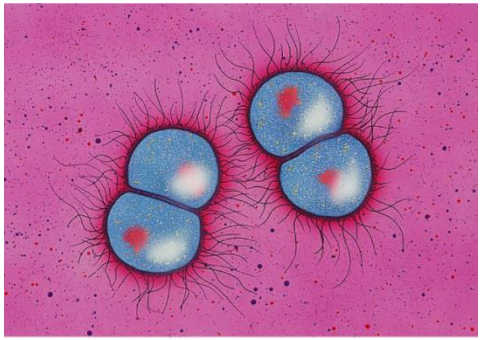
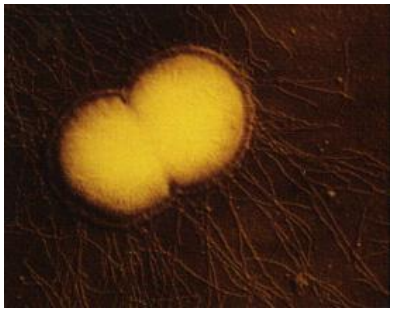
Medical Bacteriology- Lecture 14

Pathogenic Gram-Negative Cocci

Neisseriae





Gram Negative Rods- Oxidase Positive

Campylobacter

Helicobacter

Legionella

Neisseriae

- Gram-negative aerobic coccus, non motile, often arranged as **diplococci (**kidney or coffee bean shaped)
- Ferment carbohydrate producing acid but not gas- **pyogenic bacteria**
- Oxidase positive
- **The genus *Neisseria* contains two important human pathogens species:**
- ***The* gonococcus *N. gonorrhoeae* causes gonorrhea (high prevalence and low mortality)**
- ***The* meningococcus *N. meningitidis* is the cause of meningitis (low prevalence & high mortality).**

- They grow on enriched media (**chocolate blood agar**)
- The selective medium is **Thayer Martin medium** (Chocolate blood agar+ VCN antibiotic inhibitor) for primary Neisseriae isolation.
- They require extra CO2 for grow the specially up on primary isolation.
- Many normal individuals may **harbor *N. meningitidis* in the upper respiratory tract**, but ***N. gonorrhoeae*** is **never part of the normal flora** and is only found after sexual contact with an infected person (or direct contact, in the case of infections in the newborn).
- Both pathogens produce **IgA proteases** which promote virulence. (cleaves secretory IgA in mucous)
- Can easily change its surface antigens leading to evasion of the immune responses especially *N. gonorrhoeae*
- The only distinguishing structural feature between *N. meningitidis* and *N. gonorrhoeae* is the presence of a **polysaccharide capsule** in the former. The capsule is antiphagocytic and is an important virulence factor.

Neisseriae *gonorrhoeae*

- An obligate parasite of the human urogenital tract
- ***N. gonorrhoeae*** infections are acquired by **venereal transfer or fetus.** The infection may disseminate to a variuos tissues.
- **fragile,** susceptible to temperature changes, drying, uv light and other environmental stresses- Fastidious.
- Gonococci adhere to epithelial cells of the mucous membranes lining of the genital, urinary and digestive tracts of humans spreading to deeper tissue as they multiply.

- ***N. gonorrhoeae* has a wide range of virulence determinants**
- The attachment of the bacterium to epithelial cells via **pili** and production of **lipopolysaccharide endotoxin (LOS).**
- **leukocyte association factor**
- **IgA1 protease** that probably play a role in the colonization stage.
- **The LOS of the outer membrane is** (responsible for most of the symptoms of gonorrhea – damage epithelial cells - an intense inflammatory response- lysis of the phagocytes themselves- in strains that cause systemic infection, LOS binds sialic acid from the serum forming a **microcapsule of sialylated LOS**, which allows the gonococci to resist the host immune response and serum bactericidal reaction).

- Has a well-developed **iron acquisition system** that permits it to obtain iron from its host during growth that is necessary to support bacterial invasion.

