriaceae or *Bacteroides fragilis*. Although some surface proteins are candidate adhesins, no virulence factors have been discovered.

Enterococci cause opportunistic urinary tract infections (UTIs) and occasionally wound and soft tissue infections, in much the same fashion as members of the Enterobacteriaceae. Infections are often associated with urinary tract manipulations, malignancies, biliary tract disease, and gastrointestinal disorders. Vascular or peritoneal catheters are often points of entry. Respiratory tract infections are rare. There is sometimes an associated bacteremia, which can result in the development of endocarditis on previously damaged cardiac valves.

TREATMENT

The outstanding feature of the enterococci is their high and increasing levels of resistance to antimicrobial agents. Inherently relatively resistant to β-lactams and aminoglycosides, enterococci also have particularly efficient means of acquiring plasmid and transposon resistance genes from themselves and other species.

Enterococci share with streptococci a relative resistance to aminoglycosides based on failure of the antimicrobic to be actively transported into the cell. Despite this, many strains of enterococci are inhibited and rapidly killed by combinations of low concentrations of penicillin and aminoglycosides. Under these conditions, the action of penicillin on the cell wall allows the aminoglycoside to enter the cell and act at its ribosomal site.

Some strains show high level resistance to aminoglycosides based on mutations at the ribosomal binding site or the presence of aminoglycoside-inactivating enzymes. These strains do not demonstrate synergistic effects with penicillin.

Recently, resistance to vancomycin, the antibiotic most used for penicillin-resistant strains has emerged. Vancomycin resistance is due to a subtle change in peptidoglycan precursors.

Enterobacteriaceae

Enterobacteriaceae organisms are distributed worldwide and found in the soil, water, and plants. They are also present as part of the normal intestinal flora of humans and animals. Members of this family are nonsporing, nonacid fast, and moderately sized Gram-negative bacilli. They are motile by peritrichous flagella or nonmotile without any flagella. They are aerobic and facultatively anaerobic and grow readily on ordinary media, ferment sugars with production of acid and gas or acid only, reduce nitrate to nitrite, and are catalase- positive but oxidase-negative. The oxidase test is an important test by which the members of the Enterobacteriaceae can be distinguished from many other fermentative and nonfermentative Gram-negative bacilli. Members of the family show a very wide biochemical and antigenic heterogeneity among themselves. Enterobacteriaceae organisms cause a variety of diseases in humans .

Classification

The characteristics of the colonies on commonly used medium (such as the MacConkey medium) were used to identify and classify the members of family Enterobacteriaceae. The colonies were classified as lactose-fermenting bacteria (*Escherichia* spp., *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., etc.) or nonlactose-fermenting bacteria (*Salmonella* spp., *Shigella* spp., *Proteus* spp., etc.) depending on the ability of the bacteria to ferment lactose. This was used as a practical method in a routine diagnostic laboratory.

The current practice, however, is to classify bacteria on the basis of a number of morphological, biochemical, serological, and DNA-based characteristics. As per these methods, the family Enterobacteriaceae is divided into many major groups or tribes. Each tribe consists of one or more genera or subgenera. Each genus consists of many species, which are classified into different types, such as biotypes, serotypes, colicin types, bacteriophage types, etc. The family Enterobacteriaceae has been classified into eight tribes as given in Table 1.

Table 1

Ewing's classification of the family Enterobacteriaceae

Tribe		Genus/Genera
Tribe I	Escherichieae	*Escherichia*
		Shigella
Tribe II	Edwardsielleae	*Edwardsiella*
Tribe III	Salmonelleae	*Salmonella*
Tribe IV	Citrobactereae	*Citrobacter*
Tribe V	Klebsielleae	*Klebsiella*
		Enterobacter
		Serratia
		Hafnia
Tribe VI	Proteeae	*Proteus*
		Morganella
		Providencia
Tribe VII	Yersinieae	*Yersinia*
Tribe VIII	Erwinieae	*Erwinia*

Important properties distinguishing different genera of the family Enterobacteriaceae are summarized in Table 2.

Table 2

Important properties distinguishing members of the family Enterobacteriaceae

	Escherichia	*Klebsiella*	*Enterobacter*	*Salmonella*	*Shigella*	*Serratia*	*Hafnia*	*Edwardsiella*	*Citrobacter*	*Proteus*
Motility	+	–	+	+	–	+	+	+	+	+
Acid from glucose	+	+	+	+	+	+	+	+	+	+
Gas from glucose	+	+	+	+	–	V	+	+	+	V
Indole	+	–	–	–	–	–	–	+	V	V
H_2S	–	–	–	+	–	–	–	+	V	+
Urease	–	+	V	–	–	V	–	–	V	V
Citrate	–	+	V	+	–	V	+	–	+	+
PPA	–	–	–	–	–	–	–	–	–	+
Lysine decarboxylase	+	+	V	+	–	V	+	+	–	–
Arginine dihydrolase	V	–	V	V	–					
Ornithine decarboxylase	V	–	+	+	–	V	+	+	V	V

Note: V means variable results in different species or strains. Salmonella Typhi does not produce gas from sugars. *Shigella sonnei* ferments lactose and sucrose late.

Human infections caused by common members of the family Enterobacteriaceae are summarized in Table 3 and figure 1 .

Table 3

Human infections caused by common members of the family Enterobacteriaceae

Bacteria	Diseases
Escherichia spp.	Gastrointestinal tract infections
	Urinary tract infections
	Blood stream infections
	Lower respiratory tract infections
	Central nervous system infections
Shigella spp.	Gastrointestinal tract infections
Salmonella spp.	Gastrointestinal tract infections
	Blood stream infections
Klebsiella spp.	Urinary tract infections
	Lower respiratory tract infections
	Blood stream infections
Proteus spp.	Urinary tract infections
Enterobacter spp.	Lower respiratory tract infections
	Blood stream infections
Morganella spp.	Urinary tract infections
Yersinia spp.	Gastrointestinal tract infections
	Blood stream infections

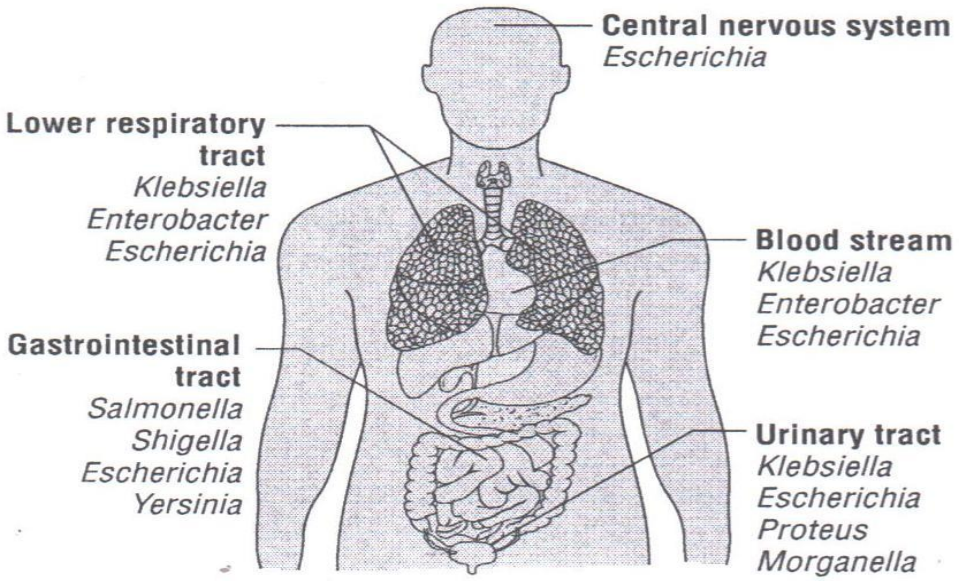


FIG. 1 . Schematic diagram showing variety of diseases caused by the members of the family Enterobacteriaceae in humans.

The genus *Escherichia*

Escherichia are animal and human intestinal pathogens. The genus *Escherichia* consists of five species: *E. coli, Escherichia fergusonii, Escherichia hermanii, Escherichia vulneris,* and *Escherichia blattae*. Of these species, *E. coli* is the most common and most important species causing infection in humans. *E. coli* is further subdivided into biotypes and serotypes based on O, H, and K antigens.

Escherichia coli

Morphology

E. coli shows the following features*:*

■ *E. coli* is a Gram-negative bacillus, which measures around 1–3 _ 0.4–0.7 µm in size.

■ The bacilli are arranged singly or in pairs.

■ They are motile due to the presence of peritrichous flagella.

■ Some strains are nonmotile. Some strains of *E. coli* may be fimbriated.

■ Some strains of *E. coli* isolated from extraintestinal infections possesses polysaccharide capsule. They do not form any spores.

Culture

E. coli is an aerobe and a facultative anaerobe. It grows at a temperature range of 10–40°C (optimum 37°C) and a pH of 7.2. The bacteria grow on a wide range of media including Mueller– Hinton agar, nutrient agar, blood agar, and MacConkey agar. Primary isolation can be made on nutrient agar and blood agar.

Nutrient agar: *E. coli* on nutrient agar after 18 hours of incubation at 37°C produces large, circular, low convex, grayish white, moist, smooth, opaque colonies. **MacConkey medium:** *E. coli* produces bright pink flat colonies due to lactose fermentation. Many strains, especially those isolated from pathologic conditions, produce beta-hemolytic colonies on **blood agar**. They do not grow on selective media, such as DCA (deoxycholate citrate agar) or SS (Salmonella– Shigella) agar, used for the culture of salmonellae and shigellae.

Liquid broth culture: *E. coli* produces turbid growth with a deposit, which disperses completely on shaking.

Biochemical reactions

E. coli shows following reactions:

1. *E. coli* ferments lactose, glucose, mannitol, maltose, and many other sugars with the production of acid and gas. They do not ferment sucrose.

2. They do not liquefy gelatin, do not produce hydrogen sulfide (H2S), or do not utilize urea.

3. The indole, methyl red (MR), Voges–Proskauer (VP), and citrate utilization tests, generally referred to as the "IMViC" tests, are four important biochemical tests widely used in the classification of enterobacteria. *E. coli* is indole and MR positive, and VP and citrate negative (IMViC+ + – –).

E. coli is inhibited by the presence of 7% sodium chloride in salt media used for isolation of staphylococci. Growth of the bacteria is also inhibited by sodium selenite in selenite broth, sodium tetrathionate in tetrathionate broth, and brilliant green in brilliant green tetrathionate broth.

Cell Wall Components and Antigenic Structure

The heat-stable **lipopolysaccharide (LPS)** is the major cell wall antigen of *E. coli*.

E. coli organisms possess four major antigens: H or flagellar antigen, O or somatic antigen, K or capsular antigen, and F or fimbrial antigens.

H or flagellar antigen: The H antigens are heat- and alcohol-labile proteins present on the flagella. The H antigens are genus specific and usually are not shared by other enterobacteria.

O or somatic antigen: O antigens occur on the surface of the outer membranes and are determined by specific sugar sequences on the cell surface. O antigen is an LPS complex and is an integral part of the cell wall. It is heat stable, resistant to boiling up to 2 hours and 30 minutes. Till now, 173 (1, 2, 3, etc. up to 173) O antigens have been described.

(Somatic O polysaccharide antigen shows cross-reactions with related genera (*Shigella*, *Salmonella*, *Yersinia*, and *Citrobacter*) in the family Enterobacteriaceae).

K or capsular antigen: The heat-labile K antigen is the acidic polysaccharide antigen present in the "envelope" or microcapsule (K for *Kapsel*, German for capsule) of the bacteria.

K antigen encloses the O antigen and may interfere with detection of the O antigens. This problem is overcome by boiling of the bacterial suspension to remove the K antigens. K antigens may also contribute to virulence by inhibiting phagocytosis.

F or Fimbrial antigens: These antigens are present on the fimbriae and are heat-labile proteins.These fimbrial antigens also contribute to virulence of the bacteria.

Pathogenesis

E. coli is an invasive bacterium. It colonizes the human intestine and, under specific conditions, directly invades the intestinal mucosa or produces toxins to cause intestinal infections. The bacteria can enter the blood stream and cause septicemia, meningitis, and other systemic manifestations. The bacteria, under certain conditions, directly invade urinary tract causing UTIs or cause intra-abdominal infections.

E. coli produces several virulence factors summarized in (Table 4), which include

Common virulence factors associated with Enterobacteriaceae:

These include following factors: (*a*) fimbriae, (*b*) endotoxin, (*c*) capsule, and (*d*) sequestration of growth factors.

Specialized virulence factors associated specifically with E. coli : These include adhesins and exotoxins.

Most infections, such as UTIs and sepsis, are endogenous and are caused by the *E. coli* present in large numbers in the gastrointestinal tract of the same host. Other *E. coli* infections, such as gastroenteritis and neonatal meningitis, are caused by exogenous infections, i.e., acquired from outside.

Table 4 Virulence factors of *Escherichia coli*

Virulence factors	Biological functions
Fimbriae	Adherence of bacteria to gastrointestinal tract; of importance in urinary tract infections; and cause mannose-resistant hemagglutination
Endotoxin	Systemic manifestations of endotoxic shock, and protect the bacillus from phagocytosis and from the bactericidal effects of complement
Capsule	Antiphagocytosis protects *Escherichia coli* from phagocytosis. Protect the organism from serum killing
Sequestration of growth factors	The capability of *Escherichia coli* to compete for nutrients in host cells
Adhesins	Adhesins facilitate firm adhesion of *Escherichia coli* to the gastrointestinal or urinary tract mucosa, thereby preventing the bacteria being eliminated by the flushing action of voided urine or intestinal motility
Hemolysins (HlyA)	Important in the pathogenesis of disease caused by uropathogenic strains of *Escherichia coli*
Enterotoxins	
Shiga toxins	Cytotoxins
Heat stable toxin	Causes increased secretion of fluids
Heat labile toxin	Watery diarrhea due to hypersecretion of fluid into the lumen of the gut
Siderophores	Removes iron from mammalian iron transport proteins like transferring and lactoferrin.

1- Urinary tract infections:

E. coli serotypes that are normally found in the feces are commonly responsible for urinary tract infections. UTI is an ascending infection in which the bacteria that originate from the intestinal tract contaminate the urethra, ascend into the bladder, and may spread to the kidney or prostate. most strains of *E. coli* can cause UTI, disease is more common with certain specific *E. coli* serogroups. These serogroups that cause UTI are known as **nephritogenic strains**, these include *E. coli* serotypes O1, O2, O4, O6, O7, O18, etc. These serotypes cause UTI, particularly because of their ability to

produce adhesins (primarily P pili), which bind to cells lining the bladder and upper urinary tract. This prevents elimination of the bacteria in voided urine. They also produce hemolysin which lyses erythrocytes and also other cells, leading to release of cytokines and stimulation of an inflammatory response.

2- Gastroenteritis:

Gastroenteritis is caused by exogenous infections acquired from water, food, or vegetables contaminated with fecal *E. coli*. The strains of *E. coli* that cause gastroenteritis are classified into the following six groups: **(*a*) enteropathogenic *E. coli* (EPEC), (*b*) enterotoxigenic *E. coli* (ETEC), (*c*) enteroinvasive *E. coli* (EIEC), (*d*) enterohemorrhagic *E. coli* (EHEC), (*e*) enteroaggregative *E. coli* (EAEC), and (*f*) diffusely adherent *E. coli* (DAEC)** (Table 31-6).

Enteropathogenic E. coli: EPEC is the major cause of infant diarrhea in tropical countries. Disease is rare in older children and adults. EPEC strains include O26, O55, O86, O111, O114, O119, O125, O126, O12, O128, and O142. EPEC causes infection by adhering to epithelial cells of the small intestine followed by destruction of the microvillus. The bacteria initially form microcolonies on the epithelial cell surface, in which the bacteria are attached to the host cells. Subsequently, the attached bacteria multiply and cause microvilli destruction, resulting in diarrhea.

Enterotoxigenic E. coli: Diarrhea caused by ETEC is endemic in the developing countries, among all age groups of the population. This is also responsible for causing traveler's diarrhea in which individuals from developed countries visiting endemic areas often suffer from ETEC diarrhea. The disease is caused by consumption of fecally contaminated food or water. Person-to-person spread does not occur. diarrhea is caused by certain specific ETEC serogroups (O6, O8, O15, O25, O27, O167). These serotypes cause diarrhea because of their ability to produce heat-labile enterotoxins (LT-I, LT-II). LT-I, which is structurally similar to cholera toxin, produces cholera-like diarrhea in patients.

Enteroinvasive E. coli: EIEC strains closely resemble shigellae in many ways: (*a*) EIEC strains are nonmotile, (*b*) they lactose ferment late with production of acid only, and (*c*) These strains show cross-reactivity with O antigen of shigellae. Named EIEC because they have the capacity to invade interstitial epithelial cells and also penetrate HeLa cells in tissue culture. The EIEC strains have the ability to invade and destroy the colonic epithelium, producing a disease characterized initially by watery diarrhea. This continuous process of epithelial cell destruction with inflammatory infiltration leads to the development of ulcers in intestine. Specific

serogroups commonly associated with outbreaks of EIEC include O28 ac, O112 ac, O124, O136, O143, O114, O152, and O154.

Enterohemorrhagic E. coli: EHEC strains are the most common cause of gastrointestinal infections in the developed countries. These strains produce diarrheal disease, ranging in severity from mild uncomplicated diarrhea to fatal hemorrhagic colitis. Hemolytic uremic syndrome (HUS) is a serious life-threatening complication in 10% of infected children below 10 years. The ingestion of as few as 100 bacilli can cause the disease. EHEC disease is most common in children below 5 years and in summer months. The condition occurs as a result of ingestion of water, unpasteurized milk or fruit juices, uncooked vegetables, and fruits contaminated with human or animal feces. The disease also occurs on consumption of undercooked ground beef or other meat products. Serotypes O157:H7 and O26:H1 are the EHEC strains that commonly cause the disease. These strains produce Shiga toxins (i.e., Stx-l, Stx-2, or both), which are primarily responsible for the diarrheal diseases. Stx-2 is most commonly associated with HUS, a disorder characterized by acute renal failure, hemolytic anemia. Stx-2 causes destruction of glomerular endothelial cells, resulting in reduced glomerular filtration and acute renal failure. The toxins also stimulate production of tumor necrosis factors.

Enteroaggregative E. coli: EAEC strains are so called because they show a typical "*stacked brick"* arrangement on glass due to their autoagglutination. fimbriae of the bacteria mediate this process. These EAEC strains secrete a low-molecular-weight, heat-stable enterotoxin called enteroaggregative heat-stable enterotoxin-1. EAEC increases mucus secretion, which forms a layer overlying the epithelium of the small intestine. This layer of biofilm traps the bacteria in epithelium of the small intestine. These strains are associated with persistent, watery diarrhea with dehydration in infants, especially in developing countries.

Diffusely adherent E. coli: DAEC strains cause watery diarrhea found primarily in children between 1 and 5 years of age. These strains are identified by their ability to adhere to cultured cells. They cause elongation of the microvilli.

3- Septicemia:

Invasion of blood stream by *E. coli* may lead to septicemia. Septicemia is caused by *E. coli* strains associated with UTIs or intra-abdominal infections, such as peritonitis and abscesses following intestinal perforation. The mortality due to *E. coli* septicemia is high for patients with

immunocompromised status, or for patients in whom the primary infection is in the abdomen or central nervous system (CNS).

4- Neonatal meningitis:

E. coli along with group B streptococci are the major causes of infection of the CNS in infants of age 1 month. The disease is caused by *E. coli* strains that possess the KI capsular antigen, which are commonly present in the gastrointestinal tracts of pregnant women and newborn infants.

Laboratory Diagnosis

Laboratory diagnosis of *E. coli* infections is based on

1. Isolation of *E. coli* by culture.

- **Specimens**

Urine is the specimen of choice for diagnosis of UTI caused by uropathogenic. Other specimens include feces or rectal swabs for gastroenteritis, blood for septicemia, cerebrospinal fluid (CSF) for meningitis, sputum for pneumonia, or other body fluids, such as pus from wound, and peritoneal abscesses caused by *E. coli*.

- **Culture**

Definitive diagnosis is based on the isolation of *E. coli* from various clinical specimens by culture. Urine culture is a very useful procedure for diagnosis of UTI. Stool culture is widely used to isolate diarrheagenic *E. coli*. Culture of blood, CSF, and other specimens is also carried out depending on the clinical diseases caused by *E. coli*, as mentioned earlier.

After incubation overnight at 37°C, pink, flat colonies of *E. coli* on the MacConkey agar and beta-hemolytic colonies on blood agar are identified by various biochemical tests.

Significant bacteriuria concept suggested on the fact that a colony count exceeding 100,000 (10^5) bacteria/mL of urine denotes significant bacteriuria and is suggestive of active UTI. Counts of 10,000 bacteria or less per milliliter are of no significance and are due to contamination of urine.

2. Demonstration of different diarrheagenic *E. coli*

Laboratory diagnosis of diarrhea caused by diarrheagenic *E. coli* can be made by demonstration of the bacilli in feces by culture. it is essential to perform various diagnostic tests in order to consider it as diarrheagenic pathogenic *E. coli* strain. These strains are identified by:

(*a*) serotyping, (*b*) animal inoculation, (*c*) cytopathic effects in cell cultures, or (*d*) molecular methods.

Identification of EPEC: Specific serogroups of *E. coli* (O26, O55, O86, O111, O114, O119, O125, O126, O12, O128, and O142) are identified by

agglutination tests with specific antisera. In a positive test, if *E. coli* colonies show agglutination with a specific serogroup (for example, O111) then the isolate is identified as *E. coli* of that serogroup (O111).

Identification of ETEC: Diagnosis of ETEC diarrhea depends on the demonstration of enterotoxin in *E. coli* isolates from stool, because toxin production is not associated with specific serogroups of *E. coli*. A strain of ETEC may produce either LT . The presence of LT in isolates of *E. coli* can be demonstrated Showing fluid accumulation in rabbit ileal loop method and other methods.

Identification of EIEC: Many of the EIEC strains are atypical *E. coli* strains. They are nonmotile and do not ferment lactose, or ferment it late with production of acid, but without producing any gas. They also do not decarboxylate lysine.

Identification of EHEC: *E. coli* O157:H7 is the most common serotype associated with the clinical disease caused by EHEC strains. The strain typically does not ferment sorbitol; hence sorbitol MacConkey medium is frequently used for isolation of the strain from stool by culture.

Identification of other strains: EAEC strains are identified by agglutination tests with specific antisera. Most of them are not typed by O antisera, but by specific H antisera.

Treatment

E. coli infections are best treated based on antibiotics susceptibility testing results. Third-generation cephalosporins, such as ceftriaxone, are recommended for meningitis and pneumonia caused by *E. coli*.

The genus *Klebsiella*

The genus *Klebsiella* belongs to the tribe Klebsielleae in the family Enterobacteriaceae. The bacteria are named after Edwin Klebs, who demonstrated the bacteria for the first time. Members of the genus *Klebsiella* are Gram-negative, rodshaped, nonmotile bacteria, with a polysaccharide capsule. The classification of *Klebsiella* has undergone various modifications. Earlier, the genus *Klebsiella*, based on biochemical reactions, was classified into three main species. Currently based on DNA homology, they have been divided into seven species, namely: (*a*) *Klebsiella pneumoniae*, (*b*) *Klebsiella ozaenae*, (*c*) *Klebsiella rhinoscleromatis*, (*d*) *Klebsiella oxytoca*, (*e*) *Klebsiella planticola*, (*f*) *Klebsiella terrigena*, and (*g*) *Klebsiella ornithinolytica.*

In recent years, Klebsielleae organisms are emerging as important agents of nosocomial infections. *K. pneumoniae* is the most important species of the group to cause infections in humans. *K. oxytoca* and *K. rhinoscleromatis* have also been associated with human infections.

Klebsiella pneumoniae

K. pneumoniae, also known as *Friedlander's bacillus*, was first isolated by Friedlander in 1883, from fatal cases of pneumonia. *K. pneumoniae* are Gram-negative, short and straight rods measuring about 1–2 x 0.5–0.8 µm in size. They are nonmotile and nonsporing. They are arranged singly or in pairs. Freshly isolated strains show a well-defined polysaccharide capsule. The capsule is often prominent and can be made out even in Gram stained smears as haloes around the bacilli, and is produced well when grown in media enriched with carbohydrates. The capsule can also be demonstrated by India ink preparation and Quellung's reaction. Accumulation of extracellular polysaccharides as a loose slime gives mucoid appearance to *Klebsiella* colonies. They are fimbriated.

They are nonmotile and nonsporing. They are lactose-fermenting, urease- positive, and indole-negative organisms. They do not produce hydrogen sulphide, and they are both VP and MR tests positive. They grow well on ordinary media, such as nutrient agar and MacConkey agar at 37°C, forming large, mucoid colonies. They produce lactose-fermenting red colonies on MacConkey agar. *Klebsiella* possesses 77 capsular polysaccharides (K antigens) and 8 somatic LPSs (O antigens).

The members of Klebsielleae have been classified into over 80 serotypes based on the capsular K antigens and somatic O antigens. All serotypes are

of the same virulence. Klebsielleae consists of invasive bacteria. They possess many virulence factors:

1. Capsule is the main virulence factor. The capsule prevents the bacteria from phagocytosis. The capsule also prevents bacterial death caused by bactericidal serum factors by inhibiting the activation or uptake of complement components.

2. Multiple adhesins are other virulence factors. These adhesins help the bacteria to adhere to host cells, which is crucial to initiate the disease process.

3. LPS is another factor that prevents membrane damage and death of bacteria.

Klebsielleae organisms cause a variety of clinical syndromes in humans. These are (*a*) community-acquired pneumonia, (*b*) UTI, (*c*) nosocomial infection, and (*d*) bacteremia and sepsis.

1. Community-acquired pneumonia Lobar pneumonia characteristically is associated with massive mucoid inflammatory exudate of lobar or lobular distribution, involving one or more lobes of the lung. Necrosis and abscess formation are more frequent than in pneumococcal pneumonia. *K. pneumoniae* serotypes 1, 2, and 3 are usually associated with the condition. Patients present with an acute onset of high fever and chills, flu-like symptoms, and productive cough, and bloody sputum. Blood culture is positive in about 25% of the cases.

2. UTI caused by *K. pneumoniae* is a common problem in patients with indwelling catheters. UTIs caused by *K. pneumoniae* cannot be distinguished clinically from those caused by *E. coli* and other common bacteria.

3. *K. pneumoniae* are emerging as important agents of **nosocomial infections** in hospitals. The presence of invasive devices, contamination of respiratory support equipment, use of urinary catheters, and use of antibiotics greatly increases the likelihood of nosocomial infections in hospitalized patients. In addition, poor health status and treatment in an intensive care unit or nursing home are other factors. UTI, pneumonia, bacteremia, wound infection, and catheter-associated bacteriuria are the common nosocomial infections associated with *K. pneumoniae*. Other rare nosocomial infections include meningitis, endocarditis.

4. *Klebsiella* **bacteremia and sepsis** produce clinical manifestations similar to those caused by *E. coli* and other Gram negative enteric organisms. In neonatal units, outbreaks of infection caused by extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella* strains present a more serious problem and may be associated with high mortality. ESBL strains of *Klebsiella*

show the following features: (*a*) these are highly virulent, (*b*) they possess capsular antigen, and (*c*) they have an extraordinary ability to spread.

Diagnosis of *K. pneumoniae* infection is made by isolation of bacteria from clinical specimens obtained from possible sites (e.g., wounds, peripheral or central intravenous access sites, urinary catheters, respiratory support equipment) and by culture. *Klebsiella* organisms may also be isolated from urine, blood, pleural fluid, and wounds. Serological tests are not useful for the detection of infection with *K. pneumoniae*.

The choice of a specific antimicrobial agent depends on antibiotics susceptibility patterns of isolated strains. A wide range of beta-lactams, aminoglycosides, quinolones, and other antibiotics are useful for treatment of *Klebsiella* infections.

Cephalosporins are widely used as monotherapy and in combination with aminoglycosides. Cephalosporins are not used for ESBL strains of *K. pneumoniae*. The carbapenems, especially imipenem, are effective against such ESBL strains.

Aztreonam and quinolones are recommended for patients allergic to penicillin. Hand washing holds the key to prevent transmission from patient to patient via medical personnel.

Klebsiella rhinoscleromatis

Rhinoscleroma caused by *K. rhinoscleromatis* is a chronic inflammatory disease involving the nasopharynx. Infection with *K. rhinoscleromatis* has a worldwide distribution and is usually observed in areas of southeastern Europe, Central America, and India. Patients present with a purulent nasal discharge with formation of crusts and nodules that may lead to respiratory obstruction. The bacilli are seen intracellularly in lesions, which can be isolated and identified by biochemical reactions. Diagnosis is by positive blood culture supplemented with histology. Rifampin has been used for treatment of rhinoscleroma.

Klebsiella ozaenae

Ozena, caused by *K. ozaenae*, is a chronic atrophic rhinitis characterized by necrosis of nasal mucosa and nasal discharge. It often occurs in elderly persons. Nasal congestion and a constant nasal bad smell are the common symptoms. However, unlike rhinoscleroma, nasal congestion is not a prominent feature. Patients may also complain of headache and symptoms

attributable to chronic sinusitis. Identification of *K. ozaenae* is difficult due to wide variations in the biochemical reactions of isolated strains.
Trimethoprim and sulfamethoxazole are used for treatment of ozena.

