NEISSERIA, HAEMOPHILUS, BORDETELLA

1. Neisseria:

   a. Introduction:

   Neisseria are gram-negative diplococci.

   *Neisseria meningitidis* causes meningitis and sepsis. Like *N. gonorrhoeae*, it sticks to mucosal cells (nasopharynx) by means of pili and other adhesins. However, it has a polysaccharide capsule which allows it to avoid phagocytosis. In addition, it has the remarkable ability to invade and multiply in the bloodstream and central nervous system (CNS). The lipopolysaccharide (LPS; endotoxin) of *N. meningitidis* is a potent stimulator of inflammation resulting in sepsis.

   *Neisseria gonorrhoeae* causes the sexually transmitted disease gonorrhea. It produces an intense local and acute inflammatory reaction. Some strains have virulence characteristics which promote bloodstream invasion. Infection begins when the organism adheres to genitourinary epithelium mediated by pili and other surface proteins. The organism exhibits genetic change by altering the protein structure of pili and by switching production of other adhesins on and off, thereby evading the immune response.

2. *Neisseria meningitidis*:

   a. Morphology, staining, and structural characteristics: *Neisseria meningitidis* are gram-negative “kidney bean” shaped diplococci.

      i. Polysaccharide capsule
         1. Responsible for division into 13 serogroups. A, B, C, Y, W-135
      ii. Outer membrane proteins
      iii. Lipopolysaccharide (LPS) / lipooligosaccharide (LOS) on surface (endotoxin)

   b. Virulence factors and determinants of pathogenicity:

      i. Pili – mediate attachment to non-ciliated nasopharyngeal epithelial cells
      ii. Traverse the cell in a vesicle to the submucosa
      iii. Multiplication within respiratory epithelium followed by destabilization and invasion of mucosal cells
      iv. Polysaccharide capsule aids in bloodstream invasion, bacterial survival, and, possibly, penetration into the central nervous system (CNS)
      v. LPS and other bacterial products causes systemic inflammation, activation of complement system, cell damage >> sepsis, shock, death

   c. Cultural and biochemical characteristics
i. Growth enhanced in CO₂
ii. Can be fastidious, needs enriched media (chocolate agar)
iii. See table 1 below

d. Epidemiology

i. Most commonly causes sporadic cases but can also be associated with small outbreaks in closed populations of children and young adults (e.g., residents of college dormitories, military barracks, etc.)
ii. Transmitted via respiratory droplets
   1. 1,000 fold higher attack rate in household contacts
   2. Antibiotic prophylaxis of exposed household contacts is often recommended (rifampin, ciprofloxacin, ceftriaxone)
iii. Respiratory tract colonization either leads to clinical disease or a transient carrier state (can still spread)

e. Primary Clinical Diseases

i. Meningitis
   1. Severe, fulminant, acute onset of headache, fever, neck stiffness/rigidity, altered mental status
   2. Can see seizures and cranial nerve deficits

ii. Meningococcemia
   1. Can occur in conjunction with meningitis or separately
   2. Sepsis that rapidly progresses to septic shock
   3. Petechiae on extremities, especially legs > progresses rapidly to purpuric (violaceous) hemorrhagic bullae
   4. Can see bilateral adrenal hemorrhage (Waterhouse-Frederichson syndrome) and acute adrenal insufficiency

iii. Chronic meningococcemia
   1. Very rare and less severe form of meningococcemia
   2. Relapsing fever, arthritis, and rash that mimics vasculitis

f. Diagnosis, treatment, and prevention

i. Gram stain and culture of clinical specimens – CSF, blood, skin
ii. Culture on chocolate agar, enhanced with CO₂, oxidase + (glucose and maltose)
iii. Nucleic acid amplification testing (PCR) available for CSF as part of a multiplex panel that also tests for many other common bacterial, viral, and fungal causes of meningitis
iv. Preferred treatment is ceftriaxone IV
v. Polysaccharide vaccine (includes A, C, Y, W-135) conjugated to diptheria toxoid for all adolescents age 11-12 (booster age 16) and for adults who are at risk
3. **Neisseria gonorrhoeae:**

**a. Morphology, staining, and structural characteristics:** *Neisseria gonorrhoeae* are gram-negative “kidney bean” shaped diplococci.

i. No true polysaccharide capsule  
ii. Lots of outer membrane proteins  
   1. Pilin – facilitates bacterial adherence to host cells  
   2. PorB – outer membrane protein I (porins/channels) – facilitates epithelial cell invasion  
   3. Opa – facilitates bacterial adherence to host cells – gives ‘opaque’ appearance to colonies in culture  
   4. Rmp – binds host antibodies to protect bacteria (?)  
   5. Transferrin – obtain iron from host cell for metabolism  
iii. LPS / LOS (endotoxin)

