Gram Negative Bacilli
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THE GENUS VIBRIO
• The genus *Vibrio* consists of:
  • Gram-negative straight or curved rods.
  • Motile by means of polar flagellum.
  • Facultative anaerobe.
  • Oxidase-positive.

• Vibrios are found in marine and fresh water worldwide.

• Most Vibrios are halophilic (tolerate NaCl).
Medically Important Vibrios
• **V. cholerae**: the agent of cholera, the most important.

• **V. parahaemolyticus**: is an invasive organism affecting primarily the colon.

• usually transmitted by ingestion of raw sea-food.

• The organism grows best in high concentrations of salt.

• A non-bloody diarrhea is observed but it is not as severe as cholera.

• **Vibrio vulnificus**: is an emerging pathogen of humans.

• It causes wound infections and gastroenteritis.
Vibrio cholera
• The causative agent of cholera.

• **Antigenic structure:**
  
  • Flagellar antigens
  
  • **O antigens:**
    
    • There are more than 139 known O serotypes.
    
    • A single serotype, designated O1, has been responsible for epidemic cholera.
    
    • O1 antigen are subtyped into three serotypes (Ogawa, Inaba and Hikojima).

• **Two biotypes of *V. cholerae* are described: Classic and El Tor.**

• The Bengal strain (O139) is a new serological strain with a unique O-antigen (which is "non-O1"), that caused large epidemics of cholera.
Cholera enterotoxin

• It is the main virulence factor of V.cholera.
• *V. cholerae* is Potent polypeptide exotoxin composed of 2 subunits:
  • An active (A) subunit and binding (B) subunit.
• Cholera toxin activates the adenylate cyclase enzyme in cells of the intestinal mucosa leading to increased levels of **intracellular cAMP**, and the secretion of $\text{H}_2\text{O}, \text{Na}^+, \text{K}^+, \text{Cl}^-, \text{and } \text{HCO}_3^-$ into the lumen of the small intestine responsible for the watery diarrhea with flakes of mucus and epithelial cells ("rice-water stool").
CHOLERA

• Cholera is a severe diarrheal disease caused by *V. cholerae*.

• Transmitted by water or food contaminated by human feces.

• Humans are the only natural host for this organism.
• Large infecting dose ($10^8 \text{ - } 10^{10}$ organisms) should be ingested to overcome the acidity of the stomach.

• Cholera is non invasive disease, organisms do not reach the blood.

• *V.cholera* penetrate the mucus layer covering of intestinal mucosa by secretion of neuraminidase and proteases.

• Adhere to the mucosal cell by fimbriae and outer proteins where they subsequently produce toxin.
• Toxin causes:
  • Extensive fluid.
  • Potassium, bicarbonate loss.
  • which results in:
  • Dehydration.
  • Hypokalemia.
  • Metabolic acidosis.
  • Anuria.
Host defences

• Gastric acidity plays an important role in preventing cholera infection.

• Secretory antitoxin IgA.
Laboratory diagnosis

• Diagnosis of first case in non-endemic area

• a) Specimens: mucus flecks from rice water stools.

• b) Direct examination of the feces:
  • Wet smear to detect rapidly motile bacteria on direct bright-field, or dark-field microscopic
  • By film stained by gram to show the comma shaped gram negative rods.

1/4/2011
c) Culture:

- V. cholerae is highly aerobic and grows on simple media.
- Growth is favored by alkaline pH (8-9).
- Mucus flecks from stools are inoculated on alkaline peptone water pH 8.5.
- Subculture from the surface pellicle after 6-8 hrs on TCBS or alkaline agar.
- On TCBS medium (thiosulphate citrate bile sucrose), they give yellow colonies as they ferment sucrose.

1/4/2011

VIBRIO CHOLERA ON TCBS AGAR

Oxidase positive

Photo by Karen M. Kiser
d) Colonies are identified by:

• 1 - Film stained by Gram: *V. cholera* is comma shaped Gram-negative rods motile by single, polar flagellum, (darting motility).

• 2 - Biochemical reactions:
  - *V. cholera* ferments glucose, maltose, mannite and sucrose with production of acid only.
  - Gives a positive cholera-red reaction and is oxidase positive.

• 3 - Agglutination with *V. cholera* O group polyvalent antiserum
• **e) PCR can be used in specialized laboratories to detect cholera toxin gene.**

• **N.B:**

  • Vibro El Tor and V. cholerae can be differentiated by:
    • *Tests for hemolysis.*
    • *Chicken cell hemagglutination.*
    • *Polymyxin sensitivity.*
    • *Susceptibility to phage IV.*
II-Diagnosis of second case during an epidemic

- Cases can be diagnosed by microscopic examination of stools for *comma shaped bacilli*
- With characteristic motility which can be immobilized by specific antisera.
Treatment of cholera

• Rapid intravenous replacement of the lost fluid and ions.

• Most antibiotics have no value in cholera therapy.

• But moderate or broad spectrum antibiotics may be used to reduce the output of viable organisms.
Control

• 1- Sanitary.
• 2- Vaccination:
  • a) Killed whole cell vaccine: injected intramuscular in two doses one week apart its efficiency is 50 % and duration of protection is 3-6 months.
  • b) Two recently developed oral vaccines for cholera
    • 1) whole cell killed vaccine that includes B-subunit of toxin: given in 3 doses, its efficiency is 62 % and duration of protection is two years.
    • 2) Live attenuated vaccine: given as single dose, efficiency is 60 % and duration of protection is 6 months.
  • C) Purified LPS fractions have also been given as vaccines with variable success
## Difference between *Vibrio cholera* and *Vibrio eltor*

<table>
<thead>
<tr>
<th>Feature</th>
<th><em>V. cholera</em></th>
<th><em>V. eltor</em></th>
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<tbody>
<tr>
<td>Haemolysis on sheep blood agar</td>
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<td>Haemagglutination of chiken RBCs</td>
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<td>Arabinose fermentation</td>
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<td>Vogues praskawer</td>
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<td>Indole</td>
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<td>Susceptibility to polymyxin B</td>
<td>Sensitive</td>
<td>Resistant</td>
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<tr>
<td>Susceptibility to cholera phage (IV)</td>
<td>Sensitive</td>
<td>Resistant</td>
</tr>
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</table>
Campylobacter
• They have long been known as animal pathogens.