Table 1 **Important properties used for differentiation of *Klebsiella* species**

Properties	*Klebsiella pneumoniae*	*Klebsiella ozaenae*	*Klebsiella rhinoscleromatis*	*Klebsiella oxytoca*
Indole	–	–	–	+
Urease	+	–	–	+
Citrate	+	V	–	+
ONPG	+	+	–	+
Malonate	+	–	+	+
Lysine decarboxylase	+	V (40%)	–	+
Ornithine decarboxylase	–	–	–	–
MR	–	+	+	V (20%)
VP	+	–	–	+

The genus *Edwardsiella*

The genus *Edwardsiella* differs from the genus *Escherichia* by its ability to produce hydrogen sulfide. The genus *Edwardsiella* includes *Edwardsiella tarda*, the only pathogenic species for humans. *E. tarda* inhabits the intestines of snakes and other cold-blooded animals. The name *tarda* refers to slow or weak fermentation of sugars by the bacteria. *E. tarda* is a Gram-negative, noncapsulated, motile bacillus with weak fermentative powers. It ferments only glucose and maltose with production of acid and some gas. It is indole, H2S, and citrate positive, and it decarboxylates lysine and ornithine.

E. tarda is an occasional human pathogen isolated from wounds, blood, and CSF in cases of fatal meningitis. The bacteria have also been isolated from stool of normal healthy people and that of patients with diarrhea.

The genus *Citrobacter*

Citrobacter is a normal inhabitant of the intestine of humans. The genus *Citrobacter* consists of three species, namely, *Citrobacter freundii*, *Citrobacter amalonaticus*, and *Citrobacter koseri* (formerly *C. diversus*). They grow well on nutrient agar and other ordinary media producing smooth and convex colonies.

The colonies are not pigmented. On MacConkey and DCA media, they produce pale colonies. *Citrobacter* spp. are motile, H2S positive, MR positive, citrate positive, and indole variable. They do not decarboxylate lysine, but most strains decarboxylate ornithine. They ferment lactose late or do not ferment at all. Differences between three species in their biochemical characteristics are summarized in Table 1.

They show extensive antigenic sharing with salmonellae, hence may be mistaken for salmonellae. Certain strains possess a Vi antigen, closely related to the antigen of *Salmonella* Typhi and *Salmonella* Paratyphi. *Citrobacter* spp. may cause infections of the urinary tract, gall bladder, and middle ear. *C. koseri* may occasionally cause meningitis in neonates.

Table 1

Important properties used for differentiation of *Citrobacter* species

Properties	*Citrobacter freundii*	*Citrobacter koseri*	*Citrobacter amalonaticus*
Indole	−	+	+
H_2S production	+	−	−
Acid from salicin	−	+	+
Acid from malonate	−	+	−
Acid from adonitol	−	+	−

The genus *Enterobacter*

The genus *Enterobacter* includes 12 species, of which *Enterobacter cloacae* and *Enterobacter aerogenes*, followed by *Enterobacter sakazakii* are the most frequently isolated species causing human infections. Other species rarely associated with human infections include *Enterobacter asburiae*, *Enterobacter gergoviae*, *Enterobacter taylorae*, and *Enterobacter hormaechei. E. cloacae* and *E. aerogenes* are two most important *Enterobacter* species responsible for a variety of nosocomial infections. Differences between *E. cloacae* and *E. aerogenes* are summarized in Table 2.

Table 2 **Differentiation of *Enterobacter* species**

Properties	*Enterobacter aerogenes*	*Enterobacter cloacae*
Gas from glycerol	+	–
Aesculin hydrolysis	+	–
Arginine dihydrolase	+	–
Lysine decarboxylase	+	–

Enterobacter are Gram-negative bacilli, which belong to the tribe Klebsielleae, and are aerobic and facultatively anaerobic. On sheep blood agar, *Enterobacter* produces large, gray, and dry or mucoid colonies; on MacConkey agar, lactose-fermenting pink colonies. The bacteria ferment glucose with production of acid. They differ from *Klebsiella* by being motile, urease negative, and ornithine decarboxylase-positive. Endotoxin of the bacteria is known to play a major role in the pathogenesis of sepsis and its complications.

Enterobacter species rarely cause disease in otherwise healthy people. These are opportunistic pathogens. The patients who stay in hospital, especially in the ICU, for prolonged periods are at high risk to aquire *Enterobacter* infections. The patients treated earlier with antimicrobial agents and those with serious underlying conditions (e.g., diabetes, malignancies, burns, mechanical ventilation), with foreign devices (e.g., such as intravenous catheters), and with immunosuppression are also at increased risk of infection by the bacteria. In these patients, they cause frequent and severe nosocomial infections, such as UTIs, lower respiratory tract infections, skin and soft tissue infections, bacteremia, endocarditis,

intraabdominal infections, septic arthritis, and osteomyelitis. These infections are associated with:

■ prolonged hospitalization,

■ use of a variety of different surgical and nonsurgical procedures, and

■ use of recent and expensive antimicrobial agents. These bacteria cause significant morbidity and mortality, and infection management is complicated by multiple antibiotic resistances shown by the bacteria. These bacteria possess inducible beta-lactamases, which are not detectable *in vitro*, but are responsible for resistance during treatment. The sources of infection may be endogenous or exogenous.

■ The endogenous *Enterobacter* infections originate from the skin, gastrointestinal tract, or urinary tract colonized by the bacteria.

■ The hands of medical personnel, intravenous solutions, endoscopes, blood products, are the frequently reported sources for exogenous infections caused by *Enterobacter*.

Diagnosis is made by repeated culture from appropriate clinical specimens. Blood culture is useful in isolation of the bacteria from bacteremia patients. Carbapenems, new quinolones, and trimethoprim–sulfamethoxazole (TMP–SMX) are the most frequently used antibiotics against *Enterobacter* infections.

Genus *Bordetella*

Genus *Bordetella* includes the bacteria that are extremely small, strictly aerobic, nonfermentative, and Gram-negative coccobacilli. These are obligate respiratory tract pathogens of warmblooded animals including birds.

The genus *Bordetella* consists of seven recognized species; of which, three are responsible for human diseases (Table 1). These are *Bordetella pertussis*, agent responsible for whooping cough, or pertussis; *Bordetella parapertussis*, responsible for disease similar to pertussis but the disease is milder; and *Bordetella bronchiseptica*, responsible for pertussis-like symptoms in humans. *Bordetella avium* is a pathogen of birds, which causes infection in turkey poultry, but does not cause any infection in humans.

Table 1

Human infections caused by *Bordetella* species

Bacteria	Diseases
Bordetella pertussis	Whooping cough or pertussis
Bordetella parapertussis	Milder form of pertussis
Bordetella bronchiseptica	Pertussis-like symptoms in humans

B. pertussis

B. pertussis (*pertussis*, Latin for intense cough) is the causative agent of whooping cough, an infectious bacterial illness that affects the respiratory tract. Whooping cough is one of many diseases that can be prevented by vaccine.

Morphology

B. pertussis shows the following features*:*

■ *B. pertussis* are extremely small ovoid coccobacilli measuring 0.2–0.5 µ 1 µm.

■ Characteristically, they demonstrate pleomorphism in their morphology.

■ They are Gram negative, occurring in singles or in pairs, and are nonmotile and nonsporing.

■ Toluidine blue staining of the bacteria demonstrates characteristic bipolar metachromatic granules.

■ Freshly isolated strain of *B. pertussis* possesses a poorly defined capsule and also fimbriae.

■ In culture smears, the bacilli are arranged in loose clumps with clear spaces in between, giving a thumbprint appearance.

Culture

B. pertussis is a strict aerobe with an optimum temperature for growth 35℃. The bacteria are nutritionally fastidious. They do not grow on common laboratory media, such as blood agar and nutrient agar. Even on blood agar, the bacteria grow slowly and require 3–6 days to form pinpoint colonies. The bacteria are usually grown on a rich medium supplemented with charcoal, starch, blood, albumin, and growth factors, such as nicotinamide. The latter is absolutely essential for the growth of the bacteria. Blood or albumin present in the medium is used apparently not to provide nutrition for the growth of bacteria but to neutralize toxic substances, such as fatty acids present in the agar. Bordet–Gengou agar with 15–20% of blood is a common medium used for primary isolation of *B. pertussis*. This medium consists of glycerol, potato, agar, and 15–20% of blood. After 48–72 hours of incubation, *B. pertussis* produces small, smooth, opaque, grayish white colonies resembling bisected pearls or mercury drops. A hazy zone of hemolysis is present around the colonies. Charcoal agar with 10% blood has also been used for primary isolation of *B. pertussis*. The bacterium does not grow on MacConkey medium, but other *Bordetella* species grow on this medium.

Biochemical reactions

B. pertussis shows the following biochemical reactions:

■ It is oxidase positive and catalase positive but biochemically inert.

■ The bacteria do not ferment carbohydrates.

■ The bacteria also do not produce indole, do not reduce nitrate, do not split urea, and do not utilize citrate.

Susceptibility to physical and chemical agents: *B. pertussis* is killed by heating at 55℃ for 30 minutes. It is also killed by drying and also by standard disinfectants. If left on culture plates, the bacteria die within few days, but they can survive in dry droplets for up to 5 days and for 3 days on cloth.

Cell Wall Components and Antigenic Properties

B. pertussis is a Gram-negative bacterium that possesses lipopolysaccharide (LPS) in the outer membrane. The LPS of *B. pertussis* is unusual. The role of their unusual LPS in the pathogenesis of whooping cough is unknown.

B. pertussis possesses two types of antigens. These are genus specific somatic O antigen and strain-specific capsular K antigen. The O antigen is protein in nature, heat-stable, and is of single antigenic type. It is present in most of the strains of *Bordetella.* The K antigen is heat labile. These K antigens are of different types and are used for differentiating *B. pertussis* isolates in epidemiological studies.

Pathogenesis

Whooping cough is also known as pertussis. *B. pertussis* colonizes the cilia of mammalian respiratory epithelium. The organism usually does not invade the tissues. In addition, *B. pertussis* causes pertussis or whooping cough in two stages. The first stage is characterized by colonization, multiplication of bacteria, and production of localized tissue damage. The second stage of toxemia is characterized by manifestation of systemic toxicity produced by a toxins.

B. pertussis produces several virulence factors (Table 2). These include (*a*) filamentous hemagglutinin (FHA), (*b*) pertussis toxin, (*c*) invasive adenylate cyclase, (*d*) lethal toxin, and (*e*) tracheal cytotoxin.

Table 2 Virulence factors of *Bordetella pertussis*

Virulence factors	Biological functions
Filamentous hemagglutinin	Binds to galactose residues on a sulfated glycolipid called sulfatide, present on the surface of the ciliated cells; binds to CR3, a receptor on the surface of polymorphonuclear leukocytes; and mediates the attachment of *Bordetella pertussis* to ciliated epithelial cells of the respiratory tract
Pertussis toxin	Causes adhesion of *Bordetella pertussis* to tracheal epithelium; S2 subunit binds to glycolipid present on ciliated epithelium; S3 subunit binds to ganglioside receptor on the surface of phagocytic cells; and S1 subunit inhibits the eukaryotic adenyl cyclase, killing of phagocytes, and migration of monocytes
Invasive adenylate cyclase or hemolysin	It has adenylate cyclase activity and a binding component that mediates attachment to host cell surface
Lethal toxin	Causes inflammation and lethal necrosis around the site of adherence of the bacteria
Tracheal cytotoxin	Kills ciliated respiratory cells; also stimulates release of cytokine IL-1 and inhibits ciliary movement

Filamentous hemagglutinin: FHA is the most important virulence factor, which mediates the attachment of *B. pertussis* to the ciliated epithelial cells of the respiratory tract.

Pertussis toxin: Pertussis toxin is also involved in adhesion of *B. pertussis* to tracheal epithelium.

Lethal toxin: Lethal toxin was earlier known as dermonecrotic toxin. It is a heat-labile protein. The role of the toxin in the disease is unknown.

Tracheal cytotoxin: Tracheal cytotoxin is not a classical exotoxin, because it is not composed of protein, but is composed of a low-molecular-weight peptidoglycan

fragment that kills ciliated respiratory cells. It also responsible for fever. Tracheal cytotoxin is toxic for ciliated respiratory epithelium and is responsible for inhibition of ciliary movement.

Infection with *B. pertussis* is initiated by attachment of the bacteria to ciliated epithelial cells of the respiratory tract. The attachment is mediated primarily by two bacterial adhesions, FHA and pertussis toxin. The bacteria multiply at the site of infection, where they produce several toxins that paralyze the tiny cilia and cause inflammation of the respiratory tract.

Pertussis toxin mediates both the colonization and toxemic stage of the disease. The increase in the cyclic AMP levels results in increased respiratory secretions and mucus production, which characterizes the paroxysmal stage of whooping cough. The S1 subunit of pertussis toxin causes inflammation in the respiratory tract. The S2 subunit of the pertussis toxin binds to a glycolipid receptor on ciliated respiratory cells.

Further many other toxins produced by *B. pertussis* also contribute to the pathogenesis of the disease. Adenylate cyclase toxin also inhibits leukocyte chemotaxis, phagocytosis, and killing of the bacteria.

An attack of whooping cough gives lifelong immunity to a child. Secondary attack is extremely rare. The immunity is conferred by specific serum antibodies against *B. pertussis*.

Incubation period varies from 7 to 10 days. The disease typically has three stages: (*a*) catarrhal stage, (*b*) paroxysmal stage, and (*c*) convalescent stage.

Catarrhal stage: This is the first stage of whooping cough. This stage resembles that of upper respiratory tract infection with running nose, nasal congestion, sneezing, malaise, and occasional cough. A low-grade fever may also be seen. This phase typically lasts 1–2 weeks. This stage is highly infectious because of the production of large number of bacteria during this stage of the disease.

Paroxysmal stage: This is the second stage of the disease. This stage is characterized by classic whooping cough or paroxysms. Each paroxysm consists of a series of repetitive intense and drawn out bouts of cough followed by an whoop. The attack is more frequent at night with an average 15 attacks in a period of 24 hours. The paroxysm may end frequently with vomiting and exhaustion. During this stage, production of mucus in the respiratory tract is much frequent and is partially responsible for causing obstruction of the respiratory tract. Duration of this stage is highly variable, lasting within 1–6 weeks up to 10 weeks.

Convalescent stage: This is the third stage of the disease, which may last for weeks or months. It is characterized by a chronic cough that becomes less paroxysmal. This stage is marked by many complications. These include subconjunctival hemorrhage, respiratory distress, secondary bacterial pneumonia, and neurological complications,

such as convulsions. These neurological complications may result in permanent sequelae, such as epilepsy, paralysis, blindness, and deafness.

Laboratory Diagnosis

- **Specimens**

Nasopharyngeal aspirates are the specimen of choice for demonstration of bacteria by microscopy and culture. These samples should be collected during the first stage or early in the second stage of disease, because the organisms are most abundant in the respiratory secretions during these stages of disease. Also, the specimen for culture should be collected before administration of antibiotics. The culture becomes negative 5 days after treatment with antibiotics. The specimens are usually not collected by using cotton swabs because the growth of bacteria is inhibited by certain fatty acids in the cotton, which are toxic to *B. pertussis*. The specimens for culture are collected by the following methods:

The pernasal swab: In this method, a sterile swab on a flexible wire is passed gently along the floor of the nasal cavity, and the mucus and pus is collected by the swab.

Cough plate method: In this method, specimens are directly coughed out by the patient on a culture medium, during a bout of spontaneous or induced coughing in an infected child. The culture plate is held 10–15 cm in front of the patient's mouth. During the process of coughing by children, infected nasopharyngeal secretions are directly deposited on the medium.

West's postnasal swab: The West's postnasal swab is usually employed to collect posterior pharyngeal wall secretions through oral cavity. Contamination with saliva should be avoided for better results. The swab containing the mucus and pus is inoculated immediately on freshly prepared medium, such as Bordet–Gengou. In case of delay, they are transported in 0.2–0.5 mL casamino acid solution (pH 7.2) . The transport media are transported immediately to laboratory for processing, because even in these media the bacteria cannot survive for long time.

- **Microscopy**

Microscopic diagnosis is made by a direct fluorescent antibody (DFA) technique for demonstration of *B. pertussis* in respiratory secretions. In this method, a smear is made from a specimen on a glass slide, air dried, and heat fixed. This smear is then stained with fluorescent labeled antibody against *B. pertussis*. DFA is a rapid and sensitive method. This method is positive in more than 75% of cases of whooping cough.

- **Culture**

The specimens are cultured on freshly prepared Bordet–Gengou medium or charcoal horse blood agar medium and incubated in moist environment at 35°C. Incubation even up to 7 days is required, because the colonies are observed only after 3 or more days of incubation.

- **Serology**

Indirect hemagglutination are frequently used to demonstrate IgG and IgA antibodies against FHA and IgG antibodies against pertussis toxin in the patient's sera.It is also useful, especially in culture-negative cases.

Treatment

Erythromycin is the drug of choice. Tetracycline, chloramphenicol, and ampicillin are also effective. These antibiotics are effective (*a*) in eradicating the bacteria from the respiratory tract and (*b*) in reducing the duration of infectivity of the patients. Treatment of pertussis is, however, primarily supported by good nursing care.

Prevention and Control

Vaccination of infants and children with pertussis vaccine is very effective. The vaccination is usually given for children below 7 years of age. Vaccines used against pertussis are of two types:

1. Whole cell inactivated vaccine

2. Acellular vaccine

B. parapertussis

B. parapertussis is a less frequent cause of whooping cough. The bacteria are responsible for only about 5% of cases of whooping cough. They relatively cause mild disease. *B. parapertussis* organisms differ from *B. pertussis* by their ability to grow on nutrient agar and to produce larger colonies, but the former do not produce pigment (Table 39-3). They are antigenically different from those of *B. pertussis*. Pertussis vaccine does not confer any protection against *B. parapertussis*.

B. bronchiseptica

B. bronchiseptica is a motile bacterium by virtue of presence of peritrichate flagella. The bacteria grow on nutrient agar and are antigenically related to *B. pertussis* and *Brucella abortus*. It differs from other *Bordetella* species in the biochemical and other properties (Table 3). *B. bronchiseptica* is responsible for causing very small proportion (0.1%) of cases of whooping cough.

Table 3 **Differential characteristics of *Bordetella* species**

Characteristics	*Bordetella pertussis*	*Bordetella parapertussis*	*Bordetella bronchiseptica*
Growth on:			
MacConkey agar	–	+/–	+
Sheep blood agar	–	+	+
Bordet-Gengou medium	3-6 days	1-2 days	1 day
Motility	Nonmotile	Nonmotile	Motile
Oxidase	+	+	–
Urease	–	+	+
Nitrate to nitrite	–	–	+
Citrate utilization	–	+/–	+

Salmonellae

Salmonellae are human and animal pathogens. *Salmonella* spp. include Gram-negative, flagellated, and facultative anaerobic bacilli characterized by the presence of O, H, and Vi antigens. The taxonomic classification of the genus *Salmonella* is complex . Based on DNA homology and host range, the genus *Salmonella* is classified into two species: *Salmonella* Enterica and *Salmonella* Bongori. *S.* Enterica is further subdivided into six subspecies I, II, IIa, IIIb, IV, and VI. Most of the salmonellae that are pathogenic to human beings belong to the subgroup I of *S.* Enterica subsp. *enterica.* This includes the typhoid and paratyphoid bacilli and most other serotypes responsible for diseases in mammals.

Additionally, each of the *Salmonella* isolates is serotyped according to the presence of particular somatic O, flagellar H, and surface Vi antigens. Presently more than 2400 serotypes are described.

Each *Salmonella* serotype is considered as a species.

Salmonellae serotypes are named as, for example, *S. Enterica* subsp. *enterica* serotype Enteritidis. However, it is abbreviated as *S.* Enteritidis . In addition, serotypes are not mentioned in italic but in Roman.

Morphology

Salmonellae are Gram-negative bacilli measuring 1–3 _m in size. They are motile with the presence of peritrichous flagella (*Salmonella* Gallinarum and *Salmonella* Pullorum are exceptions which are nonmotile). They do not form spores and capsules. Some strains of salmonellae may produce fimbriae, but most strains of *Salmonella* Paratyphi A and few strains of *Salmonella* Paratyphi B, *Salmonella* Typhi, and *Salmonella* Typhimurium are nonfimbriated.

Culture

They are aerobic and facultatively anaerobic; they grow at an optimum temperature of 37ºC in a pH of 6–8 on a variety of nonselective (Mueller–Hinton agar) and selective (Wilson and Blair's bismuth sulfite medium) media.

1. Nonselective solid media: On ***nutrient agar*** and ***blood agar***, *Salmonella* spp. produce gray white moist colonies with smooth convex surface after 18–24 hours of incubation. Rough strains produce opaque and granular colonies with irregular surface. Some strains of *S.* Paratyphi B produce large mucoid colonies due to the production of loose polysaccharide slime. On ***MacConkey agar***, they produce pale colorless colonies because they do not ferment lactose. *S.* Typhi do not grow on this medium. The colonies on ***deoxycholate citrate agar*** are similar to those produced on MacConkey

agar. Sometimes after incubation of 48 hours or more, they produce colonies with a black center.

2. Selective solid media: ***bismuth sulfite agar*** is the medium of choice for *Salmonella* spp., especially *S.* Typhi*.* The growth of *Shigella* spp., *Proteus* spp., and coliforms is inhibited on this medium. On this medium, salmonellae produce black colonies surrounded by a metallic sheen due to production of hydrogen sulphide. *S.* Paratyphi A and other species, which do not produce H2S, form green colonies. ***XLD (xylose, lysine deoxycholate agar)*** is another selective medium used for isolation of *Salmonella* spp. On this medium, *Salmonella* spp. produce pink colonies with black centers as a result of H2S production.H2S-negative *Salmonella* serotypes produce red colonies without black centers.

3. Liquid media: Selenite F and tetrathionate broth are commonly used enrichment media. ***Selenite F broth*** is frequently used for enrichment of *Salmonella* spp. from clinical specimens. However, sometimes this medium inhibits growth of some salmonellae, such as *S.* Paratyphi B and *Salmonella* Choleraesuis. ***Tetrathionate broth***, although is used for salmonellae but at times the broth allows the growth of *Shigella* spp. and also that of *Proteus* spp*.* Tetrathionate broth with brilliant green, although inhibits the growth of *Proteus* spp., sometimes is inhibitory to *Salmonella* spp.

<u>Biochemical reactions</u>

Salmonellae show following reactions:

1. Salmonellae ferment glucose, mannitol, and maltose, forming acid and gas. *S.* Typhi is an exception, which does not ferment the sugars.

2. They do not ferment lactose, sucrose, or salicin.

3. They do not produce indole.

4. Most salmonellae except *S.* Paratyphi A, *S.* Choleraesuis, and some other species produce H2S.

5. They do not hydrolyze urea. They are MR positive and VP negative and citrate positive. *S.* Typhi and *S.* Paratyphi, however, do not grow in Simmon's citrate media as they need tryptophan as the growth factor.

6. Salmonellae decarboxylate lysine, ornithine, and arginine, but not glutamic acids. However, *S.* Typhi do not decarboxylate ornithine and *S.* Paratyphi A does not decarboxylate lysine.

7. Salmonellae are catalase positive and oxidase negative.

The biochemical characteristics are useful for distinguishing different *Salmonella* spp. (Table 32-2).

Susceptibility to physical and chemical agents: The bacilli are killed at a temperature of 55ºC in 1 hour or at 60ºC in 15 minutes. They are also killed

by 0.2% mercuric chloride or 5% phenol in 5 minutes. Boiling, chlorination of water, and pasteurization of milk kill the bacteria. They survive for weeks in polluted water and soil, and for months in ice. Cultures may be viable for years if prevented from drying.

TABLE 1 Biochemical reactions of common *Salmonella* spp.

	Salmonella Typhi	*Salmonella* Paratyphi A	*Salmonella* Paratyphi B	*Salmonella* Paratyphi C
Glucose	A	AG	AG	AG
Mannitol	A	AG	AG	AG
Lactose	–	–	–	–
Sucrose	–	–	–	–
Indole	–	–	–	–
Citrate	–	–	+	+
MR	+	+	+	+
VP	–	–	–	–
H_2S	+	–	–	+
Xylose	d	–	AG	AG
D-Tartrate	A	–	–	AG
Mucate	d	–	AG	–

A, acid; AG, acid and gas; d, delayed.

Antigenic properties

Salmonella possess three major antigens:

1. H or flagellar antigen

2. O or somatic antigen

3. Surface antigens (Vi antigen, M and N antigen, and F antigens)

H or flagellar antigen: This antigen is present on the flagella and is heat and alcohol labile. The antigens are destroyed by boiling or by treatment with alcohols and acid, but they are preserved in 0.2–0.4% formaldehyde. The H antigens of *Salmonella* are genus specific and are not shared by other enterobacteria. The antigens are destroyed by boiling or by treatment with alcohols and acid but they are preserved in 0.2–0.4% formaldehyde. The H antigen is strongly immunogenic and is associated with the formation of antibodies following infection or immunization.

O or somatic antigen: O antigens occur on the surface of the outer membranes and are determined by specific sugar sequences on the cell surface. O antigen is an LPS complex and is an integral part of the cell wall. It is heat stable, resistant to boiling up to 2 hours and 30 minutes. It is also alcohol stable, resistant to treatment with 96% ethanol at 37ºC for 4 hours

and is also resistant to 0.2% formaldehyde. This antigen can be extracted from cell wall by treatment with trichloroacetic acid. Antigen is less immunogenic than H antigen. Generally, the O antibody titer produced after infection or immunization is lower than that of H antibodies. *Salmonella* has been classified into 46 "O" serogroups.

Surface antigens: These include (*a*) Vi antigen, (*b*) M and N antigens, and (*c*) F antigens, and are discussed below.

Vi antigen: Vi antigen is a surface antigen overlying the "O" antigen. Felix and Pitt, who first described this antigen, believed that it was related to virulence and gave it the name 'Vi antigen.' It is analogous to the K antigens of coliforms. The antigen is present only in few serotypes, the most important being *S.* Typhi. This antigen is also present in some strains of *Salmonella* Paratyphi C, *Salmonella* Dublin, and *Citrobacter freundii*. The presence of this antigen on the surface renders these bacteria inagglutinable by their specific O antiserum but agglutinable by Vi antisera. The Vi antigen is heat labile and is destroyed by boiling within 1 hour.

***M and N antigens*:** These antigens are present on the surface of bacteria and are polysaccharide in nature. These antigens prevent agglutination by O antiserum. Boiling for two and half hours destroys these antigens. The presence of M antigen is responsible for mucoid nature of *Salmonella* colonies.

***F antigen*:** These antigens are present on the fimbriae.

Pathogenesis

S. Typhi is an invasive bacterium. It colonizes the human intestine and, under specific conditions, directly invades the intestinal mucosa or multiplies for several days within the mononuclear phagocytic cells in the liver, spleen, lymph nodes, and Peyer patches of the ileum before invasion. The bacteria subsequently enter the blood stream and cause the disease manifestations.

Virulence factors

Virulence factors of salmonellae are complex .

Type III secretion systems: Type III secretion systems (TTSS) consist of nearly 20 proteins, which facilitate secretion of virulence factors of *Salmonella* into host cells. These are encoded by several *Salmonella* pathogenicity islands, such as *Salmonella* pathogenicity-island 1 (SPI-1) and *Salmonella* pathogenicityisland 2 (SPI-2). Absence of these pathogenicity islands renders the organism avirulent. TTSS mediate uptake of the bacteria into epithelial cells. SPI-1 mediates nonphagocytic cell invasion and SPI-2 facilitates survival and replication of *Salmonella* within macrophages.

Endotoxin: Endotoxin is responsible for many of the systemic manifestations of the disease caused by *Salmonella* spp.

Fimbriae: The species-specific fimbriae mediate binding of *Salmonella* to M (microfold) cells present in Peyer patches of the terminal part of the small intestine.

Enzymes: Catalase and superoxide dismutase are the enzymes that protect the bacteria from intracellular killing in macrophages.

Pathogenesis of enteric fever

The severity of disease in individuals infected with salmonellae is dependent on the virulence factors of the infecting strain as well as on the human host.

Infective dose: The infection is acquired by ingestion of food or water contaminated with salmonellae. The infective dose (ID50) in human volunteer experiments has been found to be about 103–106 bacilli. Although the infectious dose varies among strains, a large inoculum is necessary to overcome stomach acidity and to compete with normal intestinal flora. Large inocula are also associated with higher rates of illness and shorter incubation periods. However, lower infectious doses may be adequate to cause infection if:

■ these organisms are coingested with foods that rapidly transit the stomach (e.g., liquids) or that increase gastric pH (e.g., cheese, milk);

■ antacids are used concomitantly.

■ these bacteria are ingested by individuals with defective immune systems.