**b. Virulence factors and determinants of pathogenicity:**

i. Pili –  
   1. **Antigenic variation:** genetic recombination by transfer of variable gene sequences from unexpressed (silent) loci, *pilS*, to expression locus, *pilE*  
ii. Opa –  
   1. **Phase variation:** can switch on and off as many as 11 different Opa genes throughout the genome to express different Opa proteins on the surface  
iii. Pili, Opa, and PorB proteins mediate attachment to urogenital tract epithelium and epithelial cell invasion  
   1. Antigenic variation and phase variation help bacteria evade the immune response and allow people to be infected over and over again  
iv. LPS and other bacterial products causes local inflammatory response and tissue damage (especially neutrophils)  
v. Direct extension of infection to prostate, epididymis, and paracervical glands - can possibly be carried by sperm to fallopian tubes  
vi. Some strains can invade the bloodstream and cause disseminated infection

c. **Cultural and biochemical characteristics**

i. Growth enhanced in CO₂  
ii. Can be fastidious, needs enriched media (chocolate agar)  
   1. Growth is improved with addition of antibiotics to chocolate agar  
iii. *See table 1 below*

d. **Epidemiology**

i. Mucosal transmission via genital tract, mouth, anus, and eye via direct contact  
   1. 20-50% risk of infection with each contact
ii. 50% of women and 5-10% of men have asymptomatic infection and can spread infection

e. Clinical Diseases

i. Local – urethritis in men, cervicitis in women, proctitis in either gender
   1. Rarer is pharyngitis and prostatitis
   2. Neonatal conjunctivitis – 2-5 days after birth to an infected mother; prevented by erythromycin eye ointment to all babies at birth
ii. Disseminated – gonococcemia and septic arthritis with characteristic skin lesions
   1. Also, local dissemination with epididymitis in men and pelvic inflammatory disease (PID) in women
   2. Dermatitis
   3. Endocarditis and meningitis are very rare

f. Diagnosis, treatment, and prevention

i. Gram stain and culture of clinical specimens – urethral swab, cervical swab
ii. Nucleic acid amplification testing (PCR) from clinical specimens (urine, cervical, rectal) has greater sensitivity and specificity
iii. Antibiotic resistance has become a severe problem – gonorrhea is one of the CDC Drug Resistance Threats
iv. Preferred treatment is ceftriaxone IM x 1 dose in combination with azithromycin or doxycycline for co-infection with *Chlamydia trachomatis*

Table 1. Biochemical characteristics of *Neisseria*

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<thead>
<tr>
<th>Species</th>
<th>Oxidation / fermentation</th>
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<tr>
<td></td>
<td>Glucose</td>
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<td><em>N. meningitidis</em></td>
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<td><em>N. gonorrhoeae</em></td>
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4. Haemophilus

a. Introduction

*Haemophilus* species are small, Gram-negative coccobacilli which are found in the respiratory tract. The major human pathogen is *Haemophilus influenzae* which may be encapsulated and can invade and cause a variety of systemic infections including bacterial meningitis. The widespread use of an effective childhood vaccine has greatly reduced the number of serious infections with this pathogen. Other *Haemophilus* species producing disease are *H. parainfluenzae, H. aphrophilus, H. aegyptius and H. ducreyi.*
Mechanisms of Human Disease  Neisseria, Haemophilus, and Bordetella  
Wednesday, August 30, 2017  Margaret A. Fitzpatrick, M.D., M.S.

b. **Structural Characteristics and Pathogenesis:**
   
i. **Morphology/staining:** The bacteria are short (0.5-1.5 microns) gram-negative coccobacilli. Non-motile and non-spore forming.
   
ii. Some *Haemophilus* species have polysaccharide capsules which allows classification into antigenic serotypes a-f
   
   - This is major virulence factor that allows bloodstream invasion and promotes more severe infection
   
iii. Pili, outer membrane proteins (adhesins), and LPS/LOS (endotoxin) also present

c. **Cultural/biochemical characteristics:**
   
i. Oxidase and catalase positive
   
ii. Grow best on chocolate agar supplemented with key growth factors called “X” (hemin or hematin) and “V” (nicotinamide adenine dinucleotide)

d. **Epidemiology:**
   
i. Colonizes the throat and nasopharynx and is transmitted via respiratory droplets
   
ii. An encapsulated strain of *H. influenzae* (type B or “Hib”) was responsible for > 95% of invasive infections until vaccine introduced
   
iii. Now more than half of all infections are now caused by non-typeable (non-encapsulated) strains
   
iv. Peak incidence in children < age 5 and especially 6 months – 2 years

e. **Clinical Disease:**
   
i. **Invasive disease** – historically Hib but can also be non-encapsulated strains
   