• The most important human species are *C. jejuni* and *C. coli*.

• *C. jejuni* is among the commonest cause of enterocolitis especially in children.
Morphology

- *Campylobacter* are Gram-negative.
- Microaerophilic.
- Motile rods with single polar flagellum.
- Motility is of darting type with cork screw-like movement.

*C. jejuni* with single flagellum at each end (bipolar)
Cultural characters

• They are microaerophilic.

• *Grow best in presence of* 5% oxygen and 10% CO₂.

• **Skirrow's medium** containing vancomycin, polymyxin and trimethoprim is a selective medium used for their isolation from stools.
C jejuni

• It is an important cause of diarrhea, in children and young adults.
• The reservoir for C jejuni is gastrointestinal tract of animals.
• There is probable zoonotic transmission of campylobacter to humans.
• Human infection results from the ingestion of contaminated water, milk or undercooked foods.
Virulence factors:

- *C. jejuni* has lipopolysaccharide with endotoxin activity.
- Cytopathic extracellular toxins.
- Enterotoxin.
- A small infecting dose is required to cause illness as few as 800 bacteria.
• Illness generally occurs following 2-4 days incubation period.
• Symptoms are:
  • Fever.
  • Diarrhea.
  • Nausea.
  • Abdominal pain.
• The illness is generally self-limiting but may last a week.
Diagnosis:

• **1-Wet smear from stool:**
  • examined by **dark-field or phase-contrast microscopy** for darting motility.

• **By Gram staining** to detect morphology curved ("seagull" or "comma") shaped gram negative organisms.
2-Isolating the organism from a fecal culture

• C. jejuni is **microaerophilic**.

• Cultures must be incubated in an atmosphere of **reduced oxygen**, optimally between **5 and 10 %**.

• The optimal temperature for growth is **42°C** for *C. jejuni*. 
Campylobacter blood agar is a selective medium which can be used for isolating C. jejuni. PCR-based techniques have been developed:

- For rapid detection.
- Culture confirmation.
- For typing of C. jejuni strains.
Control

- Interrupting the transmission of the organism to humans from animals, food of animal origin, or contaminated water by properly cooking and storing meat and dairy products.
- Avoiding contaminated drinking water and unpasteurized milk.
- Washing hands after contact with animals or animal products.
  - **Vaccine**: None available.
  - **Chemotherapeutic**: Erythromycin or tetracycline can be used for severe or prolonged illness.
HELICOBACTER
Morphology:

• *H. pylori* are microaerophilic.
• Non sporulating.
• Gram-negative curved rods.
• Motile with multipolar flagella.
Diagnosis

- Specimen: gastric biopsy
- Identified in freshly prepared gastric biopsy smears by phase-contrast microscopy to detect characteristic motility.
- Histologic sections from gastric biopsies stained with Gram, Giemsa, acridine orange stains or hematoxylin and eosin stains to show the morphology.

Immunohistochemical staining of *H. pylori* from a gastric biopsy.
b) Culture of gastric biopsies

- On nonselective media as chocolate agar.
- or antibiotic-containing selective media as Skirrow's medium and incubate for 2 to 5 days.
- Colonies can be identified by gram stained film and by biochemical reactions.
- \textit{H.pylori} is strong urease, oxidase and catalase positive.
• **c) Urease test:** For direct detection of Urease in gastric biopsies.

• **d) DNA probe and PCR:** can be used for identification of *H pylori* in gastric biopsies.

• **e) Urea breath test:** $^{13}$C or $^{14}$C labeled CO$_2$ is detected in the breath after feeding labeled urea.
GENUS HAEMOPHILUS
• The genus *haemophilus*:
  • Gram-negative coccobacilli.
  • Haemophilic.

• Important species are:
  • *Influenzae,*
  • *Parainfluenzae.*
  • *aegyptius and ducreyi.*
Haemophilus influenzae

- *Haemophilus influenzae*: the major pathogen.
  - are classified into:
    - **Encapsulated or typable strains** (have polysaccharide capsule) of which there are seven types.
    - **Unencapsulated or nontypable strains** (they lack a capsule).
• The non encapsulated strains is present in the nasopharynx of approximately 75% of healthy children and adults.

• But only about (3-7%) of healthy individuals intermittently harbor *H. influenzae type b (Hib)* in the upper respiratory tract.

• **Pharyngeal carriage** of *Hib* is important in the transmission of the bacterium.
Morphology:

- Short bacilli (coccobacilli).
- Non motile.
- Non sporulated.
- Capsulated.
Cultural characters

• Facultative anaerobes.
• CO2 tension (5% CO2).
• No growth on ordinary media.
• Need two growth factors for growth:
  • Hemin (factor X)
  • Nicotinamide adenine dinucleotide (NAD+) (factor V) for growth.
• H. influenzae requires both factors X and V so it grows on chocolate agar but not on blood agar.

• It can grow on a blood agar as small colonies called satellite colonies around colonies of other bacteria that can lyse red blood cells as S. aureus.

• Other Haemophilus species require only NAD+ and can grow on blood agar.
Virulence factors

• The polyribosyl ribitol phosphate (PRP) capsule is the most important virulence factor resistant to phagocytosis.

• All virulent strains have:
  • Fimbriae for adhesion.
  • Endotoxin.
  • Neuraminidase.
  • IgA protease.
Diseases caused by H influenzae

- **Type b H influenzae**: causing:
  - Blood stream invasion.
  - Meningitis in children younger than 2 years.
- **Type b Haemophilus influenzae** also cause:
  - Epiglottitis.
  - Bacteremia.
  - Cellulitis.
• **Nontypable H influenzae** can cause:

• Localized infections as Otitias media.
• Sinusitis.
• Tracheobronchitis.
• Pneumonia in infants, children, and adults.
Diagnosis of meningitis

- **Specimens:**
  - **1** - Direct smear stained by Gram to detect gram-negative cocco bacilli.
  - **2** - Detection of capsular material in the cerebrospinal fluid by Quelling reaction or latex agglutination.
  - **3** - Culture: on chocolate agar but not on blood agar in presence of 5% CO2.

