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 various 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 transferrin)

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 37°C
- 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 meninges, 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. gonorrhoea* and *N. meningitidis*

<table>
<thead>
<tr>
<th>Feature</th>
<th><em>N. gonorrhoea</em></th>
<th><em>N. meningitidis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Site of infection</strong></td>
<td>Urethra/cervix</td>
<td>Meninges</td>
</tr>
<tr>
<td><strong>Route of infection</strong></td>
<td>Sexual/ fetus</td>
<td>Inhalation</td>
</tr>
<tr>
<td><strong>Disease</strong></td>
<td>Gonococcal</td>
<td>Meningococca/Meningococcemia</td>
</tr>
<tr>
<td><strong>Specimen of choice</strong></td>
<td>Urethral/Cervical swab</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td><strong>Biochemical reaction</strong></td>
<td>Oxidase positive</td>
<td>Oxidase positive</td>
</tr>
<tr>
<td></td>
<td>Ferment glucose only</td>
<td>Ferment glucose &amp; maltose</td>
</tr>
<tr>
<td><strong>Virulence factors</strong></td>
<td>1-leukocyte association factor</td>
<td>1- Capsule</td>
</tr>
<tr>
<td></td>
<td>2-pili</td>
<td>2- Pili</td>
</tr>
<tr>
<td></td>
<td>3-endotoxin</td>
<td>3-endotoxin</td>
</tr>
<tr>
<td></td>
<td>4-iron acquired system</td>
<td>4-leukocyte association factor</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-iron acquired system</td>
</tr>
<tr>
<td><strong>Normal flora</strong></td>
<td>Never part of normal flora</td>
<td>Part of normal flora</td>
</tr>
<tr>
<td><strong>Penicillinase producing</strong></td>
<td>Resistant to penicillin</td>
<td>Sensitive to penicillin</td>
</tr>
</tbody>
</table>

### 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**

- 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 epithelium and produce inflammation causing appears of RBCs and WBCs in stool resembling *shegillossis* (*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
# Campylobacter Species Associated with Human Disease

<table>
<thead>
<tr>
<th>Species</th>
<th>Reservoir Host</th>
<th>Human Disease</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. jejuni</td>
<td>Poultry, pigs, bulls, dogs, cats, birds, minks, rabbits, insects</td>
<td>Gastroenteritis, septicemia, meningitis, spontaneous abortion, proctitis, Guillain-Barré syndrome</td>
<td>Common</td>
</tr>
<tr>
<td>C. jejuni subsp. doylei</td>
<td>Humans</td>
<td>Gastroenteritis, gastritis, septicemia</td>
<td>Uncommon</td>
</tr>
<tr>
<td>C. coli</td>
<td>Pigs, poultry, bulls, sheep, birds</td>
<td>Gastroenteritis, septicemia, gastroenteritis, spontaneous abortion, meningitis</td>
<td>Uncommon</td>
</tr>
<tr>
<td>C. upsaliensis</td>
<td>Dogs, cats</td>
<td>Gastroenteritis, septicemia, abscesses</td>
<td>Uncommon</td>
</tr>
<tr>
<td>C. fetus</td>
<td>Cattle, sheep</td>
<td>Septicemia, gastroenteritis, spontaneous abortion, meningitis</td>
<td>Uncommon</td>
</tr>
<tr>
<td>C. fetus subsp. venerealis</td>
<td>Cattle</td>
<td>Septicemia</td>
<td>Uncommon</td>
</tr>
<tr>
<td>C. byointestinalis</td>
<td>Pigs, cattle, hampsters, deer</td>
<td>Gastroenteritis</td>
<td>Rare</td>
</tr>
<tr>
<td>C. concisus</td>
<td>Humans</td>
<td>Periodontal disease, gastroenteritis</td>
<td>Rare</td>
</tr>
<tr>
<td>C. spatorum subsp.</td>
<td>Humans, cattle, pigs</td>
<td>Abscesses, gastroenteritis</td>
<td>Rare</td>
</tr>
<tr>
<td>spatorum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. curvus</td>
<td>Humans</td>
<td>Periodontal disease, gastroenteritis</td>
<td>Rare</td>
</tr>
<tr>
<td>C. rectus</td>
<td>Humans</td>
<td>Periodontal disease</td>
<td>Rare</td>
</tr>
<tr>
<td>C. showae</td>
<td>Humans</td>
<td>Periodontal disease</td>
<td>Rare</td>
</tr>
<tr>
<td>C. lari</td>
<td>Poultry, birds, dogs, cats, monkeys, horses, seals</td>
<td>Gastroenteritis, septicemia</td>
<td>Rare</td>
</tr>
</tbody>
</table>
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

**Survival of H. pylori**
Ability to Diversify

- pH <4
  - Produces Urease
    - Metabolize urea (which is normally secreted into the stomach) to carbon dioxide and ammonia
      - Neutralizes gastric acid
    - Ammonia is toxic to the epithelial cells, and with other products of H. pylori, including protease, catalase, and phospholipases, causes damage to those cells.

- pH 5
  - Produces a more complex O-chain forming Lewis antigen(x), and terminated by a Le(y) unit.
    - Mimics human gastric mucosal cell-surface glycoconjugates
    - May cause "invisibility" of H. pylori to the host, thus aiding persistence of infection.

- pH 7
  - Lewis antigen(x)
    - Anti-Lewis antibodies may be induced that bind to gastric mucosa and cause damage

**Induces auto-reactive T cells to initiate autoimmune disease**

**MUCOSAL LESION**

**Autoimmunity**

© David Peterson, DC

*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. meningitides?
• Helicobacter pylori can withstand of stomach acidity. How? two example of infections?
• Compare between Legionnaires disease and Pontiac fever?