الجامعه التقنيه الوسطى

كليه التقنيات الصحية والطبيه / بغداد

قسم تقنيات المختبرات الطبية

المرحله الثانيه

علم البكتيريا الطبية (2)

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الفصل الدراسي الثاني علم البكتيريا الطبية (2)

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1. *Neisseria*

- Gram-negative cocci, occur in pairs (diplococci).

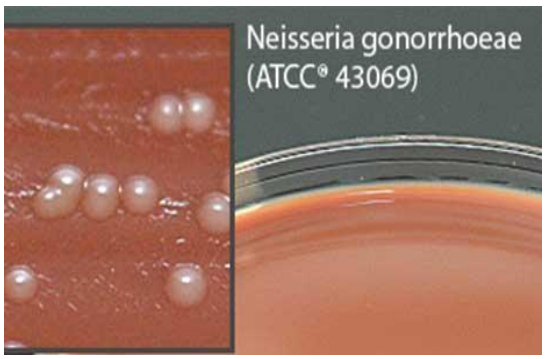
Neisseria gonorrhoeae (**gonococci**) and *Neisseria meningitidis* (**meningococci**) are exclusively pathogenic for humans and typically are found associated with or inside polymorphonuclear cells (PMNs).

Morphology and Identification

A. Typical Organisms: Neisseria is (aerobic, Gram-negative, nonmotile diplococcus, approximately 0.8 µm in diameter. Individual cocci are **kidney bean** shaped; when the organisms occur in pairs, the flat or **concave sides** are **adjacent**.

B. Culture: -grow on **sheep blood agar**, **chocolate agar**, and **selective agar media** (eg, modified Thayer-Martin agar, Martin-Lewis agar and New York City medium).

N. meningitis grows on **sheep blood agar** as well as selective media.



N. gonorrhoeae **requires enriched chocolate** agar and/or selective media for optimal growth.

The selective media contain:

-**vancomycin** (suppression of Gram-positive bacteria).

- **colistin** (suppression of Gram-negative bacteria).

- and other **inhibitory** substances to suppress the growth of many of the commensal microorganisms from these clinical sites.

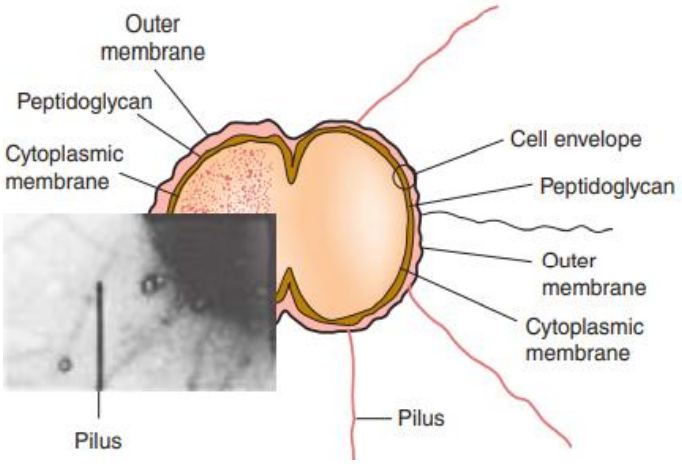
(*N. gonorrhoeae, N. meningitides*, and *N. lactamica* are **colistin-resistant**, and are therefore able to grow on these selective media).

C. Growth Characteristics: The neisseriae grow best under **aerobic** conditions; however, some Neisseria species (eg, *N. gonorrhoeae*) are capable of growing under **anaerobic** conditions as well. The neisseriae produce **acid** but **not gas** by **oxidation** of various carbohydrates (not by fermentation!); the **oxidase** test is hence a key test for identifying neisseriae. Furthermore, all Neisseria species, with the exception of *N. elongata*, are **catalase positive**.

Neisseria species are grow **best** on media containing complex organic substances, such as **heated blood**, **hemin**, and **animal proteins**, and in an atmosphere containing **5% CO_2**. These organisms are also rapidly killed by **drying**, prolonged exposure to **sunlight**, **moist heat**, and many **disinfectants**. They produce **autolytic enzymes** that result in rapid **swelling** and **lysis** in vitro at **25**°C and at an **alkaline** pH.

NEISSERIA GONORRHOEAE Gonococci oxidize only **glucose** and differ antigenically from the other neisseria.

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



A. **Pili (Fimbriae):** Pili are the hairlike appendages. They **enhance attachment** to host cells and **resistance** to phagocytosis. They are made up of stacked **pilin** proteins
B. **Por:** Por protein extends through the gonococcal cell membrane. It forms **pores** in the surface through which some **nutrients enter** the cell. Por proteins may impact **intracellular** killing of gonococci **within** neutrophils by **preventing phagosome– lysosome fusion**.
C. **Opa Proteins: adhesion** of gonococci within **colonies** and in **attachment** of gonococci to **host cell receptors**.
D. **Rmp (Protein III): is a** reduction-modifiable protein (Rmp) and changes its apparent MW when in a **reduced state**. It **associates** with Por in the formation of pores in the cell surface.
E. **Lipooligosaccharide:** In contrast to the enteric Gram-negative rods, gonococcal lipopolysaccharide (LPS) does not have long O-antigen side chains and is called a lipooligosaccharide (LOS). **Toxicity** in gonococcal infections is largely attributable to the **endotoxic** effects of LOS.
F. **Other Proteins:** Lip (H8) is a surface exposed protein that is heat modifiable like Opa. The Fbp (ferric-binding protein).

Pathogenesis, Pathology, and Clinical Findings

Gonococci that form **opaque colonies** are isolated from **men** with **symptomatic urethritis** and from **uterine cervical** cultures at **midcycle**. Gonococci that form **transparent** colonies are frequently isolated from **men** with **asymptomatic urethral** infection, from **menstruating** women, and from patients with **invasive** forms of **gonorrhea**, including **salpingitis** and **disseminated infection**. Gonococci attack **mucous membranes** of the genitourinary tract, eye, rectum, and throat, producing

acute suppuration that may lead to **tissue invasion**; this is followed by **chronic inflammation** and **fibrosis**.

Men usually have **urethritis**, with yellow, creamy pus and painful urination. Gonococcal **bacteremia** leads to skin lesions (especially hemorrhagic papules and pustules) on the hands, forearms, feet, and legs and to tenosynovitis and suppurative arthritis, usually of the knees, ankles, and wrists.

Gonococci can be cultured from blood or joint fluid of only 30% of patients with **gonococcal arthritis**.

Gonococcal **endocarditis** is an uncommon but severe infection.

Gonococcal ophthalmia neonatorum, an infection of the eye in newborns, is acquired during passage through an infected birth canal.

Diagnostic Laboratory Tests

A. **Specimens: Pus** and **secretions** are taken from the urethra, cervix, rectum, conjunctiva, throat, or synovial fluid for culture and smear. **Blood** culture is necessary in systemic illness.

B. **Smears**: Gram-stained smears of urethral or endocervical exudates typically reveal many diplococci within PMNs, therefore providing a presumptive diagnosis.

C. Culture Immediately after collection, pus or mucus is streaked on enriched selective medium (eg, modified Thayer-Martin medium [MTM]) and incubated in an atmosphere containing 5% CO_2 at 37°C. To avoid overgrowth by contaminants, selective media contain antimicrobial drugs (eg, vancomycin, colistin, nystatin, and trimethoprim). If immediate incubation is not possible, the specimen should be placed in a CO_2 - containing transport-culture system. Forty-eight hours after culture, identified presumptive identification can be achieved by the organisms' appearance on a Gram-stained smear and by a positive oxidase test. The definitive species level of the sub-cultured bacteria may be determined by their ability to produce acid from certain carbohydrates by oxidation; the only carbohydrate used by *N. gonorrhoeae* is glucose

NEISSERIA MENINGITIDIS

Antigenic Structure

Capsular polysaccharides: The six most important serogroups associated with disease in humans, worldwide, are A, B, C, X, Y, and W-135. Incorporation of human sialic acid derivatives such as NANA into the meningococcal capsules allows the organism to be overlooked by the host immune system (often referred to as "molecular mimicry").

The outer membrane of *N. meningitidis* consists of proteins and LPS that play major roles in organism virulence. There are two porin proteins (Por A and Por B), interact with host cells.

The opacity proteins (Opa) are comparable to Opa of the gonococci and play a role in attachment. Meningococci are piliated and these structures initiate binding to nasopharyngeal epithelial cells and other host cells such as endothelium and erythrocytes. The lipid A disaccharide of meningococcal LPS is responsible for many of the toxic effects found in meningococcal disease. The highest levels of endotoxin.

Pathogenesis, Pathology, and Clinical Findings:

The **nasopharynx** is the portal of entry. There, the organisms **attach** to epithelial cells with the aid of **pili**; they may form part of the transient microbiota without producing symptoms and/or disease. **Invasive meningococcal diasease** (IMD) occurs in only a small number of individuals who acquired the organism and are transient carriers; infants and adolescents have the highest incidence of IMD in developed countries. From the **nasopharynx**, organisms may reach the **bloodstream**, producing meningococcal **bacteremia**; the initial symptoms during this stage of the actual infection may be similar to those of an upper respiratory tract, "flu-like" infection, but IMD quickly ensues. IMD typically presents as **meningitis**, **sepsis** (ie, meningococcemia), or as a combination of both. **Meningitis** is the most common complication of menigococcal bacteremia.

It usually begins suddenly with an **intense headache**, **vomiting**, **photophobia**, **confusion**, and **stiff neck**; it may progress to **coma** within a few hours. Fulminant meningococcemia is more severe, presenting with a high **fever** and a **hemorrhagic rash**; the patient may also develop disseminated intravascular coagulation and ultimate circulatory collapse with bilateral **hemorrhagic necrosis** of the adrenal glands with subsequent adrenal failure (Waterhouse-Friderichsen syndrome).

In meningitis, the meninges are acutely inflamed, with thrombosis of blood vessels and exudation of polymorphonuclear leukocytes, so that the surface of the brain is covered with a thick purulent exudate. The exact mechanisms that transform an asymptomatic colonization of the nasopharynx into meningococcal bacteremia, subsequently leading to meningococcemia and meningitis, are not very well understood.

Diagnostic Laboratory Tests

A. Specimens: The typical specimens for isolation of *N. meningitides* include blood for culture and cerebrospinal fluid (CSF) for smear and culture. Puncture material or biopsies from petechiae may be taken for smear and culture. Nasopharyngeal swab cultures are suitable for carrier surveys.

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

C. Culture: Although neisseriae are inhibited by certain **toxic factors** present in media and **polyanethole sulfonate** (anticoagulant) present in commercial blood culture broths, this seems to be of a lesser problem for the ability to recover *N. meningitis* from blood cultures, compared to *N. gonorrhoeae*. CSF specimens are plated on sheep blood agar and chocolate agar and then incubated at 37°C in an atmosphere of 5% CO_2.

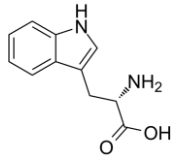
A MTM agar favors the growth of neisseriae, inhibits many other bacteria, and is used for nasopharyngeal cultures. Colonies of *N. meningitidis* are gray, convex, and glistening, with entire edges; a positive oxidase test together with a Gram-stain showing Gram-negative diplococci provides presumptive organism identification. Spinal fluid and blood generally typically yield pure cultures that can be further identified by carbohydrate oxidative reactions and subsequent agglutination with type-specific or polyvalent serum.

D. Serology: Antibodies to meningococcal polysaccharides can be measured by latex agglutination or hemagglutination tests or by their bactericidal activity.

2. Enteric Gram-negative rods: *Escherichia coli*

- The general characteristics of Enterobacteriaceae

- **Gram-negative** bacilli.
- Found as commensals in the intestinal tract of mammals.
- They are also referred to as **coliforms** or **enteric** bacteria.
- **Aerobic** and **facultative anaerobic** growth.
- Optimal growth normally at **37 C**°.
- Grow readily on **simple** media.
- **Ferment** wide range of **carbohydrates**.
- **According to the Lactose fermantation they are classified into:**
  - **Lactose fermenter** **Fermentation** of **lactose** to **produce pink colonies** on MacConkey's agar is characteristic of ***Escherichia***, ***Enterobacter*** and ***Klebsiella***.
  - **Non-lactose fermenter** ***Salmonella***, ***Shigella***, ***Serratia***, ***Proteus*** and ***Yersinia*** **do not** ferment **lactose** and form **pale** شاحب **colonies** on MacConkey's agar.
  - **Late Lactose fermenters**, *Shigella sonnei*.
- **Oxidase-negative**.
- Some are **motile (**Motile except *Shigella* and *Klebsiella*).
- **Bile** tolerant and grow readily on **bile-salt** containing media, e.g. **MacConkey's** agar.
- **Some** of them produce **urease.** (which splits urea with release of ammonia).
- **Some** of them produce **Hydrogene sulphide.**
- **Some** of them **decarboxylase amino-acids.**
- **Some** of them **derive** the **indole ring** from the amino acid tryptophan.



- **None-spore forming.**
- **None acid fast.**
- **Ferment** glucose with **acid** production
- **Reduce nitrates** into **nitrites**
- Non-capsulated except *Klebsiella*
- Non-fastidious

Enterobacteriaceae possess a variety of **antigens**:

- **lipopolysaccharide** somatic antigen('O'),
- **flagellar** antigen('H')
- **capsular polysaccharide** ('K') antigens.

Escherichia coli

Morphology

- *E. coli* is Gram-negative (-ve) rod-shaped bacteria.
- It is 1-3 x 0.4-0.7 µm in size and 0.6 to 0.7 µm in volume.
- It is arranged singly or in pairs.
- It is motile due to **peritrichous** flagella (see classification of flagella).
- Some **strains** are **non-motile**. هذا استثناء نادر
- **Some strains** may be **fimbriated**. The fimbriae are of type 1 (hemagglutinating & mannose-sensitive) and are present in both motile and non-motile strains.
- **Some strains** of *E. coli* isolated from extra-intestinal infections have a **polysaccharide** capsule.
- They are non-sporing.
- They have a thin cell wall with only 1 or 2 layers of peptidoglycan.
- They are facultative anaerobes.
- Growth occurs over a wide range of temperatures from 15-45°C.

Antigenic Structure and Pathogenicity

- ✓ Specific **fimbriae** (**adhesins**) facilitate **adherence** to **mucosal surfaces** and **colonization** of the **intestinal** and **urinary** tracts.
- ✓ *E. coli* possesses 4 antigens; H, O, K and F.

A. Flagellar or (H) Antigen

- Heat and alcohol labile **protein**
- Present on the flagella
- Genus specific
- Present as monophasic
- 75 ‘H’ antigens have been recognized

B. Somatic or (O) Antigen

- **Heat stable**, resistant to **boiling**.
- Occur on the surface of the outer membrane
- An integral part of the cell wall
- 173 ‘O’ antigens have been recognized

C. Capsular or (K) Antigen

- Heat labile

- Acidic polysaccharide (polysaccharides that contain carboxyl groups, phosphate groups and/or sulfuric ester groups) antigen presents in the envelope
- Boiling removes the K antigen
- Inhibit phagocytosis
- 103 'K' antigens have been recognized

D. Fimbrial or (F) Antigen

- Heat labile proteins
- Present in the fimbriae
- K88, K99 antigens

✓ The **heat stable lipopolysaccharide** (**endotoxin**) "in the cell wall is liberated when Gram-negative bacteria lyse", resulting in production of **inflammatory mediators** and **complement activation "** plasma proteins that can be activated directly by pathogens or indirectly by pathogen-bound antibody". This results in **endotoxic shock** and **intravascular coagulopathy**.

- **Different protein toxins** (**exotoxins**) produced by *E. coli*.

➢ **Verocytotoxin-producing E. coli (VTEC), also known as (Shiga toxin-producing *E. coli***)(**STEC**) particularly the **O157:H7** serotype, are an **important** cause of **diarrhoea** and **hemolytic uremic syndrome** (HUS).

➢ **Enteropathogenic** (EPEC): cause of **infantile diarrhoea**, **non-invasive**.

➢ **Enterotoxigenic** (ETEC): **travelers' diarrhoea**, **non-invasive**.

➢ **Enteroinvasive** (EIEC): causes **dysentery-like** illness.

➢ **Enteroaggregative** (EAEC): **watery diarrhoea without** fever.