On reaching the intestine, the salmonellae attach themselves by fimbriae or pili to cells lining the ileal mucosa. The bacteria selectively attach to

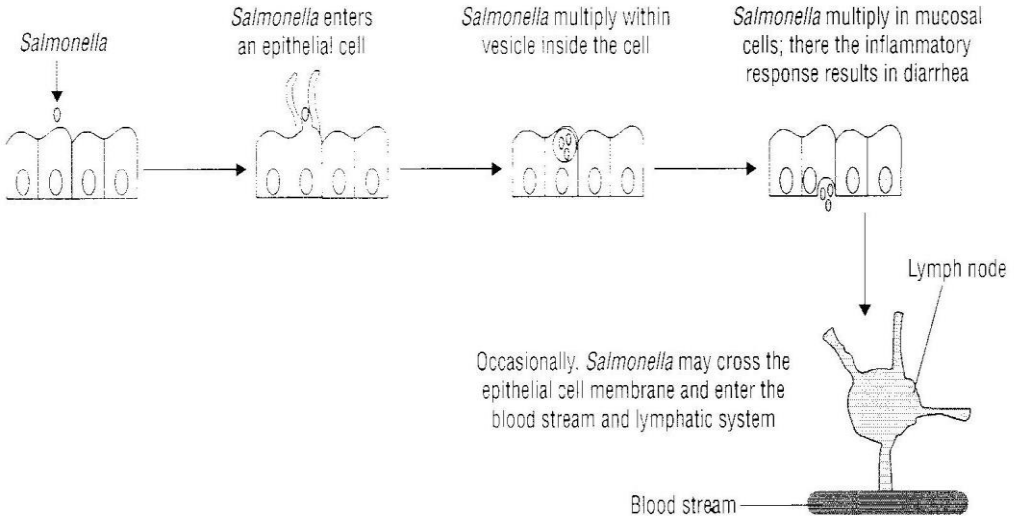


FIG.1 Pathogenesis of *Salmonella* infection.

specialized epithelial cells (M cells) of the Peyer patches. *Salmonella* TTSS mediate the initial invasion of *S.* Typhi into the intestinal mucosa. SPI-1 introduces *Salmonella* secreted invasion proteins into the M cells resulting in membrane ruffling. The ruffled membranes surround and swallow salmonellae, leading to intracellular replication in the phagosome with subsequent death of host cells and spread to adjacent epithelial cells and lymphoid tissue. Catalase and superoxide dismutase are other factors that protect the bacteria from intracellular killing.

The bacteria are then transported within phagosomes to the lamina propria, where they are released. In the lamina propria, typhoidal strains of salmonellae induce production of macrophages, while nontyphoidal strains induce production of neutrophils (nontyphoidal strains). Subsequently, *S.* Typhi and other virulent *Salmonella* strains invade deeper tissues via lymphatics and capillaries and elicit a major immune response.

The organisms travel from the submucosa to the mesenteric lymph nodes, multiply, and then enter the blood stream to spread to other tissues.

During this bacteremic phase, the bacteria may invade any organ but most commonly are found in reticuloendothelial tissues of the liver, spleen, bone marrow, gallbladder, and Peyer patches in the terminal ileum. The gallbladder is infected via the liver.

The infected bile renders stool cultures positive. Preexisting gallbladder disease predisposes to chronic biliary infection, leading to long-term fecal carriage.

Invasion of Peyer patches occurs during either the primary intestinal infection or secondary bacteremia, and further spread of bacteria occurs through infected bile. The Peyer patches

become inflamed cells, which may lead to necrosis of the superficial layer and ulcer formation, with potential hemorrhage from blood vessel.

Enteric fever is generally an acute illness manifested by fever, headache, and abdominal symptoms. The incubation period is usually .The condition is associated with a soft, palpable spleen and an enlarged

liver. These symptoms are present for a week or more and are followed by gastrointestinal symptoms. This phase corresponds to an initial bacteremic phase, which is followed by colonization of gallbladder and then reinfection of the intestines.

Intestinal perforation, severe hemorrhage, and circulatory collapse are most important complications. hepatitis, pancreatitis, arthritis, and myocarditis are other complications.

S. Typhi causes typhoid fever, while *S.* Paratyphi A, *S.* Paratyphi B, and *S.* Paratyphi C cause a mild form of this disease referred to as paratyphoid fever. The term enteric fever includes both typhoid and paratyphoid fever caused by these *Salmonella* spp.

S. Typhi and *S.* Paratyphi (A, B, and C) are strict human pathogens. They are not found in any other animal hosts. They colonize the small intestine. Other salmonellae are parasitic in various domestic animals, rodents, reptiles, and birds. *S.* Typhimurium have a wide host range affecting animals, birds, and humans.

The infected patient and, more frequently, carriers are important reservoirs of infection for enteric fever. About 2–4% of patients become chronic carriers. The bacilli persist in the gallbladder and are excreted in feces (*fecal carrier*) or persist in the kidney and are secreted in the urine (*urinary carrier*).

Laboratory Diagnosis

Laboratory diagnosis of enteric fever is based on the following methods:

1. Isolation of *Salmonella* spp. by culture,

2. Serodiagnosis by demonstration *Salmonella* antibodies and antigens, and

3. Molecular diagnosis by DNA probes and PCR.

Specimens

Blood, blood clot, bone marrow, and stool are common specimens used for isolation of typhoidal bacilli for culture. Other specimens include the cerebrospinal fluid, peritoneal fluid, mesenteric lymph nodes, resected intestine, pharynx, tonsils, abscess, bone, and urine.

Culture

Blood culture: Blood culture is a very useful procedure for diagnosis of enteric fever. It is positive in approximately 90% of cases in the first week of fever, 75% of cases in the second week, 60% in the third week, and 25% thereafter till the subsidence of pyrexia. Blood cultures, however, rapidly become negative on treatment with antibiotics.

Identification of bacteria

Colonies are identified by carrying out motility test, biochemical tests, and slide agglutination with specific *Salmonella* antisera .

Serodiagnosis

Serodiagnosis of enteric fever is based on detection of specific *Salmonella* antibodies in the serum, or antigen in the serum and also in urine by various serological tests.

Demonstration of serum antibodies

Widal test: Widal test is the traditional serologic test used for the diagnosis of typhoid fever. The test measures agglutinating antibodies against flagellar (H) and somatic (O) antigens of *S.* Typhi for typhoid and paratyphoid bacilli in the patient's sera. The H and O antigens of *S.* Typhi and the H antigens of *S.* Paratyphi A and B are used in the test. The paratyphi O antigens are not used because they cross-react with the typhoid O antigen .

The results of the Widal test should be interpreted, taking into account the following:

1. Antibodies against H and O antigens usually appear by 7th–10th day of the illness and increase steadily till the third or the fourth week, after which it declines gradually. Hence, the blood collected before 7–10 days will be negative for antibodies.

2. Sample collected in the first week and second sample is collected in the third week, is more useful than demonstration of antibodies in a single serum.

3. A titer of 1/100 or more for O antibodies and 1/200 or more for H antibodies is usually suggestive of enteric fever.

4. An elevated level of antibodies may be present in sera of patients suffering from enteric fever in past and in sera of individuals with inapparent infection or vaccination against the enteric fever.

5. Serum from an individual vaccinated with TAB vaccine may show high titers of antibodies to *S.* Typhi and *S.* Paratyphi A and B. However, in case of infection, high titers of antibodies will be seen only against the infecting species. H antibodies persist for many months after vaccination,
but O antibodies disappear earlier within 6 months.

6. Patients treated early with antibiotics, such as chloramphenicol,
may show a poor antibody response.

Treatment

Chloramphenicol was the antibiotic of choice for treatment of enteric fever since its introduction in 1948. It acts by binding to 50S bacterial-ribosomal subunits and inhibits bacterial growth by inhibiting protein synthesis. Because of low cost, for sensitive *S.* Typhi strains, chloramphenicol is still used to treat typhoid fever.

Salmonella gastroenteritis:

Salmonella gastroenteritis is the most common form of salmonellosis. *Salmonella* gastroenteritis or food poisoning is generally a zoonotic disease, caused by certain species of nontyphoidal salmonellae, which are primarily animal pathogens. *S.* Typhimurium is the most common species causing the disease in many parts of the world. Some other common species include *S.* Enteritidis, *Salmonella* Newport, and *S.* Anatum.

Human infection usually occurs by consumption of contaminated foods. The most common sources of salmonellae are milk and milk products, meat, poultry, and eggs. Improperly prepared fruits, vegetables, dairy products, and shellfish may cause infection if contaminated through manure or by unhygienic handling.

The incubation period is 6–72 hours. Nausea, vomiting, and loose watery stools are the common symptoms. Fever, abdominal cramps, myalgias, and headache are also common. Fever, which rarely exceeds 39ºC, occurs in approximately one-half of infected patients. Symptoms usually resolve spontaneously in 2–7 days.

Laboratory diagnosis is made by isolating the salmonellae from the feces by culture. In outbreaks of food poisoning, isolation of salmonellae from the food confirms the diagnosis.

Treatment

Ampicillin, amoxicillin, trimethoprim-sulfamethoxazole, cefotaxime, and ceftriaxone are effective for the treatment of the condition.

TABLE 2

Human infections caused by *Salmonella* spp.

Bacteria	Diseases
Salmonella Typhi	Typhoid fever, *Salmonella* bacteremia
Salmonella Paratyphi A, B, and C	Paratyphoid fever, *Salmonella* bacteremia
Salmonella Cholerasuis	*Salmonella* bacteremia
Salmonella Typhimurium	*Salmonella* gastroenteritis
Salmonella Enteritidis	*Salmonella* gastroenteritis
Salmonella Hadar	*Salmonella* gastroenteritis
Salmonella Heidelberg	*Salmonella* gastroenteritis
Salmonella Agona	*Salmonella* gastroenteritis
Salmonella Virchow	*Salmonella* gastroenteritis
Salmonella Seftenberg	*Salmonella* gastroenteritis
Salmonella Indiana	*Salmonella* gastroenteritis
Salmonella Newport	*Salmonella* gastroenteritis
Salmonella Anatum	*Salmonella* gastroenteritis

Shigella

Shigella is the most common cause of bacillary dysentery, which occurs worldwide. The disease is spread through fecal–oral transmission, and humans are the only natural reservoir of the bacteria.

Based on a combination of biochemical and serological characteristics, shigellae are classified into four species or subgroups, consisting of more than 45 O antigen-based serogroups and each species consisting of different serotypes.

***Shigella dysenteriae* (group A):** the bacillus originally described by Shiga, hence known as Shiga's bacillus.

***Shigella flexneri* (group B):** This group is named after Flexner (1900).

***Shigella boydii* (group C):** This group is named after Boyd, who first described these strains from India (1931).

***Shigella sonnei* (group D):** This group is named after Sonne, who first described these strains from Denmark (1915).

‣ Morphology

Shigella shows following features:

■ *Shigella* are short, Gram-negative rods, about 0.5 x1–3 µm in size.

■ They are nonmotile, nonsporing, and noncapsulated.

■ *Shigella* species with exceptions of *S. flexneri*, serotype 6, and some strains of other serotypes possess fimbriae.

‣ Culture

Shigella are aerobes and facultative anaerobes. They grow at a temperature range of 10–40°C with an optimum temperature of 37°C and pH 7.4.

1. Nutrient agar: They grow on ordinary media, such as nutrient agar or Mueller–Hinton agar. *Shigella* colonies on nutrient agar, after overnight incubation, are small, circular, convex, smooth, and translucent. Occasionally on primary isolation and frequently in subcultures, a proportion of the colonies may be of the rough type.

2. MacConkey agar: *Shigella* spp. on MacConkey agar produce nonlactose-fermenting pale, colorless colonies. However, *S. sonnei* (which ferments lactose late) forms pale pink colonies on prolonged incubation.

3. Selective media: Deoxycholate citrate agar (DCA), xylose lysine deoxycholate (XLD) agar, Salmonella–Shigella (SS) agar, and Hektoen enteric (HE) agar are frequently used selective media for isolation of *Shigella* species. DCA is a useful selective medium for isolation of *Shigella* spp. from

feces. On this medium, *Shigella* spp. produce small colonies, which on prolonged incubation produce lactose-fermenting pink colonies.
XLD agar is another selective medium, which is less inhibitory to *S. dysenteriae* and *S. flexneri*. *Shigella* spp. forms red colonies on this medium. *Shigella* spp. on SS agar form colorless colonies. *Shigella* spp. on HE agar forms green colonies.
4. Liquid media: Selenite F and Gram-negative (GN) broth are commonly used enrichment media. Enrichment of feces in GN broth for 4–6 hours followed by subculture on XLD or HE medium is useful for isolation of *Shigella* from clinical specimens.

‣ Biochemical reactions

Shigella shows following reactions:

■ *Shigella* ferments mannitol, forming acid but no gas. Mannitol fermentation test is an important biochemical test, which is used to classify shigellae into mannitol-fermenting and -nonfermenting species. *S. flexneri*, *S. boydii*, and *S. sonnei* are mannitol-fermenting species, while *S. dysenteriae* is mannitolnonfermenting species. However, exceptions are not that uncommon.

■ *Shigella* also ferments glucose, producing acid but without gas. Newcastle and Manchester biotypes of *S. flexneri* type 6, and some strains of *S. boydii* types 13 and 14 are exceptions, which do not ferment glucose.

■ They do not ferment lactose, sucrose, salicin, adonitol, or inositol. However, *S. sonnei* ferments lactose and sucrose late.

■ They reduce nitrates to nitrites and do not form H2S.

■ They are MR positive, citrate negative, and oxidase negative.

■ They are catalase positive with exception of *S. dysenteriae* type 1, which is catalase negative.

The biochemical characteristics that are useful for distinguishing different *Shigella* species are listed in .

Susceptibility to physical and chemical agents: Shigellae are killed at a temperature of 55°C in 1 hour or by 1% phenol in 30 minutes. In feces, they die within a few hours due to acidity produced by the growth of intestinal bacteria. They remain viable in moist environments for days, but die rapidly on drying. *S. sonnei* is in general more resistant to unfavorable environmental conditions than the other *Shigella* species.

Table 1

Differentiation of *Shigella* species

Characteristics	*Shigella dysenteriae*	*Shigella flexneri*	*Shigella boydii*	*Shigella sonnei*
Number of serotypes	12	6 + 2 variants	18	2 Phases; 26 colicin types
Lactose	—	—	—	—
Sucrose	—	—	—	—
Mannitol	—	+	+	+
Dulcitol	—	—	V	—
Xylose	—	—	V	V
Indole	V	V	V	—
Lysine decarboxylase	—	—	—	—
Ornithine decarboxylase	—	—	—	+

Cell Wall Components and Antigenic Structure

The cell wall of shigellae, like other Gram-negative bacilli, contains a lipopolysaccharide (LPS) structure. The LPS is liberated during lysis of the cell and, to some extent, during culture. The LPS moiety functions as an endotoxin and is an important component of the virulence of the bacteria.

‣ Antigenic structure

The antigenic structure of shigellae is simple, unlike the complex antigenic structure of salmonellae. Shigellae possess somatic O antigens and certain strains possess K antigens. *Shigella* K antigens, when present, may sometimes interfere with agglutination by O antisera. Shigellae strains also possess fimbrial antigens. Common fimbrial antigens are also found particularly in *S. flexneri*. *S. flexneri* is biochemically heterogeneous and antigenically the most complex among shigellae. *S. flexneri*, based on antigens, have been classified into six serotypes (1–6) and several subtypes (1a, 1b; 2a, 2b; 3a, 3b, 3c; 4a, 4b; 5a, 5b).

Two antigenic variants, called X and Y, which lack the type specific antigens are also recognized in addition to this. *S. flexneri* serotype 6 is always indole negative and is classified into three biotypes:
Boyd 88, Manchester, and Newcastle.

S. sonnei is antigenically homogeneous but may occur in two forms: phase I and phase II. Phase I strains produce smooth colonies, while phase II colonies form large, flat, and more irregular colonies. Cultures contain a mixture of both forms. Usually, phase II strains are isolated more frequently from carriers than from patients.

Pathogenesis

- **Virulence factors**

Virulence in *Shigella* species involves many virulence factors (Table 2).

Table 2 **Virulence factors of *Shigella* species**

Virulence factors	Biological functions
Endotoxins	Invasion, multiplication, and resistance of *Shigella* to phagocytosis by tissue macrophages
Intestinal adherence factor	Colonization of *Shigella*
Shiga toxin	Disrupts protein synthesis and produces endothelial damage

Endotoxins: The LPS moiety functions as an endotoxin and is an important component of the virulence of the bacteria. The endotoxin plays an important role in resistance of *Shigella* to nonspecific host defense encountered during tissue invasion.

The toxin helps in invasion, multiplication, and resistance of *Shigella* to phagocytosis by tissue macrophages. The endotoxin increases the cytotoxic activity of Shiga toxin on human vascular endothelial cells.

Intestinal adherence factor: Intestinal adherence factor is a 97-kDa outer membrane protein. This mediates colonization of *Shigella* spp. in infected human hosts and in animal models.

Shiga toxin: Shiga toxin is an exotoxin produced by *S. dysenteriae*. It is a heat-labile protein and acts as enterotoxin and neurotoxin. Shiga toxin (Stx) is a group of cytotoxins that contain two major immunologically non–cross-reactive groups called Stx1 and Stx2. Shiga toxins have one A subunit and five B subunits:

■ The main function of B subunit is to bind toxins to host cell glycolipid surface receptor, present on the epithelial cell of the intestines. It also mediates transfer of the A subunit into the cell.

■ Subunit A is a 32-kDa polypeptide. It cleaves the 28S rRNA in the 60S ribosomal subunit, thereby preventing the binding of aminoacyl-transfer RNA and disrupting protein synthesis.

The Shiga toxin shows three types of toxic activities:

1. *Neurotoxic activity*: This activity is demonstrable by paralysis and death of experimental animal following injection with the toxin. Although called

neurotoxin, the primary site of its action is not the neural tissue but is the blood vessels, neurological manifestations being secondary.

2. *Enterotoxic activity*: These toxins are enterotoxic for ligated rabbit intestinal segments with induction of fluid accumulation in ligated rabbit ileal loop.

3. *Cytotoxic activity*: This is demonstrated by cytotoxicity of toxin for vero, HeLa, and some selected endothelial cells, such as human renal vascular endothelial cells. This appears to be the same as vero toxin 1 (or Shiga-like toxin) produced by certain strains of *Escherichia coli* (VTEe).

The primary manifestation of Shiga toxin is damage to the intestinal epithelium of the infected host, causing diarrhea and dysentery. However, in a small number of patients, Shiga toxin can mediate damage to endothelial cells, resulting in hemolytic urinary syndrome.

‣ Pathogenesis of bacillary dysentery

Shigella spp. produce a serious disease known as bacillary dysentery. Infection occurs by ingestion. The infectivity dose (ID) is extremely low. As few as 10 *S. dysenteriae* bacilli can cause clinical disease, whereas 100–200 bacilli are needed for *S. sonnei* or *S. flexneri* infection.

Shigella spp. cause disease by invading and replicating in cells lining the intestinal mucosa of the colon. Structural proteins, such as intestinal adhesion factor, endotoxin, and exotoxin mediate the adherence of the bacteria to the cells as well as their invasion, intracellular replication, and cell-to-cell spread. The bacilli infect the epithelial cells of the villi in the large intestine and multiply inside them. Subsequently, bacteria spread laterally to involve adjacent cells and penetrate into the lamina propria (Fig. 1). Shigellae lyse the phagocytic vacuole and multiply in the host cell cytoplasm. Shigellae survive phagocytosis by inducing programed cell death or apoptosis. This mechanism also leads to the release of interleukin-1β resulting in the attraction of polymorphonuclear leukocytes into the infected tissues. This in turn alters the integrity of the intestinal wall and allows the bacteria to reach the deeper epithelial cells. Shiga toxin produced by the bacteria also plays an important role in progression of mucosal lesions after invasion of the colonic cells. The toxin also induces vascular damage in the colonic mucosa.

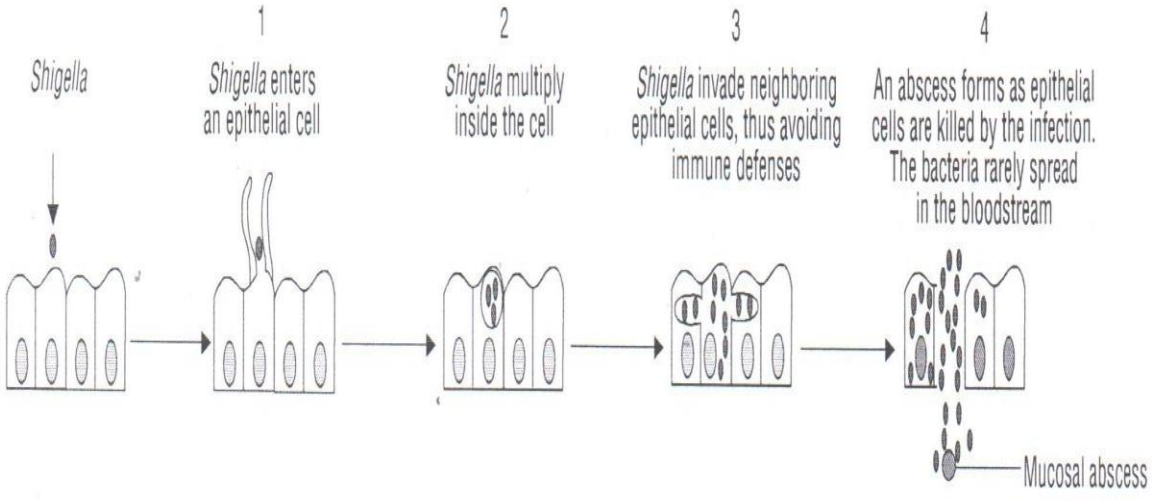


Fig. 1 Schematic diagram showing pathogenesis of bacillary dysentery.

Clinical Syndromes

Shigella spp. cause shigellosis, a clinical syndrome the whole spectrum of disease caused by the bacteria. Bacillary dysentery is a severe clinical form of the shigellosis.

Bacillary dysentery: It is an acute gastrointestinal illness manifested by fever, vomiting, abdominal cramps, and tenesmus. Incubation period is usually short. It lasts from 12 hours to 7 days, usually 48 hours, and is inversely related to the load of ingested bacilli. The condition manifests with a sudden onset of high fever along with abdominal cramp, scanty feces containing frank blood and mucus.

Infection is usually self-limiting. In a small number of patients, asymptomatic colonization of shigellae occurs in the colon, which makes the patient a persistent reservoir for infection. Complications are most often associated with *S. dysenteriae* type 1 infection. These include:

■ Arthritis, toxic neuritis, conjunctivitis.

■ Hemolytic uremic syndrome may also occur following infection with *S. dysenteriae* because of vasculopathy mediated by Shiga toxin.

■ *Shigella* septicemia is rare, except in malnourished children with *S. dysenteriae* infection.

S. sonnei causes the mildest form of bacillary dysentery in many patients; the species may cause only a mild diarrhea. *S. flexneri* and *S. boydii* causes more severe illness than that caused by *S. sonnei*.

Shigellosis occurs worldwide. Estimated 150 million cases occur annually worldwide. The incidence of shigellosis in developing countries is nearly 20 times more than in developed countries.

■ It is estimated that 30% of these infections are caused by *S. dysenteriae*.

■ *S. flexneri* is the most common cause of shigellosis in developing countries.

■ *S. sonnei* is the most common cause in the industrial world.

Shigella species are strict human pathogens. They are found in the large intestine of infected human hosts. They are not found in any other animal hosts.

Shigellosis is transmitted by:

■ ***Fecal–oral route*** by hand-to-mouth infection through contaminated fingers. Because as few as 10–200 bacilli can cause disease, shigellosis spreads rapidly in areas where sanitary standards and the level of personal hygiene are low.

■ ***Contaminated food and water:*** Food and water contaminated with human feces containing *Shigella* spp. is the main source of infection.

Laboratory Diagnosis

- **Specimens**

Stool is the specimen of choice. Diagnosis of shigellosis is made by isolating *Shigella* spp. from feces. Fresh feces are inoculated without delay or transported in a suitable medium, pH 7.0–7.4. Also, rectal swabs may be taken from the site of ulcer .

- **Microscopy**

Routine microscopy of stool may reveal clumps of polymorphonuclear leukocytes. Fecal blood or leukocytes are detectable in the stool in approximately 70% of cases of shigellosis.

- **Culture**

A sample for stool is obtained in all suspected cases of shigellosis for culture. Usually, more than one stool or rectal swab is collected and inoculated immediately on at least two different culture media, such as MacConkey, XLD, DCA, or eosin-methylene blue agars. For enrichment, one tube each of selenite F and GN broth are inoculated and incubated at 37°C for 12–18 hours before subculture onto selective media.

After overnight incubation, *Shigella* produces pale nonlactose fermenting colonies on MacConkey and DCA media and red colonies on XLD medium and colorless colonies on SS agar.

- **Identification of bacteria**

Pale non–lactose-fermenting colonies on MacConkey agar are identified by carrying out motility test, biochemical tests, and slide agglutination test with specific *Shigella* antisera.

- **Serodiagnosis**

Serological tests are not useful in the diagnosis of shigellosis.

Treatment

Uncomplicated shigellosis is a self-limited condition and patients usually recover spontaneously in a few days. Hence, no antibiotics are recommended for these cases. The dehydration observed in acute cases, particularly in infants and young children, needs adequate replacement of fluids and electrolytes by oral fluid and salts. Antibiotic treatment for *Shigella* infection is recommended (*i*) for severe or toxic cases and (*ii*) for the very young, debilitated, and the aged individuals. Antibiotic treatment is recommended to decrease the duration of illness, person-to-person spread, and cases in household contacts.

Trimethoprim–sulfamethoxazole, ampicillin, tetracycline, and the quinolones, such as nalidixic acid and ciprofloxacin, are frequently used antibiotics. Trimethoprim–sulfamethoxazole is very effective for shigellosis. The antibiotics act against *Shigella* by producing a sequential blockade in folic acid synthesis. This is the drug of choice when antibiotics susceptibility of the bacteria is not known. However, ampicillin is still the drug of choice if *Shigella* isolate is susceptible to this drug.

Most of these strains were resistant to antibiotics. Hence, it is essential to treat the cases of shigellosis with the results of *in vitro* antibiotic susceptibility testing of *Shigella*.

The genus *Proteus*

The genus *Proteus* along with two other genera *Morganella* and *Providencia* belongs to the tribe Proteeae. The name "*Proteus*" (after the Greek god Proteus who could assume any shape) refers to their property of pleomorphism. All the members of the tribe Proteeae with few exceptions are Gram negative, noncapsulated, pleomorphic, and motile bacilli. Most of these bacteria, except for some strains of *Providencia,* produce the enzyme urease which rapidly hydrolyses urea to form ammonia and carbon dioxide.

They are MR positive and VP negative, degrade tyrosine, and grow in the presence of KCN. They do not decarboxylate amino acids, such as arginine or lysine or dehydrogenase ornithine. They do not ferment lactose or dulcitol, and do not utilize malonate. The formation of the enzyme phenyl alanine deaminase, which converts phenyl alanine to phenyl pyruvic acid (PPA reaction), is the characteristic feature of the tribe Proteeae by which they are differentiated from other members of the family Enterobacteriaceae. The differentiating features of different genera in the tribe Proteeae are summarized in Table 1 .

Table 1 Differentiation of genera of the Tribe Proteeae

Biochemical properties	*Proteus mirabilis*	*Proteus vulgaris*	*Morganella morganii*	*Providencia stuartii*	*Providencia rettgeri*
Indole	−	+	+	+	+
H_2S	+	+	−(20%)	−	−
Citrate	+(65%)	V(15%)	−	+	+
Fermentation of sucrose	−	+	−	V(50%)	−
Fermentation of maltose	−	+	−	−	−
Fermentation of mannitol	−	−	−	−	+
Fermentation of trehalose	+	V(30%)	−	+	−
Ornithine decarboxylase	+	−	+	−	−

The genus *Proteus* has four species: *Proteus mirabilis*, *Proteus vulgaris*, *Proteus penneri*, and *Proteus myxofaciens. P. mirabilis* is the most important

species, which causes 90% of *Proteus* infections and is associated with community-acquired urinary tract and wound infection. *P. vulgaris* and *P. penneri* are usually associated with hospital-acquired infections. They are isolated those who are immunocompromised.

‣ Morphology

Proteus shows following features:

■ Proteeae organisms are Gram-negative and noncapsulated coccobacilli measuring 1–3 x 0.6 µm in size.
■ They are arranged as single, in pairs, or in short chains.
■ Many of them form long, curved, and filamentous forms in young cultures.
■ Most of them, with few exceptions, are motile due to the presence of peritrichous flagella.
■ They are fimbriated.

‣ Culture

Proteeae organisms are aerobic bacteria, which grow well on ordinary media, such as nutrient agar. *Proteus* colonies on the medium emit a characteristic putrefactive ("fishy") odor.

