Neisseria, Haemophilus, and Bordetella

Margaret Fitzpatrick, MD, MS
Division of Infectious Diseases
September 5, 2018

• Gram-negative cocci
  – Cocccobacilli (Haemophilus, Bordetella)
  – Diplococci (Neisseria)
• Contain lipopolysaccharides (LPS) in their outer membrane – also referred to as ‘endotoxin’
• Potential to cause severe, life-threatening infections
• Incidence of disease has been significantly reduced with vaccination

Lecture Objectives

• Distinguish the unique culture and growth characteristics of Neisseria from that of other bacteria
• Identify the major virulence factor of Neisseria meningitidis
Neisseria

• Structural characteristics
  – Small gram-negative diplococcus (intracellular)
  – Outer membrane: LPS complexed with many other antigens/proteins
  – Type IV pili

Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 8th edition. 2010.

Neisseria

• Culture and growth
  – Difficult to grow – require enriched media enhanced by CO₂
  – Chocolate agar that contains antibiotics (modified Thayer-Martin medium)
  – Oxidase positive

http://www.microbiologyinpictures.com/

Neisseria

• Two species that cause human disease:
  – *Neisseria meningitidis*
  – *Neisseria gonorrhoeae*
• Immune response to *Neisseria* requires intact complement system
  – 6,000 fold increase risk for disseminated meningococcal and gonococcal disease in persons with complement deficiency (C5-C8)
**Neisseria meningitidis: pathogenicity and virulence**

- Polysaccharide capsule
  - 13 serogroups are currently recognized, with 6 serogroups (A, B, C, W135, X, and Y) associated with endemic and epidemic disease
  - bloodstream invasion, CNS tropism
- LPS
- Type IV pilus

- Attaches selectively to columnar cells of nasopharynx
- Bacteria multiply and form large aggregates
- Within a few hours, pili undergo posttranslational modification = destabilized aggregates
- Bacteria penetrate into the host cells and release into the airways
  - person-to-person spread is increased

![Image of Neisseria meningitidis infection](image-url)
Lecture Objectives:

- Identify the primary mode of transmission of *Neisseria meningitidis*
- From a clinical description, recognize the two most common clinical presentations of disease caused by *Neisseria meningitidis*

**Neisseria meningitidis: epidemiology**

- High attack rate due to respiratory droplet transmission
  - family members in household, college students in dormitories, soldiers in military barracks
- Developed world – sporadic
  - 2011: < 800 cases of invasive meningococcal disease in US
  - Serogroup B, C, or Y
- Developing world – epidemics
  - 2009: ~ 90,000 cases of meningitis were reported in 14 African countries
  - Serogroup A or W135

**Meningococcal disease in US**

![Graph](image)

*Figure 1. Rates of meningococcal disease in the United States, According to Age, 1995–2005.*

*Figure 1 from Feikin et al. 2007. Infectious Disease*
Increased Risk for Meningococcal Disease Among Men Who Have Sex With Men in the United States, 2012–2015

- 2010–2015: clusters of serogroup C meningococcal disease among MSM in major U.S. and European cities
  - disproportionately affected MSM with HIV
- Incidence of meningococcal disease among MSM was 4 times higher than non-MSM

**Neisseria meningitidis**: clinical manifestations

- Starts with respiratory tract colonization
  - Progresses to overt clinical disease or asymptomatic carrier state
- Meningococemia
  - Septic shock
  - Petechial and purpuric rash with bullae
  - Hemorrhage and disseminated intravascular coagulation
- Meningitis
  - Can occur with or without meningococcemia (most without)
  - Severe headache, neck stiffness, confusion, fever, neurologic deficits
- Less common: pneumonia, arthritis, urethritis

**Neisseria meningitidis**: diagnosis and treatment

- Microbiology
  - Gram stain: Gram neg diplococci readily seen in CSF of patients with meningitis
  - Bacterial culture – blood, CSF, respiratory secretions
  - Nucleic acid testing – multiplex PCR from CSF
- Treatment
  - Ceftriaxone is treatment of choice
  - Penicillin G can be used if bacteria is susceptible
  - Prophylaxis of close contacts recommended with rifampin, ciprofloxacin, or single dose of ceftriaxone
**Neisseria meningitidis**: vaccination