تم حذف التفاصيل الخاصة بكل نوع

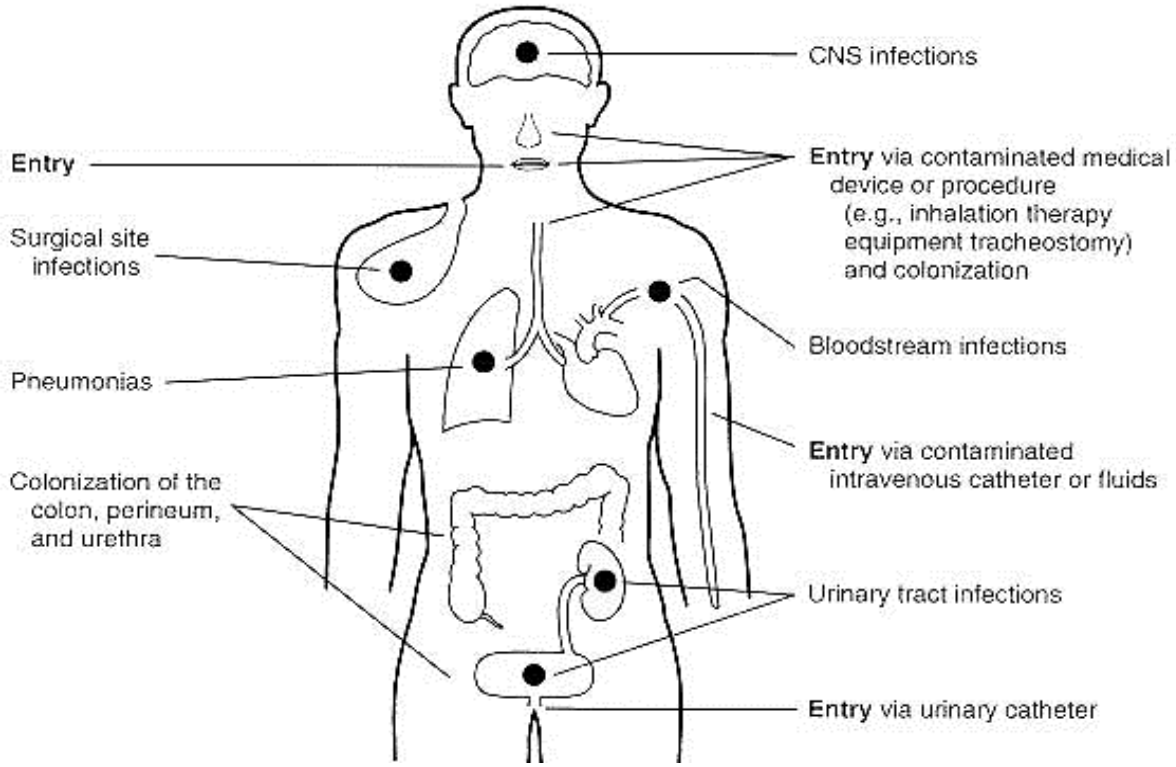
Pathogenicity of *E. coli*

Most infections (with the **exception** of **neonatal meningitis** and **gastroenteritis**) are **endogenous**; that is, the *E. coli* that are part of the patient's normal microbial flora are able to establish infection when the patient's defenses are compromised (e.g., through trauma or immune suppression).

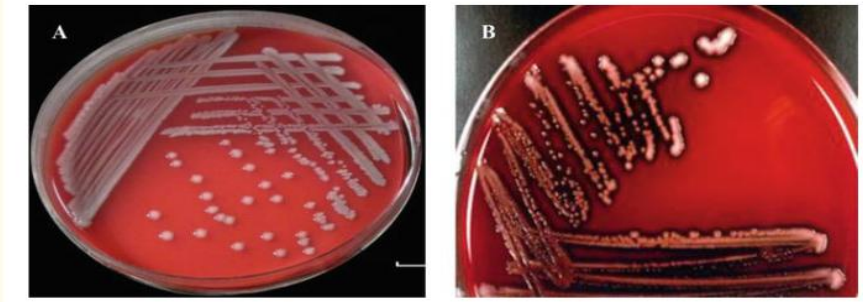
This organism is associated with a variety of diseases, including **gastroenteritis** and **extra-intestinal infections** such as **UTIs**, **meningitis**, and **sepsis**.

Clinical Feature of *E. coli*

1. Gastroenteritis **2. Urinary Tract Infection** **3. Sepsis** **4. Meningitis**



Laboratory Diagnosis

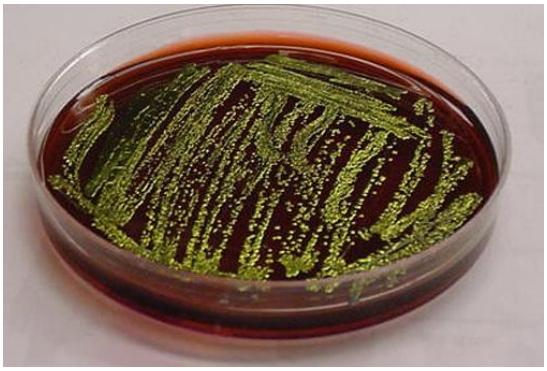


***E. coli* on Blood Agar**

1. Colonies are big, circular, gray, and moist.
2. Non-hemolytic colonies (gamma-hemolysis) (Above Figure) OR Beta (β)hemolytic (Below Figure) colonies are formed.
3. Many pathogenic strains are haemolytic on blood agar.

***E. coli* on MacConkey Agar**

1. Colonies are **circular**, **moist**, **smooth**, and of **entire** margin.
2. Colonies appear **flat** and **pink**.
3. They are **lactose** fermenting colonies.



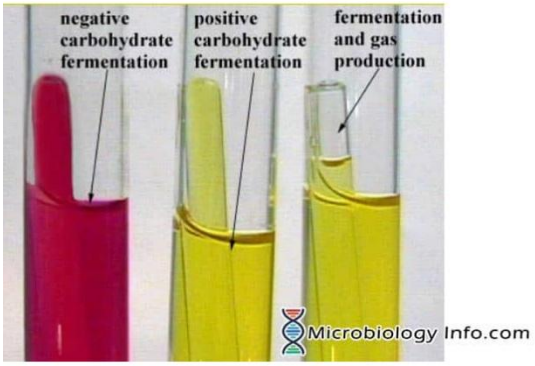
***E. coli* on Eosin Methylene Blue (EMB) Agar**

1. **Green Metallic** sheen colonies are formed.

تم الاعتماد على الأوساط شائعة الاستعمال

***E. coli* Biochemical Characters,**

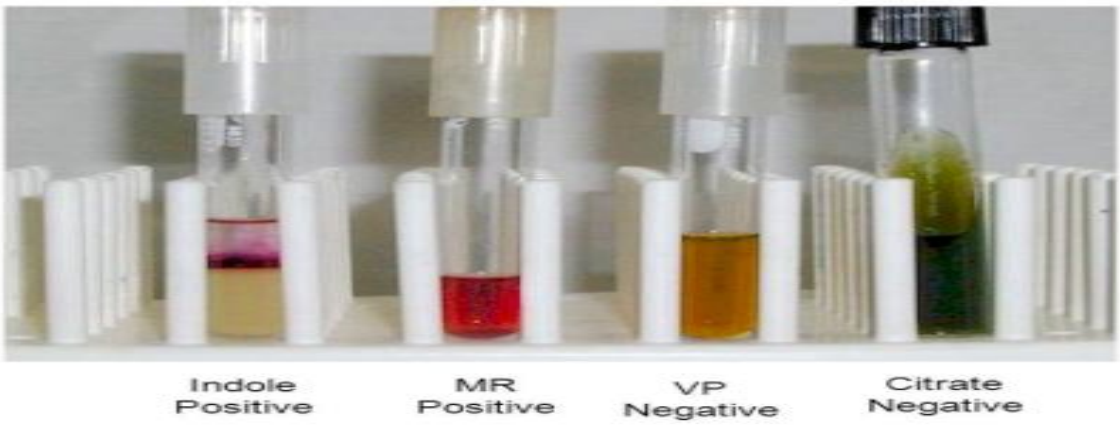
- **Glucose**, **Lactose**, **Mannitol**, **Maltose** fermented with **Acid** and **Gas**.



- Indole (+ve)
- Methyl Red (+ve)
- Voges Proskauer (-ve)
- Citrate (-ve)
- Urease not produced.
- H_2S (-ve)
- Motility test (+ve)

IMViC (+ + - -)

E. coli



3-*Klebsiella*

The genus was originally divided into 3 main species based on biochemical reactions. Today, 7 species with demonstrated similarities in DNA homology are known. These are (1) *Klebsiella pneumoniae*, (2) *Klebsiella ozaenae*, (3) *Klebsiella rhinoscleromatis*, (4) *Klebsiella oxytoca*, (5) *Klebsiella planticola*, (6) *Klebsiella terrigena*, and (7) *Klebsiella ornithinolytica*

Klebsiella pneumoniae

General characteristics:

- *K. pneumoniae* is typically colonizes **human mucosal** surfaces of the **oropharynx** and **gastrointestinal** (GI) tract. It is recorded to be associated with **pneumonia** in the alcoholic and diabetic patient population. *K. pneumoniae* is also a well-known cause of **community-acquired** pneumonia.
- It is mostly commonly isolated **Gram-negative**, **non-motile** bacteria possesses a **polysaccharide capsule**, which **protects against phagocytosis** and antibiotics and makes the colonies **moist** and **mucoid**. has a distinctive "**yeasty**" odor.

Antigenic Structure

Members of the genus *Klebsiella* form large capsules consisting of **polysaccharides (K antigens)** covering the **somatic** (O or H) antigens and can be identified by capsular swelling tests with specific antisera.

Cultural and biochemical characteristics

Klebsiella species exhibit **mucoid** growth, large polysaccharide capsules Table 3.1 and Figure 3.1, and **lack of motility**, and they usually give **positive test** results for **lysine decarboxylase** and **citrate**. *Klebsiella*, species usually give positive **Voges-Proskauer** reactions Table 3.2.

Table 3.1 Cultural Characteristics of *Klebsiella pneumoniae* on some laboratory media

Cultural Characteristics	Nutrient Agar Medium (NAM)	MacConkey Agar medium	Blood Agar Medium	EMB Agar medium
Shape	Circular	Circular	Circular	Circular
Size	2-3 mm	2-3 mm	2-3 mm	2-3 mm
Elevation	Dome-shaped	Convex	Dome-shaped	Convex
Surface	Mucoid	Mucoid	Mucoid	Mucoid
Color	Greyish white	Pink – Red	Greyish white	Pink – Purple
Structure	Translucent–Opaque	Opaque	Translucent–Opaque	Translucent–Opaque
Hemolysis	-----	-----	γ-Hemolysis (Non-hemolytic)	-----

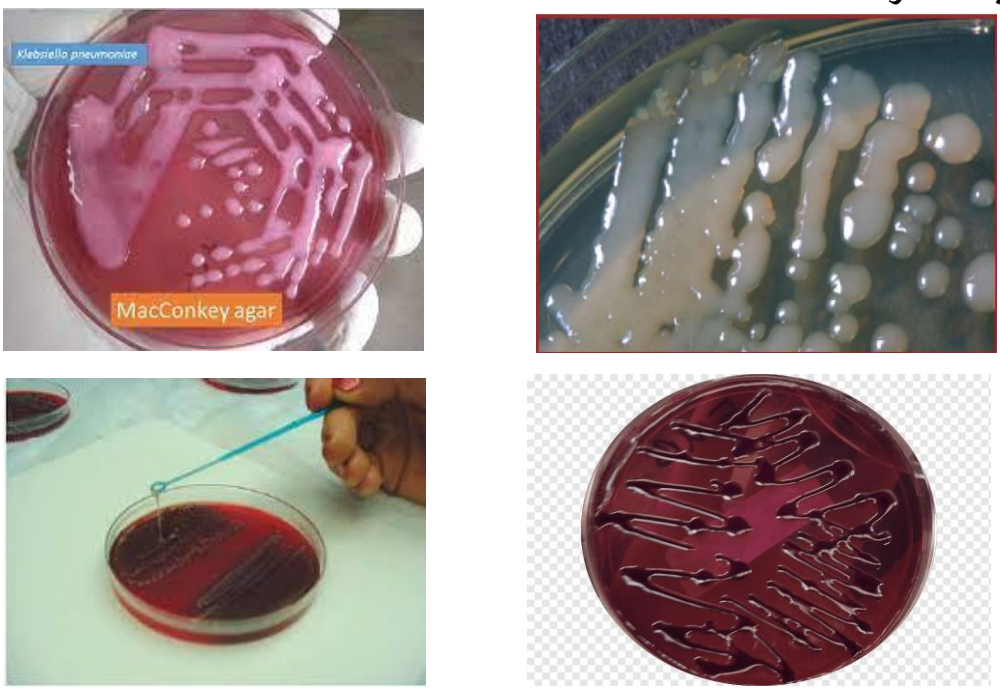


Figure 3.1 *Klebsiella pneumoniae* Growing on MacConkey, Nutrient, Blood and Eiosin Methylene Blue agar plates respectively.

Table 3.2 Biochemical tests of *Klebsiella pneumoniae*

Characteristics	*Klebsiella pneumoniae*
Capsule	+ve
Catalase	+ve
Citrate	+ve
Gelatin Hydrolysis	-ve
Gram Staining	-ve
H_2S	-ve
Indole	-ve
Motility	-ve
MR (Methyl Red)	-ve
Nitrate Reduction	+ve
Oxidase	-ve
Pigment	-ve
Shape	Rod
Spore	-ve
TSIA (Triple Sugar Iron Agar)	A/A
Urease	+ve
VP (Voges Proskauer)	+ve

Pathogenesis and Clinical Findings

Klebsiella species are present in the nasopharynx and feces of about 5% of normal individuals. The most commonly isolates species are *K. pneumoniae* and *K. oxytoca*. While *K. pneumoniae* may be isolated more frequently than *K. oxytoca* by clinical laboratories, both species are important human pathogens.

- *K. pneumoniae* can produce a **lobar pneumonia**, the production of "**currant jelly**" sputum.
- *Klebsiella* species also cause **urinary tract infections**, **wound** and **soft tissue infections**, and **bacteremia**/sepsis.
- *K. pneumoniae* has emerged as a cause of **community-acquired pyogenic liver abscess.**
- *Klebsiella* species responsible for **hospital-acquired infections**.
- *Klebsiella granulomatis* (formerly *Calymmatobacterium granulomatis*) causes a **chronic genital ulcerative disease**, and is thought to be a **sexually transmitted disease**.

Proteus, Morganella and Providencia

- Normal flora of the GI tract (except Providencia).
- Non-lactose ferment
- **All motile**, with Proteus **swarming** (Figure 3.2) motility with **peritrichous** flagella, **non-spore** forming,
- **Phenylalanine Deaminase** Test Positive (PA^{+}**Phenylalanine Agar**)
- Lysine deamination $^{+}$ (LIA (**Lysine Iron Agar**) Lysine Iron Agar (LIA) is used to **differentiate** enteric bacilli based on their ability to **decarboxylate** or **deaminate** lysine and produce **hydrogen sulfide** (H_2S). LIA also is used in combination with **Triple Sugar Iron Agar** to identify members of *Salmonella* and *Shigella* R/A)
- **Urea**se production was positive for most members and it's **strongly** $^{+}$ for **Proteus**
- TSI variable for every genus
- Indole test positive except ***P. mirabilis*** **is -ve**

Proteus species

- *P. mirabilis* and *P. vulgaris* are widely recognized human pathogens.
- The **spot-indole test** is useful for differentiation between the two most common Proteus species: is *P. vulgaris* indole **positive**, whereas *P. mirabilis* is **negative**.
- Isolated from urine, wounds, and ear and rarely from bacteremia
- Both produce **swarming** (Rauss phenomenon) colonies on **non-selective media** and have a distinctive "**burned chocolate**" odor
- Both are **strongly urease positive**
- Both are **phenylalanine deaminase positive**

Figure 3.2 Swarming phenominon of *Proteus* species

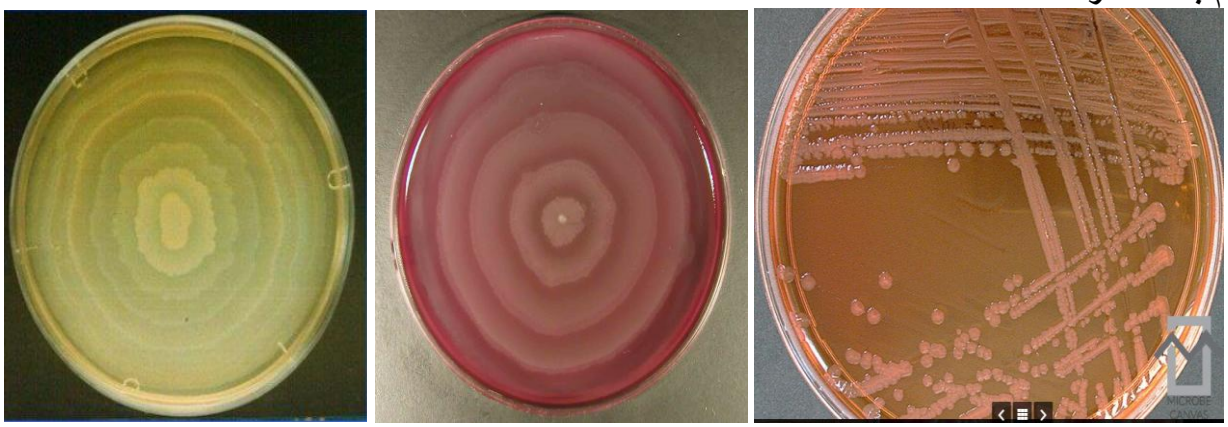
The swarming of Proteus can be inhibited by:

- **Increasing** the concentration of **agar** from 1-2% to 6%.
- **Incorporation** of **sodium azide**, **boric acid**, or **chloral hydrate** in the medium.
- The addition of **growth inhibitors** like **sulphonamides** to the medium.