Swarming: *P. mirabilis* and *P. vulgaris* typically spread or swarm on surface of the medium. They spread on the surface of the plate in successive waves to form a thin filmy layer in concentric circles. This is known as swarming (Fig. 1). The exact mechanism responsible for swarming shown by *Proteus* species is not known. Swarming, shown by *Proteus*, is a problem when mixed growth on a solid medium is obtained in which *Proteus* bacilli are present with other bacteria. Hence, several methods are available to inhibit swarming; these include the use of increased concentration of agar in the medium from 1–2% to 6%, and the use of chloral hydrate (1:500), sodium azide (1:500), alcohol (56%), sulfonamide, surface active agents, or boric acid (1:1000). Swarming does not occur on MacConkey medium, on which *Proteus* produces colorless nonlactose- fermenting colonies. It is because bile salts present in the MacConkey medium inhibit the swarming.

‣ Biochemical properties

Proteus shows following reactions:

■ *Proteus* species ferment glucose with production of acid only.
■ They are urease and PPA positive.

■ They do not ferment lactose, mannitol, mannose, inositol, adonitol, dulcitol, sorbitol, raffinose, and arabinose.
■ They reduce nitrate to nitrite but do not utilize malonate.
■ They do not decarboxylate amino acids, such as lysine or arginine.
■ *Proteus* species show variable reactions in the production of hydrogen sulfide and indole. *P. mirabilis* is indole negative, while *P. vulgaris* is indole positive. Biochemical characteristics of *Proteus* species are summarized in Table 1.

Cell Wall Components and Antigenic Properties

Motile *Proteus* strains possess somatic O and flagellar H antigens. Somatic O antigens are heat-stable proteins, which are resistant to heating at 100 °C. They are also resistant to treatment with ethanol and dilute hydrochloric acid. Thirty two different O antigens are found in *P. mirabilis*, 22 in *P. vulgaris*, and five in *P. penneri* and *P. myxofaciens.*
■ The O antigen contains both alkali-labile and alkali-stable fractions. The alkali-stable component is polysaccharide in nature and shows cross-reactivity with certain rickettsial antigens. The sharing of antigens with rickettsial antigens was first observed by Weil and Felix. They observed that certain nonmotile strains of *P. vulgaris* called the "X strains" were agglutinated by sera from typhus fever patients. This heterophilic agglutination by certain *Proteus* strains formed the basis of the Weil–Felix reaction for the diagnosis of some rickettsial infections. Three nonmotile *Proteus* strains OXl9 (*P. vulgaris* serotype O1), OX2 (*P. vulgaris* serotype O2), and OXK (*P. mirabilis*) are used in the Weil–Felix agglutination test.
■ Flagellar antigens are heat-labile proteins sensitive to ethanol and to dilute hydrochloric acid. On the basis of their O antigens, *P. mirabilis* and *P. vulgaris* have been classified into 54 O groups. These O groups are further subdivided into a large number of O types depending on their flagellar or H antigens.

Pathogenesis

Proteus organisms are invasive bacteria.

‣ Virulence factors

Proteus possesses following virulence factors:

Pili: Fimbriae or pili are the important virulence factors that facilitate adherence of *P. mirabilis* bacteria to host tissue sites, such as the urinary tract epithelium.

LPS or endotoxin: This causes a series of host inflammatory responses and is responsible for Gram-negative endotoxin induced sepsis caused by *Proteus* species.

Urease production: The ability of *Proteus* organisms to produce urease is an important factor in pathogenesis of UTI caused by *Proteus* species.

Hydrolysis of urea to ammonia makes the urine alkaline, which provides a suitable environment for *Proteus* to survive. Subsequently, alkalization of urine leads to precipitation of organic and inorganic compounds, which in turn leads to formation of stones in renal calculi. These stones are composed of a combination of magnesium ammonium phosphate (struvite) and calcium carbonate—apatite.

- **Pathogenesis of UTI**

Pathogenesis of *Proteus* infection depends on the interaction between the bacteria and the host defense mechanisms. Adherence of the bacteria to host tissue mediated by fimbriae is the first step in the disease process. The attachment of *Proteus* species to uroepithelial cells causes secretion of interleukin-6 and interleukin-8. The infection of the urinary tract is facilitated further by production of the enzyme urease and also motility of the bacteria. Urease splits urea into ammonia and carbon dioxide. The ammonia/ammonium buffer pair has a pH of 9.0, leading to excretion of highly alkaline urine, rich in ammonia. The alkalinity of urine contributes to production of renal stones, which is characteristically observed in patients suffering from UTI due to *Proteus* species.

Clinical Syndromes

Patients with multiple antibiotic treatments, urinary tract obstruction, or infection developing after catheterization or instrumentation frequently become infected with *Proteus* spp. or other bacteria, such as *Enterobacter* spp., *Klebsiella* spp., *Serratia* spp., and *Acinetobacter* spp. *Proteus* species cause (*a*) urinary tract infections, (*b*) hospital-acquired infections, and (*c*) other miscellaneous infections.

- **Urinary tract infections**

UTIs are the most common clinical manifestation of *Proteus* infections. *Proteus* is responsible for nearly 1–2% of UTIs in healthy women and 5% of hospital-acquired UTIs. It is responsible for 20–45% of UTIs associated with catheterization. Patients with UTI may present with urethritis, cystitis, prostatitis, or pyelonephritis. Chronic UTI is associated with chronic, recurring stones. Urine sediments show multiple magnesium ammonium phosphate crystals.

- **Hospital-acquired infections**

Hospital-acquired infections are usually transmitted from attending doctors or other healthcare workers and are caused by interruption of the closed sterile system by hospital staff.

- **Miscellaneous infections**

Proteus species is an important agent of wound infections. The species also causes infection of the umbilical stump in neonates, which often leads to sepsis neonatorum, bacteremia, and meningitis. *Proteus* spp. also causes nonclostridial anaerobic myonecrosis, a condition which involves subcutaneous tissue, fascia, and muscle. This condition usually occurs in association with other aerobic Gram-negative bacilli (*E. coli*, *Klebsiella* spp., or *Enterobacter* spp.) and anaerobes. *Proteus* organisms like that of *Pseudomonas* can cause Gram-negative endotoxin-induced sepsis, resulting in systemic inflammatory response syndrome, which has a mortality rate of 20–50%.

Proteus is widely distributed as saprophytes in nature. They are commonly found in sewage, in manure soil, in human and animal feces, and in decomposing animal products. *Proteus* species are most commonly found as part of normal human intestinal flora, along with *E. coli* and *Klebsiella* species. They are also present on the moist areas of the skin. In hospital settings, they most commonly colonize the skin and oral mucosa of patients and hospital personnel. Infection to patients primarily occurs from these reservoirs.

Laboratory Diagnosis

Urine is the specimen of choice for diagnosis of UTIs. Urine is collected in the same way as described earlier for UTI caused by *E. coli*. Other specimens are collected depending on the nature of infections. These include pus for wound infections, blood for septicemia, CSF for meningitis, etc. Definitive diagnosis is based on the isolation of *Proteus* spp. from various clinical specimens by culture. Urine culture is carried out in the same way as described earlier for *E. coli* and other Gram-negative bacteria.

After incubation overnight at 37°C, pale, nonlactose-fermenting colonies of *Proteus* on the MacConckey agar and those on blood agar are identified by various biochemical tests and agglutination reactions. Culture of blood, CSF, and other specimens is also carried out depending on the clinical diseases caused by *Proteus.*

Typing

Proteus species can be typed by (*a*) serotyping, (*b*) phage typing, (*c*) bacteriocin (proticin) typing.

Treatment

The choice of a specific antimicrobial agent depends on antibiotics susceptibility patterns of isolated strains. *P. mirabilis* is susceptible to nearly all antimicrobials except tetracycline. *P. mirabilis* is sensitive to ampicillin; broad-spectrum penicillins, such as ticarcillin, piperacillin; first-, second-, and third- generation cephalosporins; imipenem; and aztreonam. Resistance to these antibiotics is not a significant problem; only 10–20% of strains develop resistance to ampicillin and first-generation cephalosporins. Development of resistance to extended-spectrum beta-lactams is uncommon.

P. vulgaris and *P. penneri* are sensitive to trimethoprim and sulfamethoxazole, quinolones, imipenem, aminoglycosides, and fourth-generation cephalosporins. They are resistant to ampicillin and first-generation cephalosporins. The resistance is mediated by activation of an inducible chromosomal beta-lactamase occurring in up to 30% of these strains.

The genus *Morganella*

The genus *Morganella* belongs to the tribe Proteeae. The genus *Morganella* has only one species, *Morganella morganii* with two subspecies, *morganii* and *sibonii. M. morganii* was classified earlier under the genus *Proteus* as *Proteus morganii. M. morganii* are small, Gram-negative, motile bacilli, but unlike *Proteus* species do not produce swarming on the solid media. They are facultatively anaerobic and nonencapsulated. They grow on blood agar or on MacConkey agar. They are oxidase negative and catalase and indole positive. *M. morganii* ferments glucose and mannose but not lactose. The bacteria decarboxylate ornithine, hydrolyze urease, and reduce nitrates. They do not liquefy gelatin and do not produce hydrogen sulfide.

M. morganii is commonly found in human and animal feces and rarely causes severe invasive diseases. It is most often found as an opportunistic pathogen in patients who are hospitalized, particularly those on prolonged antibiotic therapy. *M. morganii* causes UTIs, which are often associated with an alkaline urine pH. The bacteria have also been occasionally reported to cause sepsis, pneumonia, wound infections, pericarditis, spontaneous bacterial peritonitis, and CNS infections. Nosocomial *M. morganii* strains are usually susceptible to cefepime, imipenem, meropenem, piperacillin, aminoglycosides, and fluoroquinolones. These have also shown resistance to ceftazidime and other third-generation cephalosporins.

The genus *Providencia*

The genus *Providencia* consists of five species: *Providencia stuartii*, *Providencia rettgeri*, *Providencia alcalifaciens*, *Providencia rustigianii*, and *Providencia heimbachae. Providencia* spp. are Gram-negative, motile bacilli but do not show swarming on solid media. They produce a fruity smell and on DCA form yellow to orange colonies.

All the species typically deaminate phenylalanine; only *P. rettgeri* hydrolyses urea consistently. *Providencia* species have been isolated from urine, stool, and blood, as well as from the throat, and wounds from humans.

P. stuartii is the most common species causing infection in humans. *P. stuartii* and, to a lesser extent, *P. rettgeri* are commonly found in patients with long-term indwelling urinary catheters.

Older people are at higher risk of infection by *P. stuartii* or *P. rettgeri,* because these infections are associated with the use of urinary catheters,

and the use of such catheters is much more common in the older people. *P. stuartii* constitutes nearly 60% of all bacterial pathogens isolated from urine of these patients. *P. stuartii* possesses an adhesin, which allows it to adhere to the urinary catheter. From urine, *P. stuartii* may invade to blood, causing infection of the blood stream, which is common in elderly patients and in immunocompromised patients.

P. alcalifaciens, *P. rettgeri*, and *P. stuartii* also may cause invasive diarrhea. These species are emerging as important causes of traveler's diarrhea in adults. Diagnosis of UTI and diarrhea is made by routine urine and feces culture. Blood culture is useful for diagnosis of suspected blood stream infections. Antibiotics susceptibility testing is useful for treatment with suitable antibiotics, because many *Providencia* species show resistance to multiple antibiotics.

P. stuartii is the most resistant species of all *Providencia* species. It is resistant to tetracyclines, older penicillins, cephalosporins, fluoroquinolones, aminoglycosides, and TMP–SMX. It is susceptible to late-generation cephalosporins, aztreonam, and carbapenems.

P. alcalifaciens and *P. rustigianii* are usually susceptible to antibiotics. They usually are susceptible to fluoroquinolones, aminoglycosides, late-generation cephalosporins, aztreonam, carbapenems and TMP–SMX. They are resistant to tetracyclines, older penicillins, and cephalosporins.

The genus *Pseudomonas*

Pseudomonas and related bacteria are obligatory aerobic nonfermentative and mostly oxidase-positive bacteria. Most of them are motile by presence of one or two flagella. They are primarily saprophytic, and are found in soil, water, and in other moist environment. All these bacteria belong to family Pseudomonadaceae, which contains over 200 species. Most of them are pathogenic to plants. A few species cause disease in humans. These are *Pseudomonas aeruginosa*, *Pseudomonas fluorescence*, *Pseudomonas putida*, *Pseudomonas stutzeri*, *Burkholderia cepacia*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Stenotrophomonas maltophilia*, *Acinetobacter baumannii*, and *Moraxella catarrhalis* (Table 1).

Table 1 **Human infections caused by *Pseudomonas* and other genera of the family pseudomonadaceae**

Bacteria	Diseases
Pseudomonas aeruginosa	Respiratory tract infections, skin infections, urinary tract infections, ear infections, eye infections, bacteremia, and endocarditis
Pseudomonas fluorescence	Opportunistic infections
Pseudomonas putida	Opportunistic infections
Pseudomonas stutzeri	Opportunistic infections and urinary tract infections
Burkholderia cepacia	Opportunistic respiratory infections in patients with cystic fibrosis or chronic granulomatous disease and urinary tract infections in patients with indwelling catheters
Burkholderia mallei	Acute or chronic infection with infection localized to the skin, subcutaneous tissue, or respiratory tract
Burkholderia pseudomallei	Melioidosis
Stenotrophomonas maltophilia	Nosocomial infections (pneumonia, meningitis, wound infection, etc.)
Acinetobacter baumannii	Nosocomial pathogen of respiratory tract, urinary tract, and wound
Moraxella catarrhalis	Bronchitis and bronchopneumonia seen in patients with chronic pulmonary disease and in elderly patients

Pseudomonas

Pseudomonas are found worldwide. They are found in soil, vegetations, water, plants, and animals with a predilection to moist environments. They are also commonly found in hospital environment. They are opportunistic pathogens often causing hospital or nosocomial infections. These organisms are develop innate resistance to many antibiotics.

P. aeruginosa

P. aeruginosa is the most important species associated with human infection. It is a most common human saprophyte, but it rarely causes disease in healthy individuals. *P. aeruginosa* causes most of human infections in immunocompromised human host.

Morphology

P. aeruginosa shows the following morphological features:

■ *P. aeruginosa* is a straight or slightly curved, Gram-negative bacillus measuring 0.5–1.0 µ 1.5–5.0 µm in size arranged singly, in pairs, or in short chains.

■ It is motile by the presence of a polar flagellum. Occasionally, strains may possess two or three polar flagella.

■ *Pseudomonas* spp. is noncapsulated. Although the bacteria are noncapsulated, many strains appear mucoid by production of an abundant of extracellular polysaccharide composed of alginate polymers. This slime layer forms a loose capsule or glycocalyx around the bacillus. These strains are particularly isolated from patients with cystic fibrosis.

■ *Pseudomonas* spp. is nonsporing and fimbriated.

Culture

Pseudomonas spp. are strictly aerobic bacteria. *P. aeruginosa* grows over a wide range of temperatures (5–32°C), the optimum temperature being 37°C. *P. aeruginosa* grows on commonly used routine media including nutrient agar, blood agar, MacConkey agar, and deoxycholate citrate agar (DCA).

1. Nutrient agar: *P. aeruginosa* after incubation for 24 hours at 37°C on nutrient agar produces large (2–3 mm in diameter), opaque, translucent, and irregularly round colonies. These colonies emit a characteristic musty to fruity odor due to production of aminoacetophenone from the amino acid tryptophan. It produces hemolytic colonies on blood agar.

2. MacConkey agar: The organism produces colorless non– lactose-fermenting colonies on MacConkey media.

3. Cetrimide agar: Cetrimide agar is a selective medium for culture of *P. aeruginosa.*

4. Nutrient broth: In nutrient broth, it produces a dense turbidity with surface pellicle.

P. aeruginosa produces different types of pigments, such as (*a*) pyocyanin, (*b*) pyoverdin, (*c*) pyorubin, and (*d*) pyomelanin. However, some strains of *Pseudomonas* are not pigmented.

● ***Pyocyanin*** is specifically produced by *P. aeruginosa,* which diffuses into the surrounding medium. The pigment is soluble in chloroform and water.

● ***Pyoverdin***, or fluorescein, is produced by *P. aeruginosa* as well as by many other *Pseudomonas* species. These pigments give a yellow tinge to the colonies of bacteria

and are best demonstrated in microscope using ultraviolet source of light. Fluorescein is soluble in water, but insoluble in chloroform.

● ***Pyorubin*** is a red pigment, which is soluble in water but insoluble in alcohol.

● ***Pyomelanin*** is a brown pigment.

Biochemical reactions

■ They are oxidase positive. Oxidase test is an important test for identification of *P. aeruginosa*. All the strains are oxidase positive within 30 seconds of performing the test.

■ They are nonfermentative bacteria. They utilize sugars by an oxidase metabolism, with oxygen as the terminal electron acceptor. Special media, such as oxidationfermentation (OF) media, are used to demonstrate the low quantity of acid produced during oxidative breakdown of sugars. *P. aeruginosa* utilizes glucose, forming acid only.

■ They do not utilize lactose and maltose.

■ They reduce nitrates to nitrite, which is further broken down to gaseous nitrogens.

■ They are catalase positive.

■ They are arginine dihydrolase positive.

■ They are indole, MR, VP, and H2S negative.

Susceptibility to physical and chemicals agents: *P. aeruginosa* is heat-labile bacterium, readily killed at 55°C in 1 hour. It is also highly susceptible to acid, silver salts, 2% alkaline, and disinfectants (such as Dettol and cetrimide). However, *P. aeruginosa* is very strongly resistant to common antiseptics and disinfectants, such as chloroxylenol, hexachlorophene, and quaternary ammonium compounds.

Cell Wall Components and Antigenic Structure

These include pili, slime layer capsule, lipopolysaccharide, and pyocyanin as described in Table 2.

Pili: Pili of *P. aeruginosa* are similar to those present in *Neisseria gonorrhoeae*. They are important in mediating adhesion of the bacteria to the epithelial cells.

Slime layer: *P. aeruginosa* cell wall is surrounded by loose slime layer. The loose slime layer, also known as alginate coat or glycocalyx, protects the bacteria from phagocytosis and against activity of many antibiotics.

Lipopolysaccharide: The cell wall of *P. aeruginosa* like that of other Gram-negative bacteria contains LPS. The LPS are endotoxins, which constitute a major component of the cell wall and contribute to the sepsis caused by the bacteria.

Pyocyanin: Pyocyanin is a pigment that catalyzes the production of superoxide and hydrogen peroxide, and causes tissue damage. This pigment also contributes to inflammation associated with the disease.

Table 2 Virulence factors of *Pseudomonas aeruginosa*

Virulence factors	Biological functions
Toxins	
Exotoxin A	Acts by prevention of synthesis of proteins in eukaryotic cells; causes tissue damage in chronic pulmonary infection, dermatonecrosis in burns wound, and destruction of cornea in ocular infection; causes immunosuppression
Exoenzymes S and T	These toxins show adenosine diphosphate ribosyl transferase activity, inhibit protein synthesis, and cause immunosuppression
Enzymes	
Elastase	Destroys elastin present in elastin-containing tissues (skin, lung tissue, blood vessels, etc.), immunoglobulins, and complement factors
Alkaline protease	Tissue destruction, inactivation of interferon
Phospholipase C	Causes tissue destruction by breaking down lipids and lecithin
Rhamnolipid	Breaks down lecithin-containing tissues
Cell wall components	
Pili	Adhesion of the bacteria to the epithelial cells
Capsule	Inhibits antibiotics killing of the bacteria
Lipopolysaccharide	Endotoxic activity, sepsis
Pyocyanin	Causes tissue damage, inflammation
Alginate-like exopolysaccharide	Responsible for mucoid phenotype

- **Antigenic structure**

P. aeruginosa consists of O and H antigens.

O antigen: Somatic or O antigens are the group-specific antigens. *P. aeruginosa* possesses 19 distinct group-specific O antigens, on the basis of which the organism has been classified into 19 serogroups.

H antigen: Flagella or H antigens are found in the flagella of *P. aeruginosa*.

Pathogenesis

P. aeruginosa is an invasive pathogen. It is invasion is mediated by many virulence factors including toxins, enzymes, and structure of the cell wall (Table 2)

P. aeruginosa are opportunistic pathogens as well as true pathogens. *P. aeruginosa* cause infections of the respiratory tract. They also cause infection of urinary tract in patients with catheterization. *P. aeruginosa* as opportunistic pathogens causes most of human infections in immunocompromised host.

- **Respiratory tract infections**

P. aeruginosa infection of the lower respiratory tract occurs almost exclusively in patients with malignancies and immunodeficiencies. This colonization is seen in patients with cystic fibrosis, other chronic disease, and in those with neutropenia. The condition is associated with a high mortality rate of 70%.

- **Skin infections**

P. aeruginosa can cause a variety of skin infections, such as infections of burn wound,and others. Infection of burn wounds is the most common recognized condition caused by *P. aeruginosa*. Pseudomonal wound infection is characterized by the presence of dark brown eschar associated with edema and hemorrhagic necrosis. *Pseudomonas* skin infections are commonly seen in patients who are exposed to moisture. *P. aeruginosa* causes infection of the nail in individuals whose hands are more exposed to water.

- **Urinary tract infections**

P. aeruginosa causes urinary tract infections in persons with indwelling urinary catheters and in persons undergoing instrumentation and surgery of urinary tract. These infections usually are hospital acquired.

- **Ear infections**

P. aeruginosa is one of the common agents causing external otitis in patients with history of swimming. The species is also responsible for causing malignant external otitis, a virulent form of disease that occurs primarily in patients with diabetes or acquired immunodeficiency syndrome (AIDS) and in elderly patients.

- **Eye infections**

P. aeruginosa causes pseudomonal endophthalmitis. The condition may occur following trauma, intraocular surgery, or posterior perforation of corneal ulcer. Persons wearing contact lens are at increased risk of developing pseudomonal infection.

- **Endocarditis**

Cardiovascular infections, such as pseudomonal infectious endocarditis, are caused by involvement of both normal and abnormal valves on both sides of the heart. This condition leads to destruction of heart valves and subsequent heart failure.

- **Nosocomial infections**

Most infections caused by *P. aeruginosa* are opportunistic infections seen in immunocompromised host.

Laboratory Diagnosis

Laboratory diagnosis of pseudomonal infection is based on isolation of *P. aeruginosa* from feces or other clinical specimens containing mixed microbial flora by culture on selective medium, such as cetrimide agar. Since *P. aeruginosa* is frequently

present as a contaminant in the clinical specimen, hence not a single isolation but repeated isolations are essential to confirm *P. aeruginosa* as the causative agent of condition.

Nonlactose-fermenting and beta-hemolytic green pigmented colonies which are rapid oxidase positive, are identified by biochemical properties, production of pyocins, and susceptibility to phage typing, serotyping.

- **Pyocin typing**

Typing of *P. aeruginosa* based on the production of pyocin is the most commonly used method for typing *P. aeruginosa* strains. These are used in epidemiology studies. Pyocins are bacteriocins produced by *P. aeruginosa*. Three types of pyocins are produced. The capability to produce these pyocins is observed in more than 90% of strains of *P. aeruginosa*. Those strains that produce pyocins are resistant to their own pyocins, but are sensitive to those produced by other strains.

Treatment

Treatment of *Pseudomonas* infection is by specific antimicrobial therapy. However, *P. aeruginosa* shows a considerable degree of resistance to many of the commonly used antibiotics. *Pseudomonas* are susceptible to cefotaxime, ceftazidime, gentamicin, tobramycin, carbenicillin, azlocillin, and ticarcillin. Ciprofloxacin is most frequently used antibiotic, because it is active against *P. aeruginosa* in most tissues. *Pseudomonas* infections are treated best with combination of at least two antipseudomonal antibiotics. A combination of aminoglycosides or quinolone with another antipseudomonal antibiotic is effective for most of the *Pseudomonas* infections.

Treatment with a combination of two antibiotics is usually not recommended for single urinary tract infection, local skin infection.

The Genus *Haemophilus*

The members of the genus *Haemophilus* require one or both the accessory growth factors, namely, X and V present in the blood. Hence, the genus derived its name from its essential growth requirement of certain factors, such as X and V present in the blood (*haemophilus*: *haem*, blood; *philus*, loving). Koch in the year 1883 isolated *Haemophilus aegyptius*, first member of the genus from a case of conjunctivitis in Egypt. *Haemophilus* spp. are obligate bacteria present in the mucous membranes of the humans and certain species of animals.

Haemophilus influenzae and *Haemophilus ducreyi* are two major species associated with disease in humans. *H. influenzae* causes meningitis, pneumonia, epiglottitis, bronchitis, and otitis media. *H. ducreyi* is the causative agent of sexually transmitted disease, soft chancre, or chancroid. *Haemophilus aphrophilus* is less frequent but an important cause of endocarditis. Other *Haemophilus* species are rarely pathogenic. They are responsible primarily for opportunistic infections (Table 1).

Table 1 Human infections caused by *Haemophilus* species

Bacteria	Diseases
Haemophilus influenzae	Meningitis, epiglottitis, cellulitis, pneumonia, otitis media, bronchitis and conjunctivitis
Haemophilus ducreyi	Soft sore or chancroid
Haemophilus aphrophilus	Subacute endocarditis, brain abscess, pneumonia, and sinusitis
Haemophilus parainfluenzae	Endocarditis, opportunistic infections
Haemophilus aegyptius	Conjunctivitis
Haemophilus haemolyticus	Occasional opportunistic infection
Haemophilus parahaemolyticus	Occasional opportunistic infection
Haemophilus segnis	Occasional opportunistic infection

H. influenzae

H. influenzae is the species most commonly associated with human disease. It is an important cause of meningitis in children and also of respiratory tract infection in children as well as in adults.

Morphology

H. influenzae is a small, pleomorphic, Gram-negative bacillus. It measures 1 µ 0.3 µm. In fresh cultures, the bacteria are usually coccobacilli, while in older cultures, long filamentous forms are seen. The bacteria are nonmotile, nonsporing, and nonacid fast. Some strains of *H. influenzae* possess polysaccharides capsule. The capsule can be detected by India ink preparation or by capsular swelling reaction using type-specific antiserum.

Culture

H. influenzae is a fastidious bacterium. It is facultative anaerobe, grows better in anaerobic condition. The optimal temperature is 35–37°C. They do not grow below 22°C. Presence of 5–10% CO2 enhances the growth of the bacteria.

Growth factors: *H. influenzae* requires two erythrocytic growth factors, X factor and V factor. These factors are released following lysis of blood cells, thereby allowing growth of fastidious *H. inf luenzae* on the chocolate agar. *H. influenzae* cannot grow on nutrient agar, because it lacks these growth factors.

X factor: It is a heat-stable factor present in blood. It is a hemin. X factor is required for the synthesis of iron-containing bacterial enzymes, such as catalase, cytochrome oxidase, and peroxidase.

V factor: It is a heat-labile factor present in red blood cells and in other animal and plant cells. This factor is destroyed at 120°C in 30 minutes. This factor is also synthesized by *Staphylococcus aureus* and also by some fungi. Earlier this factor was thought to be a vitamin, hence was named V factor. This has now been identified as NAD or NADP coenzyme I. The V factor appears to act as hydrogen acceptor in oxidation–reduction process in a replicating bacterial cell. *H. influenzae* produces large colonies on the blood or chocolate agar by any of the following methods:

■ *Chocolate agar:* V factor is released from erythrocytes by heating blood agar at 80–90°C in order to prepare chocolate agar. The released V factor from the erythrocytes supplements the growth of colonies.

■ *By using S. aureus as source of V factor:* V factor is produced by staphylococci; hence growth on blood agar can be promoted by providing *S. aureus* as source of V factor. Blood agar with *S. aureus* streak is routinely used for culture and identification of *H. influenzae*. In this procedure, a suspected isolate of *H. influenzae* is streaked on a blood agar plate. Then *S. aureus* is streaked across the same blood agar plate and incubated at 37°C for 18–24 hours. After incubation, the colonies of *H. influenzae*

nearer to the *S. aureus* are larger than those away from it. This phenomenon is known as ***satellitism***. This demonstrates that V factor is available in increased concentration near the staphylococcal colony and in a lower concentration away from it.

Fildes' agar or Levinthal's agar are clear and transparent media used for primary isolation and culture of *H. influenzae*. These are prepared by boiling and filtering a mixture of blood and nutrient broth (Levinthal's agar) or by adding a peptic digest of blood to nutrient agar (Fildes' agar).

The capsulated strains of *H. influenzae* produce larger, 1–3-mm diameter, high convex, and mucoid colonies on the media containing blood (chocolate agar, Fildes' agar, or Levinthal's agar) or supplemented with V factors (blood agar showing satellitism). Capsulated strains of *H. influenzae* produce translucent colonies with a conspicuous iridescence on Levinthal's agar. Fildes' agar is used best for primary isolation of *H. influenzae.* In contrast, on these media, noncapsulated strains produce relatively smaller, low convex, smooth, and transparent colonies.

Chocolate agar containing penicillin and bacitracin is usually used for isolation of *H. influenzae* from clinical specimens, which consist of normal bacterial flora. Growth of *H. influenzae* on ordinary blood agar is scanty and the colonies are small because the V factor is not freely available being found inside the red blood cells. *H. influenzae* does not produce any hemolysis on blood agar.

H. influenzae colonies show a smooth to rough variation associated with loss of capsular antigen and subsequent loss of virulence. Nonencapsulated strains of *H. influenzae* can become capsulated by transfer of genetic matter that codes for the capsule.