- **As a few as 100 pairs of cell are enough to cause disease**
- Gonorrhoea is difficult to treat because of **resistance to lots of antibiotics**, especially in developing countries
- **Penicillinase-producing *N. gonorrhoea*** (PPNG) strains are resistant to penicillin.
- **Drug of choice:** Ciprofloxacin

Disease progression

Deposit on mucosal surfaces

↓

Adhere to epithelial cells (pili, adhesion factor)

↓

Limited penetration of epithelium

↓

Epithelial cells damaged by endotoxin

↓

Acute inflammatory response

↓

↓ **Resistance to phagocytosis**

↓ (Leukocyte association factor, and pili)

↓ **Protease which cleaves IgA**

Exudate thickness, phagocytosis increase

↓

Over in 30 days (there is no long lasting immunity)

↓

Septicemia 1 % (iron from serum trasferrin)

Endocarditis, meningitis, dermatitis and polyarthritis

Neisseriae meningitidis

The major virulence factor is **antiphagocytic capsule**.

- **lipooligosaccharide,LOS,** its mechanism is endotoxic (highly toxic)
- Attachment is mediated by **fimbriae** and possibly by other outer membrane components
- the organism is extremely susceptible to temperatures above or below 37c
- The healthy human nasopharynx is the only known reservoir of *N. meningitidis*
- Meningococci are spread via respiratory droplets & transmission requires aspiration of infective particles

- **Meningitis:** refers to the inflammation the meninges of the brain or spinal cord. (meninges are any of the three membranes that envelope the brain and spinal cord).
- The disease **meningitis** is caused by a different bacteria and viruses. Bacterial causes include *Haemophilus influenzae, E. coli, Strep. pneumoniae, Strep. pyogenes, S. aureus,* and *N. meningitidis.*

- Like its relative *N. gonorrhoeae,* the organism tends to occur **intracellularly** in the **cytoplasm of neutrophils** which are attracted to the site of inflammation in the mininges, so this type of infection is called **pyogenic (pus-forming).**
- Cause lifethreating disease when the bacteria invade the blood or CFS
- Most common cause of meningitis in individual under 20
- **Initial symptoms include** fever, sore throat, headache, stiff neck, vomiting, photophobia- can produce blood coagulation and the formation of hemorrhagic lesions
- **Treatment, prevention : Penicillin**, admistrated intravenously, is the drug of choice
- Vaccination

Disease progression

Carrier

↓ droplet

Susceptible host

↓

Local nasopharyngeal infection

↓

Lymphatic channels

↓

Blood

↓

Acute meningitis

Fulminating meningococcemia

Chronic meningococcemia

Genital infection

Metastatic lesions: Dissemination of meningococci may result in metastatic lesions with the hemorrhage and necrosis in the : lungs, Joints, Ears, Vascular system, Skin, Virtually any organ system, Central nervous system (permanent nerve damage)

Comparison features of *N. gonorrhea* and *N. meningitidis*

Feature	*N. gonorrhea*	*N. meningitides*
Site of infection	Urethra/cervix	Meninges
Route of infection	Sexual/ fetus	Inhalation
Disease	Gonococcal	Meningococca/Meningococcemia
Specimen of choice	Urethral/Cervical swab	Cerebrospinal fluid
Biochemical reaction	Oxidase positive Ferment glucose only	Oxidase positive Ferment glucose & maltose
Virulence factors	1-leukocyte association factor 2-pili 3- endotoxin 4- iron acquired system	1- Capsule 2- Pili 3- endotoxin 4-leukocyte association factor 5- iron acquired system
Normal flora	Never part of normal flora	Part of normal flora
Penicillinase producing	Resistant to penicillin	Sensitive to penicillin

Other species of Neisseria

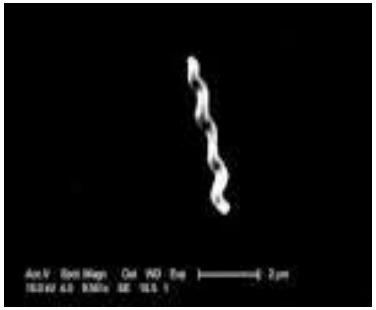
- ***N. flavescens*** – rarely can cause outbreak meningitis or septacemia.

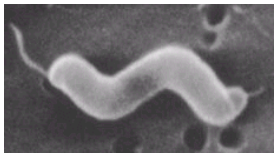
N. mucosa – commonly normal flora in rhinopharynx.

N. sicca – normal flora of naso/rhinopharynx. Also found in sputum/saliva.

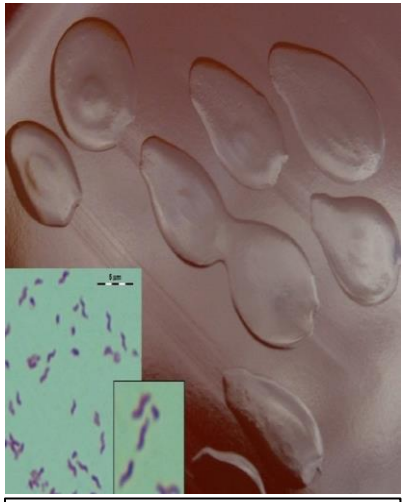
N. subflava – normal flora of rhino/nasopharynx. Very rarely it will cause meningitis.