H. Influenza on chocolate agar
• NB:

Blood culture can be used for isolation of H influenzae as many cases are accompanied by bacteremia.
Treatment of *H. influenzae* meningitis

- The recommended treatment is **Ampicillin** for strains that do *not* make β-lactamase.

- A third-generation cephalosporin or **chloramphenicol** for strains that do.
Vaccines

• **1) polyribosyl ribitol phosphate (PRP):**

  • Consists of **type b** capsular polysaccharide.
  • Not always effective in **very young children**.
2) protein-conjugated PRP

- Which couple the polysaccharide to a protein.
- Effective in younger infants who are at higher risk for the disease.
- The vaccines are given by injections. More than 90% of infants obtain long term immunity with 2-3 doses of the vaccine.
- This vaccine has reduced the frequency of infection due to type b H. influenzae. But it does not protect against diseases caused by other types of Haemophilus. Or against meningitis caused by other types of bacteria.
 OTHER SPECIES

• *H. parainfluenzae*: causes pneumonia and endocarditis.

• *H. aegyptius*: Cause pink eye (conjunctivitis) and is spread very easily, especially among children.

• *H. ducreyi*: Causes Chancroid, a sexually transmitted disease characterized by painful genital ulcers and swelling of lymph nodes in the inguinal area.

• *H. ducreyi* does not require the V factor for growth.

• Not ferment glucose.
GENUS BORDETELLA

- **Species:**
  - B. pertussis
  - B. parapertussis
  - B. bronchiseptica
• **Bordetella pertussis**: causes whooping cough (pertussis).

• **B. parapertussis**: cause a milder form of bronchitis.

• **B. bronchiseptica**: causes respiratory disease in various animals and occasionally in humans.
Bordetella pertussis

- It is the most important member of the genus Bordetella.
- It causes wooping cough which is a disease of children.
- B. pertussis is strict human pathogen.
- Not present in normal human flora.
Virulence characters

• **A) Adherence mechanisms of B. pertussis:**
  • involve a "filamentous hemagglutinin" which is a fimbrial-like structure on the bacterial surface, and cell-bound pertussis toxin (PTx). which help the bacterium bind to the host cell surface.

• **B) Toxins Produced by B. pertussis:**
  • B. pertussis produces a variety of substances with toxic activity in the class of exotoxins and endotoxins.
Endotoxin

• The heat-stable Bordetella lipooligosaccharide (LOS) endotoxin is similar in Structure.
• Chemical composition,
• Biologic activity to other endotoxins of Gram-negative.
Exotoxins

• 1) Invasive adenylate cyclase (hemolysin):
  • This toxin acts locally to reduce phagocytic activity and helps the organism to initiate infection.
  • The adenylate cyclase was identified as a hemolysin because it will lyse red blood cells.
  • It is responsible for hemolytic zones around colonies of *B. pertussis* growing on blood agar.
• 2) Lethal toxin: (formerly called dermonecrotic toxin):
  • causes inflammation and local necrosis.

• 3) Tracheal cytotoxin:
  • destroys the ciliated cells of trachea.
4) Pertussis toxin

• An **exotoxin** which enters target cells and increase intracellular levels of **cAMP**.
• This leads to decrease **phagocytic activities of phagocytes**.
• Alteration of hormonal activities that are regulated by **cAMP**, such as **increased insulin production**, **increased sensitivity to histamine**.
• Alters both **antibody and cell mediated immunity responses**.
WHOOPING COUGH

• It is an acute respiratory disease of children caused by B. pertussis.
• Common and dangerous childhood disease in unvaccinated children.
• Transmission is by:
  • Inhalation of droplets expelled in cough spray.
  • OR by contaminated objects.
The disease pertussis has two stages:

- After an incubation period of 1 to 2 weeks, whooping cough begins with the:
  - **Catarrhal phase (colonization)**: which lasts 1 to 2 weeks.
  - Is characterized by:
    - Fever
    - Malaise
    - Rhinitis
    - Cough
  - Patient is highly infectious.
• The second paroxysmal (toxemic) phase:
  • lasting 2 to 4 weeks, toxins cause injury of ciliated cells.
  • Characterized by:
  • Paroxysmal coughing that often ends in a characteristic *inspiratory gasp (whoop).*
Diagnosis:

- **a) Specimens:** Nasopharyngeal swabs or nasopharyngeal secretions.

- **b) Culture of samples:**
  - Strict aerobe, slowly growing, need incubation for 3-7 days
  - *B. pertussis* is nutritionally fastidious, no growth on common laboratory media and need rich media supplemented with blood.
  - The widely used medium is **Bordet-Gengou agar containing blood, potato extract and glycerol or charcoal-horseblood agar (Regan-Lowe).**
• Colonies are glistening with appearance of bisected pearl and narrow zone of haemolysis around.

• Colonies are identified by film stained by Gram to show the morphology, biochemicals and slide agglutination with specific antisera.
Morphology

• Gram negative coco-bacilli with bipolar staining.
• Non-motile
• Non-capsulated
• Non-spore forming.
Biochemical reactions

• B. pertussis is oxidase positive

• Urease negative.
• **c)PCR:** Detection of *B. pertussis* DNA by PCR.

• **d)Serological tests:**
  • Circulating antibodies appearing as late as week 3 of illness and reaching their maximum at **weeks 8 to 10**, have been demonstrated by agglutination and complement fixation tests and ELISA.
  
  • The detection of specific IgA and IgM antibodies, however, is **indicative of recent infection.**
Prevention

• 1. Killed whole bacterial vaccine is administered as **DPT** combination.

• 2. Cellular pertussis vaccines.
YERSINIA PESTIS

• It is a facultative intracellular parasite.

• Yersinia pestis is the cause of plague in animals and humans.
Virulence factors

• Many factors as endotoxin, invasiveness, and exotoxin.