Addition of **Teepol** (a surface-active agent), which is present in **Teepol Lactose agar**

Table 3.3: Culture Characteristics of *Proteus vulgaris*

Cultural Characteristics	Nutrient Agar Medium (NAM)	MacConkey Agar medium	Blood Agar Medium	EMB Agar medium
Shape	Irregular (due to swarming)	Circular	Irregular (due to swarming)	Circular
Size	1-2 mm	2-3 mm	1-2 mm	2-3 mm
Elevation	Effuse	Low Convex	Effuse	Effuse
Surface	Glistening	Smooth	Glistening	Glistening
Color	Greyish white	Colorless or Pale colored	Greyish white	Colorless
Structure	Translucent	Transparent	Translucent – Opaque	Transparent
Hemolysis	-----	-----	γ-Hemolysis (Non-hemolytic)	-----



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https://microbe-canvas.com/Bacteria/gram-negative-rods/facultative-anaerobic-3/catalase-positive-3/oxidase-negative/colistin-resistant/proteus-vulgaris.html

Antigenic Structure

The Proteus possess **thermostable**, somatic (O), and **thermolabile** flagellar (H) antigens upon which, several serotypes have been recognized.

Pathogenicity and Pathogenesis

The two species to most commonly produce infections in humans are ***P. mirabilis*** and ***P. vulgaris***. Both species produce **urease**, resulting in rapid **hydrolysis** of **urea** with liberation of **ammonia**.

- Thus, in urinary tract infections with *Proteus* species, the urine becomes **alkaline**, promoting **stone** formation and making **acidification** virtually impossible.
- The rapid **motility** of Proteus may also contribute to its **invasion** of the urinary tract.
- *P. mirabilis* causes urinary tract infections and occasionally other infections, such as **bloodstream** infection (frequently secondary due to a UTI) and respiratory tract infections.
- *P. vulgaris* is probably more frequently implicated in **wound** and **soft tissue infections** than UTIs.

Biochemical tests and identification

Basic Characteristics	Properties (Proteus mirabilis)
Capsule	Negative (-ve)
Catalase	Positive (+ve)
Citrate	Positive (+ve)

Basic Characteristics	Properties (Proteus mirabilis)
Flagella	Positive (+ve)
Gas from Glucose	Positive (+ve)
Gelatin Hydrolysis	Positive (+ve)
Gram Staining	Negative (-ve)
H_2S	Positive (+ve)
Indole	Negative (-ve)
Motility	Positive (+ve)
MR (Methyl Red)	Positive (+ve)
Nitrate Reduction	Positive (+ve)
Oxidase	Negative (-ve)
Pigment	Negative (-ve)
Shape	Rods
Spore	Negative (-ve)
Urease	Positive (+ve)
VP (Voges Proskauer)	Negative (-ve)
Fermentation of	
Glucose	Positive (+ve)
Lactose	Negative (-ve)
Enzymatic Reactions	
Acetate Utilization	Negative (-ve)
Esculin Hydrolysis	Negative (-ve)
Lipase	Positive (+ve)
Lysine decarboxylases	Negative (-ve)
Phenylalanine Deaminase	Positive (+ve)
Tryptophan Deaminase	Negative (-ve)

4. *Pseudomonads* and *Acinetobacter*

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

In the general population *P. aeruginosa* is carried by very few people but this can rise to over 30% after a stay in hospital.

The invasive potential of this organism means that it causes disease in a wide range of **hospital patients**. It is a particular problem to the **neutropenic patient** where it can cause fulminant septicaemia and death.

Patients undergoing **artificial ventilation** for **extended periods** in intensive therapy units may become colonized with *P. aeruginosa* and **secondary lower respiratory tract infection may follow**. **Extensive burns** become colonized and **septicaemia** develops in a proportion of patients. Multidose optical solutions can be contaminated by *P. aeruginosa* which, when used, can produce a rapidly *progressive corneal infection* which ends in **ocular perforation**.

Pseudomonas aeruginosa is an important pathogen for patients with *cystic fibrosis* where colonization with this organism is inevitable. Skin infection may arise in healthy subjects exposed to high infective doses such as **deep sea divers** and **users of contaminated hydrotherapy pools and Jacuzzi.**

***Pseudomonas aeruginosa*.**

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

Classification: There are more than 100 species in the genus Pseudomonas. There are two primary pathogens, ***P. pseudomallei*** and ***P. mallei***.

Morphology and Identification:

A. Typical Organisms *P. aeruginosa* is **motile** (except **P. mallei**) and **rod shaped**, measuring about 0.6 ×2 μm . It is **Gram-negative** and occurs as **single** bacteria, in **pairs**, and occasionally in **short chains**.

B. Culture *P aeruginosa* is an **obligate aerobe** that grows readily on many types of culture media, sometimes producing a **sweet** or **grape-like** or **corn taco–like odor**. Some strains **hemolyze** blood. *P aeruginosa* forms **smooth round colonies** with a **fluorescent greenish color <u>pyoverdin</u>** which gives a greenish color to the agar. It often produces the **non-fluorescent bluish pigment <u>pyocyanin</u>**, which **<u>diffuses</u>** into the agar. Other *Pseudomonas* species do not produce **<u>pyocyanin</u>**, Some strains **produce** the **dark red pigment <u>pyorubin</u>** or the **<u>black pigment pyomelanin</u>**.

C. Growth Characteristics *P aeruginosa* grows well at 37–42°C; its growth at 42°C helps differentiate it from other *Pseudomonas* species that produce fluorescent pigments. It is oxidase positive. It does not ferment carbohydrates, but many strains oxidize glucose.

Antigenic Structure and Toxins:

- Pili: Adhere to epithelial cells
- Exopolysaccharide: Anti-phagocytic property/ inhibit pulmonary clearance.
- Lipopolysaccharide: Endotoxic effect Enzymes
- Elastases: Digests protein (elastin, collagen, IgG)
- Proteases
- Hemolysins
- Phospholipases C (heat labile): Degrade cytoplasmic membrane components

Exotoxin A: Cytotoxic by blocking protein synthesis.

Endotoxin: like that of other gram-negative bacteria, causes the symptoms of sepsis and septic shock.

Pathogenesis: *Pseudomonas aeruginosa* is primarily an opportunistic pathogen that causes infections in hospitalized patients (e.g., those extensive burns), with in whom the skin host defenses are destroyed; in those with chronic respiratory disease (e.g., cystic fibrosis), in whom the normal clearance mechanisms are impaired; in those who are immunosuppressed;

- Urinary tract infection- chronic, complicated Urinary tract infection and associated with indwelling catheter.
- Wound infection of burn sites, pressure sores and ulcers.
- Septicaemia- “Ecthyma gangrenosum” skin lesion (haemorrhagic skin necrosis)

- Otitis externa- Malignant external ear infection in poorly treated diabetic patients.
- Pneumonia- Infection of the lung in patients with cystic fibrosis.
- Eye infection- Secondary to trauma or surgery.

Laboratory diagnosis:

Isolation Bacteria of the genus Pseudomonas grow readily on simple media such as **nutrient** or **blood** agar, and will also grow on the less inhibitory selective media such as **MacConkey**.

Specimen: pus, urine, sputum, blood, eye swabs, surface swabs Smear: Gram-negative rods. *Pseudomonas pseudomallei* is usually isolated from sputum, blood or pus from abscesses.

Culture: Obligate aerobe, grows readily on all routine media over wide range of temperature (5-42 ^{O}C). Bluish-green pigmented large colonies with characteristic "fruity" odor on culture media. Media can be made **selective for Pseudomonas** by the incorporation of one or more of the **antibiotics** or **disinfectants** to which it is naturally resistant such as **irgasin**, **cetrimide** or **nalidixic acid**.

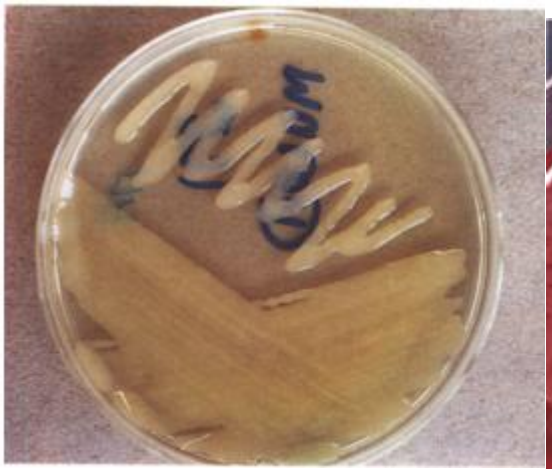
Colonies of *P. aeruginosa* are morphologically diverse and dwarf, rough, mucoid, rugose, coliform-like colonies and the more commonly encountered large convex, flat, oval colonies are described.

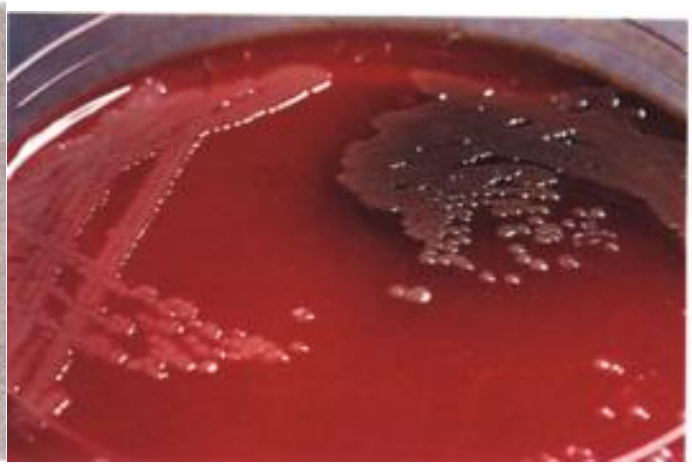
A culture of *P. aeruginosa* has a characteristic musty odour. The colonies of *P. pseudomallei* and *P. mallei* are slower to appear and are typically wrinkled with a **faint pinkish colour** developing after about five days.

P. aeruginosa are lactose and fructose oxidation, arginine dehydrolase, gelatinase and lysine decarboxylase.

In **Centrimide** agar: *Pseudomonas aeruginosa* colonies (greenish-blue in color) are medium sized and characterized by an irregular growth

In blood agar: Colonies of *Pseudomonas aeruginosa* surrounded by a wide zone of beta-hemolysis. Cultivation 48 hours in an aerobic atmosphere, 37°C.





***Pseudomonas aeruginosa* may produce the characteristic blue-green pigment or none at all**

Biochemical reactions: Oxidase positive Catalase-positive Citrate-positive Indole-negative Produce acid from carbohydrate by oxidation, not by fermentation.

Examples of biochemical tests used in the identification of Pseudomonas spp.

Species	*Oxidase*	*Lactose*	*ADH*	*ODC*	*Gelatin*
P. aeruginosa	+	–	+	–	+
P. pseudomallei	+	+	+	–	+

ADH: Arginine dehydrolase, ODC: Ornithine decarboxylase

Acinetobacter *Acinetobacter* species are aerobic, Gram-negative bacteria that are widely distributed in soil and water and can occasionally be cultured from skin, mucous membranes, secretions, and the hospital environment. *A baumannii* is the species most commonly isolated. *Acinetobacter lwoffii* and other species are isolated occasionally.

A. Morphology and Identification: Acinetobacters are usually coccobacillary or coccal in appearance; they resemble neisseriae on smears, because diplococcal forms predominate in body fluids and on solid media. Rod-shaped forms also occur, and occasionally the bacteria appear to be Gram-positive.

B. Culture: *Acinetobacter* grows well on most types of media used to culture specimens from patients. *Acinetobacter* recovered from patients with meningitis, bacteremia, female genital, sputum, skin, pleural fluid, and urine, usually.

5- ***Shigella and Salmonella***

Genus: Shigella

Familly: Enterobacteriaceae

Tribe: Escherichia

Genus: Salmonella

Discovered by Kiyoshi **Shiga** in 1898.

It is the causative agent of **human shigellosis**.

Classification: There are more than **40 serotypes**. The classification of shigellae relies on **biochemical** and **antigenic** characteristics (O antigens). The pathogenic species are *Shigella sonnei, Shigella flexneri, S. dysenteriae, and Shigella boydii*.

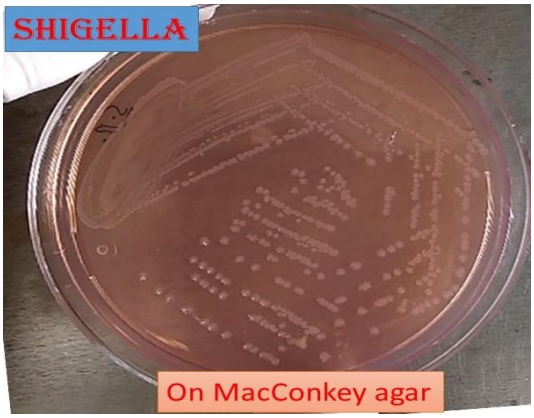
Important Properties:

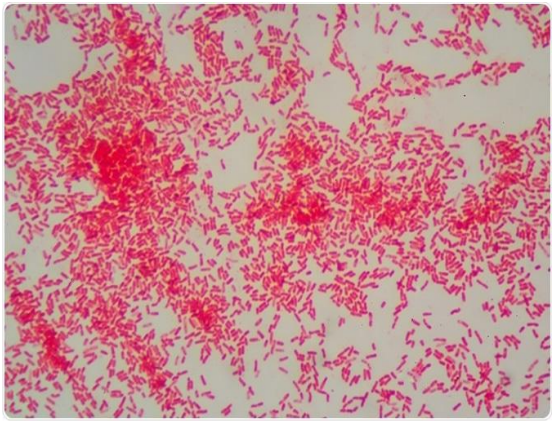
Shigellae are

- **short Gram-negative rods**.
- **non–lactose-fermenting.**
- **Resistant to bile salts**
- **Divided into four groups: A, B, C, and D according to (O) antigen.**

Shigella can be distinguished from **salmonellae** by three criteria:

- They produce **no gas** from the fermentation of **glucose**
- They **do not** produce **H_2S**
- They are **non-motile**.

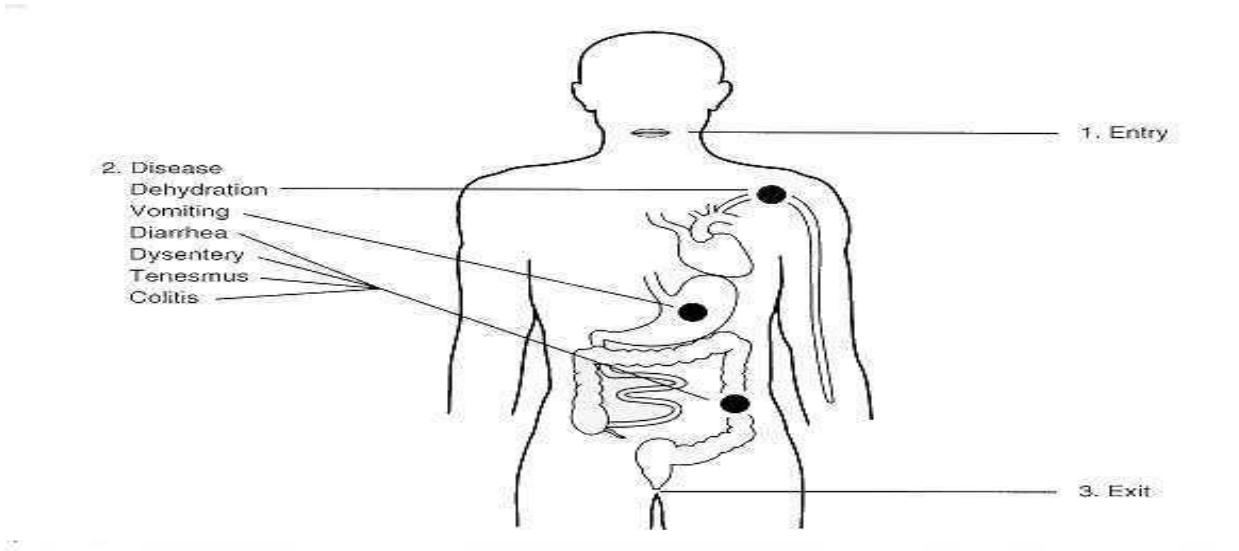




Virulence Factors:

1. K. capsular antigen
2. O. antigen (HL)
3. Shiga toxin: with **cytotoxic** and **neurotoxic** activity.

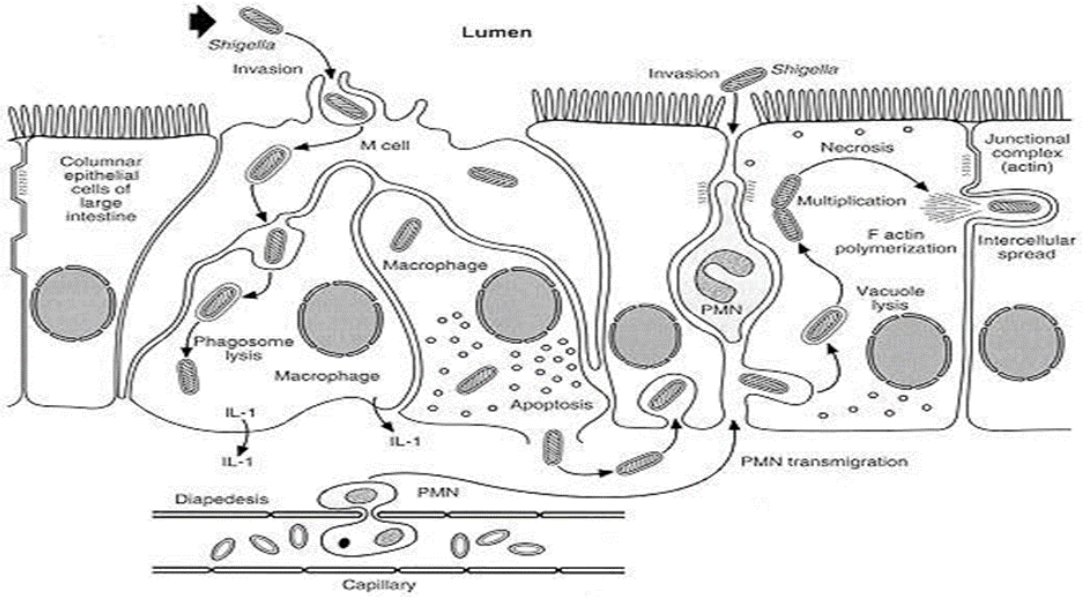
Pathogenesis of Shigella:



• Shigella causes **bacillary** dysentery , **Low** infective dose **< 200 bacilli** (can be transmitted **easily** unlike salmonella (**More serious and virulent** than salmonella)

• Incubation period = 1-3 days

• Upon ingestion, the bacteria **pass** through the **gastrointestinal** tract until they reach the **small intestine**. There they begin to **multiply** until they reach the **large intestine**. In the large intestine, the bacteria cause cell **injury** and the beginning stages of Shigellosis via two main mechanisms: **direct invasion of epithelial cells** in the large intestine and **production of enterotoxin** 1 and **enterotoxin** 2. High fever, chill, abdominal cramp and pain accompanied by tenesmus, bloody stool with mucus & WBC and HUS are involved.