Biochemical reactions

H. influenzae shows the following biochemical reactions:

■ It is catalase and oxidase positive.

■ It ferments glucose and galactose, but does not ferment lactose, sucrose, and mannitol.

■ It reduces nitrates to nitrites.

Susceptibility to physical and chemical agents: *H. influenzae* is a delicate bacillus. It is readily killed within 30 minutes by moist heat at 55°C. It dies within a few days at 4°C as well as in culture plates and dies in less than 2 days in dried clinical secretions. Bacteria are sensitive to commonly used disinfectants and also to desiccation. Cultures are difficult to maintain due to autolysis. Cultures may be preserved by frequent subcultures on chocolate agar. Lyophilization is a good method for long term preservation of *H. influenzae* culture.

Cell Wall Structures and Antigenic Properties

The cell wall of *H. influenzae* is typical of other Gram-negative bacilli; lipopolysaccharide with endotoxin activities is present in the cell wall. Three major surface antigens are present in *H. influenzae*. They are (*a*) capsular polysaccharide antigen, (*b*) outer membrane protein (OMP), and (*c*) lipooligosaccharide.

Pathogenesis

Haemophilus are the obligate bacteria present in the mucous membranes of humans and certain species of animals. The capsulated strains, are usually associated with invasive conditions, such as pneumonia, meningitis, septicemia, cellulitis, septic arthritis, etc. The uncapsulated strains primarily cause infections at mucosal surfaces, including otitis media, conjunctivitis, bronchitis, and sinusitis.
H. influenzae produces following virulent factors (Table 2):

Table 2 Virulence factors of *Haemophilus influenzae*

Virulence factors	Biological functions
Capsular polysaccharide	Polyribosyl ribitol phosphate (PRP) of the capsule is antiphagocytic. It resists phagocytosis of the bacteria by leukocytes
Lipopolysaccharide	Causes meningococcal inflammation
IgA1 protease	Causes break down of IgA, facilitates colonization of *Haemophilus influenzae* on mucosal surface
Pili	Helps in adherence of *Haemophilus influenzae* to epithelial cells

- **Infections caused by capsulated *H. influenzae***

Meningitis: Meningitis is the most serious manifestation of infection. It occurs primarily in children of 2 months to 2 years of age. Altered mental state and fever are most common symptoms. Headache and photophobia are usually present in older children. Mortality rate is above 90% in untreated children. meningitis is rare in adults.
Septic arthritis: Septic arthritis in children is characterized by involvement of single large joint,
Pneumonia: Pneumonia typically occurs in infants and is clinically indistinguishable from other bacterial pneumonias.
Suppurative lesions: It can cause suppurative lesions, such as pericarditis, and septic arthritis. Endophthalmitis, cervical adenitis, osteomyelitis, and endocarditis are less common invasive infections caused.

‣ Infections caused by noncapsulated strains

The nontypable influenzae are the opportunistic bacteria causing infections of the upper and lower respiratory tract. These strains cause otitis media, bronchitis, pneumonia, and conjunctivitis. Nonencapsulated *H. influenzae* along with *Streptococcus pneumoniae* is the most common cause of otitis media. *H. influenzae* is a major causative agent of conjunctivitis in older children, next only to *S. pneumoniae*. This is the most common cause of community-acquired bacterial pneumonia in adults.

Laboratory Diagnosis

‣ Specimens

CSF and blood are the specimens of choice for the diagnosis of meningitis. Blood is a frequently used specimen for culture for diagnosis of cellulitis, arthritis, or pneumonia. Throat swab, pus, and aspirates from joints, middle ear, and sinuses are the other specimens collected depending on the lesions caused by *H. influenzae.* All the specimens are collected under strict aseptic conditions in sterile containers. Since *H. influenzae* is very sensitive to low temperatures, the clinical specimens are never refrigerated but are kept in incubator at 37°C before transporting to the laboratory. The specimens are transported immediately to the laboratory for processing and better isolation of the pathogen.

‣ Microscopy

Gram-staining smear of the CSF shows small, Gram-negative coccobacilli in more than 80% of the CSF from cases of untreated meningitis. Gram-stained smear is also useful as a rapid diagnostic method for demonstration of *H. influenzae* in lower respiratory tract disease and arthritis.

‣ Culture

Detection of *H. influenzae* in blood, CSF, or other body fluids by culture confirms the diagnosis of *H. influenzae* infection. CSF culture is useful in cases of meningitis. On chocolate agar or Levinthal's agar, *H. influenzae* colonies appear as 1–2-mm, smooth, and opaque colonies after 24 hours of incubation.

H. influenzae on blood agar can also be detected by satellitism as described earlier. Blood culture is useful in the cases of pneumonia.

‣ Identification of bacteria

The colonies are identified by:

1. Typical colony morphology—1–2 mm, smooth, and opaque colonies after 24 hours' incubation on chocolate agar.

2. Demonstration of requirements for X and V factors by satellitism.

3. Specific biochemical properties.

- **Antigen detection**

Agglutination tests can be used to detect polysaccharide antigen in body fluids.

- **Biotyping**

H. influenzae has been subdivided into eight biotypes on the basis of three biochemical reactions: (*a*) indole production, (*b*) urease activity, and (*c*) ornithine decarboxylase activity.

- **Phage typing**

H. influenzae is of four phage types. These are HP1, HP3, S2, and N3.

Treatment

Antibiotics are the mainstay of treatment of *H. influenzae* infections. *H. influenzae* is susceptible to sulfonamides, chloramphenicol, ciprofloxacin, ampicillin, cefotaxime, and ceftazidime. Cefotaxime and ceftriaxone are the initial drugs of choice of treatment of meningitis. These antibiotics given parenterally to patients with uncomplicated meningitis for 7–14 days are effective. Penicillins are useful in the management of mucosal infections caused by nonencapsulated *H. influenzae*. As many as 25–50% of isolates produce beta-lactamase; therefore, they are resistant to this class of drugs. Beta-lactamase-producing oral antibiotics, with activity against *H. influenzae*, include trimethoprim– sulfamethoxazole, cefuroxime axetil, cefixime, clarithromycin, azithromycin, and ciprofloxacin. These drugs are given for 10 days in cases of otitis media and for at least 14 days for sinusitis.

Prevention and Control

- **Vaccination**

The conjugate vaccine, now routinely given to infants and children, is highly effective. The vaccine has dramatically reduced the prevalence of invasive disease.

The genus*Vibrio*

Members of the family Vibrionaceae are aerobes and facultative anaerobes, nonsporing, catalase and oxidase positive. They reduce nitrates to nitrites and can grow on ordinary media. The family Vibrionaceae includes the genera *Vibrio*, *Plesiomonas*, and *Aeromonas.*Table 1.

Table 1

Differentiating features of *Vibrio*, *Aeromonas*, and *Plesiomonas* based on utilization of amino acids

Utilization of amino acids	*Vibrio*	*Aeromonas*	*Plesiomonas*
Lysine decarboxylation	+	−	+
Ornithine decarboxylation	+	−	+
Arginine Dihydrolase	−	+	−

Vibrio species are oxidase-positive, Gram-negative curved bacilli that are motile by presence of polar flagellum. The name "vibrio" is derived from the characteristic vibratory motility (from *vibrare*, meaning to vibrate). They are asporogenous and noncapsulated. Vibrios are natural inhabitants of sea water but
are also found in fresh water worldwide. The genus *Vibrio* consists of at least 33 species of curved bacilli, of which 12 species have been implicated in human infections. *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* are the most prominent species causing human infections.

V. cholerae

V. cholerae is the most important species that causes cholera, the most feared epidemic diarrheal disease. Dehydration and death can occur rapidly within a matter of hours of infection.

Morphology

V. cholerae shows following features:

■ *V. cholerae* are Gram-negative bacilli with rounded or slightly pointed ends. They measure 1–3 µm in length and 0.5–0.8 µm in diameter.

■ The bacteria are typically comma shaped. S-shaped or spiral forms may be seen due to two or more cells lying end to end.

■ They are frequently pleomorphic in old cultures. the vibrios are typically arranged in parallel rows.

■ They are actively motile by the presence of a single polar flagellum.
■ It is nonsporing, noncapsulated.

Culture

V. cholerae is strongly aerobic and grows best under aerobic conditions, whereas growth is slow under anaerobic conditions. It grows within a temperature range of 16–40°C (optimum 37°C). It grows better in an alkaline medium, the range of pH being 7.4–9.6 (optimum 8.2). Sodium chloride (0.5–1%) is required for optimal growth, though high concentrations (5% and above) are inhibitory for growth of the bacteria. Unlike other halophilic bacteria, *V. cholerae* can grow in the absence of salt.
V. cholerae grows well on a wide variety of media including nonselective media (e.g., nutrient agar, MacConkey agar, blood agar, gelatin agar, and peptone water) and special media (transport media, enrichment media, and selective media).

Enrichment media: The enrichment media for *V. cholerae* are liquid media with a high pH. The high pH of the media suppresses growth of many commensal intestinal bacteria but favors the growth of *V. cholerae*. Alkaline peptone water (APW) is examples of transport media used for *V. cholerae*. The pH of APW is 8.6. Nearly 1 g of stool or rectal swab is inoculated into 10 mL of APW in a screw-capped tube and is transported to the laboratory. The APW is incubated at 37°C for 3–6 hours, and afterwards the subculture is made on the selective media.

Selective media: These media are useful for isolation of *V. cholerae* and other vibrios from feces. These include (*a*) thiosulfate citrate bile salt sucrose (TCBS) medium, (*b*) Monsur's gelatin taurocholate trypticase tellurite agar (GTTA) medium, and (*c*) alkaline bile salt agar (BSA). All these media charcteristically have a high pH, which suppresses growth of other intestinal bacteria but favors growth of vibrios.
V. cholerae produces large, yellow convex colonies on (TCBS) medium. This is due to fermentation of sucrose by the bacteria, leading to production of acid. Accumulation of acid reduces pH of the medium, and so the color of the bromothymol blue indicator becomes yellow, thus making *V. cholerae* colonies yellow. Nonsucrose-fermenting *V. parahaemolyticus* produces blue green colonies.

Biochemical reactions

V. cholerae shows following features:
■ It is catalase positive and oxidase positive.
■ *V. cholerae* ferments sugars with production of acid only (no gas). It ferments glucose, sucrose, maltose, mannitol, and mannose. It is a late lactose fermenter ferments lactose on incubation for several days.
■ It does not ferment arabinose, inositol, and dulcitol.
■ It forms indole and reduces nitrates to nitrites.

■ *V. cholerae* shows positive cholera red reaction by producing a reddish pink color in the peptone water due to formation of nitroso indole. The two properties of formation of indole and reduction of nitrates to nitrites form the basis of cholera red reaction. This reaction is tested by adding a few drops of concentrated sulfuric acid to a 24-hour peptone water culture at 37°C.
■ It is methyl red positive and urease test negative.
■ It liquefies gelatin and decarboxylates lysine and ornithine, but not arginine.
V. cholerae biotypes Classical and Eltor show variable Voges– Proskauer reaction, hemolysis of sheep RBCs, and hemagglutination of chick RBCs. The differences between Classical and Eltor biotypes of *V. cholerae* are summarized in Table 35-2.
Susceptibility to physical and chemical agents: *V. cholerae* is most susceptible to heat, drying, acids, and common disinfectants. *V. cholerae* are killed by heating at 56°C for 30 minutes, killed in a few minutes in gastric juice of normal acidity.
They are resistant to high alkalinity. They remain viable for 1–2 weeks in fresh sea water. They survive in pure tap water for up to 30 days. On fruits, they survive for 1–5 days at room temperature and for a week in the refrigerator.

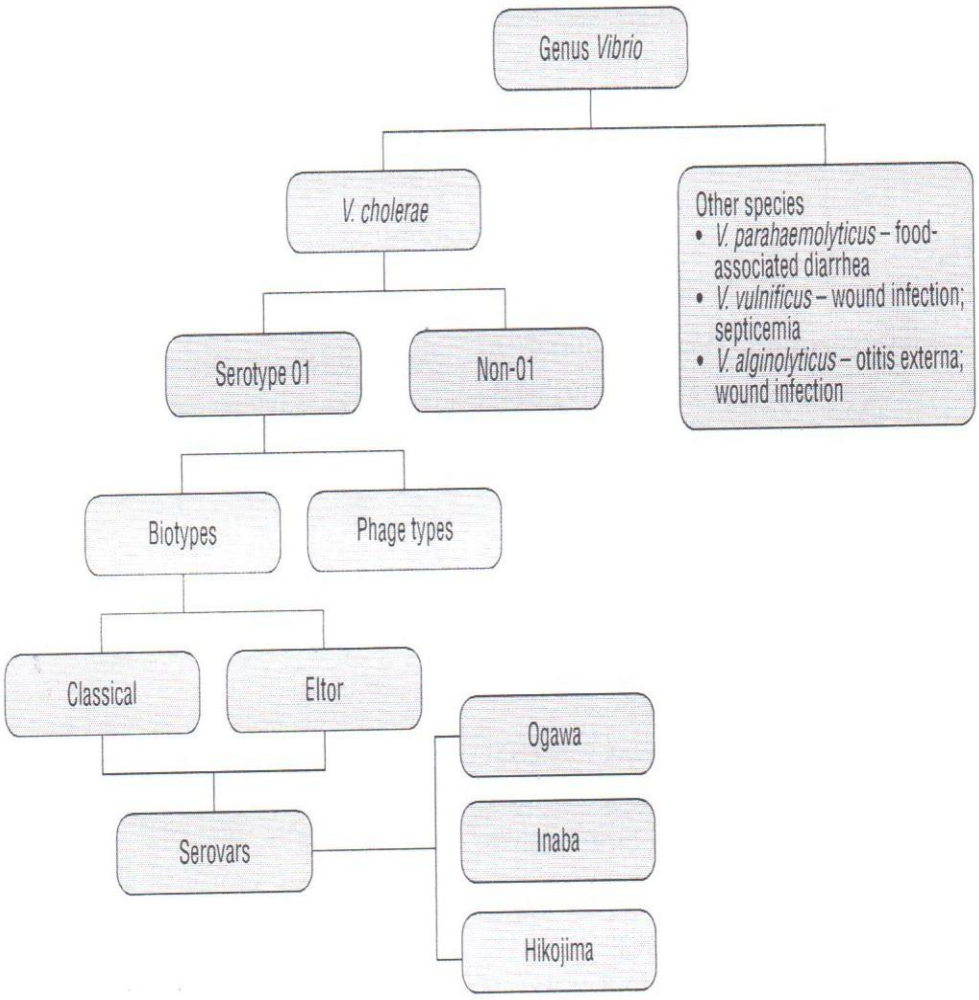
Cell Wall Components and Antigenic Structure

V. cholerae like that of Gram-negative bacilli also possesses the lipopolysaccharide (LPS). The LPS has no role in the pathogenesis of cholera but is responsible for the immunity produced by killed *V. cholerae* vaccine. *V. cholerae* possesses two antigens:
■ Somatic O antigen is present in the cell wall of the bacteria. It is a group-specific antigen.
■ Flagellar H antigen is a heat-labile antigen present in the flagella and is shared by all strains of *V. cholerae*.

Serological classification:

V. cholerae has been classified according to somatic carbohydrate O antigen into many serotypes. This serological classification was suggested by Gardner and Venkatraman (1935). More than 200 serotypes have been described, which have been classified broadly into two groups. *cholerae* O1 and non-O1 *V. cholerae*:
■ *V. cholerae* O1 agglutinate with antisera to the O1 group and are called cholera vibrios or agglutinable vibrios. *V. cholerae* O1 is subdivided into two biotypes—Eltor and Classical, on the basis of their biochemical parameters. Each biotype has
been divided further into three subtypes—Ogawa, Inaba, and Hikojima (Table 2). This classification is based on the differences in minor O antigens, such as A, B, C. Serotype Ogawa contains A and B antigens, Inaba contains A and C antigens, and Hikojima contains all three antigens A, B, and C. Hence, Hikojima strains are agglutinated by both Ogawa and Inaba antisera, whereas Ogawa and Inaba strains are agglutinated by their own specific antisera only.

■ Non-O1 *V. cholerae*, which do not agglutinate with O1 group antisera, are designated as noncholera vibrios or nonagglutinating vibrios (Fig. 1).



(Fig. 1) A schematic diagram showing classification of the genus *Vibrio*.

Table 2

Differential characteristics of *Vibrio cholerae* biotypes

Properties	*Vibrio cholerae* biotype	
	Classical	Eltor
Hemolysis of sheep RBCs	−	+
Agglutination of chick erythrocytes	−	+
Voges–Proskauer test	−	+
Polymixin B sensitivity	+	−
Susceptibility to		
Mukerjee Group IV Phage	+	−
Eltor phage 5	−	+
Vibriostatic (O/129) agent	+	−

Pathogenesis

V. cholerae is a salt water bacterium. Marine ecosystem in association with plankton is the primary habitat of the bacteria. The bacteria can multiply freely in the water. The number of bacteria in contaminated water increases during the warm and hot months of the year. *V. cholerae* is never found in normal humans.

Cholera is a toxin-mediated disease. Cholera toxin (CTx) produced by *V. cholerae* is the key virulence factor of the bacteria. Virulence factors of *V. cholerae* are showed in (Table 3).

Table 3

Virulence factors of *Vibrio cholerae*

Virulence factors	Biological functions
Cholera toxin	The toxin inhibits absorption of sodium and chloride in the intestine; causes hypersecretion of large volumes of water and electrolytes. Activation of adenylate cyclase and overproduction of cAMP.
Toxin coregulated pilus	Helps in adherence of *Vibrio cholerae* to mucosal cells of the intestine
Accessory colonization	Helps in adhesion of bacteria to the intestinal mucosa
Hemagglutination-protease (mucinase)	Induces intestinal inflammation and also helps in releasing free vibrios from the bound mucosa to the intestinal lumen
Neuraminidase	Increases toxin receptors for *Vibrio cholerae*
Siderophores	Causes sequestration of iron

V. cholerae usually enters the body orally through contaminated water and food (Fig. 35-3). Gastric acidity is the first line of defense against infection caused by *V. cholerae,* because vibrios are highly susceptible to the acidity of stomach. The conditions that reduce acidity of the stomach to pH above 5 make the host more susceptible to infection by cholera vibrios. On reaching the small intestine by using its own mechanisms, such as motility , *V. cholerae* reaches the mucous layer of the small intestine. Hemagglutinin and protease break mucin and fibronectin of the mucosa.

Vibrios, once adhered to the intestinal wall, produce cholera toxin. The toxin activates cAMP, which inhibits the absorption of sodium transport and activates the excretory chloride transport in the intestinal epithelial cells. This leads to an accumulation of sodium chloride in the lumen of intestine. The high osmolarity of the intestinal fluid is balanced by large secretion of water, which overcomes the absorptive capacity of the lumen, eventually causing diarrhea.

The incubation period is short and varies from 2 to 3 days after ingestion of the bacteria. The condition shows an abrupt onset of watery diarrhea and vomiting. Profuse watery diarrhea is the most important manifestation of cholera. The volume of diarrheic stool excreted in cholera is much more than that of diarrhea caused by any other infectious pathogen. The cholera stool is profusely watery that is colorless and odorless, free of proteins and speckled with mucous and often is described as rice water stool (in color and consistency, stool resembles water that has been washed off from cooked rice); contains few leukocytes, but no erythrocytes because *V. cholerae* does not stimulate any inflammatory response in the intestinal mucosa; and is a bicarbonate-rich electrolyte fluid, which contains little protein.

Cholera if left untreated can lead to severe loss of fluid and electrolytes due to diarrhea and vomiting. This could lead to isotonic dehydration, metabolic acidosis, Dehydration in cholera characteristically develops very fast, within hours after the onset of symptoms. This rapid development of dehydration is not seen with diarrheal diseases caused by any other enteropathogen. In patients with severe disease, diarrhea leads to shock, and patients may die of cardiac arrhythmia and renal failure.

Laboratory Diagnosis

Specimens

Fresh stool specimen collected before administration of antibiotics is the specimen of choice. Vomitus is not a useful specimen. The collected stool or rectal swab specimen should be sent to laboratory for immediate culture .If delay, the specimens may be preserved at 4°C in a refrigerator or in transport media used for *V. cholerae*.

Microscopy

Dark-field microscopy is a useful method for demonstrating characteristic motility of the bacilli and its inhibition by antisera. This is a rapid method of examination of stool collected from cases or after enrichment for 6 hours. Direct immunofluorescence is another rapid method used for demonstration of vibrios in the stool. However, microscopic examination of a Gram stained stool smear is not recommended for diagnosis of cholera.

Culture

The specimen collected in holding media is inoculated first in the enrichment media and incubated for 6–8 hours before being inoculated on selective (TCBS or GTTA) and nonselective media (BSA, MacConkey agar, and blood agar). The specimen may also be plated directly into these media before enrichment. The specimens collected in transport media are incubated for 6–8 hours including the transit time before inoculation into the suitable media. The inoculated plates are incubated at 37°C overnight. *V. cholerae* produces characteristic yellow colonies on TCBS and nonlactose-fermenting colonies on MacConkey agar.

Colonies on media are identified by performing a series of biochemical tests. These include oxidase test ,utilization of amino acids, such as lysine, ornithine, and arginine fermentation of sugars; sheep cell hemolysis; chick cell agglutination; VP test; polymyxin B sensitivity; etc.

Serodiagnosis

Suspected *V. cholerae* are tested by slide agglutination, using specific *V. cholerae* O1 antisera. In this test, the colonies are picked up with a straight wire and mixed with a drop of antisera on the slide. Agglutination of the bacteria shows that the test is positive for *V. cholerae* O1. If positive, agglutination of bacteria is repeated using specific Inaba and Ogawa sera for serotyping. Hikojima strains agglutinate well with both Inaba and Ogawa sera. If agglutination is negative, the test is repeated with at least five more colonies, as both O1 and non-O1 vibrios may coexist in the same specimen.

Treatment

Treatment of cholera includes (*a*) replacement of fluid and electrolytes and (*b*) antibiotic therapy.

V. cholerae are uniformly sensitive to tetracycline, ciprofloxacin, and erythromycin. Tetracycline or doxycycline is the drug of choice for adults and trimethoprim–sulfamethoxazole for children. Pyrazolidone is the usually recommended treatment for pregnant females suffering from cholera.

Noncholera vibrios

V. cholerae serotypes from O2 to O139 are known as noncholera vibrios, nonagglutinable vibrios, or *V. cholerae* non-O1 vibrios. They are similar to *V. cholerae* O1 biochemically and genetically. *V. cholerae* O139 is only example of noncholera vibrios that causes cholera. Noncholera vibrios other than *V. cholerae* O139 are widely found in the aquatic environments. In humans, they may cause mild to severe diarrheal disease resembling cholera. Occasionally, they may also cause wound infections, septicemia, and other extraintestinal infections.

Vibrios that require a higher concentration of sodium chloride are known as halophilic vibrios. They are natural inhabitants of sea water and marine life. *V. parahaemolyticus*, *Vibrio alginolyticus*, and *V. vulnificus* are three important halophilic vibrios species known to cause infection in humans.

Vibrio parahaemolyticus

V. parahaemolyticus is now recognized as an important cause of seafood-associated gastroenteritis throughout the world. *V. parahaemolyticus* is a curved Gram-negative bacillus that resembles *V. cholerae*. However, it differs from *V. cholerae* by having a capsule showing bipolar staining and pleomorphism. It shows pleomorphism especially in cold cultures or when grown on 3% salt agar. Like other vibrios, it has polar flagella when grown in liquid culture, but shows peritrichous flagella when grown on solid media. It has simple nutritional requirement, but requires salt for growth. The organism fails to grow in the medium in the absence of sodium chloride. It can grow well in salt concentration up to 8% and at an optimum salt concentration of 2–4%.

On MacConkey agar, it produces colorless nonlactose- fermenting colonies; on blood agar, it forms beta-hemolytic colonies. The colonies on TCBS agar are nonsucrose-fermenting green colonies.

V. parahaemolyticus is oxidase, catalase, indole, and citrate positive. It reduces nitrates to nitrites. It is a fermenter ferments glucose, mannose, maltose, mannitol, and arabinose with production of acid only. It does not ferment sucrose, lactose, and salicin. It decarboxylates lysine and ornithine but not arginine. It is VP positive.

V. parahaemolyticus is a heat-labile bacterium, readily destroyed at 60°C in 15 minutes. It is susceptible to drying, distilled water, and vinegar in which it dies within a few minutes. The organism, however, can remain viable on freezing and refrigeration.

V. parahaemolyticus contains three antigens: somatic O antigens, flagellar H antigens, and capsular K antigen. Serotyping is based on O and K antigen.

■ Thermostable direct hemolysin is the key virulence factor of *V. parahaemolyticus*. This virulence factor is found only in strains that are pathogenic to human beings.

■ Strains isolated from humans possess this toxin, hence are pathogenic, while strains isolated from environment source, such as water, fish do not possess this thermostable toxin, hence are nonpathogenic.

V. parahaemolyticus is widely distributed in estuary and marine environments. Seafood, such as fish, crabs, or oysters, is the main source and reservoir of infection. The infection is acquired on consumption of contaminated seafood.

V. parahaemolyticus in humans causes gastroenteritis. The severity of the condition can vary from mild self-limited diarrhea to an acute illness. After an incubation period of 5–72 hours, the patients with gastroenteritis manifest as eosinophilic watery diarrhea. The condition is associated with nausea, vomiting, abdominal pain, and low-grade fever, which may be present for 3 days. Stool usually does not contain any blood or mucus, but contains cellular exudates. The patient usually recovers within 3 days.

V. parahaemolyticus also causes extraintestinal infections, such as wound infections and ear and eye infections in individuals exposed to contaminated sea water.

Campylobacter

The family Campylobacteriaceae consists of two genera of medical importance: *Campylobacter* and *Helicobacter*. The genera belonging to this family are Gram-negative, spiral bacilli. They are microaerophilic and grow only in the presence of a reduced oxygen concentration. They lack the capacity to oxidize or ferment sugars.

Campylobacter infections are one of the most common bacterial infections in humans. They cause both diarrheal and other systemic diseases. The generic name *Campylobacter* is derived from the Greek word *Kampylos* meaning curved rod. The genus *Campylobacter* resembles the genus *Vibrio* in being motile by means of polar flagella and in being curved Gram-negative bacilli and oxidase positive. They, however, differ from vibrios (*a*) in being microaerophilic, (*b*) not fermenting carbohydrates.

The genus *Campylobacter* consists of 18 species and subspecies, of which 11 species are known to cause infections in humans. These species cause both intestinal and extraintestinal diseases (Table 1). *Campylobacter jejuni* and *Campylobacter fetus* are the two most important species that cause most of the *Campylobacter* infections in humans.

Table 1

Human infections caused by *Campylobacter* species

Bacteria	Diseases
Campylobacter jejuni	Gastroenteritis, septicemia, meningitis, proctitis, and Guillain–Barre syndrome
Campylobacter jejuni subsp. *doyle*	Gastroenteritis, gastritis, and septicemia
Campylobacter fetus	Gastroenteritis, septicemia, meningitis, and proctitis
Campylobacter fetus subsp. *venerealis*	Septicemia
Campylobacter coli	Gastroenteritis, septicemia, meningitis, and spontaneous abortion
Campylobacter upsaliensis	Gastroenteritis, septicemia, and abscesses
Campylobacter hyointestinalis	Gastroenteritis
Campylobacter showae	Periodontal disease
Campylobacter curvus	Periodontal disease
Campylobacter rectus	Periodontal disease
Campylobacter concisus	Periodontal disease and gastroenteritis
Campylobacter lari	Gastroenteritis and septicemia

Morphology

Campylobacter spp. shows following morphological features:

■ *Campylobacter* are small, comma-shaped, Gram-negative rods. They vary in width from 0.2 to 0.5 µm.

■ They show a rapid motility by means of a single polar flagellum.

■ They are nonsporing bacteria.

Culture

Campylobacter spp. grow more slowly than other enteric bacteria. *Campylobacter* are microaerophilic, requiring an atmosphere with decreased oxygen concentration (5%) and increased carbon dioxide concentration (5–10%) for their growth. Most species including *C. jejuni, Campylobacter coli*, *Campylobacter lari*, and *Campylobacter hyointestinalis* grow well at 42°C, hence are called thermophilic bacilli.

Selective media for *Campylobacter* species are blood-based and antibiotics-containing media, such as Skirrow's, Butzler, and Campy-BAP selective media. *Campylobacter* on these media produce well-formed colonies after 48 hours of incubation.

C. jejuni produces moist, gray or colorless, flat, and nonhemolytic colonies on this medium. Other *Campylobacter* species produce circular and convex colonies.

Biochemical reactions

Campylobacter spp. show following reactions:

■ *Campylobacter* are relatively biochemically inert.

■ They do not produce indole.

■ They are oxidase positive, catalase positive (*C. jejuni* subsp. *doyle* is an exception), and nitrate positive.

■ *C. jejuni* is the only species that hydrolyzes sodium hippurate.

Susceptibility to physical and chemical agents: *Campylobacter* spp. survive at 4°C in Cary–Blair transport medium for many weeks. *Campylobacter* spp. are sensitive to hydrochloric acid in the stomach, and so conditions that decrease or neutralize gastric acid secretion reduce the amount of inoculum needed to cause disease.