• V and W proteins: These proteins are associated with rapid spread and resistance to phagocytosis

• *Y. pestis* has capsular protein-polysaccharide complex antigen: termed envelope (F-1) antigen which is highly expressed at 37 degrees in the mammalian host but not in the flea and is anti-phagocytic.
Plague

- Plague is caused by *Yersinia pestis*.
- It is a disease of wild animals and is transmitted from rat to rat, and from rat to man by bite of infected flea.
- The flea acquires *Y. pestis* during a blood meal from infected rodents, then the flea regurgitates the organisms into next bite wounds.
• In humans, after bite of flea, Y. pestis engulfed by polymorphs will be killed as it is non capsulated but that engulfed by macrophages can survive, resynthesize their capsule, multiply and become resistant to phagocytosis.

• The resulting infection spreads to draining lymph nodes and multiply producing hemorrhagic necrosis with painful swelling known as bubo.

• Within hours organism spreads by blood to spleen, liver and lungs resulting in pneumonic plague which is fatal.
Clinical forms:

- **Bubonic plague**: with painful swelling of the groin or axilla.
- **Pneumonic plague**: which is fatal.
- **Septicaemic plague**.
Diagnosis

- Specimen: aspirate from the bubo, blood or sputum.
- Direct smear: stained by Gram or methylene blue.
- Yersinia pestis is a pleomorphic, capsulated, Gram-negative, bipolar staining, non motile and non spore forming bacillus.
• Culture of the specimen on blood agar or Macconkey.

• Yersinia pestis is facultative anaerobes, can grow on ordinary media but rapid growth on enriched media.

• Optimal temperature for growth is 28°C.

• Extreme caution is warranted in handling of the specimen, as it is highly infectious.
Prevention and Treatment

• **Hospitalization and strict isolation** are the rule.

• **Streptomycin and tetracycline** are highly effective.

• **An effective formalin-killed vaccine** is available but is recommended only for people at a high risk.

• **Control of urban plague** is based upon flea and rodent control.
BRUCELLA

• Three species are important human pathogens:
  
  • *B. abortus* affects cows.
  
  • *B. melitensis* affects goats and sheep.
  
  • *B. suis* affects pigs.

• Species are differentiated by production of urease and \( \text{H}_2\text{S} \), dye sensitivity, cell wall antigens and phage sensitivity.
Morphology

• Gram-negative cocco bacilli
• Non-spore-forming
• Non-motile
• Non capsulated.
Cultural characters

• Aerobic
• Grow best in a 5-10% CO$_2$-enriched environment.
• *Brucellae* possess a typical Gram-negative LPS endotoxin, as well as two major serological determinants; A and M.
BRUCELLOSIS

• **Brucellosis** is a severe acute febrile disease caused by Brucella species.
• Human infections are acquired from handling of infected animals.
• Consuming contaminated milk or milk products (**zoonosis**).
• No human to human transmission.
• Acquired Exposure is frequently **occupational**. Thus, veterinarians, meat workers and animal handlers are at great risk.

• In animals, brucellae affect the reproductive organs causing abortion and sterility.

• In contrast to animals, abortion is not a feature of brucellosis in pregnant women.
Pathogenesis

• Portals of entry are the mouth, conjunctivae, respiratory tract and skin abrasions.

• Brucellae are facultative intracellular parasites, multiply in monocyte-macrophage cells to reticuloendothelial system (spleen, liver, bone marrow, lymph nodes and kidneys), where they live and multiply forming granulomas in these organs.
• Release of brucella from granulomas causes recurrent bacteraemia and recurrence of fever and chills.

• These may produce an undulant fever in which intensity of fever and symptoms recur and recede at about 10 day intervals.
Diagnosis

- **a) Specimens:** Blood, biopsy of lymph node, spleen, liver and bone marrow.
- **b) Blood culture:**
  - Cultures must be incubated **3-4 weeks with added CO2 (5-10 %) at 37°C.**
  - Enriched medium is needed to support adequate Brucella growth.
  - Identification of cultures by stained film for morphology, slide agglutination with specific antiserum and growth inhibition by dyes.
- **Molecular techniques** for typing are being developed.
- **c) Serology**
- Is important of diagnosis.
- Interpretation is complicated by subclinical infections and persistent levels of antibody.
- **EIA (enzyme immunoassay) tests**, designed to differentiate between specific IgM and IgG antibodies are used.
Control

• Pasteurizing milk
• Minimise occupational exposure by observing safety precautions (protective clothing and laboratory containment).
• Eradication of infected animals and vaccination of animals by live attenuated vaccines to reduces the reservoir.
• Vaccines for humans have been developed.
• Vaccination for persons at high risk is possible, but not widely accepted.
Treatment

• Tetracycline.
• OR:
• A tetracycline/streptomycin combination is generally curative.
Main differences between members of the genus Brucella

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<th>Organism</th>
<th>Inhibition of growth by</th>
<th>CO2 required</th>
<th>H2S production</th>
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<tr>
<td></td>
<td>Basic Fuchsin 1/25000</td>
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<tr>
<td>Br melitensis</td>
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<td>Br abortus</td>
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<td>Br suis</td>
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Anaerobic Non Sporing Gram-Negative Bacilli
• There are many genera of Gram-negative anaerobic bacilli. Only Bacteroides, Prevotella, Fusobacterium and Porphyromonas are of clinical importance.

• Present in the normal flora, and may cause opportunistic infections.

• The most common infections are oral, dental, pleuropulmonary, intra-abdominal, female genital tract, skin, soft tissue and bone infections.

• The source of infection is endogenous when mucosal damage by surgery, trauma, or disease allows tissue penetration by members of the normal flora.
**Bacteroides fragilis**

- Gram negative non spore forming bacilli, has polysacaride capsule.
- Present in normal flora of alimentary and genital tracts and represents about 10\% of bacterial species in the colon, but it is the most predominant cause of infection in abdominal cavity, it represents about 35\% of all anaerobic isolates.
- Pathogenecity is due to polysacaride capsule (antiphagocytic) and extracellular enzymes as IgA protease, collagenase, neuraminidase DNase and heparinase.
• **Bacteroides fragilis** is among the most resistant of all anaerobes to antimicrobial agents as pencillin, tetracycline.

• This is related to **β-lactamase production by B fragilis**.