Laboratory Diagnosis:

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

Culture: The materials are streaked on differential media (eg, MacConkey or EMB agar) and on selective media (Hektoen enteric agar or xylose-lysine-deoxycholate agar), which suppress other Enterobacteriaceae and Gram-positive organisms. **Colorless** (lactose-negative) colonies are inoculated into TSI agar. Organisms that **fail** to produce H_2S, that **produce acid** but **not gas** in the **butt** and an **alkaline** slant in TSI agar medium.

Salmonella Shigella (SS) Agar: Shigella **Clear**, **colorless**, **transparent**.

XLD- Agar: *Shigella flexneri* **Red Colonies**

TSI-Agar: Salmonella **Alkaline** slant/**acidic** butt (K/A); **- H_2S** and **Gas-**

Genus: *Salmonella*

Introduction:. The organisms are named after the American veterinary pathologist **Daniel Elmer Salmon** in 1885. Currently, there are three recognized species: *S. enterica*, *S. bongori and S. subterranean*. Salmonella is found worldwide in cold- and warm-blooded animals (including humans), and in the environment. They cause illnesses such as **typhoid fever**, **paratyphoid fever**, and foodborne illness.

Classification:

- The members of the genus Salmonella were originally classified on the basis of **epidemiology**; **host range**; **biochemical reactions**; and **structures** of the O, H, and Vi (**when present**) antigens.
- *Salmonella* spp. have both H and O antigens. There are over 60 different O antigens, and individual strains may possess several O and H antigens; the latter can exist in variant forms, termed '**phases'**. *Salmonella* serotype **Typhi** also has a **capsular polysaccharide** antigen referred to as 'Vi' (for **virulence**), which is related to **invasiveness**

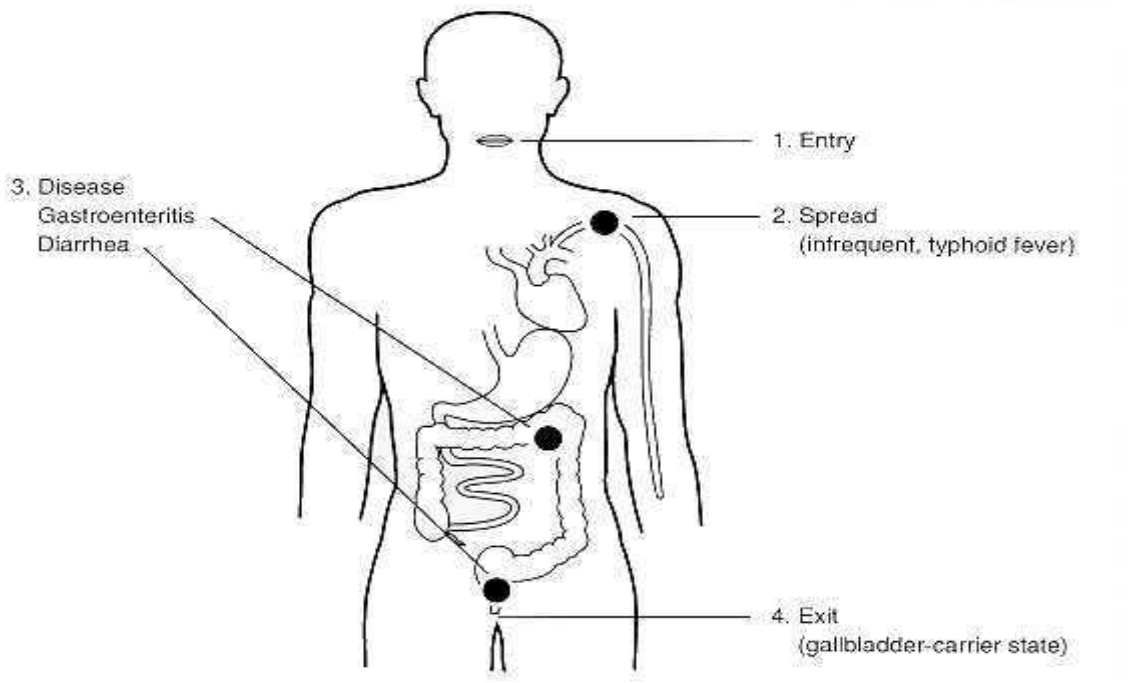
- Over 2500 serotypes are distinguished, most of which belong to the species *S. enterica*. However, many of these have been given binomial names (e.g. *Salmonella typhimurium* and *Salmonella enteritidis*), although they are not separate species. In clinical practice, laboratories identify microorganisms according to their binomial name.

Important Properties: Salmonellae are **motile** rods that characteristically ferment **glucose** and **mannose** without producing **gas** but do not ferment **lactose** or **sucrose**. Most salmonellae produce **H_2S**. They are often pathogenic for humans or animals when ingested.

Virulence Factors:

1. **Type III secretion systems:** which **facilitate** secretion of virulence factors of Salmonella into host cells.
2. **Endotoxin:** Endotoxin is responsible for many of the systemic manifestations of the disease caused by *Salmonella* spp.
3. **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**. These M cells typically **transport** foreign antigens, such as **bacteria** to the underlying **macrophages** for clearance.
4. **Acid tolerance response gene:** The acid tolerance response (ATR) gene protects *Salmonella* spp. from **stomach acids** and the **acidic** pH of the **phagosome**, thereby facilitating survival of bacteria in phagosomes
5. **Enzymes**: **Catalase** and **superoxide dismutase** are the enzymes that protect the bacteria from **intracellular killing** in **macrophages**.

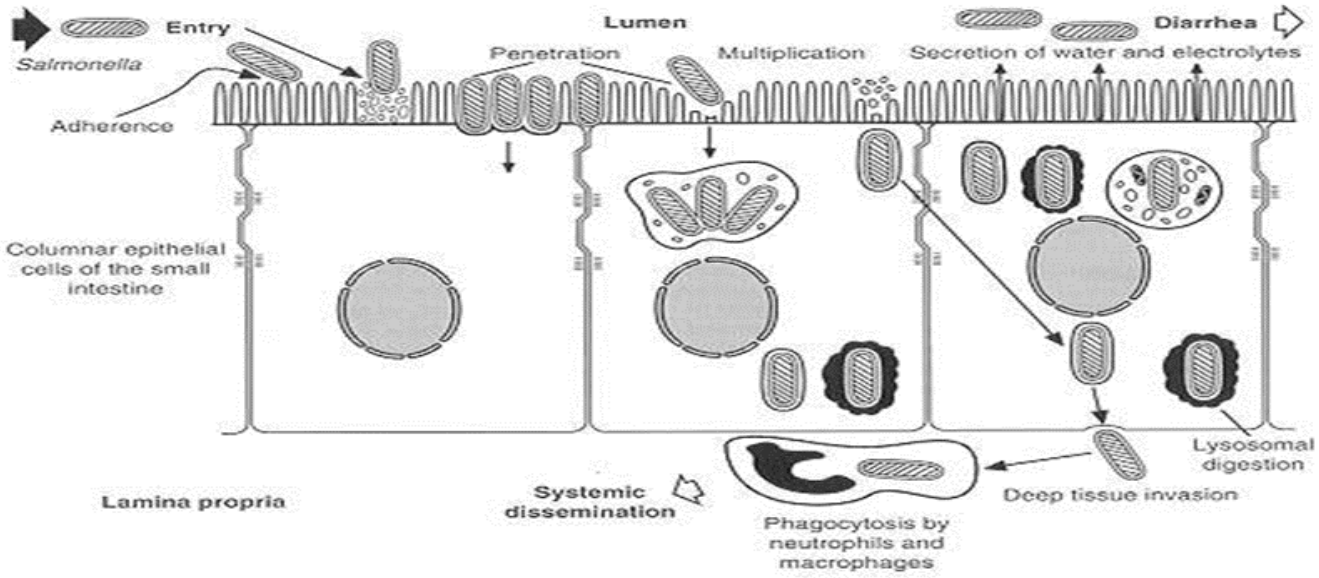
Pathogenesis of Salmonella: The three types of Salmonella infections (**enterocolitis**, **enteric fevers**, and **septicemia** وجود البكتريا بمجرى الدم وتكاثرها) have different pathogenic features.



(1) **Enterocolitis**: is characterized by an **invasion** of the **epithelial** and **sub-epithelial tissue** of the small and large intestines.

(2) In **typhoid** and other **enteric** fevers, infection begins in the **small** intestine, but few **gastrointestinal** symptoms occur.

(3) **Septicemia** accounts for only about 5−10% of Salmonella infections and occurs in one of two settings: a patient with an underlying **chronic disease**, such as **sickle cell anemia** or **cancer**, or a child with enterocolitis.



Laboratory Diagnosis:

In enterocolitis: the organism is most easily isolated from a stool sample in selective media e.g. XLD (Xylose lysine deoxycholate agar), DCA (deoxycholate citrate agar), salmonella-shigella (SS) agar, and enrichment media, e.g. **selenite** broth; identification of *Salmonella* spp. by biochemical agglutination tests. Phage typing can be used for typing individual strain.

Salmonella Shigella (SS) Agar: salmonella **colorless**, **transparent**, with a **black center** if **H_2S** is produced

XLD- Agar: *Salmonella* Typhi **red Colonies**, **black** centers.

TSI-Agar: Salmonella **Alkaline** slant/**acidic** butt (K/A); + H_2S and Gas +.

In the enteric fevers: a **blood culture** is the procedure most likely to reveal the organism during the first weeks of illness. **Stool cultures** may also be positive, especially in **chronic carriers** in whom the organism is secreted in the bile into the intestinal tract. **Urine culture** results may be positive after the second week.

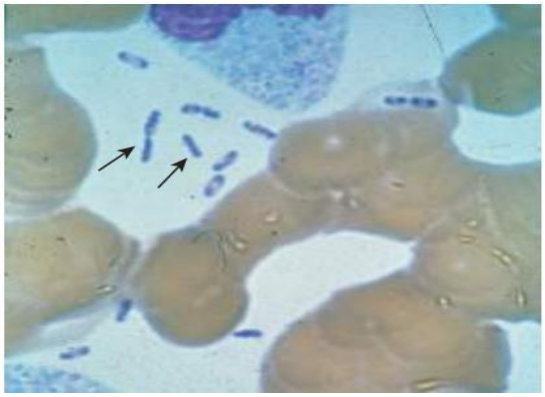
Serologic Methods:

I. Agglutination test

II. Tube dilution agglutination test (Widal test): Serum agglutinins rise sharply during the **second** and **third** weeks of *S* serotype Typhi infection.

6-Yersinia

Yersinia pestis is short, **pleomorphic**, Gram-negative rods that often exhibit **bipolar staining (**A bipolar stain is **a particular staining pattern those colors only the two opposite poles of the microorganism in question, leaving the rest of the bacterium unstained or a lighter color)** with special stains such as Wright, Giemsa, Wayson, or methylene blue, appear as single cells or as pairs or short chains in clinical material. They are catalase positive and microaerophilic or facultatively anaerobic.



Cultural and Biochemical Characteristics

It is non-motile, grows as a facultative anaerobe on many bacteriologic media, and can be readily isolated when **sterile** specimens such as **blood** or **lymph node aspirates** are plated onto **sheep blood agar**. Growth is more rapid when agar plates are incubated at **28°C**. In cultures on sheep blood agar incubated at 37°C, colonies may be smaller when compared to colonies from agar plates incubated at 28°C. Colonies of *Y. pestis* are typically **gray** to **white**, sometimes **opaque**, and are 1–1.5 mm in diameter with **irregular edges**; the organism does not produce **hemolysis**.

Antigenic Structure

All yersiniae possess

- **lipopolysaccharides** that have endotoxic activity when released.
- *Y. pestis* and *Y. enterocolitica* also produce antigens and toxins that act as virulence factors.
- They have type III secretion systems that consist of a membrane-spanning

complex that allows the bacteria to inject proteins directly into cytoplasm of the host cells.

- The virulent yersiniae produce V and W antigens.

Clinical Findings

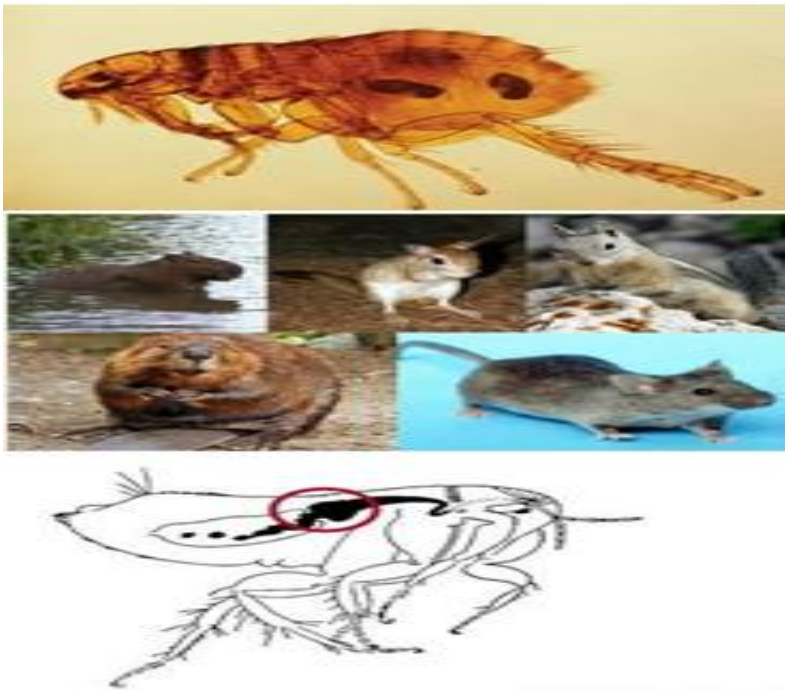
The clinical manifestations of **plague** depend on the **route** of **exposure**, and three forms of the disease have been described:

- bubonic plague,الطاعون الدبلي (the most common, incubation period of 2–7 days, sudden onset of high fever and development of painful lymphadenopathy, tender lymph nodes (buboes) in the neck, groin, or axillae).
- pneumonic plague, commonly with greatly enlarged
- septicemic plague. (Occur spontaneously or as a complication of untreated bubonic plague, *Y. pestis* multiplies intravascularly, can be seen in blood smears)
- Patients typically present with a sudden onset of high fever, chills, and weakness, progressing rapidly to septic shock with associated disseminated intravascular coagulation, hypotension (septic shock),
- altered mental status, and renal and cardiac failure.
- Bleeding into skin and organs can also occur. Vomiting and diarrhea may develop during the early stages of septicemic plague. Terminally,
- signs of pneumonia and meningitis can appear.