   - Meningitis
   
   - Epiglottitis – sudden onset fever, sore throat, cough, stridor, red/swollen
   
   - Septic arthritis, pneumonia, bacteremia, cellulitis (usually face/cheek)
   
ii. **Non-invasive (mucosal) disease** – most often non-encapsulated strains
   
   - Otitis media, sinusitis, acute exacerbations of chronic obstructive lung disease (COPD), conjunctivitis
   
   - Older children and adults

f. **Diagnosis, treatment, and prevention:**
   
i. Gram stain and culture of clinical specimens (CSF, joint fluid, blood) on chocolate agar with X and V supplements
   
ii. Nucleic acid amplification (PCR) testing available but not widely used yet
   
iii. Treatment for severe invasive disease with 2nd and 3rd gen cephalosporins
   
iv. Treatment for less severe and non-invasive disease with amoxicillin/clavulanate, macrolides, fluoroquinolones, and trimethoprim/sulfamethoxazole
   
v. Vaccine – conjugate of polysaccharide capsule antigens and other protein (diphtheria toxoid, N. meningitidis OMP, tetanus toxoid)
1. Incidence of invasive Hib has dropped 94% between 1986-1995
2. Universal immunization starting at 2 months

5. *Bordetella*

a. Introduction

*Bordetella* are tiny, aerobic, Gram-negative coccobacilli that are found in the respiratory tract. They are responsible for pertussis, a prolonged illness characterized by severe cough. Three closely related, genetically identical species, *B. pertussis*, *B. parapertussis* (milder forms of pertussis), and *B. bronchiseptica* (respiratory disease in animals—rarely in humans) are found in humans.

b. Structural Characteristics

i. Morphology/staining: gram-negative aerobic coccobacilli (0.5-1.0 microns)
   1. Difficult to grow, need agar supplemented with charcoal, starch, blood, or albumin that absorbs toxic substances and metabolites
ii. Structural/pathogenesis
   1. No polysaccharide capsule
   2. Pertactin and filamentous hemagglutinin (Fha) both bind to integrins on the membranes of ciliated respiratory epithelial cells and trigger phagocytosis and intracellular survival
   3. Toxins are major virulence factor
      a. Pertussis toxin (PT)
         i. AB subunit: A - S1 enzymatic, B – S2-S5 binding subunits
         ii. A subunit catalyzes ADP ribosylation of a cellular regulatory protein (G- protein) which prevents inactivation of adenylate cyclase while B subunit binds to receptors on ciliated respiratory cells and phagocytic cells
         iii. Biologic effects of PT include increased respiratory secretions, mucus production and lymphocytosis
      b. Adenylate cyclase toxin
         i. Causes increased cAMP levels in host cells
         ii. Interferes with chemotaxis and superoxide production of leukocytes
      c. Tracheal cytotoxin – fragment of peptidoglycan that binds to and causes extrusion of ciliated respiratory epithelial cells. The toxin also stimulates IL-1 release (fever).

c. Epidemiology

i. Spread by respiratory droplets and highly infectious; only human pathogen
ii. Adults with subclinical or milder clinical disease are the major reservoir of spread to infants who have more severe disease
d. Clinical Disease

i. Incubation 7-10 days
ii. Catarrhal stage - rhinorrhea, fever, sneezing, anorexia
iii. Paroxysmal cough - up to 50 times a day for 2-4 weeks. Inspiratory whoop, vomiting, mucoid secretions, marked lymphocytosis
iv. Convalescent stage - cough gradually fades
v. Complications - pneumonia (S. pneumoniae), atelectasis, convulsions, hemorrhage

e. Laboratory diagnosis, treatment, and prevention:

i. Difficult to grow from a nasopharyngeal aspirate wash but can be done on charcoal Regan-Lowe media
ii. Antigen detection from nasopharyngeal smears but direct immunofluorescence
iii. Nucleic acid testing (PCR) from nasopharyngeal specimen done by reference and public health dept laboratories
iv. Treatment is only beneficial in the early catarrhal stage – macrolides
v. Vaccine is combined with diphtheria and tetanus toxoids
   1. As of spring 1997, the ACIP recommends use of DTaP, which contains one of the acellular pertussis vaccines, made from purified, detoxified pertussis toxin (PT), filamentous hemagglutinin (Fha), and Pertactin (Pn)
   2. These preparations should replace the killed, partially purified, whole cell vaccines (DPT) which were associated with side effects of pain, fever, and rash and to which a host of possible neurological reactions were attributed
   3. In order to reduce the incidence of pertussis in adults, one dose of vaccine containing a reduced amount of pertussis antigens, Tdap, is recommended for all adults in place of a routine (every 10 years) Td.