Pathogenesis

Campylobacter species are invasive bacteria. In humans, they cause primarily two types of infections: intestinal and extraintestinal. The prototype species for intestinal infections is *C. jejuni* and for extraintestinal infections is *C. fetus*.

C. jejuni possesses many virulence factors, such as enterotoxins, lipopolysaccharide (LPS), adhesins, and cytotoxic enzymes (Table 2). However, specific role of these virulence factors in the pathogenesis of disease is not well-known.

Table 2 **Virulence factors of *Campylobacter* species**

Virulence factors	Biological functions
Enterotoxins	Facilitate adherence to the jejunum, ileum, and colon
Lipopolysaccharide	Adhesion
PEB1	Superficial antigen that has been found to be a major adhesion protein
Adhesion proteins	Adhesion
Cytotoxic enzymes	Cytotoxicity action
S protein	Found exclusively in *Campylobacter fetus* and is the major virulence factor. Inhibits C3b binding responsible for both the serum and phagocytic resistance of the bacteria

Human infection follows ingestion of 1000–10,000 bacteria, but the illness is infrequent with a dose of less than 10,000 bacteria. Following three mechanisms have been postulated in the pathogenesis of intestinal disease caused by *C. jejuni*:

1. Adherence and production of heat-labile enterotoxins:

C. jejuni adheres to the jejunum, ileum, and colon. Adherence to epithelial cells or mucus at these sites is possibly facilitated by flagella. LPS or other outer membrane components are also believed to contribute to adhesion. *C. jejuni* enterotoxin is a heat-labile choleralike enterotoxin, which is responsible for diarrhea observed during infections.

2. Invasion and proliferation of bacteria within the intestinal epithelium:

C. jejuni causes characteristic histologic damage in the mucosal surface of the jejunum, ileum, and colon. The organism produces diffuse, bloody, edematous, and exudative enteritis.

3. Invasion of intestinal mucosa and proliferation:

C. jejuni invades intestinal mucosa and multiplies in the lamina and mesenteric lymph nodes. This results in extraintestinal infections, such as cholecystitis, mesentery adenitis, urinary tract infection, and meningitis.

‣ Pathogenesis of disease caused by *C. fetus*

C. fetus is more frequently associated with systemic infections, such as bacteremia. A single protein, namely, S protein, found in *C. fetus* is the major virulence factor. Hence, it makes the bacteria resistant to the bactericidal effects of serum.

Laboratory Diagnosis

The diagnosis of *Campylobacter* infection is confirmed by demonstrating the bacteria by direct examination of feces or isolation of the bacteria by culture.

‣ Specimens

Diarrheic fresh stool is the specimen of choice for enteric infections. Rectal swab may also be used. Other specimens include blood, body fluids, and tissues for diagnosis of

extraintestinal infections. In case of delay, feces or rectal swab are transported in transport medium.

- **Microscopy**

Presumptive diagnosis is made by examination of wet mount of stool by dark-field microscopy or phase contrast microscopy. The bacteria are identified by their characteristic darting motility. Gram staining of the stool shows typical Gram-negative, curved rods with a sensitivity of 50–75%. Fecal leukocytes and erythrocytes can also be detected in Gram-stained smear of the stool in approximately 75% patients with *Campylobacter* enteritis.

- **Culture**

Definitive diagnosis of *Campylobacter* enteric infection is best done on isolation of the organism by stool culture. Culture of *C. jejuni* from stool requires special selective media— Butzler medium, Skirrow's medium, Preston's *Campylobacter*
selective medium, and Blaser's medium (Campy-BAP). The selective media contain antibiotics that inhibit growth of the other fecal bacteria. Fresh stool specimens collected within 24 hours are inoculated directly on their media. But old stool specimens are first enriched in an enrichment medium, such as Preston *campylobacter* enrichment broth for at least 24 hours at 4°C (cold enrichment) before inoculating on the selective media. Inoculated media are incubated in 5% O2 and 10% CO2 at 42°C for 48 hours. If *C. fetus* or other unusual *Campylobacter* species are suspected, stool specimens are inoculated on media without antibiotics and are incubated at 37°C. Other extraintestinal specimens, such as blood, body fluids, and tissue, can be inoculated on routine media for isolation of *Campylobacter* species.
Preliminary identification of colonies is made by typical Gram staining features, darting motility, and oxidase test. Definitive identification is made by biochemical tests.

- **Histology**

Examination by sigmoidoscopy shows widening or proctocolitis in up to 80% of patients with *Campylobacter* enteritis. The histopathological changes include fecal mucosal edema and hyperemia with crypt abscess formation.

- **Serodiagnosis**

ELISA is available for demonstration of specific antibodies in the serum, and a high titer of antibodies is usually seen after the symptoms are resolved. Serology may not be useful for routine diagnosis, but is useful for epidemiological studies.

Treatment

Most *C. jejuni* infections are mild and self-limited. Therefore, they do not require antibiotic therapy. Supportive treatment is adequate. Treatment with antibiotics is recommended only for persons:

■ with fever, bloody diarrhea, or

■ with symptoms persisting for more than 7 days, and
■ for immunocompromised host. Erythromycin is the antibiotic of choice. Ciprofloxacin and tetracycline are the alternative antibiotics, but are not recommended for use in young children. Recently, reports of erythromycin and ciprofloxacin resistant strains have increasingly been documented from many parts of the world.

Helicobacter

Helicobacter are *Campylobacter*-like bacteria with a spiral or helical morphology. They are motile by means of sheathed flagella. *Helicobacter* spp. inhabit stomach of humans and many other mammals, such as monkeys, dogs, cats, ferrets, mice, etc. The genus *Helicobacter* consists of many species, which are associated with human diseases (Table 3). Of these, *Helicobacter pylori*, *Helicobacter cinaedi*, and *Helicobacter fennelliae* are three important species that cause human infections. The association of *H. pylori* with gastritis, peptic ulcer, is recognized worldwide.

H. pylori is a curved, spiral, or S-shaped Gram-negative bacillus measuring 3 µm in length and 0.5µ0.9 µm in breadth. The organism is highly motile and shows cork screw motility due to presence of a unipolar flagella. It is nonsporing.

Table 3 **Human infections caused by *Helicobacter* species**

Bacteria	Diseases
Helicobacter pylori	Peptic ulcer, gastritis, and gastric adenocarcinoma
Helicobacter cinaedi	Gastroenteritis, septicemia, and proctocolitis
Helicobacter fennelliae	Gastroenteritis, septicemia, and proctocolitis
Helicobacter canis	Gastroenteritis
Helicobacter rappini	Gastroenteritis
Helicobacter canadensis	Gastroenteritis
Helicobacter pullorum	Gastroenteritis
Helicobacter heillmannii	Gastritis

The genus *Brucella*

Brucellosis is primarily a zoonotic infection caused by *Brucella* organisms infecting the domestic animals. The disease continues to be a major public health problem worldwide. The condition is known by various names, such as Malta fever, Mediterranean fever, undulant fever, etc.

The genus *Brucella* are strict parasites of domestic and wild animals, but they can infect humans, which are accidental hosts. The genus *Brucella* consists of seven species, four of which are human pathogens. These are *Brucella abortus*, *Brucella melitensis*, *Brucella suis*, and *Brucella canis*.

Morphology

Brucella shows the following features:

■ Brucellae are Gram negative, but counter stain poorly and require relatively more time for staining.

■ They are small coccobacilli, measuring 0.5–0.7 µ 0.6–1.5 µm in size.

■ These are arranged singly, sometimes in pairs, or in short chains. They do not produce spores, flagella, or capsule.

Culture

Brucellae are strict aerobes. Most biovars of *B. abortus* require 5–10% CO2 for their growth. They grow at a temperature range of 22–40°C (optimum temperature 37°C) and pH range of 6.6–7.4. Brucellae can grow on simple media, but their growth is slow and weak. Growth is improved by addition of serum, blood, liver extract, and glucose. *Brucella* organisms grow best on trypticase soy based or other enriched media. Blood agar and trypticase soy agar are the media of choice. Tryptose agar, trypticase soy agar, serum potato infusion agar, and serum dextrose agar are the other media also used for culture of Brucellae. Addition of bacitracin, polymyxin, and cycloheximide to these media makes them selective. On these solid media, *Brucella* spp. produce small, moist, translucent, and glistening colonies after 3 or more days of incubation. In liquid media, growth is uniform.

Biochemical reactions

Brucella species show following biochemical properties:

■ Brucellae are catalase and oxidase positive (except for *B. ovis* and *B. neotomae*, which are oxidase negative).

■ They reduce nitrate to nitrite and have variable urease activity.

■ Some *Brucella* species produce H2S, while some species do not produce H2S.

■ Citrate is not utilized.

■ They do not produce indole and are MR and VP test negative.
■ They do not ordinarily ferment any sugars.

Brucella species and biovars are differentiated by characteristics showed in (Table 1)

Table 1 **Differential features of *Brucella* species**

Species	Animal reservoir	Biotypes	CO_2 requirement	H_2S requirement	Urease production	Growth in presence of 20 μg/mL		
						Basic fuchsin 1:50,000	Thionine	
							1:25,000	1:50,000
Brucella melitensis	Goats, Sheep	1	–	–	Variable	+	–	+
	Goats, Sheep	2	–	–	Variable	+	–	+
	Goats, Sheep	3	–	–	Variable	+	–	+
Brucella abortus	Cattle	1	+(–)	+	1-2 hours	+	–	–
	Cattle	2	+(–)	+	1-2 hours	–	–	–
	Cattle	3	+(–)	+	1-2 hours	+	+	+
	Cattle	4	+(–)	+	1-2 hours	+	–	–
	Cattle	5	–	–	1-2 hours	+	–	+
	Cattle	6	–	+(–)	1-2 hours	+	–	+
	Cattle	9	–	+	1-2 hours	+	–	+
Brucella suis	Swine	1	–	+	0-30 minutes	–	+	+
	Swine	2	–	–	0-30 minutes	–	–	+
	Swine	3	–	–	0-30 minutes	+	+	+
	Swine	4	–	–	0-30 minutes	+	+	+
	Swine	5	–	–	0-30 minutes	–	+	+
Brucella canis	Dogs		–	–	0-30 minutes	–	+	+

Susceptibility to physical and chemical agents: The brucellae are rapidly killed at 60℃ in 10 minutes; hence they are killed by pasteurization in the milk. They are also killed by disinfectants, such as 1% phenol in 15 minutes. They are sensitive to direct sunlight and acid. The bacteria survive for 10 days in refrigerated milk, for 1 month in

ice cream, and for 4 months in butter. *B. melitensis* remains viable in urine for 6 days, in dust for 6 weeks, and in water or soil for 10 weeks.

Cell Wall Components and Antigenic Structure

Brucella like any other Gram-negative bacterium contains LPS in the outer cell membrane of the cell walls. But, the LPS of *Brucella* is different both structurally and functionally from that of other Gram-negative bacteria.

Brucella species show antigenic cross-reaction with *Salmonella* serotypes (O: 30 antigen), *Escherichia coli* (O: 116, O: 157), *Vibrio cholerae*, *Pseudomonas multocida*, *Yersinia enterocolitica*, and *Francisella tularensis*.

Pathogenesis

Brucella species have predilection for intracellular growth, hence may be demonstrated inside phagocytes. Intracellular location of the *Brucella* explains the relative resistance of the bacterium to chemotherapy.

- **Virulence factors**

LPS is the principle virulence factor of *Brucella* spp. Intracellular location of the bacteria makes them resistant to killing in serum and also by phagocytes (Table 2).

Table 2 **Virulence factors of *Brucella* species**

Virulence factors	Biological functions
LPS	Facilitates intracellular survival of the bacteria by inhibiting polymorphonuclear degranulation
Intracellular location of the bacterium	Makes it resistant to killing in serum and also by phagocytes

- **Pathogenesis of brucellosis**

Brucellosis is a systemic disease in humans that can involve almost any organ system. They enter through (*a*) abrasion or cut in the skin, (*b*) conjunctiva, (*c*) respiratory tract, and (*d*) gastrointestinal tract. Shortly after gaining entry to the body, brucellae are rapidly ingested by polymorphonuclear leukocytes, which are attracted to the site of inflammation. But inside the leukocytes, the brucellae are not killed due to the presence of enzyme superoxide dismutase, O polysaccharide of LPS, and nucleotide-like substances produced by the bacteria. Brucellae that are not killed by leukocytes spread from the site of infection to the local lymph nodes which drain the site of infection. Inside the lymph glands, the bacteria multiply and

are released to the blood stream following rupture of the cells and are phagocytosed by the macrophages.

Bacteria present in the macrophages are then carried to the organs of reticuloendothelial system, such as liver, spleen, bone marrow, lymph nodes, and kidney. In these organs, brucellae multiply in phagosomes of macrophages due to inhibits the phagolysosome fusion.

Apart from the organs of reticuloendothelial system, any other organ system (central nervous system, heart, joints, genitourinary system, pulmonary system, and skin) can be involved in brucellosis.

Immunity in brucellosis, like other obligate intracellular pathogens, is primarily cell-mediated immunity. The humoral immunity is characterized by production of antibodies against LPS of the bacteria. However, these serum antibodies against LPS do not provide any protective immunity against bacteria.

The clinical manifestations of brucellosis depend on the infecting *Brucella* species. Of the four *Brucella* species causing human infections, *B. melitensis* is the most virulent species.

The incubation period may range from 3 days to several weeks. Human infections may be of three types, as follows: (*a*) acute brucellosis, (*b*) chronic brucellosis, and (*c*) localized infection.

Acute brucellosis: Acute brucellosis is seen in approximately 50% of patients infected with *Brucella*. Patients usually complain of nonspecific symptoms, such as anorexia, fatigue, weakness, malaise, or joint pain. Fever is an important symptom and is seen in almost all patients. The fever is interm ttent and undulant
(hence known as undulant fever).

Chronic brucellosis: This condition develops in incompletely treated patients. Symptoms often last for 3–6 months and occasionally for a year or more.

Localized infection: *B. suis* is more likely to cause localized and infection.

Complications include infections of the heart, central nervous system (CNS), and the skin. Brucella endocarditis is the most dangerous complication and is responsible for 80% of deaths in brucellosis. Chronic meningoencephalitis is the usual manifestation of CNS infection.

Brucella species cause infection in a wide range of hosts, but these species show a high degree of host specificity. *B. melitensis* infects goats and sheep; *B. abortus*, cattle; *B. suis*, swines; and *B. canis*, dogs and foxes. The infected animals, such as cattle, goats, sheep, buffalos, and swine are the important reservoirs of infections.

Human brucellosis is primarily a zoonotic bacterial infection. The infection is acquired always from animals directly or indirectly.

Brucella organism is transmitted to humans by the following ways :

1. Ingestion: Brucellosis results primarily by drinking contaminated unpasteurized milk or milk products. The infection can also be transmitted by drinking water or eating raw vegetables contaminated with feces or urine of infected animals and also by eating meat of infected animals.

2. Direct contact: The infection is acquired by direct contact with the infected materials of septic abortion of the animals or at the time of slaughter of animals. *Brucella* species present in the infected materials (placenta, fetuses, vaginal discharge, urine, or infected carcasses) enter the human host through the mucosa, conjunctiva, or skin.

3. Inhalation: The infection is transmitted by inhalation of dust from wool or other dried material of infected animals. Infection by inhalation is important among veterinarians and laboratory workers.

4. Accidental inoculation: Accidental inoculation is a serious risk in laboratory workers who handle culture of the organism.

Laboratory Diagnosis

Clinical diagnosis of brucellosis is very difficult due to protean manifestation of the disease. Hence, laboratory diagnosis plays an important role in confirming the diagnosis of brucellosis.

- **Specimens**

Blood is the specimen of choice and is collected for culture and for serological test. Bone marrow and sometimes synovial fluid and pleural fluid are also collected for culture. Specimens such as liver and lymph nodes can also be cultured for isolation of *Brucella* organisms. Rarely, the bacteria can be isolated from cerebrospinal fluid (CSF), urine, sputum, breast milk, vaginal discharge.

- **Microscopy**

Gram staining is not useful for demonstration of *Brucella* organisms in clinical specimens due to their small size and intracellular location.

- **Culture**

Isolation of *Brucella* from blood and other clinical specimens is the definitive diagnostic procedure in brucellosis. Approximately, 5–10 mL of blood is collected in the 200 mL serum dextrose broth or trypticase soy broth and incubated at 37°C under 5–10% of CO2. After fourth day of incubation, subcultures are made on solid media, every 3–5 days for 8 weeks before declaring the culture as negative.

Bone marrow cultures are more sensitive than blood culture.

- **Serodiagnosis**

Serological tests are useful for diagnosis of subclinical brucellosis and for cases of acute and chronic brucellosis by demonstration of specific antibodies in patient's

serum. Specific brucella antibodies, both IgG and IgM antibodies, appear in the serum 7–10 days after infection. IgM antibodies persist for up to 3 months after which these antibodies decline.

Then IgG and IgA antibodies appear after 3 weeks of infection and persist for longer time. Hence, in acute stage or subclinical brucellosis, both IgG and IgM can be demonstrated; in chronic brucellosis, only IgG can be demonstrated, as IgM are absent.

As IgG antibodies persist for many months or years, demonstration of significant rise in the antibody titer is the definitive serological evidence of brucellosis. A fourfold increase in the titer or a single high antibody titer of 1:160 is the presumptive evidence of *Brucella* infection.

B. abortus is used as an antigen in brucella agglutination tests because the antibodies against *B. melitensis* or *B. suis* can crossreact with *B. abortus* antigen. *B. abortus* antigen not only detects specific antibodies against its own antigens but also detects antibodies produced against *B. melitensis* and *B. suis*. However, antibodies against *B. abortus* do not react with *B. canis*. Hence, specific *B. canis* antigen is used to detect antibodies in the serum of patients infected with this organism.

A number of serological tests have been developed and tested in serodiagnosis of brucellosis. Common methods are (*a*) standard tube agglutination tests, (*b*) indirect immunofluorescent tests, and (*c*) enzyme-linked immunosorbent assay (ELISA).

Standard tube agglutination test: This test remains the standard method and is the most commonly used serological test for diagnosis of brucellosis. It detects the presence of antibodies against LPS component of *Brucella*. The test uses killed strains of *B. abortus* as antigen and is useful for diagnosis of brucellosis caused by *B. abortus*, *B. melitensis*, and *B. suis*. The tube agglutination test is considered positive when antibody titers are greater than or equal to 1 in 160 .

***Modified tube agglutination test*:** In this test, 2-mercaptoethanol is added to the patient's sera before testing it. Addition of mercaptoethanol causes disruption of disulfide bond of IgM; hence only IgG is detected. This modified tube agglutination test is useful for specific detection of IgG antibodies, and titers higher than 1:80 are suggestive of active infection. A high IgG antibody titer or a titer that is higher after treatment suggests relapse or persistent infection. This test is useful for diagnosis of brucellosis during convalescence.

Nevertheless, these serological tests may show <u>erroneous results due to</u> the following factors:

1. Blocking antibodies: Blocking or nonagglutinating antibodies in the serum may contribute to a false positive result. This can be avoided by heating the serum at 55℃ for 30 minutes or by using 4% saline or diluent in the test.

2. Prozone phenomenon: It is a frequent problem in diagnosis of brucellosis. This occurs due to high level of brucella antibodies in the serum, Prozone phenomenon gives rise to a false negative test. This can be avoided by routine dilution of serum to at least 1:320 because inhibition of agglutination may occur at low dilution.

3. Cross-reactivity with *V. cholerae*, *Y. enterocolitica* serotype O9, *F. tularensis*, and *Salmonella* species can cause false positive results due to the presence of cross-reacting LPS in these organisms. Cholera-induced antibodies may be removed by 2-mercaptoethanol absorption.

- **Laboratory diagnosis in animals**

The diagnosis of brucellosis in animals is based essentially on the same method as that employed for the diagnosis of human infections. In addition, culture of milk and urine from infected animals may give positive results. Rapid latex agglutination test and Rose Bengal card test are the rapid diagnostic methods, and are also used for diagnosis of brucellosis in cattle population.

Milk ring test: This is a frequently used serological test for demonstration of antibodies in the milk of an animal. This is a screening test used to detect the presence of *Brucella* infection in infected cattle. In this test, a concentrated suspension of killed *B. abortus* or *B. melitensis* stained with hematoxylin is used as antigen. In a positive test, if antibodies are present in the milk, the bacilli are agglutinated and raised with the cream to form a blue ring at the top, leaving the milk unstained. In a negative test, the milk remains uniformly blue without formation of any colored ring.

Treatment

Brucellae are sensitive to a number of oral antibiotics and aminoglycosides. The combination of tetracycline and doxycycline is effective against most species of *Brucella*. Relapse is common on therapy with a single drug. So, the combination of antibiotics is recommended whenever possible. Combination of doxycycline with rifampin is effective. Most of the patients respond to a 6-week course of therapy with a combination of rifampin and doxycycline given orally. Only less than 10% of patients show relapse. A dosage of doxycycline for 6 weeks with combination of streptomycin for the first 3 weeks is also effective. Relapse in brucellosis is due to inadequate therapy and not due to development of resistance to antibiotics.

Prevention and Control

Pasteurization or boiling of milk is most important to prevent transmission of brucellosis in humans, as most of the human infections are acquired by ingestion of contaminated milk and milk products. Also, use of protective clothing and gloves by persons handling or coming into close contact with animals prevents transmission of infection.

Genus *Mycoplasma*

Mycoplasma belongs to class Mollicutes (Mollis, soft; cutis, skin),order Mycoplasmatales. This order contains four families Mycoplasmataceae, Acholeplasmataceae, Spiroplasmataceae,and Anaplasmataceae; of which, most *Mycoplasma* causing human infections belong to the family Mycoplasmataceae.
Family Acholeplasmataceae includes mostly saprophytic mycoplasmas; these mycoplasmas do not require sterols as growth factor. Family Spiroplasmataceae includes mostly mycoplasmas, which are parasites of arthropods and plants; they require sterols as their growth factor. Family Anaplasmataceae contains mycoplasmas that are strict anaerobes and are found in the intestinal tract of cattle and sheep.

TABLE 1 Human infections caused by *Mycoplasma* and *Ureaplasma* species

Bacteria	Diseases
Mycoplasma pneumoniae	Upper respiratory tract diseases, lower respiratory tract infections, and primary atypical pneumonia
Mycoplasma hominis	Pelvic inflammatory disease and postpartum fever
Mycoplasma fermentans	Opportunistic infections in patients with HIV
Mycoplasma pirum	Septicemia in patients with HIV
Mycoplasma salivarium	Infection unknown
Mycoplasma orale	Infection unknown
Mycoplasma genitalium	Infection unknown
Ureaplasma urealyticum	Chorioamnionitis, prematurity, vaginitis, cervicitis, acute salpingitis, and pelvic inflammatory disease

Mycoplasma pneumoniae

M. pneumoniae is the most important species causing upper respiratory tract disease. It is also known for causing walking pneumonia or primary atypical pneumonia.

Morphology

Mycoplasmas show the following morphological features:
■ yy ooplasmss eee vyyysmll l bttt rr ia messuiing 150–250 nm in dimension. They do not have a cell wall and typically their cell membranes contain sterols.

■ aa ny *Mycoplasma* can pass through 0.45 µm filter, hence were once believed to be viruses. However, they differ from viruses in the following properties:
● Thyy nnnnnnnboth ribonuclein nnid NNNAd ddddeoxyiibonuclii c acid (DNA).
● Thyy eee bble eo rr ow on lll l-free media in vitro.
● Thyy hhow both intllllll ulal ll d extracellular parasitism in vivo.
■ *Mycoplasma* species was also considered to be L form of bacteria because they lack a cell wall. However, they differ from bacteria including L forms in the following properties:
● Thyy vvve steods in the eell membnnn.
● They do not show any reversion to structure with cell walls.
● They do not share any DNA homology with other bacteria.
● They have genome with a low molecular weight.
● They have low guanine and cytosine contents.
● The absence of cell wall makes *Mycoplasma* resistant to penicillins, cephalosporins, vancomycin, and other antibiotics that interfere with the synthesis of the cell wall.
■ *Mycoplasma* species typically show pleomorphism and occur as granular and filaments of various sizes. The filaments are slender of varying length and show true branching.
■ They multiply by binary fission. However, genomic replication and cell division are often asynchronous, resulting in production of multinucleate fragments and other body forms and chains of beads.
■ They do not possess flagella or pili, but some *Mycoplasma* species including *M.pneumoniae* show gliding motility on liquid-covered surfaces.
■ *Mycoplasma* organisms stain poorly by Gram stain and are Gram negative. They are better stained by Giemsa and Diene stain.

Culture
Mycoplasmas are aerobic and facultative anaerobes. M. pneumoniae is an exception, which is a strict aerobe. They grow at 37°C and at pH range of 7.3–7.8.

1. **PPLO broth:** Pleuropneumonia-like organism (PPLO) broth is a medium widely used for isolation of *mycoplasma*. This medium is supplemented with 20% horse serum, 10% yeast extract, and glucose. Phenol red is used as a pH indicator. The high concentration of animal serum (horse serum) is used as a source of exogenous sterols (cholesterol and other lipids). Addition of agar makes the medium solid. The medium is supplemented with penicillin, ampicillin, and polymyxin B to inhibit growth of contaminating bacteria, and amphotericin B is used to inhibit contamination with fungi.
2. **PPLO agar:** The PPLO broth is solidified by addition of agar. The mycoplasmas are typically slow growers with a generation time of 1–6 hours. Mycoplasmas on PPLO agar produce small colonies, typically described as a fried-egg appearance, which consists of a central, opaque, granular area of growth surrounded by a flat, translucent,

peripheral area. Initially, the mycoplasmas multiply within the agar to produce opaque, ball-shaped colonies that grow up to the surface of the agar and then spread around it, forming a translucent peripheral zone. Colonies may be seen with a hand lens, which is bsst studied fftss staining yy Dinn'' s method.
By this staining, the fried-egg appearance colonies of *Mycoplasma* appear highly granular, with the center of the colonies stained dark blue and the periphery of the colonies staining light blue. The agar in the medium appears clear or a slightly violet. *Mycoplasma* other than M. pneumoniae becomes colorless after a period of time, because it reduces the methylene blue.
■ .. pneumoniee unlike othrr *Mycoplasma* species are very slowgrowing bacteria. They require 1–4 weeks to produce colonies on agar.
■ .. pneumoniee doss not poɑduee rried-egg appearance colonies but instead produces a colony known as mulberryshaped colonies. These colonies do not show any thin hallow unlike that of fried-egg appearance colonies.
Most *Mycoplasma* colonies produce a zone of hemolysis on blood agar. Mycoplasmas do not have the capability to synthesize cholesterol and related sterols; hence these are supplied from outside for growth of mycoplasmas. They also lack the ability to synthesize purines and pyrimidines.

Biochemical reactions
Mycoplasmas show the following biochemical reactions:
1. M. pneumoniae and other species (M. fermentans, M. genitalia, and M. agalactiae) utilize glucose and other carbohydrates as the major source of energy.
2. M. salivarium and other species (M. orale, M. hominis, and M. fermentans) utilize arginine as a major source of energy.
3. *Mycoplasma* are chemo-organotrophs, the metabolism being mainly fermentative.
The liquid culture medium used for fermentation reaction is supplemented with glucose, arginine, and urea, and phenol red as an indicator. *Mycoplasma* species fermenting carbohydrate utilize glucose to produce lactic acid, resulting in formation of acidic pH. Arginine-utilizing *Mycoplasma* species produce ammonia, CO2, and adenosine triphosphate by metabolism of arginine. Ammonia production leads to change of the medium to alkaline.

- **Other properties**

Susceptibility to physical and chemical agents: Mycoplasmas are readily killed by heating at 56°C for 30 minutes.
The bacteria are sensitive to antiseptic solutions, such as cycloheximide and cetrimide, which inhibit their growth. They are resistant to UV light and photodynamic action of methylene blue, hence M. pneumoniae can grow in presence of 0.002% of methylene blue in agar, while many other *Mycoplasma* species are inhibited at this concentration.

Cell Wall Components and Antigenic Structure

Membrane glycolipids and proteins are the major antigenic determinants of the mycoplasmas. Membrane glycolipid antigens show cross-reaction with human tissues and other bacteria.

These antigens are identified by complement fixation tests.Glycolipids with similar antigenic structure have been demonstrated in neurons in human brain. The antibodies against M.pneumoniae glycolipid may cross-react with brain cell, therefore, causing damage to neuronal cells. This cell damage possibly is responsible for neurological manifestations of M. pneumoniae infection.

M. pneumoniae possesses two major surface proteins including the adhesion protein P1, which is responsible for attachment of bacteria to cell structures. These protein antigens are identified by enzyme-linked immunosorbent assay (ELISA). The P1 protein induces production of antibodies, which not only react with P1 protein but also react with antigenic determinants of RBCs, leading to lysis of erythrocytes in autoimmune disease process.

Pathogenesis and Immunity

Mycoplasmas are primarily extracellular pathogens that adhere to surface of ciliated and nonciliated epithelial cells.