<u>Pathogenesis and Pathology</u>

When a flea برغوث feeds on a rodent قوارض infected with Y. pestis, the ingested organisms multiply in the gut of the flea and, helped by the coagulase, block its proventriculus االمعاء من امامي جزء so that no food can pass through. Subsequently, the "blocked" and hungry flea bites ferociously بشراسة, and the aspirated blood, contaminated with Y. pestis from the flea, is vomited into the bite wound. The inoculated organisms may be phagocytosed by polymorphonuclear cells and macrophages.

The Y. pestis organisms are killed by the polymorphonuclear cells but multiply in the macrophages; because the bacteria are multiplying at 37°C, they produce the antiphagocytic protein and subsequently are able to resist phagocytosis. The pathogens rapidly reach the lymphatics, and an intense hemorrhagic inflammation develops in the enlarged lymph nodes, which may undergo necrosis and become fluctuant.



Diagnostic Laboratory Tests

A. Specimens

Blood is taken for culture and aspirates of enlarged lymph nodes for smear and culture. Acute and convalescent نقاهة sera be examined for antibody levels. In pneumonia, sputum is cultured; in possible meningitis, cerebrospinal fluid is taken for smear and culture.

B. Smears

Wright, Giemsa, or Wayson stains may be more useful when staining material from a suspected buboes or a positive blood culture result because of the striking bipolar appearance (safety pin shape) of the organism using these stains that is not evident on a direct Gram-stain. More specific direct staining methods include the use of fluorescent antibody stains targeting the capsular F1 antigen.

C. Culture

All materials are cultured on blood, chocolate, and MacConkey agar plates and in brain–heart infusion broth. Growth on solid media may be slow, requiring more than 48 hours, but blood culture results are often positive in 24 hours. Y. pestis produces non-lactose-fermenting colonies on MacConkey agar, and it grows better at 28°C than at 37°C. The organism is

- ✓ catalase positive;
- ✓ indole, oxidase, and urease negative;
- ✓ Non-motile.
- ✓ Definite حتمي identification of cultures is best done by immunofluorescence or by lysis by a specific Y. pestis bacteriophage. All cultures are highly infectious and must be handled with extreme caution inside a biological safety cabinet.

7-Vibrio

Vibrios are among the most common bacteria in marine and estuarine waters, worldwide. They are comma-shaped, curved, and sometimes straight facultatively anaerobic, fermentative rods; they are catalase and oxidase positive, and most species are motile by means of monotrichous or multitrichous polar flagella. Vibrios can grow within a broad temperature range (14–40°C), and all species require sodium chloride (NaCl) for growth; hence the term halophilic ("salt loving"). *V. cholerae* serogroups O1 and O139 cause cholera in humans, and other vibrios, most commonly *V. parahaemolyticus* and *V. vulnificus*, are important human pathogens, causing skin and soft tissue infections, sepsis, or gastroenteritis.

VIBRIO CHOLERAE

The bacterium *V. cholerae* is the cause of cholera. The epidemiology of cholera closely parallels the recognition of *V. cholerae* transmission in water and the development of sanitary water systems. Cholera is associated with poor sanitation, as well as direct contact with or consumption of contaminated water and/or food (eg, water used for drinking, cooking, bathing, and crop irrigation).

Morphology and Identification

A. Typical Organisms

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

B. Culture

V. cholerae produces convex, smooth, round colonies that are opaque and granular in transmitted light. *V. cholerae* and most other vibrios grow well at 37°C on routine agar media to recover enteric bacteria (eg, blood agar and MacConkey agar); however, selective agars for *Vibrio* species, such as **thiosulfate-citrate-bile salts-sucrose (TCBS) agar** and enrichment broth (eg, alkaline peptone broth), can also be used to recover vibrios, especially from specimens (eg, stool) when a mixture of organisms is expected. All vibrios, including *V. cholerae*, grow well on TCBS agar; *V. cholerae* produces yellow colonies (sucrose fermented) on TCBS agar that are readily visible against the dark-green background of the agar. Non-sucrose-fermenting vibrios (eg, most strains of *V. parahaemolyticus* and *V. vulnificus*) produce green colonies on TCBS agar.

Characteristically, vibrios grow at a very high pH (8.5–9.5) and are rapidly killed by acid. To ensure optimal recovery of vibrios, stool specimens should be collected early in the course of the diarrheal illness; prompt inoculation onto appropriate agar media is necessary.

If processing of specimens may be delayed, the stool specimen should be mixed in a Cary-Blair transport medium and refrigerated. In areas where cholera is endemic, direct cultures of stool on selective media, such as TCBS, and enrichment

broth cultures (eg, alkaline peptone water with 1% NaCl, pH 8.5) are appropriate. In the United States and other countries where cholera is rare, routine use of TCBS agar for stool cultures in clinical laboratories is generally not necessary or cost effective; exceptions may be made if recovery of other vibrios (eg, *V. parahaemolyticus*) is a frequent and/or seasonal occurrence (eg, coastal U.S. regions with regular and frequent consumption of bivalve mollusks and crustaceans).

The Medically Important Vibrios

Organism	Human Disease
V. cholerae serogroups O1 and O139	Epidemic and pandemic cholera
V. cholerae serogroups non-O1/non-O139	Cholera-like diarrhea; mild diarrhea; rarely, extraintestinal infection
V. parahaemolyticus	Gastroenteritis, wound infections, septicemia
V. vulnificus	Gastroenteritis, wound infections, septicemia

C. Growth Characteristics

V. cholerae regularly ferments sucrose and mannose but not arabinose. A positive oxidase test result is a key step in the preliminary identification of *V. cholerae* and other vibrios. While most *Vibrio* species are halophilic, requiring the presence of NaCl (range from < 0.5–4.5%) to grow, *V. cholerae* can grow on most agar media without additional salt.

FIGURE Gram-stain of *V. cholerae.* Often they are comma shaped or slightly curved (arrows) and 1 × 2 to 4 µm. Original magnification ×1000.

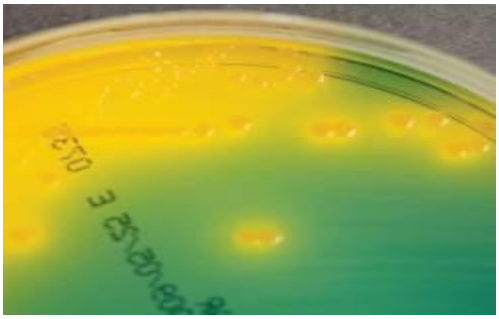


FIGURE Colonies of *V. cholerae* growing on thiosulfate, citrate, bile salts, and sucrose agar. The glistening yellow colonies are 2–3 mm in diameter and are surrounded by a diffuse yellowing of the indicator in the agar up to 1 cm in diameter. The plate is 10 cm in diameter.

Antigenic Structure and Biologic Classification

Many vibrios share a single heat-labile flagellar H antigen. Antibodies to the H antigen are probably not involved in the protection of susceptible hosts.

V. cholerae has O lipopolysaccharides that confer serologic specificity. Based on the O antigen, there are over 200 serogroups; however, only *V. cholerae* strains of serogroup O1 and O139 cause epidemic and pandemic cholera. Occasionally, non-O1/non-O139 *V. cholerae* strains have been described as causes of cholera-like diarrheal disease. Antibodies to the O antigens tend to protect laboratory animals against infections with *V. cholerae*.

The *V. cholerae* serogroup O1 antigen has determinants that make possible further subtyping; these serotypes are Ogawa, Inaba, and Hikojima. Furthermore, two biotypes of epidemic *V. cholerae* have been defined, classic and El Tor. The El Tor biotype produces a hemolysin, gives positive results on the Voges-Proskauer test, and is resistant to polymyxin B. Molecular techniques can also

be used to type *V. cholerae*. Typing is used for epidemiologic studies, and tests generally are done only in reference laboratories.

V. cholerae O139 is very similar to *V. cholerae* O1 El Tor biotype. *V. cholerae* O139 does not produce the O1 lipopolysaccharide and does not have all the genes necessary to make this antigen. *V. cholerae* O139 and other non-O1 *V. cholerae* strains, as well as *V. vulnificus* produce acidic polysaccharide capsules; however, *V. cholerae* O1 does not make a capsule.

Vibrio cholerae Enterotoxin

V. cholerae produce a heat-labile enterotoxin with a molecular weight (MW) of about 84,000, consisting of subunits A (MW, 28,000) and B (see Chapter 9). Ganglioside GM1 serves as the mucosal receptor for subunit B, which promotes entry of subunit A into the cell. Activation of subunit A 1 yields increased levels of intracellular cyclic adenosine monophosphate (cAMP) and results in prolonged hypersecretion of water and electrolytes. There is increased sodium-dependent chloride secretion, and absorption of sodium and chloride by the microvilli is inhibited. Electrolyte-rich diarrhea occurs with as much as 20–30 L/day, resulting in dehydration, shock, acidosis, and death. The genes for *V. cholerae* enterotoxin are located on the bacterial chromosome. Cholera enterotoxin is antigenically related to LT of *Escherichia coli* and can stimulate the production of neutralizing antibodies. However, the precise role of antitoxic and antibacterial antibodies in protection against cholera is not clear.

Diagnostic Laboratory Tests

A. Specimens

As stated above, stool specimens should be collected early in the course of the diarrheal illness and inoculated within 2–4 hours of collection onto appropriate agar media, to ensure optimal recovery of vibrios. If processing of specimens may be delayed, the stool specimen should be mixed in a Cary-Blair transport medium and refrigerated.

B. Smears

Direct detection of *V. cholerae* on smears made from stool samples is not distinctive of the organism, and therefore not routinely recommended. Dark-field or phase-contrast microscopy can be used to detect *V. cholerae* O1 directly from stool samples or the enrichment broth. Observation of “shooting

star" motility is suggestive of *V. cholerae* O1; if the motility is extinguished after mixing the sample with a polyvalent O1 antiserum, the organism is confirmed as *V. cholerae* O1. However, if there is no motility or the type of motility does not change after applying the antiserum, the organism is not *V. cholerae* O1.

C. Culture

Vibrios, including *V. cholerae*, grow well on most agar media (including MacConkey and blood agar) used in clinical laboratories. Some strains of *V. cholerae* may however be inhibited on MacConkey agar. Growth is rapid in alkaline peptone broth or water, containing 1% NaCl with a pH of 8.5, or on TCBS agar; typical colonies can be picked in 18 hours of growth.

For enrichment, a few drops of stool can be incubated for 6–8 hours in taurocholate peptone broth (pH, 8.0–9.0); organisms from this culture can then be stained or subcultured onto other appropriate agar media. Accurate identification of vibrios, including *V. cholerae*, using commercial systems and kit assays is quite variable. MALDI-TOF MS is a promising newer methodology for identification of vibrios, and studies have shown rapid and reproducibly accurate identification for *V. parahaemolyticus*.

8- *CAMPYLOBACTER*

Introduction:

Campylobacters cause both diarrheal and systemic diseases, and are among the most widespread causes of infection, worldwide. *Campylobacter* infections of wild and domesticated animals, which are also the natural reservoirs for these organisms, are also widespread. *C. jejuni* is the prototype organism in the group and is a very common cause of diarrhea in humans. Other campylobacters, less commonly isolated from humans, include *C. fetus*, *C. coli*, and *C. upsaliensis.*

Scientific Content:

Morphology and Identification

A. Typical Organisms

C. jejuni and other campylobacters are curved, comma-, or S-shaped, Gram-negative, non-spore-forming rods; they have also been described as having "sea gull wing" shapes. Campylobacters are motile, with a single polar flagellum at one or both ends, but some organisms may lack flagella all together.

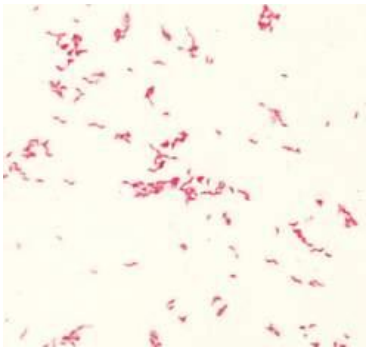
B. Culture

Campylobacter species, including *C. jejuni*, multiply at a slower rate when compared to other Gram-negative, enteric bacteria; therefore, selective media, containing various antibiotics (eg, Campy-Blood agar and Skirrow's media) are needed for isolation of campylobacters from stool specimens. *Campylobacter* species require a microaerobic atmosphere, containing reduced O2 (5–7%) and increased 10% CO2 for incubation and optimal growth.

A relatively simple way to produce the incubation atmosphere is to place the plates in an anaerobe incubation jar without the catalyst and to produce the gas with a commercially available gas-generating pack or by gas exchange. Furthermore, most campylobacters grow best at 42°C, although growth can be seen on agar media with incubation between 36°C and 42°C. Incubation of primary plates for isolation of *C. jejuni* should always be at 42°C. Several selective agar media are in widespread use for isolation of campylobacters; Skirrow's medium contains vancomycin, polymyxin B, and trimethoprim to inhibit growth of other bacteria, but this medium may be less sensitive than other commercial products that contain charcoal, other inhibitory compounds, as well as cephalosporin antibiotics.

These selective media are suitable for isolation of *C. jejuni* and *C. coli* at 42°C. However, *C. upsaliensis*, while growing at 42°C, is not recovered on selective media, and *C. fetus* shows variable growth at 42°C, and may not be recovered at that temperature.

The colonies of *Campylobacter* species may have different appearances; generally, the colonies tend to be colorless or gray. They may be watery and spreading or round and convex, and both colony types may appear on one agar plate. Hemolysis on blood-containing agar media is not observed.



Gram-stain of *C. jejuni* showing "comma"- or "gull wing"-shaped Gram-negative bacilli (*arrows*). Campylobacters stain faintly and can be difficult to visualize.

C. Growth Characteristics

Because of the selective media and incubation conditions for growth, an abbreviated set of tests is usually all that is necessary for further identification of campylobacters. *C. jejuni* as well as *C. coli* are positive for both oxidase and catalase. Campylobacters do not oxidize or ferment carbohydrates. Gramstained smears show typical morphology. Nitrate reduction, hydrogen sulfide production, hippurate hydrolysis tests, and antimicrobial susceptibilities can be used for further identification of species. A positive hippurate hydrolysis test distinguishes *C. jejuni* from the other *Campylobacter* species.

Antigenic Structure and Toxins

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

Diagnostic Laboratory Tests

A. Specimens

Diarrheal stool is the preferred specimen when attempting to isolate campylobacters in patients with gastrointestinal illness. Rectal swabs may also be acceptable specimens. *C. jejuni* and *C. fetus* may occasionally be recovered from blood cultures usually from immunocompromised or elderly patients.

B. Smears

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

C. Culture

Culture on the selective media as described earlier is the definitive test to diagnose *C. jejuni* enteritis. If *C. fetus* or another species of *Campylobacter* is suspected, an agar medium without cephalosporins should be used and incubation at 36–37°C is necessary.

9- *HELICOBACTER PYLORI*

Members of the genus *Helicobacter* are usually spiral, curved, or fusiform rod-shaped Gram-negative bacteria. *Helicobacter* species have been isolated from the gastrointestinal and hepatobiliary tract of many different mammalian hosts, including humans, dogs, cats, pigs, cattle, and other domestic and wild animals. The various helicobacters can be divides into two groups: *Helicobacter* species that primarily colonize the stomach (gastric helicobacters), and those that colonize the intestines (enterohepatic helicobacters). Humans are the primary host-reservoir for *H. pylori*, which is a spiral-shaped, Gram-negative, catalase- and oxidase-positive, and urease positive rod. *H. pylori* is associated with antral gastritis, duodenal (peptic) ulcer disease, gastric ulcers, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphomas.

Morphology and Identification

A. Typical Organisms

Helicobacter species, including *H. pylori*, have many characteristics in common with campylobacters. *Helicobacter* species are motile and have single and/or multiple monopolar flagella that are typically sheathed and can vary greatly in their flagellum morphology.

B. Culture

While *H. pylori* can be readily isolated from gastric biopsy specimens, culture sensitivity may be limited by several factors, including delayed specimen transport and processing, prior antimicrobial therapy, or contamination with other mucosal bacteria. Special transport media (eg**, Stuart's transport medium**) should be used to main the organisms' viability when transport to the laboratory is anticipated to exceed 2 hours.

H. pylori usually grows within **3–6 days when incubated at 37°C** in a microaerophilic and humid atmosphere; however, incubation of up to 14 days may be necessary before resulting the culture as negative. To achieve a higher yield for recovery of the organism, the biopsy specimen may be homogenized prior to streaking onto the agar plate. The agar media for primary isolation include enriched agar media supplemented with blood and/or blood products (eg, **Chocolate agar**) or antibiotic-containing media such as **Skirrow's medium**, in order to suppress overgrowth by other competing bacterial flora. The colonies have varying appearance on blood agar ranging from gray to translucent and are 1–2 mm in diameter.

C. Growth Characteristics

H. pylori is oxidase positive and catalase positive, and has a characteristic Gram-stain morphology; the organism is motile, and is a strong producer of urease.

Pathogenesis and Pathology

H. pylori is able to survive in the acidic environment of the stomach and ultimately establish lifelong colonization of the gastric mucosa in the absence of antimicrobial treatment. While *H. pylori* grows optimally at a **pH of 6.0–7.0**, it would be killed or not grow at the pH within the gastric lumen (**pH 1–3**). Several factors contribute to the organism's ability to overcome the acidic environment of the stomach, contributing to colonization, inflammation, changes in gastric acid production, and tissue destruction.