• Causes **abscess, abdominal cavity, genital infections and endocarditis**.
**Prevotella melaninogenica**

- Present normally in oral cavity, upper alimentary, respiratory tract and colon.

- Short to **coccoid Gram-negative rods**; they produce a distinctive pigment (brown to black).

- Produce **collagenase, fibrinolysin enzymes** and are sensitive to penicillin.

- Causes **abscess dental, sinus, pulmonary, abdominal and pelvic infections**.
**Fusobacterium**

- Are spindle shaped Gram-negative rods.
- Present in oral cavity, colon and female genital tract.
- Sensitive to **penicillin**.
- Causes **chronic ulcerative lesions in the gingiva**, **vincent angina**, and **abscess of oral cavity**.
Diagnosis of anaerobic infections

- Clinical signs as:
  - Foul-smelling discharge tissue necrosis, gas in tissues or discharges.
- A definitive diagnosis requires:
  - Isolation of the organisms responsible for the infection.
  - Specimens should be collected and transported under strict anaerobic conditions.
• **Direct Gram stain**: 
  May be helpful because of the frequently unique morphology of Gram-negative anaerobic bacilli.

• **Culture on anaerobic media**: 
  And identification of the growth and the use of gas liquid chromatography.
CHLAMYDIAE

• They are obligate intracellular bacteria, non motile and coccoid.
• They are unable to synthetize ATP and depend on the host cell to supply them with ATP and energy requirements.
• There are three species of chlamydiae: *C. trachomatis, C. psittaci, and C. pneumoniae*.
• Chlamydiae were originally thought to be viruses; however, they have cell wall and contain both DNA & RNA, ribosomes and can be affected by antibiotics, therefore are classified as bacteria.
Developmental cycle

• The chlamydiae exist in nature in two forms:
  • **1. Elementary body (EB):**
    • A non replicating, infectious particle.
    • 0.25 to 0.3 µm in diameter, that is released from ruptured infected cells and can be transmitted from one individual to another.
• **2. Reticulate body (RB):**

• an intracytoplasmic form, **0.5 to 0.6 µm** in diameter, that can replicate and grow.

• The infectious elementary body enters the cell by **endocytosis** and develops into the non infectious **reticulate body (RB)** within a cytoplasmic vacuole in the infected cell. The reticulate body contains no cell wall.

• The reticulate body divides by binary fission to form particles which after synthesis of the outer cell wall, develop into new infectious elementary body and is released from the cell to infect other cells.
Diseases caused by chlamydiae

- **Chlamydia trachomatis:**

- **1. Ocular Infections:**

- Chlamydia trachomatis causes **trachoma and inclusion conjunctivitis** which is spread to the eyes by:
  - Flies, dirty towels and fingers.
  - And in neonates: by passage through an infected birth canal.
• **2. Genital Infections:**

• Some C. trachomatis strains cause genital infections, including non gonococcal urethritis in men and acute salpingitis and cervicitis in women.

• Other strains cause *lymphogranuloma venereum*, a venereal disease with genital lesions and regional lymph node involvement (*buboes*).

• Genital infection is spread sexually.
**Chlamydia psittaci**

- **Respiratory Infections**
  - Usually causes an influenza like illness called *psittacosis*.
  - Chlamydia psittaci, the cause of *psittacosis* in birds and occasionally in humans.
  - Psittacosis is acquired from infected birds.

- **Chlamydia pneumoniae (TWAR organism)**
  - Causes atypical pneumonia in humans, *C. pneumoniae* spreads in human by droplet infections.
Infectious life cycle of Chlamydia

1. **Entry**
   - Elementary bodies

2. **Dormant phase**
   - Vacuole

3. **Elementary body metabolism**

4. **Development into reticulate body**

5. **Maturation of reticulate bodies**

6. **Release**
   - Elementary bodies
Laboratory Diagnosis of Chlamydial Infections

- **Specimens:**
  - Scrapings from eyes.
  - Urogenital tract.
  - Urethral.
  - Cervical exudates.
  - Sputum.
• **1. Microscopic examination**

• Inclusion bodies in scraped tissue cells are detected by staining with **Giemsa or iodine** or by staining with **fluorescent monoclonal antibodies**.

• Inclusion bodies of **C. trachomatis** contain **glycogen** so can be stained with **iodine** and this differs them from other species of chlamydia.
• **2. Culture:**

  • a) **On McCoy cells:** after incubation, typical cytoplasmic inclusions are seen.

  • b) **Yolk sac of embryonated egg:** have been used to isolate Chlamydia.
3. Serological tests:

a) Detection of chlamydial antigen directly in specimens by using specific immunofluorescent antibodies prepared against either C trachomatis or C psittaci.

b) Detection of anti-Chlamydia antibodies in sera and tears from infected humans by the complement fixation or microimmunofluorescence tests.
• **Molecular techniques:**
  
  • **DNA probes:** It is possible to diagnose *C. trachomatis* in tissue specimens by hybridization with a specific **DNA probe**.

  • **polymerase chain reaction (PCR):** is used for diagnosis.
Prevention and Control

• There is no available vaccines.

• It is important to take precautions against sexually transmitted infections because chlamydia is so common and it often doesn't produce symptoms.
Treatment

• Tetracycline and erythromycin are the drugs of choice.

• Penicillin is not effective.
MYCOPLASMAS

• **Mycoplasmas** are the smallest self-replicating organisms with the smallest genomes, Mycoplasmas have no cell walls.

• They are highly pleomorphic, may be spherical to filamentous.

• They can be stained with **Giemsa**. Do not stain by Gram stain.

• The **cytoplasmic membrane** contains sterols. Sterols are acquired from media or tissue and not synthetized by the organism, Mycoplasmas reproduce by **binary fission**.
Clinical manifestations

- *Mycoplasma pneumoniae*
  - causes respiratory tract infections, pharyngitis, bronchitis and Primary atypical pneumonia.
  - It affects mainly children ages 5 to 9 years.
- *Ureaplasma urealyticum*
  - causes non gonococcal urethritis in men.
  - spread through sexual contact.
• **Mycoplasma hominis**
  - It may cause pyelonephritis, pelvic inflammatory diseases such as tubo-ovarian abscess or salpingitis.