Campylobacter & Helicobacter & Legionella





Campylobacter



Colonies of *C. jejuni*. (2 days, 42°C). Hemolysis on blood agar

- Small, spirally curved gram-negative bacteria rod- , typical '**gull-wing' or S shape**.
- Motile bacteria with single polar flagellum- non-spore forming.
- Stricly microaerophilic --requiring 5-10% O2 and 10% CO2 enriched environment.
- Oxidase and catalase positive.
- One of the most common gastroenteritis in the developing world
- Animals are reservoir of the bacteria, causes sepsis, abortion or enteritis
- Human infected by contaminated food, unpasteurized milk or water.
- Infections produce bloody diarrhea that is self-limiting

- **Species of medical importance:** ***Campylobacter jejuni***
- ***Campylobacter coli***
- ***Campylobacter fetus***
- *C. jejuni* & *C. coli* found in animal feces, most common causes of human **Campylobacter enteritis**
- The organism multiply in small intestine, invade the epithellium and produce inflammation causing appears of RBCs and WBCs in stool resembling **shegillosis (dysentery-like gastroenteritis)** as well as other types of infection, including bacteremia and central nervous system infections in humans.

- **Campylobacter enteritis** manifests with fever, headache, abdominal pain and bloody mucoid diarrhea, and usually self-limited enteritis in a week period
- **Biochemical reaction:**
- ***C. jejuni*** … hydrolyzes hippurate.
- ***C. coli*** … does not hydrolyze hippurate.

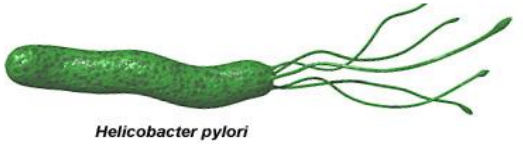
- **Treatment:** Erythromycin

(*C. jejuni* on Selective media)

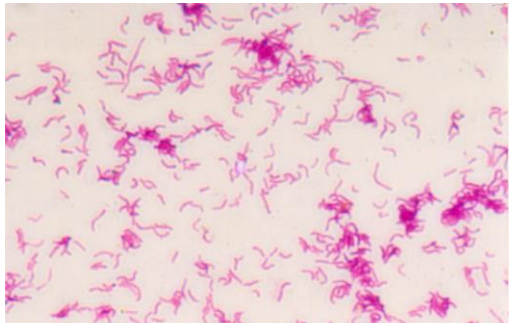
Campylobacter Species Associated with Human Disease

Species	Reservoir Host	Human Disease	Frequency
C. jejuni	Poultry, pigs, bulls, dogs, cats, birds, minks, rabbits, insects	Gastroenteritis, septicemia, meningitis, spontaneous abortion, proctitis, Guillain-Barré syndrome	Common
C. jejuni subsp. *doylei*	Humans	Gastroenteritis, gastritis, septicemia	Uncommon
C. coli	Pigs, poultry, bulls, sheep, birds	Gastroenteritis, septicemia, gastroenteritis, spontaneous abortion, meningitis	Uncommon
C. upsaliensis	Dogs, cats	Gastroenteritis, septicemia, abscesses	Uncommon
C. fetus	Cattle, sheep	Septicemia, gastroenteritis, spontaneous abortion, meningitis	Uncommon
C. fetus subsp. *venerealis*	Cattle	Septicemia	Uncommon
C. hyointestinalis	Pigs, cattle, hamsters, deer	Gastroenteritis	Rare
C. concisus	Humans	Periodontal disease, gastroenteritis	Rare
C. sputorum subsp. *sputorum*	Humans, cattle, pigs	Abscesses, gastroenteritis	Rare
C. curvus	Humans	Periodontal disease, gastroenteritis	Rare
C. rectus	Humans	Periodontal disease	Rare
C. showae	Humans	Periodontal disease	Rare
C. lari	Poultry, birds, dogs, cats, monkeys, horses, seals	Gastroenteritis, septicemia	Rare