Gastric mucus is relatively impermeable to acid and has a strong buffering capacity. On the lumen side of the mucus, the pH is low (1.0–3.0); on the epithelial side, the pH is about 5.0–7.0. After entering the stomach, *H. pylori* utilizes its urease activity to neutralize the gastric acid; intracellular urease activity as well as urease located on the bacterial cell surface allow for the **breakdown of urea into ammonia and CO 2**; NH3 is converted to ammonium (**NH4+**) and extruded from the bacterial cell leading to neutralization of the gastric acid.

Diagnostic Laboratory Tests

A. Specimens

Gastric biopsy specimens can be used for histologic examination or minced in saline and used for culture. Blood is collected for determination of serum antibodies. Stool samples may be collected for *H. pylori* antigen detection. Diagnostic testing methods are summarized in Table 1

B. Smears

The diagnosis of gastritis and *H. pylori* infection can be made histologically; this approach is generally more sensitive than culture. A gastroscopy procedure with biopsy is required. Routine stains (eg, hematoxylin & eosin stain) demonstrate acute/chronic gastritis, and Giemsa or special stains (eg, silver stains or immunohistochemical stains) can show the curved or spiral-shaped organisms.

C. Culture

Since *H. pylori* organisms adhere to the gastric mucosa, the bacteria cannot be recovered from stool specimens like other gastrointestinal pathogens. As described above, culture is usually performed when patients are not responding to treatment, and there is a need to perform antimicrobial susceptibility testing. Tissue for culture is obtained by endoscopy and biopsy of the gastric mucosa.

TABLE 1 Diagnostic Testing Methods for *H. pylori*

Testing Modality	Advantages	Disadvantages
Invasive Testing		
Histology	Allows for detection of organism and assessment of the extent of tissue damage (eg, ulceration).	Requires mucosal biopsy and sample processing in Pathology. Requires several days for results.
Urease detection in tissue	Rapid test, with most positive results being obtained within 2 hours.	Requires mucosal biopsy. May give false-positive results with bacterial overgrowth. False-negative tests when patient receives PPI treatment.
Microbiologic culture	Allows for antimicrobial susceptibility testing.	Requires several days for results (slow growth of organisms); requires special and careful specimen processing (false-negative results due to prolonged specimen transport and processing in suboptimal conditions).
Noninvasive Testing		
Serology	Noninvasive; inexpensive; "rapid" turn-around-time for results. Useful for epidemiologic purposes and evaluation of symptomatic patients.	Provides no assessment of extent of tissue damage/pathology. Not suitable to assess completion of antimicrobial therapy. Usually cannot differentiate between acute and past infection.
Urea breath test	Relatively noninvasive and rapid. Valuable for assessment of therapy (eradication of infection).	Requires expensive instrumentation for testing; less convenient than serology. False-negative results when patient receives PPI therapy. Provides no assessment of extent of tissue damage/pathology.
Stool antigen test	Relatively noninvasive, convenient, rapid, and inexpensive. Most valuable for assessment of response to antimicrobial therapy.	Not useful to assess the extent of tissue damage/pathology.

D. Antibodies

Several assays have been developed to detect serum antibodies specific for *H. pylori*. While testing for IgG serum antibodies against *H. pylori* is useful to confirm the exposure to the organism, either for epidemiologic purposes or for the evaluation of a symptomatic patient, the antibody titers do not typically correlate with the severity of the disease. Furthermore, IgM antibodies disappear rapidly during the initial course of an acute infection, and are of little diagnostic value. The relevance of IgA testing remains controversial, and both IgA and IgG serum antibodies persist even if the *H. pylori* infection is eradicated. The role of antibody testing in differentiating active *H. pylori* infection from past infection and/or completion of therapy is therefore limited.

10-*Haemophilus*

General Characteristics

The genus *Haemophilus* contains significant genetic diversity. Members of the genus are small, nonmotile, pleomorphic gram-negative bacilli. The cells are typically coccobacillary or short rods. Species of the genus *Haemophilus* require protoporphyrin IX (a metabolic intermediate of the hemin biosynthetic pathway), referred to as **X factor** and **V factor**, nicotine adenine dinucleotide (NAD), or nicotine adenine dinucleotide phosphate (NADP) for *in vitro* growth. *Haemophilus* spp. are facultative anaerobes enhanced in a 5% to 7% CO 2-enriched atmosphere. The morphologic and physiologic features of individual species are presented in the discussion of laboratory diagnosis.

Epidemiology

As presented in Table 1, except for *Haemophilus ducreyi, Haemophilus* spp. normally inhabit the upper respiratory tract of humans. Asymptomatic colonization with *Haemophilus influenzae* type b is rare. Although *H. ducreyi* is only found in humans, the organism is not part of our normal microbiota, and its presence in clinical specimens indicates infection.

TABLES-1

Organism	Habitat (Reservoir)	Mode of Transmission
Haemophilus influenzae	Normal microbiota: upper respiratory tract	Person-toperson: respiratory dropletsEndogenous strains
Haemophilus ducreyi	Not part of normal human microbiota; only found in humans during infection	Person-toperson: sexual contact
Other Haemophilus spp. ***Haemophilus parainfluenzae*** ***Haemophilus parahaemolyticus***	Normal microbiota: upper respiratory tract	Endogenous strains

Pathogenesis and Spectrum of Disease

Production of a capsule and factors that mediate bacterial attachment to human epithelial cells are the primary virulence factors associated with *Haemophilus* spp. In general, infections caused by *H. influenzae* are often systemic and life-threatening, whereas infections caused by nontypeable (do not have a capsule) strains are usually localized. Most serious infections caused by *H. influenzae* type b are biotypes I and II.

Most *H. influenzae* infections are now caused by nontypeable strains (NTHi). Transmission is often via respiratory secretions. The organism is able to gain access to sterile sites from colonization in the upper respiratory tract. Clinical infections include otitis media (ear infection), sinusitis, bronchitis, pneumonia, and conjunctivitis. Immunodeficiencies and chronic respiratory problems such as chronic obstructive pulmonary disease may predispose an individual to infection with NTHi.

Chancroid is the sexually transmitted disease caused by *H. ducreyi* . The initial symptom is the development of a painful genital ulcer and inguinal lymphadenopathy.

TABLES-2

Organism	Virulence Factors	Spectrum of Disease and Infections
Haemophilus influenzae	Capsule: Antiphagocytic, type b most common. Additional cell envelope factors mediate attachment to host cells. Unencapsulated strains: pili and other cell surface factors mediate attachment.	Encapsulated strains: Meningitis Epiglottitis Cellulitis with bacteremia Septic arthritis Pneumonia Nonencapsulated strains: Localized infections Otitis media Sinusitis Conjunctivitis Immunocompromised patients: Chronic bronchitis Pneumonia Bacteremia
Haemophilus influenzae	Uncertain; probably similar to those of other *H. influenzae.*	Purulent conjunctivitis single strain identified as the Brazilian purpuric fever, high mortality in children between ages 1 and 4; infection includes purulent meningitis, bacteremia, high fever, vomiting, purpura (i.e., rash), and vascular collapse.
Haemophilus ducreyi	Uncertain, but capsular factors, pili, and certain toxins are probably involved in attachment and penetration of host epithelial cells.	Chancroid; genital lesions progress from tender papules (i.e., small bumps) to painful ulcers with several satellite lesions; regional lymphadenitis is common.

Laboratory Diagnosis

Specimens

Specimens consist of expectorated sputum and other types of respiratory specimens, pus, blood, and spinal fluid for smears and cultures depending on the source of the infection.

Direct Observation

To increase the sensitivity of direct Gram stain examination of body fluid specimens, especially CSF, specimens may be centrifuged (2000 rpm for 10 minutes), and the smear is prepared from the pellet deposited in the bottom of the tube. Gram stains of the smears from clinical specimens must be examined carefully. *Haemophilus* spp. stain a pale pink and may be difficult to detect in the pink background of proteinaceous material often found in clinical specimens.

Antigen Detection

H. influenzae type b capsular polysaccharide in clinical specimens, such as CSF and urine, can be detected directly using commercially available particle agglutination assays.

Molecular Methods

Rapid screening procedures are very useful for patient therapy and evaluating outbreaks and have been developed for detection from CSF, plasma, serum, and whole blood. A polymerase chain reaction (PCR) for *H. influenzae* capsular types a and f has been developed.

Incubation Conditions and Duration

Most strains of *Haemophilus* spp. are able to grow aerobically and anaerobically (facultative anaerobes). Growth is stimulated by 5% to 10% carbon dioxide (CO2). It is recommended that cultures be incubated in a candle jar, CO2 pouch, or CO2 incubator.

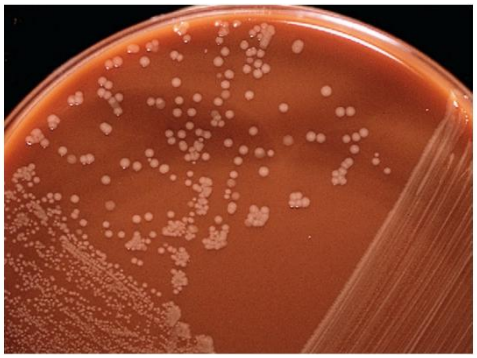


FIGURE 1. *Example of Haemophilus* influenzae growing on chocolate (CHOC) agar. Notice the tan mucoid colonies characteristic of encapsulated strains

Cultivation / Media of Choice

Haemophilus spp. typically grow on chocolate agar as smooth, flat or convex, buff or slightly yellow colonies. Chocolate agar provides hemin (X factor) and NAD (V factor), necessary for the growth of Haemophilus spp. Most strains will not grow on 5% sheep blood agar, which contains protoporphyrin IX but not NAD. Several bacterial species, including Staphylococcus aureus, produce NAD as a metabolic byproduct. Therefore, tiny colonies of Haemophilus spp. may be seen growing on sheep blood agar very close to colonies of bacteria capable of producing V factor; this is known as the **satellite phenomenon.**

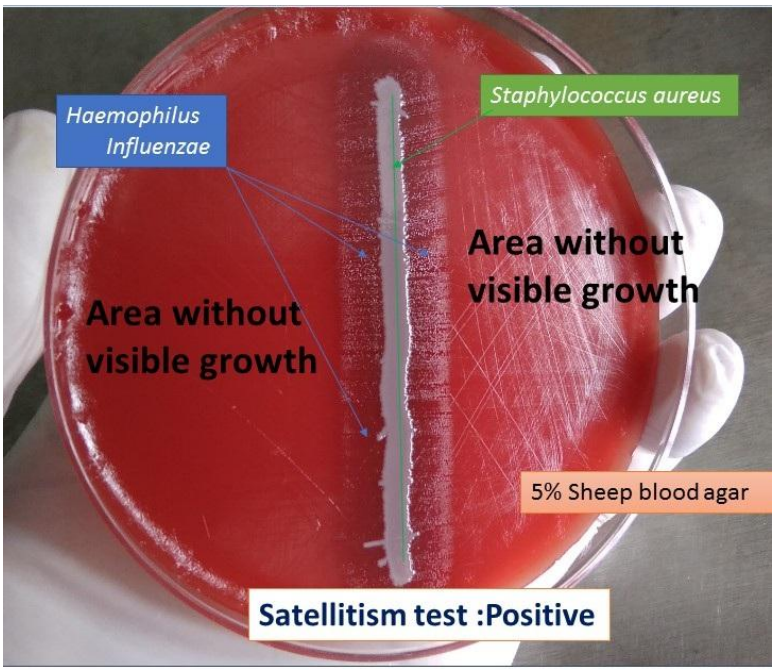


FIGURE: 2 ***Haemophilus influenzae satellite phenomenon***

Treatment

Invasive *H. influenzae* infection often requires hospitalization. The current recommended treatment of life-threatening illness caused by *H. influenzae* is cefotaxime or ceftriaxone. Alternative drugs include trimethoprim-sulfamethoxazole, imipenem, and ciprofloxacin.

11-*Bordetella and Brucella*

Bordetella

Bordetella pertussis is mesophilic coccobacillus causes **whooping cough** (pertussis). It is obligate pathogens of humans colonizing the ciliary epithelial cells of the respiratory tract. *B. pertussis* is fastidious, slow-growing organism.

- The cells of *B. pertussis* are Gram-negative minute coccobacilli ranging in size between 0.2-0.5 µm × 0.5-2.0 µm.
- The cells are occasionally **filamentous** that can elongate several µm in length, usually observed in clinical samples.
- *B. pertussis* is **non-motile** with **no flagella**.
- The cells are either **encapsulated** or surrounded by a sheath of **slime**. The capsule is usually observed in freshly isolated species whereas the slime formation occurs in vitro in the form of **biofilm**.
- Both the capsule and slime sheath are composed of **polysaccharides.**
- The surface of the cell consists of fine filamentous appendages.
- The lipopolysaccharide of *B. pertussis* is different from that of other **Gram-negative bacteria** with different **phosphate composition** than the lipid A in other bacteria.
- The lipopolysaccharide of *B. pertussis* acts as **endotoxins**.

The organisms are minute gram-negative coccobacilli resembling *H influenzae*. With toluidine blue stain, bipolar metachromatic granules can be demonstrated. A capsule is present.

Culture

Primary isolation of *B pertussis* requires **enriched media**. **Bordet-Gengou medium** (potato-blood-glycerol agar) that contains **penicillin G**, 0.5 g/mL, can be used; however, a **charcoal-containing medium**. The plates are incubated at 35–37 °C for 3–7 days in a moist environment (eg, a sealed plastic bag). The small, faintly staining gram-negative rods are identified by immunofluorescence staining. *B pertussis* is non-motile. The organism is a strict aerobe and forms acid but not gas from glucose and lactose. It does not require X and V factors on subculture. Hemolysis of blood-containing medium is associated with virulent *B pertussis.*

- The medium of **choice** for the selective isolation of *B. pertussis* is the Bordet-Gengou medium with **glycerol**. The medium is composed of a potato-extract medium without peptone containing **50**% blood.

- The **charcoal horse blood** agar is a better medium for the selective cultivation of *B. pertussis* as it has a longer shelf-life and is superior in its ability to support *B. pertussis* growth.

- Commercial media for *B. pertussis* include **Stainer-Scholte broth** and **cyclodextrin solid** medium.
- *B. pertussis* is an **obligate** aerobe with the most efficient growth at the temperature range of 30-37°C.
- The metabolism is mostly based on the oxidation of **amino acids** as these bacteria do not usually utilize **carbohydrates**.
- The growth of *B. pertussis* on artificial media is difficult due to the susceptibility of the bacteria to various compounds like unsaturated **fatty** acids, **colloidal sulfur**, and **sulfides**.

The Virulence factors

1- Adhesins such as filamentous hemagglutinin, fimbriae.
2- Pertussis toxin.
3- Adenylate cyclase,
4- Tracheal cytotoxin.

Pathogenesis & Epidemiology

Bordetella pertussis, a pathogen **only for humans,** is transmitted by **airborne droplets** produced during the severe coughing episodes. The organisms attach to the ciliated epithelium of the upper respiratory tract but do not invade the underlying tissue. Decreased cilia activity and subsequent
death of the ciliated epithelial cells are important aspects of pathogenesis.

Clinical Findings

Whooping cough is an acute trachea-bronchitis that begins with mild upper respiratory tract symptoms followed by a severe paroxysmal cough, which lasts from 1 to 4 weeks. The paroxysmal pattern is characterized by a series of hacking coughs, accompanied by production of copious amounts of mucus, that end with an inspiratory “whoop” as air rushes past the narrowed glottis.

Laboratory Diagnosis

The organism can be isolated from nasopharyngeal swabs taken during the paroxysmal stage. Bordet-Gengou1 medium used for this purpose contains a high percentage of blood (20%–30%) to inactivate inhibitors in the agar.

Treatment

Azithromycin is the drug of choice.

Prevention

There are two types of vaccines: an acellular vaccine containing purified proteins from the organism and a killed vaccine containing inactivated *B. pertussis* organisms.

Brucella

Disease

Brucella species cause brucellosis (**undulant** feverالحمى المتموجة, **Malta** Feverحمى مالطا).

Important Properties

Brucellae are **small**, **aerobic, Gram-negative** "but often stain irregularly" rods without a **capsule,** In young culture thaey varies from **cocci** to **rods** 1.2µm in **length**, with short cocco-bacillary forms predominating and they are, non-motile, and non-spore-forming.

There are three major human pathogens:

- *Brucella melitensis* (**goats** and **sheep**).
- *Brucella abortus* (**cattle**),
- *Brucella suis* (**pigs**).

Growth Characteristics

Fresh specimens from animal or human sources are usually inoculated on **trypticase-soy agar or blood culture media**. *Brucella* colonies are small, convex, smooth colonies appear on enriched media in 2–5 days. *B. abortus* requires 5–10% CO_2 for growth, whereas the other **three** species grow in **air**. Brucellae **utilize** carbohydrates but produce neither **acid** nor **gas** in amounts sufficient for classification. **Catalase** and **oxidase** are produced by the species. **Hydrogen sulfide** is produced by many strains, and **nitrates** are reduced to **nitrites**.