• **Mycoplasma genitalium**
  - Present in the human urogenital tract and may cause urethritis and pelvic inflammatory diseases.
• **Diagnosis:**
• **Diagnosis of atypical pneumonia:**
• **Specimens:**
  • Sputum.
  • Throat swab.
  • Nasopharyngeal secretions.
• **a) Culture:**

• Grow slowly on enriched fluid media or special mycoplasma agar, the center of colony grow into the center of the agar to give fried-egg shaped colonies.

• A routine mycoplasma medium consists of heart infusion, peptone, yeast extract, salts, glucose or arginine, and horse serum.

• Colonies appearing on the plates can be identified as M pneumoniae by staining directly on agar with homologous fluorescein-conjugated antibody or by demonstrating that a specific antiserum to M pneumoniae inhibits their growth on agar. Colonies of ureaplasmas are usually minute.
b) Molecular techniques:

- **DNA probes**: diagnosis can be done by hybridization with a specific DNA probe.

- **Polymerase-chain reaction (PCR)**: direct demonstration of organisms in the respiratory specimens by nucleic acid amplification techniques.
• c) Serodiagnosis

• Serum samples are examined for specific mycoplasmal antibodies by complement fixation, indirect hemagglutination and latex agglutination tests.

• The cold agglutinin test: for detection of non specific antibodies which agglutinate group O erythrocytes. It is less useful because it is positive only in one-half of patients and because it can give positive results in other conditions (non specific).
• Control:
  • There is no certified vaccine for \textit{M pneumoniasae}. 

• Treatment:
  • The mycoplasmas are sensitive to \textit{tetracyclines, macrolides, and the newer quinolones}, but are resistant to antibiotics that specifically inhibit bacterial cell wall synthesis.

  • \textbf{Tetracycline or erythromycin} is recommended for treatment of \textit{M pneumoniasae pneumonia}. 
LEGIONELLA

• *Legionella pneumophila* are thin pleomorphic Gram-negative motile bacilli possesses pili (fimbriae).

• *legionella* species are widespread in nature. prefer wet areas.
• **Clinical manifestations:**

• The most common presentation of *Legionella pneumophila* is **acute atypical pneumonia** (legionellosis).

• Less often, disease presents as a non pneumonic epidemic, influenza like illness called **Pontiac fever**.
Pathogenesis

• The source of Legionella is water, particularly the surface waters of rivers and lakes and drinking water.

• Legionella does not multiply in sterile tap water.

• Disease may occur in the community or in hospitals.

• People with compromised host defenses are at increased risk.
• Infection begins in the lower respiratory tract.

• Alveolar macrophages, engulf the bacteria.

• Legionella is a facultative intracellular parasite and multiplies freely in alveolar macrophages leading to their death and releasing a new generation of microbes to infect other cells.

• Legionella bacilli are usually transmitted to humans in aerosols.
Laboratory diagnosis

• **1. culture:**

  • The preferred diagnostic method as it is both sensitive and specific.
  
  • The medium of choice is **buffered charcoal-yeast extract medium.** This medium contains yeast extract, iron, L-cysteine, and α-ketoglutarate for bacterial growth; activated charcoal to inactivate toxic peroxides.
• 2. Direct detection of bacterial antigen in clinical specimens:
  • By direct immunofluorescence or radioimmunoassay which is faster than culture
• 3. Serologic diagnosis:

• Detection of specific antibodies to legionella in serum is moderately sensitive and specific.

• It is important to detect IgM and IgG.
Control

• Elimination of Legionella from the source is an effective control mechanism.
• This can be done by periodic superheating of water and continuous chlorination.
• There is no vaccine.
• The drug of choice is erythromycin.
SPIROCHETES

• Spirochetes are long slender organisms that appear as:
  • Helical coils.
  • Motile.
  • unicellular.
  • Spiral-shaped organisms.
• Three genera are pathogenic for man:
• *Leptospira* species, which causes *leptospirosis*.
• *Borrelia* which causes *Lyme disease*, and *relapsing fevers*.
• *Treponema pallidum*, which causes *syphilis*.
TREPONEMA

• Genus Treponema contains both pathogenic and nonpathogenic species.

• Nonpathogenic treponemes may be part of the normal flora of the intestinal tract, the oral cavity, or the genital tract.
• **Human pathogens cause:**
  • Syphilis (T. pallidum)
  • yaws (T. pertenue).
  • Endemic syphilis (T. endemicum).
  • Pinta (T. carateum).
  • Some of the oral treponemes have been associated with gingivitis and periodontal disease.
Treponema pallidum
Treponemes are slender, spiral coils (regular corkscrew-shaped cells), and they have an outer membrane which surrounds the periplasmic flagella, a peptidoglycan cytoplasmic membrane complex, and a protoplasmic cylinder. They cannot be stained by the Gram stain, but can be stained by Giemsa and Fontana stain. Treponemes in tissues can be visualized by silver impregnation methods. Live treponemes, which are too slender to be seen by light microscopy, can be seen unstained by dark-field microscopy.
Cultural characters

- Pathogenic treponemes have not yet been cultured in vitro.
- Treponema are microaerophilic.
- The cells have a high lipid content (cardiolipin, cholesterol), which is unusual for most bacteria.
- Fastidious organism with narrow optimal ranges of pH (7.2 to 7.4), and temperature (30 to 37°C).
- It is rapidly inactivated by mild heat, cold, desiccation, and most disinfectants.
- Treponemes multiply by binary transverse fission.
SYPHILIS

• **Pathogenesis:**
  • The disease caused by *T. pallidum is syphilis*, which is strictly human disease. transmitted by sexual contact.
  • Organisms penetrate mucous membranes or enter minute breaks in the skin
  • Less than 10 organisms are capable of producing infection.
• **The primary stage**
• occurs after an incubation period of **10 to 90 days**.
• The principal sign is a painless superficial ulcer with a firm base called **a hard chancre**, generally found on the genitals.
• This lesion is filled with **treponemes** and is, therefore, **highly contagious**.
• **The secondary stage**

• After an asymptomatic period of **2 to 24 weeks**.

• Organisms multiply in many different tissues.