- Virulence factors

The adhesion protein called P1 is the key virulence factor of the Mycoplasma. The bacteria usually do not cause invasion of the blood to produce systemic manifestation of the disease.

P1 antigen: P1 antigen is a membrane-associated protein,which helps in adhesion of mycoplasmas to epithelial cells.

This protein or adhesin combines specifically with sialated glycoprotein receptors present at the base of the cilia on epithelial surface. This same receptor is also present on the surface of the erythrocytes. The antibodies against P1 antigen can also act as an autoantibody against RBCs causing their agglutination.

- Pathogenesis of *Mycoplasma* infections

Following attachment, *Mycoplasma* cause direct damage to the epithelial cells in which first cilia and then ciliated epithelial cells are destroyed. Loss of the cells interferes with normal functioning of the upper respiratory tract. This results in the lower respiratory tract to become infected with microbes and mechanically irritated. The mechanical irritation causes persistent cough typically seen in patients with respiratory infection caused by M. pneumoniae.

M. pneumoniae behaves as a super antigen. This causes migration of inflammatory cells to the site of infection and produces cytokines, such as tumor necrosis factor-alpha, interleukin-1, and interleukin-6. These cytokines help in clearing of the bacteria and of

the disease. The bacteria usually do not cause invasion of the blood to produce systemic manifestation of the disease.
Mycoplasma rarely penetrates the submucosa except in rare cases of immunosuppression or following instrumentation. In these conditions, they may invade the blood stream and cause infection in different organs of the body.
Host immunity M. pneumoniae infection does not induce any protective immunity.
Individuals suffering from M. pneumoniae infection are susceptible to reinfection by the bacteria. The P1 antigen induces production of antibodies. These antibodies are found in nearly 50% of patients infected with M. pneumoniae. The antibody against P1 antigen is an autoantibody that cross-reacts with antigen I of RBCs and is not protective.

Clinical Syndromes
M. pneumoniae primarily causes respiratory infections in humans.

- Respiratory infections

Majority of the respiratory infections are mild and self- limiting.M. pneumoniae causes (a) upper respiratory tract infections, (b) lower respiratory tract infections, and (c) primary atypical pneumonia.
Upper respiratory tract infections: M. pneumoniae typically causes mild upper respiratory tract infections. The condition is characterized by low-grade fever, malaise, and headache. Nonproductive cough is a typical manifestation, which appears 2–3 weeks after exposure. The cough is initially nonproductive but may later produce small to moderated quantities of sputum, which may become mucopurulent and even blood tinged in more severe cases. Lower respiratory tract infections: These include tracheobronchitis and bronchopneumonia. The condition is characterized by primary infection of bronchi with infiltration of bronchial epithelial cells by lymphocytes and plasma cells.
Primary atypical pneumonia: The condition is also known as walking pneumonia. Incubation period varies from 2 to 3 weeks. Patients suffering from atypical pneumonia usually do not appear ill. Hence, the illness is often referred to as walking pneumonia. The pharynx is affected, becomes edematous but without any cervical adenopathy. The condition is associated with presence of patchy bronchopneumonia seen on the chest Xray. Disparity between physical findings and radiological evidence of chest is the hallmark of infection. The infection is usually self-limiting. In 5–20% of patients, pleural effusion may occur.

- Extrapulmonary infections

Extrapulmonary manifestations are not rare. Cardiac abnormalities,such as myocarditis and pericarditis are the most frequently reported extrapulmonary manifestations. Other manifestations include neurological abnormalities, otitis media, and erythema multiforme (Stevens–Johnson syndrome). M. pneumoniae infections tend to cause much more severe disease in:

h hhildeen suffiii ng foom immunosuppssssive disesse;
■ individuals with sickle cell anemia, functional asplenia and
h hhildeen with Down yyndrom..
Subclinical infection may occur in 20% of adults .
is widely documented in the European countries and in the United States. The exact information on M. pneumoniae infection is not available from the developing countries. However, the results of few seroprevalence studies indicate that the condition may be endemic in many of the developing countries.

Habitat
M. pneumoniae is a strict human pathogen. The bacteria inhabit the upper respiratory tract in an infected host. They are usually present in the mucosa, residing extracellularly on the upper respiratory tract.

- **Reservoir, host, and transmission of infection**

Humans are the usual host of M. pneumoniae and thus significant reservoir of infection. Patients with active infection are more likely to transmit M. pneumoniae infection. M. pneumoniae is most commonly transmitted by close contact through nasal secretions. The infection is transmitted by inhalation of aerosolized droplets. Person-to-person transmission usually occurs among college students and military recruits who live together in close proximity. M. pneumoniae is usually associated with pneumonia, and highest rate of infection is found in children between 9 and 10 years, and also in young adults. Infection is common among school-going children.
In recent years, the infection is also being increasingly documented in people older than 65 years. In this old population, M. pneumoniae is responsible for causing nearly 15% of community-acquired pneumonia and is second pathogen next only to Streptococcus pneumoniae as a cause of pneumonia.

Laboratory Diagnosis
Initial treatment of M. pneumoniae infection is based on clinical diagnosis of the condition. The definite diagnosis of the condition usually takes 3–4 weeks. Hence, treatment is started without waiting for the result of laboratory tests.

- **Specimens**

Respiratory specimens include throat washings, bronchial washings, and expectorated sputum. Tracheal washings are more useful than sputum specimens, because most patients with respiratory tract infections do not produce any sputum as they have a dry and nonproductive cough. The specimens are collected and transported to the laboratory immediately.
■ ff delyy is anticipated, they are usually inoculated in suitable transport media, such as SP4 transport medium to prevent desiccation.

■ The spccimnns nnn be stoeed tt -70°C if these cannot be sent immediately to the laboratory after collection.

- **Microscopy**

Microscopy is of no value in diagnosis of M. pneumoniae infections.
Mycoplasmas are stained poorly, because they lack cell wall.

- **Direct antigen detection**

Antigen capture immunoassay has been used for direct detection of M. pneumoniae in sputum specimen with high sensitivity and specificity. Direct immunofluorescence, counter-current immunoelectrophoresis, and immunoblotting with monoclonal antibodies are the tests used for detection of antigen in clinical specimens.

- **Culture**

Bacterial culture is of little practical value because of its fastidious growth requirements and need for 3–4 weeks for culture. Isolation of M. pneumoniae from clinical specimens by culture confirms the diagnosis of M. pneumoniae respiratory illness.
The specimens are inoculated into *Mycoplasma* medium, such as PPLO agar supplemented with serum (source for sterols), glucose, pH indicator, yeast extract (source for nucleic acid precursor), and antibiotics and antifungal agents (to inhibit bacteria and fungi). On this medium, M. pneumoniae grows very slowly and colonies are demonstrated at 37°C. The growth is facilitated by incubation in the presence of 95% N2 and 5% of CO2. The bacteria produce small colonies with homogenous appearance, typically described as mulberry-shape colonies.

- **Identification of bacteria**

The identifying features of M. pneumoniae colonies are summarized in Box 1. They are identified by the following tests.
Color change: The colonies are identified by noting a color change from red to yellow of phenol red due to fermentation of glucose resulting in the production of acid, which makes pH of the media acidic.
Diene test: In this method, the Diene stain (diluted 1:10 with distilled water) is added directly to the plate containing suspected colonies of Mycoplasma. The plate is then immediately rinsed with distilled water to remove the stain. This is followed by decolorizing the medium by adding 1 mL of 95% ethanol and keeping it for 1 minute. The plate is rewashed with distilled water and is allowed to dry. After drying, the colonies are observed under low power of the microscope.
By Diene staining, the fried-egg appearance colonies of *Mycoplasma* appear highly granular, with the center of the colonies stained dark blue and the periphery of the colonies staining light blue. The agar in the medium appears clear or slightly violet. *Mycoplasma* other than M. pneumoniae becomes colourless after a period of time because it reduces the methylene blue. The colonies identified are confirmed by inhibition of their growth with specific M. pneumoniae antisera.
Hemadsorption test: The test is performed by flooding M. pneumoniae colonies grown on surface of agar with 2 mL of 0.2–0.4% suspension of guinea pig erythrocytes in

Mycoplasma growth medium. The plate is incubated at 35°C for 35 minutes followed by washing with 3 mL of *Mycoplasma* growth medium by gently rotating the plate. The washing fluid is gently removed by aspiration with a pipette. M. pneumoniae colonies adsorb guinea pig erythrocytes, which is facilitated at 37°C. M.pneumoniae colonies adsorbing erythrocytes on their surface are demonstrated under the microscope. The colonies are observed under 40x magnification.

Tetrazolium reduction test: The test is based on the principle that M. pneumoniae has the ability to reduce triphenyl tetrazolium, a colorless compound, to formazan, a red-colored compound. This test is performed by flooding the colonies of M. pneumoniae on agar with a solution of 2-p-iodophenyl-3-nitrophenyl-5-phenyltetrazolium chloride and incubating it at 35°C for an hour. In a positive test, M. pneumoniae colonies appear reddish after 1 hour and may appear purple to black after 3–4 hours of incubation.

Box 1 Identifying features of *Mycoplasma*

1. Very small bacteria; poorly stained with Gram stain but stain well with Diene stain.
2. A color change from red to yellow of phenol red.
3. Colonies stained directly by applying Diene stain.
4. Colonies positive for hemadsorption test.
5. Colonies positive for tetrazolium reduction test.
6. The colonies identified are confirmed by inhibition of their growth with specific *M. pneumoniae* antisera.
7. On agar, mycoplasmas produce colonies having a "fried egg" appearance with an opaque central zone of growth within the agar and a translucent peripheral zone on the surface.

- **Serodiagnosis**

Serodiagnosis is based on demonstration of specific antibodies in serum using *Mycoplasma* antigens. M. pneumoniae glycolipid antigen extracted by chloroform and methanol is also widely used for serodiagnosis of atypical pneumonia. Complement fixation test and ELISA are the tests frequently used.

Complement fixation test: A fourfold rise in complementfixing antibody titer or a single titer of 1:64 or more is suggestive of recent infection. The complement-fixing antibodies appear after 7–10 days of infection and reach the peak after 4–6 weeks of infection. The complement-fixing antibodies are demonstrated in approximately 80% of the cases.

Enzyme-linked immunosorbent assay: IgM ELISA is most frequently used test. IgM ELISA is used to detect specific IgM antibodies in a single serum specimen. This test has a specificity of 99% and sensitivity of 97%. Recently, a quantitative, rapid, single-specimen, membrane-based ELISA has been evaluated as a rapid diagnostic method for demonstration of either IgM or IgG antibodies. The results for this test can be obtained within 30 minutes of performing the test.

Nonspecific serological tests: These tests are called nonspecific because they do not use specific *Mycoplasma* antigen; instead they use cross-reacting and nonspecific antigens. These tests include Streptococcus MG agglutination test and cold agglutination test.

■ tt eeptooocuus MG ggglutination tsst: nn this test, a hett killed suspension of Streptococcus MG is used as antigen. The antigen is mixed with serial dilution of ptt ient's unhett ed serum. The agglutination is observed after overnight incubation at 37°C. An antibody titer of 1:20 or more is suggestive of M. pneumoniae infection.

■ oo ld ggglutination tttt : nn this tsst, humnn O group ttt thooyytss are used as antigen. This is based on the principle that autoantibodies that agglutinate human O group cells at low temperatures appear in most of the cases of atypical pneumonia. The test is pfffommdd by oollccting patinnt's blood, which should never be refrigerated before separation of serum because agglutinins are readily absorbed by homologous RBCs at low tmmptttt usss. The patient's srrum is mixed with equal volume of 0.2% washed suspension of human O erythrocytes. The suspension is incubated at 4°C overnight and observed for clumping. The clumping is disassociated at 37°C. The titer of 1:32 or more is suggestive of M. pneumoniae infection.

The cold agglutinins usually appear in more than 50% of the cases by the second week of infection and reach a peak at 4–5 weeks. The antibody titer thereafter declines rapidly, and test becomes negative in about 5 month.

A fourfold rise in cold agglutinin titer of the paired serum and convalescent sera, or a single titer of 1:32 is suggestive of M. pneumoniae infection. The noted disadvantage of this test is that this is a nonspecific test, because the cold agglutinins are also demonstrated in sera of other diseases, such as rubella, infectious mononucleosis, adenovirus infections, psittacosis, tropical eosinophilia, trypanosomiasis, cirrhosis of liver, and hemolytic anemia.

■ Molecular Diagnosis

Recently, a seminested polymerase chain reaction (PCR) assay using 60S ribosomal DNA has been developed for demonstration of P1 adhesin in protein for diagnosis of M. pneumoniae infection. These tests are of high sensitivity and specificity. They have added advantage of detecting M. pneumoniae infections at an early stage. Disadvantage of the test is that it is expensive and is available only in few laboratories .

Treatment

Therapy with antibiotics is usually not necessary for treatment of upper respiratory tract infection caused by M. pneumoniae.

However, treatment with antibiotics may be helpful for management of *Mycoplasma* pneumonia, because it reduces duration of illness and also reduces the number of *Mycoplasma* in clinical specimen. It also reduces the symptoms, enhances resolution of pneumonia, and facilitates recovery from the disease. Pneumonia is usually a self-limiting disease and is not life-threatening in most patients.

M. pneumoniae remains susceptible to tetracyclines and erythromycin, because these antibiotics act on the mycoplasmas by inhibiting synthesis of protein. Tetracycline has

the additional advantage of also being active against most other mycoplasmas and chlamydiae, the common causes of nongonococcal urethritis.
Mycoplasma organisms are resistant to penicillins and cephalosporins, because these antibiotics act on the cell wall, which is lacking in mycoplasmas.

Prevention and Control
Isolation of the patients infected with M. pneumoniae is the best way to prevent the spread of the disease. Antibiotic prophylaxis with tetracyclines or erythromycin is also useful. No vaccine is available against *Mycoplasma* infections.

Genital *Mycoplasma* Species
M. hominis and *Mycoplasma* genitalis are the genital *Mycoplasma* species, which inhabit the mucosa of the urogenital tract.

***Mycoplasma* hominis**
M. hominis is a facultative anaerobe and is relatively a fast-growing Mycoplasma, which grows within 1–4 days. The bacteria metabolize arginine but do not utilize glucose. M. hominis typically produces large fried-egg appearance colonies on *Mycoplasma* medium. Inhibition of the growth of the bacteria with specific antisera to M. hominis is used to differentiate it from other genital mycoplasmas. The clinical manifestations by genital mycoplasmas vary depending on the type of infection:
■ .. hominis is sssoii tt ed with incccdion of genitourinyyy ttttt and reproductive disease. M. hominis causes genital infection, which may result in diverse manifestation, such as salpingitis, pelvic abscess, puerperal infection, septic abortion.
■ tt also aauses nongnnitll infttt ions, such as septic rr thritis, peritonitis, septic thrombophlebitis, and brain abscess.
t tt may ssso aausp piimrr y tt ypiaal pneumonin nnd mnningitis in newborns.
The incidence of colonization by genital mycoplasmas increases after puberty and is related to the sexual activity. M. hominis colonizes in approximately 15% of sexually active men and women, while Ureaplasma colonizes 45–70% of sexually active women. M. hominis organisms unlike other mycoplasmas are resistant to erythromycin and occasionally to tetracyclines. Clindamycin is useful to treat infections caused by such resistant strains of M. hominis.

***Mycoplasma* genitalis**
M. genitalis has also been implicated as a cause of nongonococcal urethritis and pelvic inflammatory disease (PID). M. genitalis is primarily a pathogen of the gastrointestinal tract, which can cause occasional infection in the genitourinary and respiratory tract. It is a very difficult organism to be isolated by culture. Their isolation may require more than 2–4 months of incubation.
These genital *Mycoplasma* species have been isolated more frequently from African American than from white men and women.
Colonization of infants by *Mycoplasma* species usually occurs during passage of the baby through the birth canal. Colonization with these genital mycoplasmas occurs only up to 2 years.

Since genital mycoplasmas have been transmitted sexually, avoidance of sexual activity or the use of proper safety procedures prevents the disease caused by these genital mycoplasmas.

Genus *Rickettsia*

Rickettsiae are obligate, intracellular, very small (0.3 X 1–2 μm), Gram-negative bacilli that multiply within cytoplasm of eukaryotic cells. They have very small genome composed of 1–1.5 million base pairs. These organisms, because of their small size, were once thought to be viruses. Nevertheless, these organisms are bacteria because they show following characteristics:

1. They have typical Gram-negative cell walls.
2. They contain both DNA and RNA, enzymes for the Kreb's cycle, and ribosomes for protein synthesis.
3. They multiply by binary fission.
4. They are susceptible to antibiotics.

Rickettsiae are primary pathogens of arthropods, such as lice, fleas, ticks, and mites. In these hosts, they are found in their intestinal tract. They are usually transmitted to humans by arthropod vectors, such as lice, mites, ticks, etc. Coxiella burnetii causing Q fever is an exception, which is transmitted usually by airborne droplets. They also infect humans in whom they are found in the reticuloendothelial cells and vascular endothelium.

TABLE 1

Bacteria	Diseases
Rickettsia prowazekii	Epidemic or louse-borne typhus; relapsing louse-borne typhus or Brill–Zinsser's disease
Rickettsia typhi	Endemic or flea-borne murine typhus
Rickettsia rickettsiae	Rocky Mountain spotted fever
Rickettsia akari	Rickettsial pox
Rickettsia conori	Boutonneuse fever (i.e., Kenya tick bite fever, African tick typhus, Mediterranean spotted fever, Indian tick typhus, and Marseilles fever)

Genus *Rickettsia*

Rickettsia organisms cause a wide variety of diseases varying considerably in severity from self-limiting illness to fulminating, life-threatening infection.

Morphology

They are small, Gram-negative coccobacilli varying from 0.3–0.6 to 0.8–2 μm in size. They are nonmotile and noncapsulated.They are stained poorly with Gram stain but are stained well with the following: deep red with Machiavello and Gimenez stain and bluish purple with Giemsa and Castaneda stain.

Culture

Rickettsiae fail to grow on cell-free media. They usually grow inside the cell, usually in the cytoplasm (most Rickettsia) or in the nucleus of the cell (Rickettsia causing spotted fever). They grow well at optimum temperature of 32–35^{O}C. They grow in various cell lines, in the developing chick embryo, and also in many laboratory animals.

Cell lines: They grow on HeLa, Hep2, Detriot-6, mouse fibroblasts, and other continuous cells lines. Cultures in the cell lines are used primarily for the maintenance of Rickettsia but are not useful for primary isolation of Rickettsia from clinical specimens.

Chick embryo: In the developing chick embryo 5–6 days old, Rickettsia spp. grow well in the yolk sac. The inoculated eggs are incubated at 35^{O}C for most Rickettsia spp. and at 33^{O}C for spotted group. The yolk sac is widely used as a source of Rickettsia for preparation of rickettsial antigens and vaccines. Rickettsia shows poor growth on chorioallantoic membrane.

Laboratory animals: Guinea pigs and mice are the commonly used laboratory animals for isolation of Rickettsia organisms from animal specimens.

- **Other properties**

Susceptibility to physical and chemical agents: The extracellular Rickettsia organisms are very delicate microorganisms.

They are rapidly killed by heating at 56^{O}C and also at room temperature. They are destroyed by usual strength of antiseptics,such as hypochlorite, 1% ethanol, 2% formaldehyde, 5% hydrogen peroxide, and 70% ethanol. They are preserved at -70^{O}C or in a lyophilized state. They are preserved better in a special medium known as SPG medium containing sucrose, potassium phosphate, and glutamate, and also in the skimmed milk.

Cell Wall Components and Antigenic Structure

Rickettsia possesses three different types of antigens as follows:

1. Group-specific antigen: This is a soluble antigen present on surface of the organisms. This is extracted from rickettsial pathogens by repeated washings and centrifugations.
2. Species- or strain-specific antigen: Species-specific or strain-specific antigen (e.g., scrub typhus) is present in the cell wall of the bacteria.
3. Alkali-stable polysaccharide antigen: This is a surface antigen found in some species of Rickettsia and in some strains of Proteus species (Proteus OX19, OX2, and OXK). This sharing of antigen between Rickettsia and Proteus forms the basis of Weil Felix

test, which is employed for diagnosis of rickettsial infections by demonstration of antibodies using Proteus strains.

Typhus Fever Group

Typhus refers to a group of infectious diseases that are caused by different rickettsial organisms. These are of three types.

1. Epidemic or louse-borne typhus caused by Rickettsia prowazekii.
2. Relapsing louse-borne typhus or Brill–Zinsser's disease caused by R. prowazekii.
3. Endemic or flea-borne murine typhus caused by Rickettsia typhi.

Rickettsia prowazekii

R. prowazekii is the causative agent of epidemic typhus, also called louse-borne typhus. This condition is an acute febrile illness transmitted by human body louse Pediculus humanus corporis. R. prowazekii is named after the scientist Von Prowazek who died of the typhus fever while studying the disease. This typhus fever is an ancient disease and has been reported from all parts of the world. This disease was widely prevalent in Russia and in Eastern Europe. This disease was also responsible for Napolean's defeat in Russia in 1812. This was one of the three diseases responsible for misery and sufferings during the Irish famine of 1845–1850.

Properties of the Bacteria

R. prowazekii organisms like other rickettsiae are small, Gramnegative, intracellular bacteria. They stain poorly with Gram stain, but stain best with Giemsa or Gimenez stain. Like other rickettsiae, they can grow in various tissue cultures (HeLa, Hep2, Detriot-6, mouse fibroblasts, and other continuous cells lines) and in yolk sac of embryonated egg.

- Other properties

Like other rickettsiae, they are susceptible to various physical and chemical agents, as described earlier.

Pathogenesis and Immunity

R. prowazekii is an invasive bacterium, which characteristically multiplies in endothelial cells of the blood vessels, leading to vasculitis.

- **Virulence factors**

The capability to multiply inside the cell is important mechanism of the disease process caused by R. prowazekii. Adhesins are the most important virulent factor of rickettsia. These are outer membrane proteins, which facilitate the entry of rickettsiae into the host cells. Once inside the cells, they remain, multiply, and accumulate in large numbers before lysing the host cell.

- **Pathogenesis of rickettsial infections**

After inoculation from the infected sites, the rickettsiae reach the circulation, multiply, and cause rickettsemia. Rickettsiae are localized in the endothelial cells of small arterial

capillary and venous vessels. At these sites, they multiply and cause endothelial cellular hyperplasia resulting in multiorgan vasculitis.
The process may end in thrombosis and development of small nodules. Thrombosis of supplying blood vessels may cause gangrene of the extremities, ear lobes, nose, and genitalia. The vasculitis process also may result in increased vascular permeability with consequent edema, loss of blood volume, hypoalbuminemia, reduced osmotic pressure, and hypotension.
Brill–Zinsser's disease is an example of a recrudescent case of typhus fever, which is observed in some people. The exact mechanism responsible for the recrudescence is not known.

Clinical Syndromes
R. prowazekii causes epidemic typhus and recrudescent typhus.

- **Epidemic typhus**

Incubation period varies from as low as 2–3 days to an average period of 8 days. Epidemic typhus is characterized by high fever, severe headache, and chills. Appearance of a petechial or macular rash on the fourth or fifth day first starting on the trunk and then spreading over to the extremities but without affecting the face, palms, and sole is the characteristic feature of the condition. This rash is seen in nearly 40% of patients.
The patient if left untreated may become stuporous and delirious. The name typhus is derived from the word "typhus", meaning cloud or smoke, which denotes cloudy state of consciousness in the disease process. Myocarditis and central nervous system (CNS) dysfunction are the noted complications of epidemic typhus. The disease is associated with mortality rate as high as 60% in old or debilitated persons.

- **Recrudescent typhus**

This condition was seen in some patients treated with antibiotics and has been apparently cured of the disease. R. prowazekii in such patients may persist in their body tissues, may reemerge, and cause a recurrence of typhus fever months, years, or even decades after antibiotic treatment. This condition is called recrudescent typhus. This disease was first noticed by Brill in 1988, and R. prowazekii were isolated from the areas in 1934.
Hence, the disease is also called Brill–Zinsser disease. Improper or incomplete antibiotic therapy, poor general health, and malnutrition are some of the risk factors that may predispose a person to recrudescence. The presentation of this disease is less severe and mortality is much lower than the epidemic typhus.

Laboratory Diagnosis
Diagnosis of rickettsial diseases, including epidemic typhus caused by R. prowazekii, is made by isolation of rickettsia in animal models or by serological tests.

- **Culture**

Rickettsiae are highly infectious pathogens; therefore, isolation of these pathogens from clinical specimens is carried out only in the laboratory equipped with high safety provision.

Rickettsiae may be isolated by growing in the cell culture. They grow better on Vero cells, in three and half days. The rickettsiae in these infected cell lines are identified by immunofluorescence, using group-specific and strain-specific monoclonal antibodies. Isolation of rickettsia in the eggs or chick embryos is usually not followed for primary isolation of rickettsia from clinical specimens.

TABLE 2 Animal inoculation test

Rickettsial pathogen	Fever	Testicular inflammation or tunica reaction	Scrotal necrosis
Rickettsia rickettsiae	+	+	+
Rickettsia typhi	+	+	−
Rickettsia conori	+	+	−
Rickettsia akari	+	+	−
Rickettsia prowazekii	+	−	−

- **Serodiagnosis**

Weil–Felix test: This is a heterologous agglutination test used since long for diagnosis of rickettsial infections. The test detects antirickettsial antibodies that cross-react with O antigens of certain nonmotile strains of Proteus. In this test, nonmotile strains of Proteus vulgaris OX19 and OX2 and Proteus mirabilis OXK are used as antigens. The Weil–Felix test becomes positive 10–20 days after infection; sera from epidemic typhus strongly react with OX19 antigen and weakly agglutinate with OX2 antigen. They do not agglutinate with OXK antigen. Weil– Felix test is negative or weakly reactive in Brill–Zinsser disease.

Reactions of Weil–Felix test in other rickettsial infections are summarized in Table 3

Complement fixation test, indirect hemagglutination, indirect immunofluorescence, latex agglutination, and enzyme immunoassay are the other tests, which employ rickettsial antigens for demonstration of rickettsial antibodies for diagnosis of rickettsial infections including R. prowazekii. Of these methods, the indirect fluorescent antibody (IFA) test is the method of choice for diagnosis of epidemic typhus.

TABLE 3 Weil–Felix test in rickettsial diseases

Rickettsial diseases	Agglutination		
	OX19	OX2	OXK
Epidemic typhus	+++	+	−
Brill–Zinsser disease	+/−	−	−
Endemic typhus	+++	+/−	−
Spotted fever	++	++	−
Scrub typhus	−	−	+++

Molecular Diagnosis

Polymerase chain reaction (PCR) is used to detect rickettsia in blood or tissue for early diagnosis of the condition.

Treatment

Tetracycline and chloramphenicol are the drugs of choice for the treatment of epidemic typhus. Antibiotic therapy in combination with treatment of louse infestation of the human host is effective.

Rickettsia typhi

R. typhi is the causative agent of endemic or murine typhus.

Properties of the Bacteria and Pathogenesis and Immunity

The morphology, cultural characteristics, and pathogenesis of the disease caused by R. typhi are similar to that caused by R. prowazekii. Infection with R. typhi can confer immunity to subsequent infection.

Clinical Syndrome

The typhus fever caused by R. typhi is a milder disease than the epidemic typhus and has a shorter duration. The incubation period varies from 7 to 14 days. The condition has a sudden onset of symptoms with fever, headache, malaise, and myalgia. A rash develops on third to fifth day of infection in approximately half of the infected patients. The rash is typically present on the chest and abdomen but may spread to palms and soles. Untreated course of the disease may last up to 3 weeks.

The endemic typhus differs from epidemic in being a mild illness of shorter duration, associated with few complications and case fatality rate less than 1%.

Laboratory Diagnosis

Weil–Felix test used for diagnosis of epidemic typhus is also used for the diagnosis of endemic typhus. IFA test using R. typhi-specific antigen is used as a specific test for serodiagnosis of endemic typhus. A single titer of 1:128 or a fourfold rise in antibody titer in paired sera is diagnostic of the disease. R. typhi is differentiated from R. prowazekii by Neil Mooser reaction and by partial DNA homology.

Treatment
Tetracycline, doxycycline, and chloramphenicol are highly effective in the treatment of endemic typhus.

Spotted Fever Group
Spotted fever group of rickettsial diseases include:
■ Rocky Mountain spotted fever caused by Rickettsia rickettsiae,
■ Rickettsial pox caused by R. akari, and
■ Boutonneuse fever (i.e., Kenya tick-bite fever, African tick typhus, Mediterranean spotted fever, Indian tick typhus, and Marseilles fever) caused by R. conori.
Rickettsiae of this group possess a common soluble group antigen. They also multiply in the nucleus as well as in the cytoplasm of the infected cells. All these species except R. akari are transmitted by ticks. A total of 12 species of rickettsiae have been associated with humans causing spotted fever and seven species have been isolated from arthropod vectors.
R. rickettsiae is the most common species belonging to the spotted fever group and is responsible for Rocky Mountain spotted fever.

Rickettsia rickettsiae
R. rickettsiae causes Rocky Mountain spotted fever, the most serious type of spotted fever. Rocky Mountain spotted fever was the first among the spotted fever group to be described. This disease was earlier called as Mediterranean disease and later boutonneuse fever by Connor who described this condition for the first time in 1910. Megaw in 1917 first described this disease in the foothills of Himalaya in India.