Virulence factors

lipopolysaccharide (LPS), T4SS secretion system and BvrR/BvrS system, which allow interaction with host cell surface

Pathogenesis

The common routes of infection in humans are the intestinal tract (ingestion of infected milk), mucous membranes (droplets), and skin (contact with infected tissues of animals). Cheese made from unpasteurized goats' milk is a particularly common vehicle. They localize in the **reticuloendothelial system,** namely, the lymph nodes, liver, spleen, and bone marrow. Many organisms are killed by macrophages, **but some survive within these cells, where they are protected from antibody.**

The host response is granulomatous, with lymphocytes and epithelioid giant cells, which can progress to form focal abscesses.

Clinical Findings

After an incubation period of 1 to 3 weeks, nonspecific symptoms such as fever, chills, fatigue, malaise, anorexia, and weight loss occur. The onset can be acute or gradual. The undulating (rising-and-falling) fever pattern that gives the disease its name occurs in a minority of patients

Enlarged lymph nodes, liver, and spleen are frequently found. Pancytopenia occurs.

Note:- *Brucella melitensis* infections tend to be more severe and prolonged, whereas those caused by *B. abortus* are more self-limited.

Treatment

The treatment of choice is tetracycline plus rifampin.

Prevention

Prevention of brucellosis involves pasteurization of milk, immunization of animals, and slaughtering of infected animals. There is no human vaccine.

12-*Chlamydia*

Order: Chlamydiales

Family Chlamydiaceae.

They are:

- **obligate intracellular bacteria** (like viruses)
- require biochemical resources of eukaryotic host cells to fuel their metabolism for growth and replication.
- *Chlamydia* spp. are similar to Gram-negative bacilli in that they have **lipopolysaccharide** (LPS) as a component of the cell wall. The chlamydial LPS, however, has little endotoxic activity.
- They have a major outer membrane protein (MOMP) that is very **diverse**.
- Chlamydiae have a unique developmental **life cycle**,
  - ✓ an **intracellular**, **replicative form**, the **reticulate body** (RB),
  - ✓ an **extracellular**, **metabolically inert**, **infective** form, the **elementary body** (EB).

The EB **cannot live** long periods of time outside of a host cell. The EB **transforms** into an RB after infecting a host cell. **Within vacuoles**, the RB divides via **binary fission**. The vacuole **enlarges** and becomes an **intracytoplasmic inclusion** as the number of RB rises. The RB then **transform back** into EB, which are then discharged from the host cell **48** to **72** hours after infection. There is evidence that, in addition to the replicative cycle associated with acute chlamydial infections, Chlamydia can persist in **vitro** in an abnormal form.

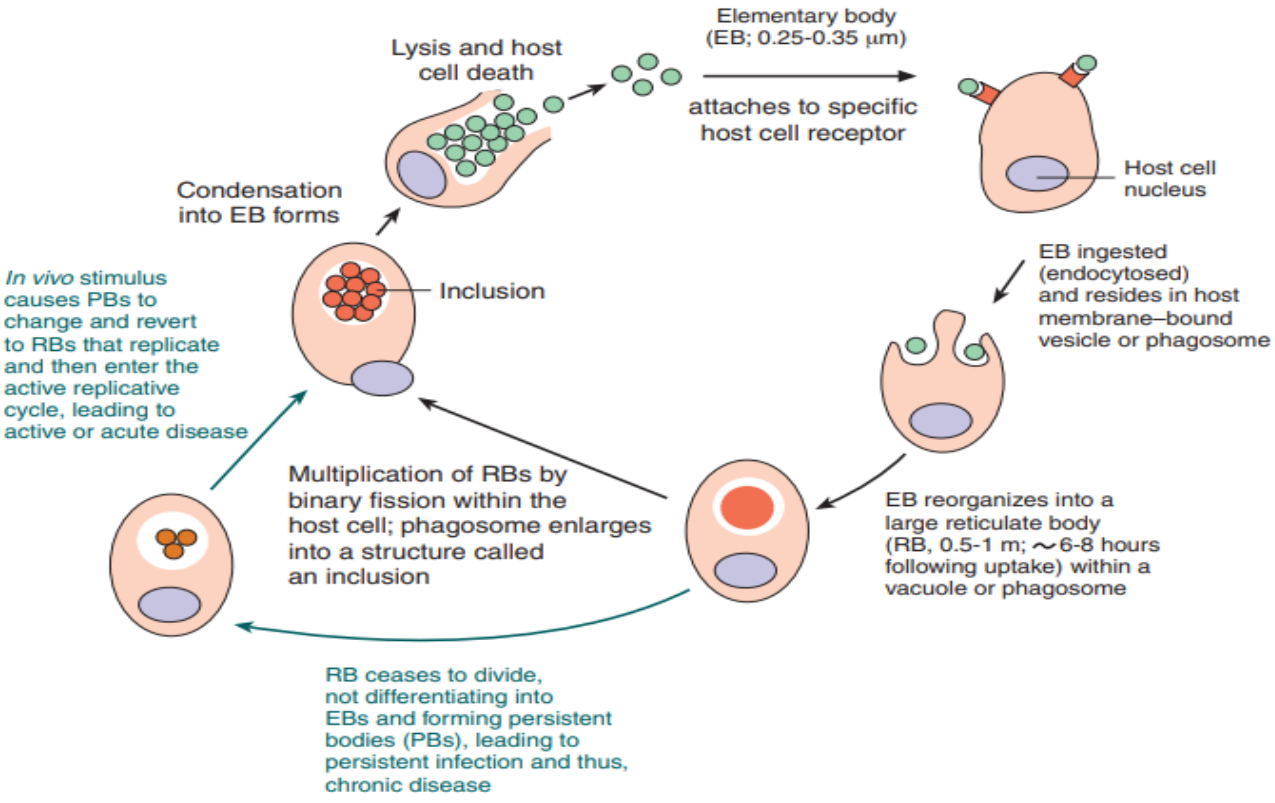


Figure 43-1 The life cycle of chlamydiae. The entire cycle takes approximately 48 to 72 hours.

Differential Characteristics Among Chlamydiae That Cause Human Disease

Property	*Chlamydia trachomatis*	*Chlamydia psittaci*	*Chlamydia pneumoniae*
Host range	Humans (except one biovar that causes mouse pneumonitis)	Birds, lower mammals, humans (rare)	Humans
Elementary body morphology	Round	Round	Pear-shaped
Inclusion morphology	Round, vacuolar	Variable, dense	Round, dense
Glycogen-containing inclusions	Yes	No	No
Plasmid DNA	Yes	Yes	No
Susceptibility to sulfonamides	Yes	No	No

DNA, Deoxyribonucleic acid.

Chlamydia trachomatis

General Characteristics *C. trachomatis* infects humans almost exclusively and is responsible for various clinical syndromes. Based on major outer membrane protein (MOMP) antigenic differences, *C. trachomatis* is divided into **18** different **serovars** that are associated with different primary clinical syndromes.

Spectrum of Disease

- **Trachoma** is manifested by a **chronic inflammation** of the **conjunctiva** and remains a major cause of preventable blindness worldwide.
- **Lymphogranuloma venereum** (LGV) is a sexually transmitted disease.

- **Oculo-genital Infections** *C. trachomatis* can cause acute inclusion conjunctivitis in adults and newborns. The organism is acquired when contaminated genital secretions get into the eyes via fingers or during passage of the neonate through the birth canal.
- **Perinatal Infections** Approximately one fourth to one half of infants born to females infected with *C. trachomatis* develop inclusion conjunctivitis. Usually, the incubation period is 5 to 12 days after birth, but it may be as long as 6 weeks

Laboratory Diagnosis

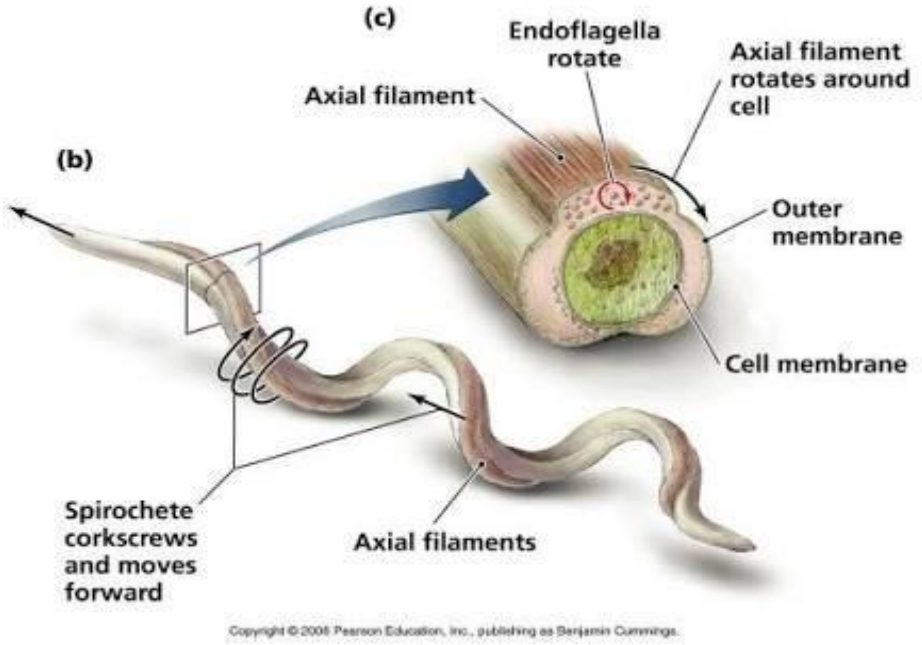
1. **Indirect method: Culture**: Several different **cell lines** have been used to isolate *C. trachomatis* in cell culture, including **McCoy, HeLa**, and **monkey kidney** cells; **cycloheximide**-treated McCoy cells are commonly used. After shaking the clinical specimens with 5-mm glass beads, centrifugation of the specimen onto the cell monolayer (usually growing on a coverslip in the bottom of a vial, commonly called a "shell vial") facilitates adherence of elementary bodies. After 48 to 72 hours of incubation, monolayers are stained with a fluorescein labeled monoclonal antibody.

2. **Direct Detection Methods**
   - **Cytologic Examination**. Cytologic examination of cell scrapings from the conjunctiva of newborns or persons with ocular trachoma can be used to detect *C. trachomatis* inclusions, usually after Giemsa staining.

   - **Antigen Detection and Nucleic Acid Hybridization**. To circumvent the shortcomings of cell culture, antigen detection methods are commercially available.

13-*Spirochetes*

The spirochetes are **long**, **slender**, **helically coiled**, **spiral**, or **corkscrew** shaped bacilli. *T. pallidum* has an **outer sheath**. Inside the sheath is the outer membrane, which contains peptidoglycan and maintains the structural integrity of the organisms. **Endoflagella** (**axial filaments**) are the **flagella-like organelles** in the **periplasmic** space coated by the outer membrane. The endoflagella begin at each end of the organism and wind around it, extending to and **overlapping** يتشابك at the **midpoint**. Inside the endoflagella is the inner membrane (cytoplasmic membrane) that provides osmotic stability and covers the protoplasmic cylinder. A series of cytoplasmic tubules (body fibrils) are inside the cell near the inner membrane. Treponemes reproduce by **transverse fission**.



TREPONEMA PALLIDUM AND SYPHILIS

Morphology and Identification

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

B. Culture Pathogenic *T. pallidum* has never been cultured continuously on artificial media, in fertile eggs, or in tissue culture.

In proper suspending fluids and in the presence of reducing substances, *T. pallidum* may remain motile for 3–6 days at 25°C. In whole blood or plasma stored at 4°C, organisms remain viable for at least 24 hours, which is of potential importance in blood transfusions.

Antigenic Structure:

- The outer membrane
- The peptidoglycan–cytoplasmic membrane complex.
- Membrane proteins are present that contain covalently bound lipids at their amino terminals.
- The lipids appear to anchor the proteins to the cytoplasmic or outer membranes.
- The endoflagella are in the periplasmic space.
- *T. pallidum* has hyaluronidase.
- The endoflagella are composed of three core proteins that are homologous to other bacterial flagellin proteins plus an unrelated sheath protein.
- Cardiolipin is an important component of the treponemal antigens.

Pathogenesis, Pathology, and Clinical Findings

Acquired Syphilis:

- Natural infection with *T. pallidum* is limited to the human host.
- Human infection is usually transmitted by sexual contact, and the infectious lesion is on the skin or mucous membranes of genitalia.
- *T. pallidum* can probably penetrate intact mucous membranes, or the organisms may enter through a break in the epidermis.
- Spirochetes multiply locally at the site of entry, and some spread to nearby lymph nodes and then reach the bloodstream. Within 2–10 weeks after infection, a papule develops at the site of infection and breaks down to form an ulcer with a clean, hard base (“hard chancre”). e. t. c.
-

B. Congenital Syphilis A pregnant woman with syphilis can transmit *T. pallidum* to the fetus through the placenta beginning in the 10th–15th weeks of gestation.

Diagnostic Laboratory Tests

A. Specimens: Specimens include tissue fluid expressed from early surface lesions for demonstration of spirochetes by either dark-field microscopy or immunofluorescence; such specimens can also be tested by nucleic acid amplification. Blood can be obtained for serologic tests; cerebrospinal fluid (CSF) is useful for Venereal Disease Research Laboratory.

B. Dark-Field Examination A drop of tissue fluid or exudate is placed on a slide, and a coverslip is pressed over it to make a thin layer.

C. Immunofluorescence Tissue fluid or exudate is spread on a glass slide, air-dried, and sent to the laboratory.

D. Serologic Tests for Syphilis These tests use either nontreponemal or treponemal antigens.

Table 48.2: Diagnostic tests for syphilis

A. Demonstration of treponemes in the exudate	1. Dark-ground microscopy 2. Direct fluorescent-antibody staining for *Treponema pallidum* (DFA- Tp) 3. Silver impregnation method (Levaditi's stain) 4. Enzyme immunoassay, Polymerase chain reaction (PCR).
B. Serological tests	**a. Nontreponemal tests** **Nonspecific *(reagin antibody)* tests using the cardiolipin antigen *(standard tests for syphilis or STS)*.** 1. Wassermann complement fixation test 2. Kahn flocculation test 3. Venereal Disease Research Laboratory (VDRL) test 4. Rapid Plasma Reagin (RPR) test 5. Toluidine red unheated serum test (TRUST) **b. Treponemal tests** **a. Group specific test using cultivable treponemal (Reiter strain) antigen** I. Reiter Protein CF (RPCF) test (1953) **b. Specific tests using pathogenic treponemes *(T. palidum)*** I. Test using live *T. pallidum* *Treponema pallidum* Immobilization (TPI) test **II. Tests using killed *T. pallidum*** a. Treponema pallidum agglutination (TPA) test b. Treponema pallidum immune adherence (TPIA) test c. Fluorescent Treponemal Antibody Absorption (FTA-ABS) test **III. Tests using *T. pallidum* extract** a. *Treponema pallidum* Hemagglutination Assay (TPHA) Microhemagglutination test for *Treponema pallidum* (MHA-TP) b. *Treponema pallidum* Enzyme Immunoassays (TP-EIA):

14-*Mycobacterium*

Table-1. Classification of mycobacteria

Tubercle bacilli
1. Human—*M. tuberculosis*
2. Bovine—*M. bovis*
3. Murine—*M. microti*
4. Aviam—*M. avium*
5. Cold blooded—*M. marinum*

Lepra bacilli
Human—*M. leprae*
Murine—*M. lepraemurium*

Mycobacteria causing skin ulcers
1. *M. ulcerans*
2. *M. balnei*

Atypical mycobacteria
1. Photochromogens
2. Scotochromogens
3. Nonphotochromogens
4. Rapid growers

Johne's bacillus
M. paratuberculosis

Saprophytic mycobacteria
M. butyricum, M. phlei, M. stercoris.

Morphology

M. tuberculosis is a slender, straight or slightly curved rod with rounded ends, about 3 μm × 0.3 μm, in pairs or as small clumps. The bacilli are non-motile, non-sporing, non-capsulated and acid-fast. They are gram-positive but are difficult to stain.

When stained with carbol fuchsin by the Ziehl-Neelsen method, they resist de-colorization by 20 percent sulfuric acid and absolute alcohol for 10 minutes **(acid and alcohol fast).** With this stain, the *Tubercle bacilli* stain **bright red,** while the tissue cells and other organisms are stained blue (Fig. 1). Organisms in **tissue** and **sputum smears** often stain irregularly and have a beaded مخرزة or barred appearance, presumably because of their **vacuoles** and **polyphosphate** content.

Acid fastness has been ascribed يعزى to the presence in the bacillus of **mycoloic acid.** It is related to the **integrity of the cell** and appears to be a property of the lipid-rich waxy cell wall. Staining may be uniform متسق or granular. In *M. tuberculosis* beaded or barred مخطط forms are frequently seen, but *M. bovis* stains more uniformly. *M. bovis* appear straighter, bolder and shorter with uniform staining.