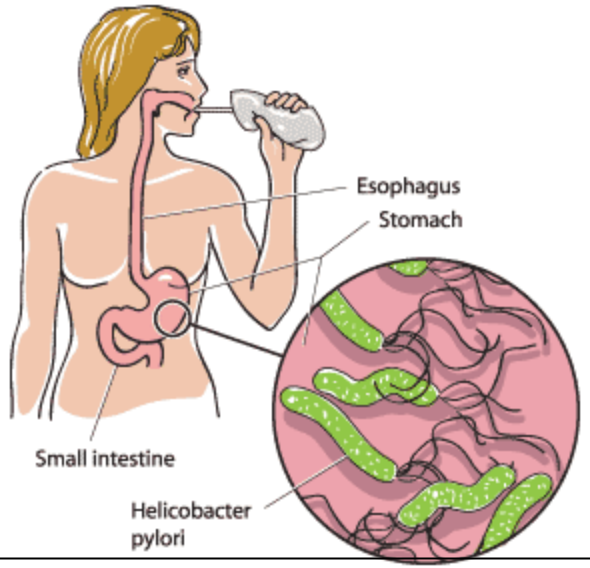
Helicobacter pylori



- Spiral-shaped gram negative, microaerophilic, highly motile rods with polar flagella, colonizes the stomach of hosts
- **Route of entry:** Ingestion of contaminated food and drinks
- Peptic ulcer disease (gastric and duodenal ulcer)
- Gastric cancer