• Clinical manifestations include mucous patches on mucous membranes and wart-like lesions called **condylomata** in moist intertriginous areas.

• All of these lesions are highly contagious.
• **The tertiary stage**

• Can affect all areas of the body and be fatal.

• Cardiovascular and neurological involvement are the most frequent causes of death.
• **Congenital syphilis**

• *T. palladium* can be transmitted through placenta to foetous which may die.

• Miscarried.

• Still birth at term.

• Or others borne live with congenital syphilis.
Diagnosis

• **a. Specimens:**
  • Exudate from chancre, mucous patches, blood for serology.

• **b. Direct smear:**
  • Examined unstained fresh with dark ground microscope for motile treponemes.
  • Stained with flouresein-labelled antitreponemal antibodies and examined by florescent microscope.
• **c. Serological tests:**

• Syphilitic patients produce antibodies which react only with treponemal antigens (specific) and regain antibodies (non specific) which can react with aqueous suspension of cardiolipin extract.

• **Serologic tests fall into two general categories:**
1. Non treponemal antigen tests:

- Which measure antibodies directed against lipid antigens, principally cardiolipin.

- Examples are the Venereal Disease Research Laboratory (VDRL).
- Rapid Plasma Reagin (RPR) tests.
- Complement fixation test (Wasserman reaction).
2. Treponemal antigen tests

• Which detect antibodies directed against protein constituents of T. pallidum

• Examples are fluorescent T. pallidum antibody-absorption, Microhemagglutination for T. pallidum tests and Treponema pallidum immobilisation test.

• The sensitivity of the non treponemal and treponemal tests varies with the stage of the disease

• The results of non treponemal tests usually parallel the extent of infection; titers tend to be highest during secondary syphilis and subside during subclinical infection (latency) or following antibiotic therapy.

• The treponemal tests often remain reactive for life.
• Treatment

• Penicillin is the drug of choice in the treatment of syphilis.

• penicillin was found to be effective in eradicating syphilis of all clinical stages as well as the congenital infection.
• **Yaws**

• *T. pertenue* is the causative agent of non-venereal tropical disease known as **yaws**.

• Yaws occur in **the tropics**. Children often become infected at an early age.

• Lesion is ulcerating papule which heals by scar with destruction of skin and bone but no visceral or nervous affection.

• Like syphilis, yaws respond dramatically to treatment with **penicillin**.
• **Pinta**

- *T. carateum* is the causative agent of Pinta, which occurs primarily in the tropics.
- The disease may be acquired at any age through contact.
- The primary lesion is non-ulcerating and is followed within 5 to 18 months by flat, erythematous skin lesions on the hands, feet and scalp.
- These lesions heal slowly after treatment (unlike syphilis & yaws).
- **Penicillin** is effective against Pinta.
BORRELIA

• Borrelia is a flexible, spiral-shaped, Gram-negative spirochete motile with internal flagella.

• Many members of the genus are commensals and others are pathogenic

• Pathogenic species:
  • B. recurrentis.
  • B. hermsii .
  • B. turicatae .
  • B. burgdorferimun.
• **Borrelia recurrentis**

• **Morphology:** are usually longer than the treponemes, their coils are irregular, wide, flexible, and can be stained by Gram and Giemsa.

• **The nutritional requirements** of the borreliae are more complex than those of leptospires. Can be cultivated on **fluid media containing blood or serum**.

• The borreliae are **microaerophilic organisms**.
RELAPSING FEVER

• The epidemic relapsing fever:
  • Caused by B. recurrentis and is transmissible from person to person through the louse. and causes epidemics in crowded, unsanitary populations.
  • There is no animal reservoir and lice do not transmit the disease trans-ovarially.
  • Humans are the reservoir host.
• **The endemic relapsing fever:**
  • Caused by various species of Borrelia (*B. hermsii* and *B. turicatae*).
  • Spread from various animal reservoirs (often rodents) to man by ticks.
  • The tick-borne relapsing fevers are **zoonoses** with rodents as the major reservoir.
Clinical presentation

• The most remarkable feature is its tendency to relapse at regular intervals.

• Incubation period of 3 to 10 days.

• The febrile period: sudden onset of fever, for 3-4 days. The organism may be recovered from the blood, urine, and the CSF. The fever then subsides and the organisms disappear from the blood.

• The afebrile period: the blood is not infectious, but after 3 to 10 days, organisms reappear again in blood and fever returns.
• The relapses are due to the ability of **borreliae** to undergo multiple cyclic antigenic variations.

• The organisms in each successive attack show antigenic change in cell surface antigens.

• And circulating antibodies specific for the organisms of each onset appear in the blood. These antibodies are responsible for the agglutination and disappearance of the spirochetes.
Diagnosis

• **a) During a febrile attack:** Usually made by microscopic examination of blood samples. The organisms can be identified by **dark-field microscopy** or in stained blood smears by Giemsa.

• **b. During afebrile attack.**

• **Animal inoculation:** Intaperitonial injection of blood into young rats or white mice will result in demonstrable bacteremia within **2–4 days** and borreliemia can be detected in blood smear of animal.

• **Serological tests:** are of little diagnostic value because of the numerous antigenic variants encountered.
• Prevention and control

• Relapsing fever responds well to penicillin and tetracyclines.

• Tick and louse-control measures are the most effective means of prevention.

• No vaccines are available.
Lyme disease

- Is caused by *B burgdorferi*.
- Lyme disease is another tick-borne illness.
- Lyme disease are zoonosis with rodents as the major reservoir.
- It is relapsing febrile disease with a characteristic skin rash flu-like symptoms, and sequelae such as arthritis, carditis, and neuritis.

**Diagnosis:** Serological tests, direct observation of spirochetes in clinical specimens and culture are diagnostic but are infrequently successful even in definite cases.
LEPTOSPIRA

• *Leptospira* are very thin.
• Tightly coiled.
• Obligate aerobic spirochetes.
• Characterized by a unique flexuous type of motility.
• The genus is divided into two species:

• **Pathogenic leptospires:** *L. interrogans* are the cause of leptospirosis.