Properties of the Bacteria
Morphology, culture, biochemical reactions, and other properties of R. rickettsiae are similar to those of other rickettsial pathogens. They are small intracellular bacteria, which multiply in the cytoplasm of the infected cells. They stain poorly with Gram stain, but stain well with Giemsa or Gimenez stain.

Pathogenesis and Immunity
R. rickettsiae like other rickettsiae multiply within the endothelial cells of the small blood vessels and invade the blood streams. Subsequently, they cause vasculitis and vascular lesions, which are found in almost all organs but are commonly found in the skin and in the adrenal glands, liver, heart, and CNS. The condition progresses to hypoalbunemia, hyponatremia, and hypovolemia due to loss of plasma into the tissues.

Clinical Syndrome
R. rickettsiae cause Rocky Mountain spotted fever.

- **Rocky mountain spotted fever**

Incubation period is 7 days. The condition is characterized by development of fever, severe headache, chills, and myalgia. A rash may develop after three or more days and typically appears initially on wrist, ankles, and palms and soles and then spreads to the trunk. The rash is maculopapular early in the disease but may later become petechial and hemorrhagic.
This is a serious disease associated with many complications, such as respiratory failure, encephalitis, and renal failure.
The patient may die within 5 days of onset of symptoms. The overall mortality rate is nearly 4% despite effective antibiotic therapy. The deficiency of enzyme glucose-6-phosphate dehydrogenase (G6PD) is usually associated with more severity of infection.

Laboratory Diagnosis

- **Specimens**

These include skin biopsy for antigen detection and serum for serodiagnosis.

- **Culture**

R. rickettsiae can be isolated in embryonated egg or tissue cultures, but cultures are rarely attempted because of associated risk of infection.

- **Direct detection of rickettsial antigen**

Direct detection of R. rickettsiae in skin biopsy specimens of the rash from infected patients by direct fluorescent antibody test using the specific antirickettsial antibodies is a rapid and specific method for confirming diagnosis of Rocky Mountain spotted fever. This test is recommended for its use prior to therapy or within the first 48 hours after the antibiotic therapy.

- **Serodiagnosis**

Definite diagnosis of Rocky Mountain spotted fever is made by employing serological tests that detect R. rickettsiae immunoglobulin G (IgG) antibodies. IFA and enzyme linked immunosorbent assay (ELISA) are new serological tests that are used for early diagnosis of the condition.
The IFA is most commonly used test. This test uses groupspecific heat-labile proteins and the LPS antigens of scrub typhus group. Therefore, the test is not species specific. The IFA is 95–100% sensitive and 100% specific. Demonstration of a fourfold rise in antibody titers between acute and convalescent sera or demonstration of antibody titre of 1:64 or more in a single serum is diagnostic of the disease. These antibodies are detected in the serum 2–3 weeks after the onset of the disease and remain in the serum after a very long period of time.

Molecular Diagnosis

PCR has been used to detect R. rickettsiae DNA in the skin biopsy specimens of the rash with good sensitivity and high specificity.

Treatment

Tetracyclines, chloramphenicol, and fluoroquinolones, such as ciprofloxacin are effective against R. rickettsiae.

Other Rickettsial Species in the Spotted Fever Group

Six other rickettsial species in the spotted fever group have been associated with human disease. These include tick-borne diseases, such as boutonneuse fever caused by R. conori, Australian tick typhus caused by Rickettsia australis, and Siberian tick typhus caused by Rickettsia siberica. All these rickettsial pathogens are maintained in wild animals and ixodid ticks. Humans are the accidental hosts. The clinical diseases produced by these rickettsiae are similar to the Rocky Mountain spotted fever but are relatively milder.

Rickettsia akari

Rickettsial pox caused by R. akari is prevalent worldwide. It has been reported from Russia, South Africa, and Korea. Common house mouse (Mus musculus) is the natural reservoir. R. akari is transmitted from mouse to mouse by the bite of the mouse mite (Liponyssoides sanguineus). The infection in the mite is transmitted to the progeny transovarially (Fig 1).

The incubation period is 7 days. The condition manifests by development of papule at the site of the bite by the mite.

Subsequently, the papule progresses to an ulcer and then it leads to the formation of eschar. This is followed by development of fever, headache, malaise, and myalgia in 3 –10 days.

A generalized papular vesicular rash usually appears 3–4 days after the emergence of fever. The illness lasts for a short duration of 10–14 days, after which recovery occurs. Complete healing of rash occurs within 2–3 weeks without treatment.

The rickettsial pox is clinically is a mild form of infection.

Like other rickettsial infections, diagnosis of the condition is clinically supported by serology. This disease is differentiated from other rickettsial infections by:

■ The presence of an eschar at the site of bite of mouse mite,

■ The presence of a vesicular pustular eruption, and

■ A negative Weil–Felix reaction. Rodents are the natural host of the mite transmitting rickettsial pox in different parts of the world. Treatment with doxycycline or chloramphenicol is highly effective for rickettsial pox infections.

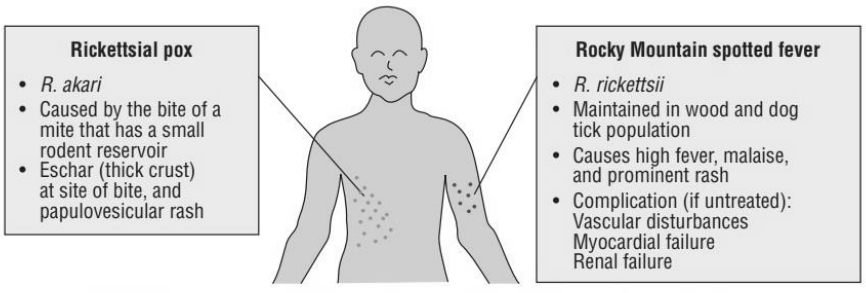


FIG. 1 Comparison of rickettsial pox and Rocky Mountain spotted fever.

Genus *Chlamydia*

Mycoplasma belongs to class Mollicutes (Mollis, soft; cutis, skin),order Mycoplasmatales. This order contains four families Mycoplasmataceae, Acholeplasmataceae, Spiroplasmataceae,and Anaplasmataceae; of which, most *Mycoplasma* causing human infections belong to the family Mycoplasmataceae.
Family Acholeplasmataceae includes mostly saprophytic mycoplasmas; these mycoplasmas do not require sterols as growth factor. Family Spiroplasmataceae includes mostly mycoplasmas, which are parasites of arthropods and plants; they require sterols as their growth factor. Family Anaplasmataceae contains mycoplasmas that are strict anaerobes and are found in the intestinal tract of cattle and sheep.

TABLE 1 Human infections caused by *Mycoplasma* and *Ureaplasma* species

Bacteria	Diseases
Mycoplasma pneumoniae	Upper respiratory tract diseases, lower respiratory tract infections, and primary atypical pneumonia
Mycoplasma hominis	Pelvic inflammatory disease and postpartum fever
Mycoplasma fermentans	Opportunistic infections in patients with HIV
Mycoplasma pirum	Septicemia in patients with HIV
Mycoplasma salivarium	Infection unknown
Mycoplasma orale	Infection unknown
Mycoplasma genitalium	Infection unknown
Ureaplasma urealyticum	Chorioamnionitis, prematurity, vaginitis, cervicitis, acute salpingitis, and pelvic inflammatory disease

Mycoplasma pneumoniae

M. pneumoniae is the most important species causing upper respiratory tract disease. It is also known for causing walking pneumonia or primary atypical pneumonia.

Morphology

Mycoplasmas show the following morphological features:
■ Mycoplasmas are very small bacteria measuring 150–250 nm in dimension. They do not have a cell wall and typically their cell membranes contain sterols.

■ Many *Mycoplasma* can pass through 0.45 µm filter, hence were once believed to be viruses. However, they differ from viruses in the following properties:
● They contain both ribonucleic acid (RNA) and deoxyribonucleic acid (DNA).
● They are able to grow on cell-free media in vitro.
● They show both intracellular and extracellular parasitism in vivo.
■ *Mycoplasma* species was also considered to be L form of bacteria because they lack a cell wall. However, they differ from bacteria including L forms in the following properties:
● They have sterols in the cell membrane.
● They do not show any reversion to structure with cell walls.
●They do not share any DNA homology with other bacteria.
● They have genome with a low molecular weight.
● They have low guanine and cytosine contents.
● The absence of cell wall makes *Mycoplasma* resistant to penicillins, cephalosporins, vancomycin, and other antibiotics that interfere with the synthesis of the cell wall.
■ *Mycoplasma* species typically show pleomorphism and occur as granular and filaments of various sizes. The filaments are slender of varying length and show true branching.
■ They multiply by binary fission. However, genomic replication and cell division are often asynchronous, resulting in production of multinucleate fragments and other body forms and chains of beads.
■ They do not possess flagella or pili, but some *Mycoplasma* species including *M.pneumoniae* show gliding motility on liquid-covered surfaces.
■ *Mycoplasma* organisms stain poorly by Gram stain and are Gram negative. They are better stained by Giemsa and Diene stain.

Culture
Mycoplasmas are aerobic and facultative anaerobes. M. pneumoniae is an exception, which is a strict aerobe. They grow at 37°C and at pH range of 7.3–7.8.

1. **PPLO broth:** Pleuropneumonia-like organism (PPLO) broth is a medium widely used for isolation of *mycoplasma*. This medium is supplemented with 20% horse serum, 10% yeast extract, and glucose. Phenol red is used as a pH indicator. The high concentration of animal serum (horse serum) is used as a source of exogenous sterols (cholesterol and other lipids). Addition of agar makes the medium solid. The medium is supplemented with penicillin, ampicillin, and polymyxin B to inhibit growth of contaminating bacteria, and amphotericin B is used to inhibit contamination with fungi.
2. **PPLO agar:** The PPLO broth is solidified by addition of agar. The mycoplasmas are typically slow growers with a generation time of 1–6 hours. Mycoplasmas on PPLO agar produce small colonies, typically described as a fried-egg appearance, which consists of a central, opaque, granular area of growth surrounded by a flat, translucent,

peripheral area. Initially, the mycoplasmas multiply within the agar to produce opaque, ball-shaped colonies that grow up to the surface of the agar and then spread around it, forming a translucent peripheral zone. Colonies may be seen with a hand lens, which is best studied after staining by Diene's method.

By this staining, the fried-egg appearance colonies of *Mycoplasma* appear highly granular, with the center of the colonies stained dark blue and the periphery of the colonies staining light blue. The agar in the medium appears clear or a slightly violet. *Mycoplasma* other than M. pneumoniae becomes colorless after a period of time, because it reduces the methylene blue.

■ M■pneumoniae unlike other *Mycoplasma* species are very slowgrowing bacteria. They require 1–4 weeks to produce colonies on agar.

■ M. pneumoniae does not produce fried-egg appearance colonies but instead produces a colony known as mulberryshaped colonies. These colonies do not show any thin hallow unlike that of fried-egg appearance colonies.

Most *Mycoplasma* colonies produce a zone of hemolysis on blood agar. Mycoplasmas do not have the capability to synthesize cholesterol and related sterols; hence these are supplied from outside for growth of mycoplasmas. They also lack the ability to synthesize purines and pyrimidines.

Biochemical reactions

Mycoplasmas show the following biochemical reactions:

1. M. pneumoniae and other species (M. fermentans, M. genitalia, and M. agalactiae) utilize glucose and other carbohydrates as the major source of energy.
2. M. salivarium and other species (M. orale, M. hominis, and M. fermentans) utilize arginine as a major source of energy.
3. *Mycoplasma* are chemo-organotrophs, the metabolism being mainly fermentative.

The liquid culture medium used for fermentation reaction is supplemented with glucose, arginine, and urea, and phenol red as an indicator. *Mycoplasma* species fermenting carbohydrate utilize glucose to produce lactic acid, resulting in formation of acidic pH. Arginine-utilizing *Mycoplasma* species produce ammonia, CO2, and adenosine triphosphate by metabolism of arginine. Ammonia production leads to change of the medium to alkaline.

- **Other properties**

Susceptibility to physical and chemical agents: Mycoplasmas are readily killed by heating at 56°C for 30 minutes.

The bacteria are sensitive to antiseptic solutions, such as cycloheximide and cetrimide, which inhibit their growth. They are resistant to UV light and photodynamic action of methylene blue, hence M. pneumoniae can grow in presence of 0.002% of methylene blue in agar, while many other *Mycoplasma* species are inhibited at this concentration.

Cell Wall Components and Antigenic Structure

Membrane glycolipids and proteins are the major antigenic determinants of the mycoplasmas. Membrane glycolipid antigens show cross-reaction with human tissues and other bacteria.

These antigens are identified by complement fixation tests.Glycolipids with similar antigenic structure have been demonstrated in neurons in human brain. The antibodies against M.pneumoniae glycolipid may cross-react with brain cell, therefore, causing damage to neuronal cells. This cell damage possibly is responsible for neurological manifestations of M. pneumoniae infection.

M. pneumoniae possesses two major surface proteins including the adhesion protein P1, which is responsible for attachment of bacteria to cell structures. These protein antigens are identified by enzyme-linked immunosorbent assay (ELISA). The P1 protein induces production of antibodies, which not only react with P1 protein but also react with antigenic determinants of RBCs, leading to lysis of erythrocytes in autoimmune disease process.

Pathogenesis and Immunity

Mycoplasmas are primarily extracellular pathogens that adhere to surface of ciliated and nonciliated epithelial cells.

- Virulence factors

The adhesion protein called P1 is the key virulence factor of the Mycoplasma. The bacteria usually do not cause invasion of the blood to produce systemic manifestation of the disease.

P1 antigen: P1 antigen is a membrane-associated protein,which helps in adhesion of mycoplasmas to epithelial cells.

This protein or adhesin combines specifically with sialated glycoprotein receptors present at the base of the cilia on epithelial surface. This same receptor is also present on the surface of the erythrocytes. The antibodies against P1 antigen can also act as an autoantibody against RBCs causing their agglutination.

- Pathogenesis of *Mycoplasma* infections

Following attachment, *Mycoplasma* cause direct damage to the epithelial cells in which first cilia and then ciliated epithelial cells are destroyed. Loss of the cells interferes with normal functioning of the upper respiratory tract. This results in the lower respiratory tract to become infected with microbes and mechanically irritated. The mechanical irritation causes persistent cough typically seen in patients with respiratory infection caused by M. pneumoniae.

M. pneumoniae behaves as a super antigen. This causes migration of inflammatory cells to the site of infection and produces cytokines, such as tumor necrosis factor-alpha, interleukin-1, and interleukin-6. These cytokines help in clearing of the bacteria and of

the disease. The bacteria usually do not cause invasion of the blood to produce systemic manifestation of the disease.
Mycoplasma rarely penetrates the submucosa except in rare cases of immunosuppression or following instrumentation. In these conditions, they may invade the blood stream and cause infection in different organs of the body.
Host immunity M. pneumoniae infection does not induce any protective immunity.
Individuals suffering from M. pneumoniae infection are susceptible to reinfection by the bacteria. The P1 antigen induces production of antibodies. These antibodies are found in nearly 50% of patients infected with M. pneumoniae. The antibody against P1 antigen is an autoantibody that cross-reacts with antigen I of RBCs and is not protective.

Clinical Syndromes
M. pneumoniae primarily causes respiratory infections in humans.

- Respiratory infections

Majority of the respiratory infections are mild and self- limiting.M. pneumoniae causes (a) upper respiratory tract infections, (b) lower respiratory tract infections, and (c) primary atypical pneumonia.
Upper respiratory tract infections: M. pneumoniae typically causes mild upper respiratory tract infections. The condition is characterized by low-grade fever, malaise, and headache. Nonproductive cough is a typical manifestation, which appears 2–3 weeks after exposure. The cough is initially nonproductive but may later produce small to moderated quantities of sputum, which may become mucopurulent and even blood tinged in more severe cases. Lower respiratory tract infections: These include tracheobronchitis and bronchopneumonia. The condition is characterized by primary infection of bronchi with infiltration of bronchial epithelial cells by lymphocytes and plasma cells.
Primary atypical pneumonia: The condition is also known as walking pneumonia. Incubation period varies from 2 to 3 weeks. Patients suffering from atypical pneumonia usually do not appear ill. Hence, the illness is often referred to as walking pneumonia. The pharynx is affected, becomes edematous but without any cervical adenopathy. The condition is associated with presence of patchy bronchopneumonia seen on the chest Xray. Disparity between physical findings and radiological evidence of chest is the hallmark of infection. The infection is usually self-limiting. In 5–20% of patients, pleural effusion may occur.

- Extrapulmonary infections

Extrapulmonary manifestations are not rare. Cardiac abnormalities,such as myocarditis and pericarditis are the most frequently reported extrapulmonary manifestations. Other manifestations include neurological abnormalities, otitis media, and erythema multiforme (Stevens–Johnson syndrome). M. pneumoniae infections tend to cause much more severe disease in:

■ children suffering from immunosuppressive disease;
■ individuals with sickle cell anemia, functional asplenia and
■ children with Down syndrome.
Subclinical infection may occur in 20% of adults .
is widely documented in the European countries and in the United States. The exact information on M. pneumoniae infection is not available from the developing countries. However, the results of few seroprevalence studies indicate that the condition may be endemic in many of the developing countries.

Habitat
M. pneumoniae is a strict human pathogen. The bacteria inhabit the upper respiratory tract in an infected host. They are usually present in the mucosa, residing extracellularly on the upper respiratory tract.

- **Reservoir, host, and transmission of infection**

Humans are the usual host of M. pneumoniae and thus significant reservoir of infection. Patients with active infection are more likely to transmit M. pneumoniae infection. M. pneumoniae is most commonly transmitted by close contact through nasal secretions. The infection is transmitted by inhalation of aerosolized droplets. Person-to-person transmission usually occurs among college students and military recruits who live together in close proximity. M. pneumoniae is usually associated with pneumonia, and highest rate of infection is found in children between 9 and 10 years, and also in young adults. Infection is common among school-going children.
In recent years, the infection is also being increasingly documented in people older than 65 years. In this old population, M. pneumoniae is responsible for causing nearly 15% of community-acquired pneumonia and is second pathogen next only to Streptococcus pneumoniae as a cause of pneumonia.

Laboratory Diagnosis
Initial treatment of M. pneumoniae infection is based on clinical diagnosis of the condition. The definite diagnosis of the condition usually takes 3–4 weeks. Hence, treatment is started without waiting for the result of laboratory tests.

- **Specimens**

Respiratory specimens include throat washings, bronchial washings, and expectorated sputum. Tracheal washings are more useful than sputum specimens, because most patients with respiratory tract infections do not produce any sputum as they have a dry and nonproductive cough. The specimens are collected and transported to the laboratory immediately.
■ If delay is anticipated, they are usually inoculated in suitable transport media, such as SP4 transport medium to prevent desiccation.

■ The specimens can be stored at -70°C if these cannot be sent immediately to the laboratory after collection.

- **Microscopy**

Microscopy is of no value in diagnosis of M. pneumoniae infections.
Mycoplasmas are stained poorly, because they lack cell wall.

- **Direct antigen detection**

Antigen capture immunoassay has been used for direct detection of M. pneumoniae in sputum specimen with high sensitivity and specificity. Direct immunofluorescence, counter-current immunoelectrophoresis, and immunoblotting with monoclonal antibodies are the tests used for detection of antigen in clinical specimens.

- **Culture**

Bacterial culture is of little practical value because of its fastidious growth requirements and need for 3–4 weeks for culture. Isolation of M. pneumoniae from clinical specimens by culture confirms the diagnosis of M. pneumoniae respiratory illness.
The specimens are inoculated into *Mycoplasma* medium, such as PPLO agar supplemented with serum (source for sterols), glucose, pH indicator, yeast extract (source for nucleic acid precursor), and antibiotics and antifungal agents (to inhibit bacteria and fungi). On this medium, M. pneumoniae grows very slowly and colonies are demonstrated at 37°C. The growth is facilitated by incubation in the presence of 95% N2 and 5% of CO2. The bacteria produce small colonies with homogenous appearance, typically described as mulberry-shape colonies.

- **Identification of bacteria**

The identifying features of M. pneumoniae colonies are summarized in Box 1. They are identified by the following tests.
Color change: The colonies are identified by noting a color change from red to yellow of phenol red due to fermentation of glucose resulting in the production of acid, which makes pH of the media acidic.
Diene test: In this method, the Diene stain (diluted 1:10 with distilled water) is added directly to the plate containing suspected colonies of Mycoplasma. The plate is then immediately rinsed with distilled water to remove the stain. This is followed by decolorizing the medium by adding 1 mL of 95% ethanol and keeping it for 1 minute. The plate is rewashed with distilled water and is allowed to dry. After drying, the colonies are observed under low power of the microscope.
By Diene staining, the fried-egg appearance colonies of *Mycoplasma* appear highly granular, with the center of the colonies stained dark blue and the periphery of the colonies staining light blue. The agar in the medium appears clear or slightly violet. *Mycoplasma* other than M. pneumoniae becomes colourless after a period of time because it reduces the methylene blue. The colonies identified are confirmed by inhibition of their growth with specific M. pneumoniae antisera.
Hemadsorption test: The test is performed by flooding M. pneumoniae colonies grown on surface of agar with 2 mL of 0.2–0.4% suspension of guinea pig erythrocytes in

Mycoplasma growth medium. The plate is incubated at 35°C for 35 minutes followed by washing with 3 mL of *Mycoplasma* growth medium by gently rotating the plate. The washing fluid is gently removed by aspiration with a pipette. M. pneumoniae colonies adsorb guinea pig erythrocytes, which is facilitated at 37°C. M.pneumoniae colonies adsorbing erythrocytes on their surface are demonstrated under the microscope. The colonies are observed under 40x magnification.

Tetrazolium reduction test: The test is based on the principle that M. pneumoniae has the ability to reduce triphenyl tetrazolium, a colorless compound, to formazan, a red-colored compound. This test is performed by flooding the colonies of M. pneumoniae on agar with a solution of 2-p-iodophenyl-3-nitrophenyl-5-phenyltetrazolium chloride and incubating it at 35°C for an hour. In a positive test, M. pneumoniae colonies appear reddish after 1 hour and may appear purple to black after 3–4 hours of incubation.

Box 1 Identifying features of *Mycoplasma*

1. Very small bacteria; poorly stained with Gram stain but stain well with Diene stain.
2. A color change from red to yellow of phenol red.
3. Colonies stained directly by applying Diene stain.
4. Colonies positive for hemadsorption test.
5. Colonies positive for tetrazolium reduction test.
6. The colonies identified are confirmed by inhibition of their growth with specific *M. pneumoniae* antisera.
7. On agar, mycoplasmas produce colonies having a "fried egg" appearance with an opaque central zone of growth within the agar and a translucent peripheral zone on the surface.

- **Serodiagnosis**

Serodiagnosis is based on demonstration of specific antibodies in serum using *Mycoplasma* antigens. M. pneumoniae glycolipid antigen extracted by chloroform and methanol is also widely used for serodiagnosis of atypical pneumonia. Complement fixation test and ELISA are the tests frequently used.

Complement fixation test: A fourfold rise in complementfixing antibody titer or a single titer of 1:64 or more is suggestive of recent infection. The complement-fixing antibodies appear after 7–10 days of infection and reach the peak after 4–6 weeks of infection. The complement-fixing antibodies are demonstrated in approximately 80% of the cases.

Enzyme-linked immunosorbent assay: IgM ELISA is most frequently used test. IgM ELISA is used to detect specific IgM antibodies in a single serum specimen. This test has a specificity of 99% and sensitivity of 97%. Recently, a quantitative, rapid, single-specimen, membrane-based ELISA has been evaluated as a rapid diagnostic method for demonstration of either IgM or IgG antibodies. The results for this test can be obtained within 30 minutes of performing the test.

Nonspecific serological tests: These tests are called nonspecific because they do not use specific *Mycoplasma* antigen; instead they use cross-reacting and nonspecific antigens. These tests include Streptococcus MG agglutination test and cold agglutination test.

■ Streptococcus MG agglutination test: In this test, a heatkilled suspension of Streptococcus MG is used as antigen. The antigen is mixed with serial dilution of patient's unheated serum. The agglutination is observed after overnight incubation at 37°C. An antibody titer of 1:20 or more is suggestive of M. pneumoniae infection.

■ Cold agglutination test: In this test, human O group erythrocytes are used as antigen. This is based on the principle that autoantibodies that agglutinate human O group cells at low temperatures appear in most of the cases of atypical pneumonia. The test is performed by collecting patient's blood, which should never be refrigerated before separation of serum because agglutinins are readily absorbed by homologous RBCs at low temperatures. The patient's serum is mixed with equal volume of 0.2% washed suspension of human O erythrocytes. The suspension is incubated at 4°C overnight and observed for clumping. The clumping is disassociated at 37°C. The titer of 1:32 or more is suggestive of M. pneumoniae infection.

The cold agglutinins usually appear in more than 50% of the cases by the second week of infection and reach a peak at 4–5 weeks. The antibody titer thereafter declines rapidly, and test becomes negative in about 5 month.

A fourfold rise in cold agglutinin titer of the paired serum and convalescent sera, or a single titer of 1:32 is suggestive of M. pneumoniae infection. The noted disadvantage of this test is that this is a nonspecific test, because the cold agglutinins are also demonstrated in sera of other diseases, such as rubella, infectious mononucleosis, adenovirus infections, psittacosis, tropical eosinophilia, trypanosomiasis, cirrhosis of liver, and hemolytic anemia.

■ Molecular Diagnosis

Recently, a seminested polymerase chain reaction (PCR) assay using 60S ribosomal DNA has been developed for demonstration of P1 adhesin in protein for diagnosis of M. pneumoniae infection. These tests are of high sensitivity and specificity. They have added advantage of detecting M. pneumoniae infections at an early stage. Disadvantage of the test is that it is expensive and is available only in few laboratories .

Treatment

Therapy with antibiotics is usually not necessary for treatment of upper respiratory tract infection caused by M. pneumoniae.

However, treatment with antibiotics may be helpful for management of *Mycoplasma* pneumonia, because it reduces duration of illness and also reduces the number of *Mycoplasma* in clinical specimen. It also reduces the symptoms, enhances resolution of pneumonia, and facilitates recovery from the disease. Pneumonia is usually a self-limiting disease and is not life-threatening in most patients.

M. pneumoniae remains susceptible to tetracyclines and erythromycin, because these antibiotics act on the mycoplasmas by inhibiting synthesis of protein. Tetracycline has

the additional advantage of also being active against most other mycoplasmas and chlamydiae, the common causes of nongonococcal urethritis.
Mycoplasma organisms are resistant to penicillins and cephalosporins, because these antibiotics act on the cell wall, which is lacking in mycoplasmas.

Prevention and Control
Isolation of the patients infected with M. pneumoniae is the best way to prevent the spread of the disease. Antibiotic prophylaxis with tetracyclines or erythromycin is also useful. No vaccine is available against *Mycoplasma* infections.

Genital *Mycoplasma* Species
M. hominis and *Mycoplasma* genitalis are the genital *Mycoplasma* species, which inhabit the mucosa of the urogenital tract.

***Mycoplasma* hominis**
M. hominis is a facultative anaerobe and is relatively a fast-growing Mycoplasma, which grows within 1–4 days. The bacteria metabolize arginine but do not utilize glucose. M. hominis typically produces large fried-egg appearance colonies on *Mycoplasma* medium. Inhibition of the growth of the bacteria with specific antisera to M. hominis is used to differentiate it from other genital mycoplasmas. The clinical manifestations by genital mycoplasmas vary depending on the type of infection:
■ M. hominis is associated with infection of genitourinary tract and reproductive disease. M. hominis causes genital infection, which may result in diverse manifestation, such as salpingitis, pelvic abscess, puerperal infection, septic abortion.
■ It also causes nongenital infections, such as septic arthritis, peritonitis, septic thrombophlebitis, and brain abscess.
■ It may also cause primary atypical pneumonia and meningitis in newborns.
The incidence of colonization by genital mycoplasmas increases after puberty and is related to the sexual activity. M. hominis colonizes in approximately 15% of sexually active men and women, while Ureaplasma colonizes 45–70% of sexually active women.
M. hominis organisms unlike other mycoplasmas are resistant to erythromycin and occasionally to tetracyclines. Clindamycin is useful to treat infections caused by such resistant strains of M. hominis.

***Mycoplasma* genitalis**
M. genitalis has also been implicated as a cause of nongonococcal urethritis and pelvic inflammatory disease (PID). M. genitalis is primarily a pathogen of the gastrointestinal tract, which can cause occasional infection in the genitourinary and respiratory tract. It is a very difficult organism to be isolated by culture. Their isolation may require more than 2–4 months of incubation.
These genital *Mycoplasma* species have been isolated more frequently from African American than from white men and women.
Colonization of infants by *Mycoplasma* species usually occurs during passage of the baby through the birth canal. Colonization with these genital mycoplasmas occurs only up to 2 years.

Since genital mycoplasmas have been transmitted sexually, avoidance of sexual activity or the use of proper safety procedures prevents the disease caused by these genital mycoplasmas.