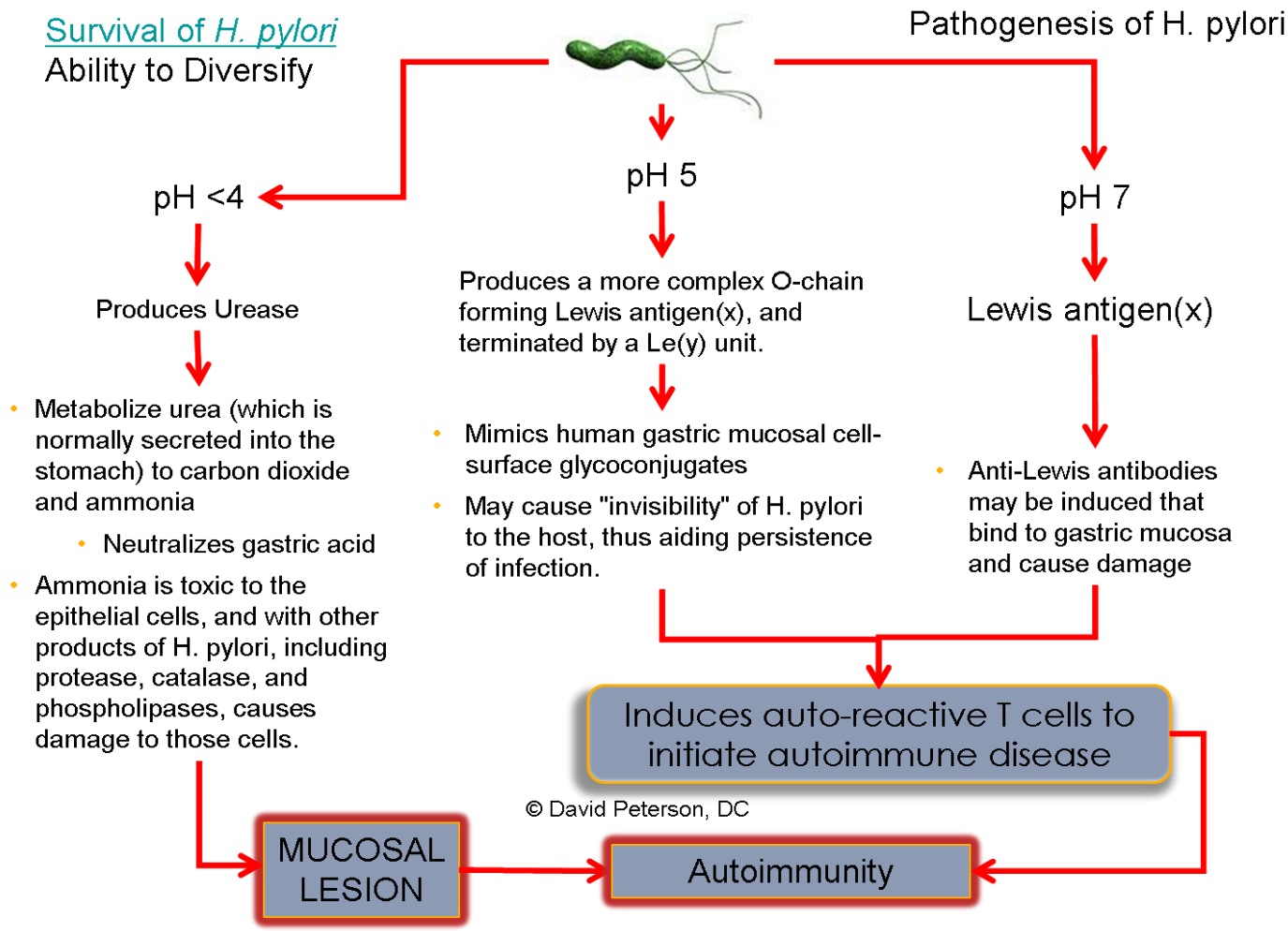


- **Virulence factor:**
- Urease
- Flagella for adhesions
- Protease
- toxins (inhibit stomach acid production)
- LPS
- **Biochemical reaction:** Catalase positive
- Oxidase positive
- **Urease positive**
- **Special test:** urea breath test
- **Serology:**
- Detection of antibodies in the serum specific for *H. pylori*
- Detection of *H. pylori* antigen in stool specimen



The test contains *urea*, which is attached to a carbon atom. The bacteria break down the urea thereby releasing the carbon, which is eventually exhaled in the breath. The breath is then tested to check for the carbon.

Pathogenesis



H. pylori are a stomach bacteria that resides in the mucosal lining of the gastrointestinal tract is responsible for gut inflammation, gastric and duodenal ulcers, gastric mucosa-associated lymphoid tissue lymphoma, and gastric cancer. Its infection can induce autoimmune processes against the gastrointestinal mucosal lining, with consequent autoimmune gastritis. It is also involved in non-gastrointestinal conditions such as heart disease, hair loss and type 1 diabetes.

H. pylori are one of the most common chronic infections worldwide. It affects approximately 50% of the world population. The majority of those infected never develop clinical symptoms. H. pylori specifically colonize the stomach lining and causes chronic inflammation in the upper digestive tract.

Legionella

- ***L. pneumophila***
- Fastidious, aerobic, gram negative intracellular rods- non spore forming-Growth on BCYE (buffered charcoal yeast extract) media with gray-white colonies
- Ubiquitous in warm moist environment
- Direct fluorescent antibody test (fluoresceinated antibody specific for *Legionella)* can be directly performed on sputum samples

- **Virulence Factors** **Proteases- Phosphatases- Lipases- Dnase- RNase**
- **Major secretory protein (Metalloprotease**): Possess cytotoxic and hemolytic property
- **Route of transmission:** Inhalation of aerosols from contaminated cooling towers, tap water and potable water following chlorination

- **1. Legionnaires disease:** causes **Pneumonia** (infection of the lung)- severe- with high fever, chills, dry cough, hypoxia, diarrhea, and altered mentation
- **2. Pontiac fever: mild respiratory illness without pneumonia which resembles acute influenza,** fever, chills, headache, malaise, altered mentation

- **Treatment:** Erythromycin
  Rifampin

Legionell colonies on selective media

Review Questions

- Write the Latin name of the bacteria that cause stomach ulcer? And how it can be diagnosed? What its virulence factors?
- What is the role of IgA protease, give example of which bacteria produce it?
- Gram-negative aerobic coccus, non motile, often arranged as diplococci (kidney shaped or coffee bean shaped), write the bacterial name ?
- Compare between two pathogenic *Neisseria meningitides* and *N. gonorrhoeae* (both side of similarity & differentiate). Their selective media?
- Why cannot treatment *N. gonorrhoeae* by Penicillin antibiotics, and what is the best choice of antibiotic against *Neisseria meningitides*?
- Give three examples of bacteria that causes meningitis ?
- Give two examples of non fragile Neisserial normal flora?
- Gram-negative non-spore forming motile rods- typical 'gull-wing' or S shaped. Requires selective media like skirrow's media for isolation of the bacteria from fecal specimen. What is the bacteria?
- What is the major virulence factor for *N. meningitis*?
- *Helicobacter pylori* can withstand of stomach acidity. How ? two example of infections?
- Compare between Legionnaires disease and Pontiac fever?