• **Free-living leptospire:** Serotypes of *L. biflexa* exist in water and soil.
• **Morphology**

• Leptospira is thin tightly coiled spiral-shaped, Gram-negative motile with internal flagella, one or both ends are hooked.

• Can not be visualized with the bright-field microscope.

• Can be seen by dark-field or phase contrast microscopy.
• Cultural characters

• The leptospires are the most readily cultivated of the pathogenic spirochetes.

• They are obligate aerobes and can be grown readily in a variety of artificial media supplemented with 10% heat-inactivated rabbit serum.
• **Serological characters**

• *Leptospira* are divided into different serotypes based on their antigenic composition.

• The serotype *Leptospira interrogans icterohaemorrhagiae* is the main strains isolated from humans and animals.
LEPTOSPIROSIS

• **Leptospirosis**: is a zoonosis affecting many wild and domestic animals.

• Humans acquire the infection by contact with the urine of infected animals.

• Human-to-human transmission is extremely rare.
Pathogenesis

• The primary reservoir hosts are wild animals and domestic animals which secrete leptospires in urine.

• Human can be infected by swimming, drinking, bathing in water contaminated with leptospiras.

• Workers in miners, farmers, sewage workers, and swimmers in stagnant pools and canals are at great risk.

• Leptospires enters the host through mucous membranes or small breaks in skin.
• The serotype *Leptospora interrogans icterohaemorrhagiae* is responsible for Weil’s disease, which consists of jaundice, haemorrhagic tendencies, and involvement of the kidneys.

• After incubation period of **7 to 14 days**, leptospiras invade blood causing fever, headache, muscle pain, involve the central nervous system, kidneys, and liver and jaundice occurs.
Diagnosis

• **a) Specimens:** Blood or urine

• **b) Direct microscopic examination:** of the blood by dark field microscopy or stained by Giemsa.

• **c) culture:** The organisms can be isolated from blood or urine by culture on broth or agar media enriched with 10% serum and incubation for several weeks.

• **d) Animal inoculation:** intraperitoneally inoculation into young guinea pigs or hamsters with fresh plasma or urine. Leptospirosis can be detected in peritoneal cavity after few days.

• **e) Serodiagnosis:** Antibodies can be detected by agglutination or haemagglutination tests.
• **Treatment:** *Penicillin and tetracyclines are the drugs of choice.*

• **Preventive measures:**
  - Leptospirosis in domestic animals can be controlled through vaccination with inactivated whole cells or an outer membrane preparation.

  • Vaccines for human use are not available.
  • Measures to diminish the chances of contact with contaminated water.
THE FAMILY *RICKETTSIACEAE*

**RICKETTSIA**

- Obligate intracellular Gram negative bacteria that infect mammals.
- These organisms are small, pleomorphic coccobacilli. Their structure is typical of Gram-negative bacteria.
- Has both RNA and DNA and ribosome.
- Multiply by simple binary fission. Rickettsia replicate in the cytoplasm and nucleus of their host cell. Coxiella replicate only in the phagolysosome.
- Rickettsiae cannot be cultivated on inanimate media, but only in viable eukaryotic host cells (*in cell culture, embryonated eggs, or susceptible animals*).
- They include the genera Rickettsiae, Ehrlichia, Bartonella, Orientia, and Coxiella.
Diseases caused by Rickettsia

- *Rickettsia prowazekii* (epidemic typhus).
- *Rickettsia typhi* (endemic typhus),
- *Rickettsia rickettsii* (spotted fever),
- *Bartonella (formerly Rickettsia) quintana* causes trench fever.
- *Coxiella burnetii* (Q fever)
- *Orientia (formerly Rickettsia) tsutsugamushi* causes scrub typhus.
• **Pathogenesis**

• *Typhus, spotted fever and trench fever* are transmitted via arthropod vectors; From the portal of entry in the skin, rickettsiae spread via blood to infect endothelial cells where they multiply by binary fission causing lysis of host cell and spread to other cells.

• **Characteristic triad of symptoms include**
  
  • Fever
  • Headache
  • Rash.
• Epidemiology

• **Epidemic typhus and trench fever** are transmitted from human to human via the louse.

• **Endemic (murine) typhus** is primarily maintained in rodent and is transmitted via the flea.

  • Humans are an accidental host.

• **Spotted fever** is found in animals and is transmitted by the tick.

  • Humans are accidental hosts.
Laboratory diagnosis

• a) The Weil-Felix test: Proteus OX19, OX2 or OXK antigens are used as antigen for detection of rickettsial antibodies but it is not always reliable as it is nonspecific and insensitive.

• b) Detection of antibodies to rickettsial antigens themselves: (indirect fluorescence antibody test or latex agglutination), which are specific.

• c) Isolation of rickettsia: By inoculation into tissue culture or chick embryo and grown over 4-7 days but this is very hazardous to laboratory personnel.

• d) The use of immunofluorescent antibodies: to examine a biopsy can be diagnostic.
• Treatment of Rickettsial disease

• The treatment of choice for all of Rickettsial disease is: Tetracycline

• Chloramphenicol as the second choice.
Prevention of transmission by breaking the chain of infection

• **For epidemic typhus:** massive delousing with insecticide, formalin killed vaccine is available and is used for military personnel during war.

• **For endemic typhus:** Rat proof buildings, a live attenuated vaccine against epidemic typhus has proved successful.

• **For Q fever:** Proper pasteurization of milk, a formalin killed vaccine containing killed phase I organism shows promise in protecting against infection e.g veterinarians, sheperds and abattoir workers.

• **For Spotted fever:** Reduce exposure by wearing protective clothings, using tick repellents.

• No vaccine is available.
COXIELLA

- **Coxiella burnetii** causes **Q fever**, which present as acute febrile illness with pneumonia but no rash.
- **Q fever is zoonosis** found mostly in animals (*cattle, sheep, goats, rodents*), coxiella multiply in placenta and at birth contaminate earth as it can survive in dust for months.
- Humans acquire disease primarily by inhalation of contaminated aerosols or ingestion of contaminated milk or food.
- The disease is difficult to diagnose clinically, and cultivation poses a biohazard.
- Therefore, serology is the mainstay of laboratory diagnosis.
Thank You