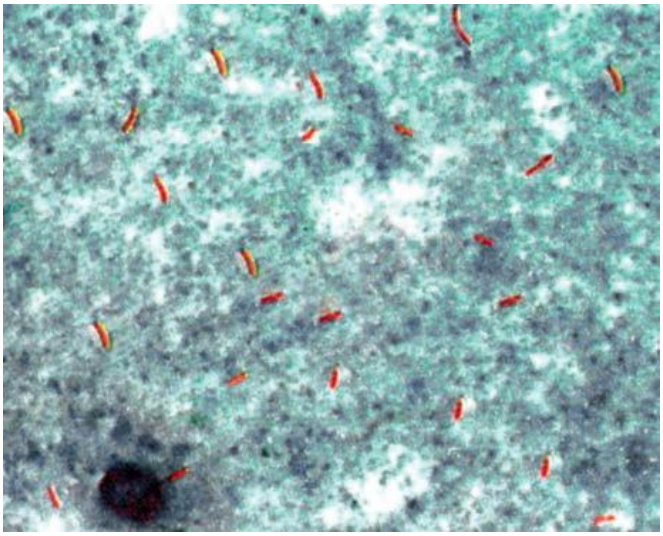


Fig. 1- *Mycobacterium tuberculosis* in Ziehl-Neelsen stained smear

Cultural Characteristics

M. tuberculosis is an **obligate aerobe** while *M. bovis* is **microaerophilic** on primary isolation, becoming aerobic on subculture. The optimal growth temperature of tubercle bacilli is 35 to 37°C but they fail to grow at 25°C or 41°C. Most other mycobacteria grow at one or other, or both, of these temperatures. Optimum pH is 6.4 to 7.0. The bacilli grow slowly, the **generation time** *in vitro* being **14** to **15** hours. Colonies appear in about **two weeks** and may sometimes take up to **eight weeks**.

The solid medium most widely employed for routine culture is **Lowenstein-Jensen (LJ) medium** without starch.

Human tubercle bacilli produce visible growth on LJ medium in about 2 weeks, although on primary isolation from clinical material colonies may take up to 8 weeks to appear. On solid media, *M. tuberculosis* forms **dry**, **rough**, **raised**, **irregular** colonies with a **wrinkled** مجعدsurface. They are **creamy white**, **becoming yellowish or buff** **اصفر برتقالي****colored on further incubation**. They are tenacious دبق، متماسك and not easily emulsified يمكن استحلابه . *Mycobacterium tuberculosis* has a luxuriant وافر growth **(eugenic محسن النسل growth***)* as compared to *Mycobacterium bovis* which grows poorly on LJ glycerol medium **(dysgenic growth)** and colonies, in comparison are **flat** مسطح, **smooth**, **moist**, **white** and **break up** بسهولة **تتقطع** easily when touched. The growth of *M. bovis* is much better on LJ pyruvate medium (media containing sodium pyruvate in place of glycerol).

Antigenic Structure

Mycobactria contain many unique immune-reactive substances, most of which are components of the cell wall. Mycobacteria possess two types of antigens, **cell wall** (insoluble) and **cytoplasmic** (soluble) antigens.

1. **Cell wall antigens**

The basic structure of the cell wall is typical of gram-positive bacteria: an inner cytoplasmic membrane overlaid with a thick peptidoglycan layer and no outer membrane. The cell wall consists of **lipids**, **proteins** and **polysaccharides**. These lipids constitute **60%** of the cell wall weight and contributes to several biological properties. Lipids of the cell wall particularly **mycolic acid** fraction جزء are responsible for acid-fastness of bacteria and the cellular reaction of the body. The cell wall is made up of four distinct layers (Fig. 2).

(i) Peptidoglycan (murein) layer

(ii) Arabinogalactan layer

(iii) Mycolic acid layer

(iv) Mycosides (peptidoglycolipids or phenolic glycolipids)

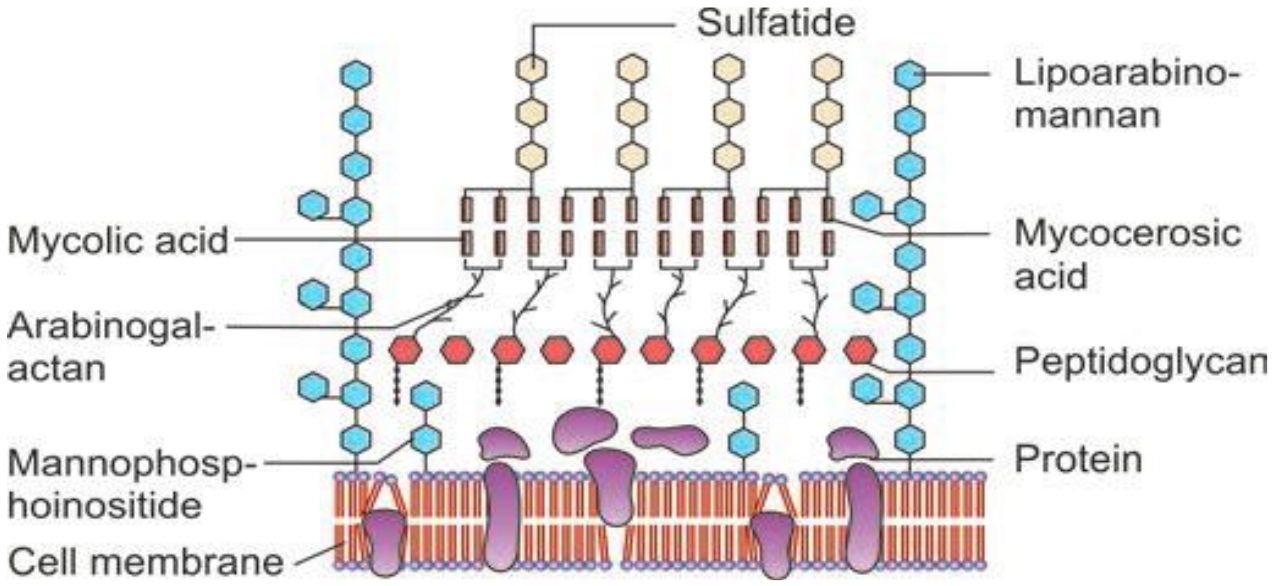


Fig.2- Cell wall of *Mycobacterium tuberculosis*

Test	*M. tuberculosis*	*M. bovis*	*Atypical mycobacteria*
Production of niacin	+	−	−
Binding of neutral red	+	+	+/−
Hydrolysis of Tween 80	−	−	+
Production of enzymes:			
• Nitrate reduction	+	−	+/−
• Arylsulphatase	−	−	−/+
• Catalase at room temp	−	−	+
at 68°C	−	−	+
• Catalase-Peroxidase	Weak +	Weak +	Strong +
Nicotinamidase	+	−	−
• Pyrazinamidase	+	−	+/−
Susceptibility to:			
• Pyrazinamide	+	−	−
Uptake of iron	−	−	−/+

Specimen Collection

Persons suspected of having pulmonary or laryngeal TB should have at least three sputum specimens examined by smear and culture. It is best to obtain a series سلسلة of early-morning specimens collected on 3 consecutive متعاقب days. Specimens should be obtained in an isolated, well-ventilated area or a sputum collection booth. For patients unable to cough up sputum, deep coughing may be induced by inhalation of an aerosol of warm, hypertonic (5%-15%) saline. Patients should be given time — 15 minutes is usually sufficient — to produce sputum, which is usually brought up by a deep cough. Because induced sputum is very watery and resembles saliva, it should be labeled "induced" to ensure that the laboratory staff do not discard it. Bronchoscopy can be done if there is suspicion مشكوك فيه of TB and the patient cannot cough up sputum. Gastric aspiration can also be used to obtain specimens of swallowed sputum. During specimen collection, patients produce an aerosol that may be hazardous to health care workers or other patients in close proximity. For this reason, precautionary measures for infection control must be followed during sputum induction, bronchoscopy, and other common diagnostic procedures. Because TB can occur in almost any anatomical site, a variety of clinical specimens other than sputum (e.g., urine, cerebrospinal fluid, pleural fluid, pus, or biopsy specimens) may be submitted يخضع for examination when extra-pulmonary TB disease is suspected. Tissue specimens for the culture of *M. tuberculosis* should be placed in a transport medium (e.g., Dubos) or a normal saline solution. Formalin or other preservatives should not be used because these solutions kill or inhibit the growth of *M. tuberculosis*.

15-*Mycoplasma and Rickettsia*

Mycoplasma

Mycoplasmas are the smallest prokaryotes capable of binary fission, and they grow, albeit slowly, on inanimate media. There are more than 200 species of these cell wall-free bacteria considered to be parasite living within eukaryotic cells. They cause both human and animal diseases and are normal commensals of the human mucous membranes, including the oral cavity.

Characteristics of Mycoplasma

1. Not seen with Gram stain because it lacks peptidoglycan cell wall
2. Plastic , pleomorphic shape (neither rods nor cocci)
3. Cell membrane is a sterol- containing lipid bilayer
4. Colonies may have fried egg appearance
5. Rarely cultured for diagnostic purposes.

Mycoplasma pneumoniae , causes:

- **Primary atypical pneumonia**

Primary atypical pneumonia (lower respiratory tract disease) takes the form of fever, non-productive cough, severe headache, weakness and tiredness; it is an important cause of community-acquired pneumonia. The acute illness lasts for about 2 weeks, but in a majority, the symptoms last longer.

M. pneumoniae may cause skin rashes and ulcerations of both the oral and vaginal mucosa. These appear as maculopapular, vesicular or erythematous eruptions. The skin lesions, which often affect the extremities, have a target or iris appearance (target lesions).

In the oral mucosa, erythematous patches may appear first, quickly becoming bullous and erosive. This leads to extensive blood encrustations, especially the labial lesions. When the oral ulceration is associated with the skin rash and conjunctivitis,

- **mucocutaneous eruptions, including the oral mucosa**
- **haemolytic anaemia.**

Virulence factors:

- P1 membrane-associated protein (*pneumoniae)*
- Movement of cilia ceases (ciliostasis)
- Peroxide and superoxide
- Superantigen (*pneumoniae)*

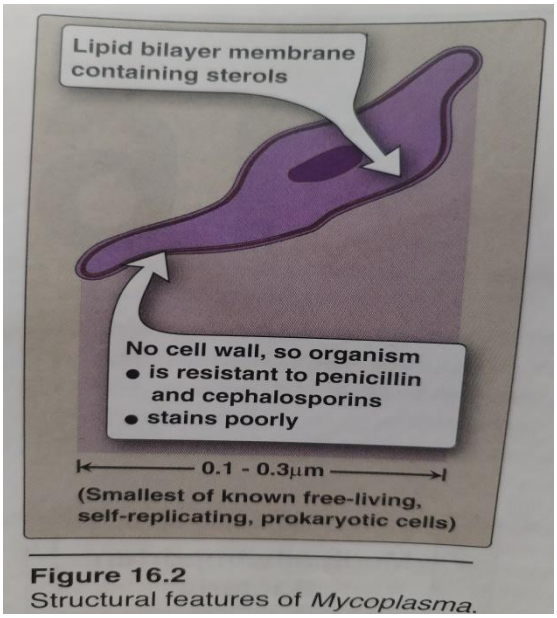


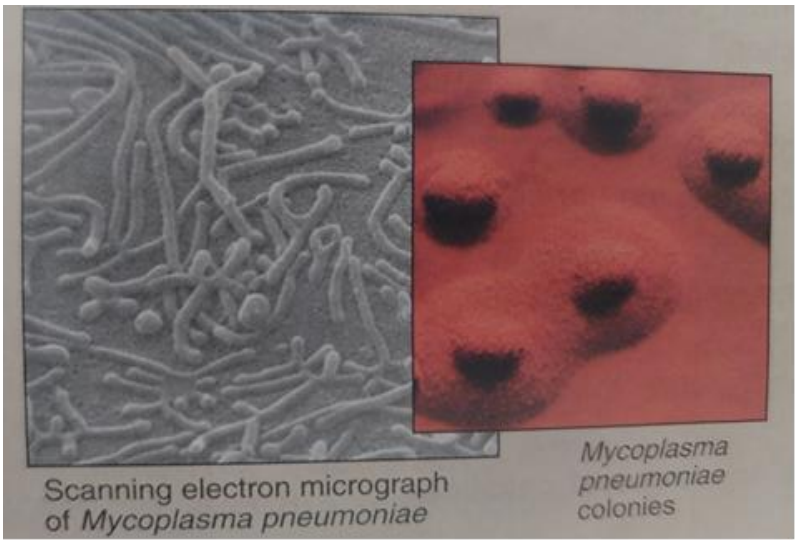
Figure 16.2
Structural features of *Mycoplasma*.

Laboratory identification

☒ Direct microscopic examination of clinical material for *M.pneumoniae* of limited value.

☒ Sputum samples or throat swabs can be cultured on special media , isolation of the organism usually requires eight to fifteen days .

☒ Serologic tests .



Rickettsia

Rickettsiae are coccobacilli smaller but similar to Gram- negative bacteria resembling them structurally and metabolically; they do not stain with Gram stain. They, like Chlamydia and viruses, are obligate intracellular parasites. The best-known human rickettsial disease is typhus, which spreads wildly in conditions of malnutrition and poverty.

Characteristics of Rickettsia

1. Small, rod-like or coccobacillary, with a multilayered outer cell wall resembling that of Gram- negative bacteria
2. able to infect many species, including arthropods, birds and mammals; members of the genus are transmitted to humans via bites of infected arthropods
3. obligate intracellular parasites, grow only inside living host cell
4. visible by light microscope when stains are used (e.g., Giemsa)
5. Doxycycline is the drug of choice for treatment

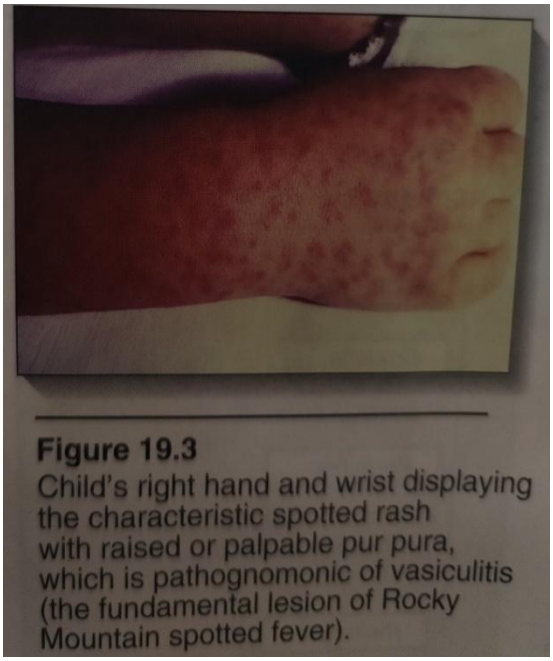


Figure 19.3
Child's right hand and wrist displaying the characteristic spotted rash with raised or palpable pur pura, which is pathognomonic of vasiculitis (the fundamental lesion of Rocky Mountain spotted fever).

Rickettsial diseases

1.Typhus an acute febrile illness, now rare, with a maculopapular rash transmitted by the rat flea; the fatality rate is frequently high as a result of haemorrhagic complications

2.spotted fevers Rocky Mountain spotted fever and other tick-borne fevers.

3.Louseborne (epidemic) typhus

Laboratory identification

- ☒ Not routinely culture because of obligate intracellularity
- ☒ Serological Test
- ☒ Detected by immunofluorescence or histochemical procedures on some clinical samples.

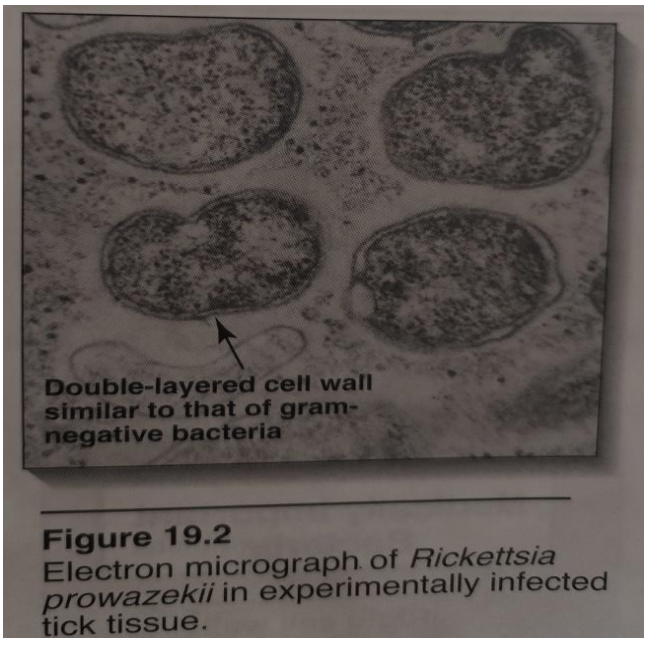


Figure 19.2
Electron micrograph of *Rickettsia prowazekii* in experimentally infected tick tissue.