- **Serogroups A, C, Y W135 (Menactra, Menveo)**  
  - conjugated polysaccharide vaccines  
  - Adolescents: everyone age 11-12, booster age 16  
  - Adults: complement deficiency, functional or anatomic asplenia, microbiologists, outbreak, military recruit, HIV, travel

- **Serogroup B (Trumenba, Bexsero)**  
  - newer recombinant protein vaccines  
  - Adolescents: consider age 16-18  
  - Adults: complement deficiency, functional or anatomic asplenia, microbiologists, outbreak

**Neisseria**

- Two species that cause human disease:  
  - Neisseria meningitidis  
  - Neisseria gonorrhoeae

**Lecture Objectives:**

- Recognize the three proteins that mediate Neisseria gonorrhoeae host cell attachment and penetration and the importance of antigenic variation  
- Identify the primary mode of transmission of Neisseria gonorrhoeae
**Neisseria gonorrhoeae: pathogenicity and virulence**

- No true polysaccharide capsule
- Many proteins on outer membrane:
  - Pilin – mediates epithelial cell attachment
  - Opa – mediates epithelial cell attachment
  - Rmp – protects other outer membrane proteins
  - PorB – prevents phagolysosome fusion
  - Transferrin – bacterial iron metabolism

**Neisseria gonorrhoeae: pathogenicity and virulence**

- Pili, PorB, and Opa proteins mediate attachment and penetration into host cells
  - then pass into the subepithelial space where infection spreads
- Antigenic variation of pili allow bacteria to evade long-term immunity

**Neisseria gonorrhoeae: epidemiology**

- Transmission occurs across mucosal surfaces by direct contact
  - Anogenital tract most common but also oropharyngeal
- High rates of infection in adolescents and young adults (peak incidence age 15-24)
- Can have asymptomatic infection
  - 50% women, 5-10% of men
- Women have 50% risk of acquiring infection after exposure; men only 20% risk
Neisseria gonorrhoeae: epidemiology

Lecture Objectives:

• Distinguish between primary (localized) and secondary (disseminated) disease caused by Neisseria gonorrhoeae
• Choose the best laboratory test for the diagnosis of Neisseria gonorrhoeae

Neisseria gonorrhoeae: clinical manifestations

Primary (localized disease)
- Urethritis
- Cervicitis
- Proctitis
- Pharyngitis
- Neonatal conjunctivitis

Secondary (local invasion or disseminated)
- Epididymitis/prostatitis
- Osteomyelitis (Pelvis Inflammatory Disease)
- Gonococcal endocarditis and septic arthritis
- Dermatitis
Gonococcal cervicitis and urethritis

Disseminated gonococcemia

*Neisseria gonorrhoeae*: diagnosis

- Gram stain - urethra, cervix, joint fluid
  - 95% sensitive from male urethra
  - 50-70% sensitive from uterine cervix
- Culture
  - Requires chocolate agar and CO2
- Nucleic acid amplification (NAAT – PCR) - direct detection from clinical specimens
  - Genital, oral, anal, urine
  - Easy to collect and high sensitivity and specificity = preferred test
  - Cannot test antibiotic susceptibility
Neisseria gonorrhoeae: treatment

- Ceftriaxone IM injection x 1 dose is preferred treatment
- Given with treatment for possible co-infection with Chlamydia trachomatis (either azithromycin or doxycycline)
- Drug resistance is becoming a global health problem
  - Widespread resistance to quinolones and azithromycin with increasing resistance reported to cephalosporins

A 21-year-old woman was admitted to the hospital with a 2-day history of fever, chills, and a few painful erythematous pustules over her arms and legs. She had also noted a painful, red, hot, swollen right knee for the past 2 days. She had an elevated white blood cell count. Blood cultures drawn at the time of admission were positive 10 hours later with gram-negative diplococci.

Which of the following is true regarding microbiologic culture and identification of this bacteria?

A. Requires supplemental O₂
B. Grows best on chocolate agar
C. Oxidase test negative
D. Nucleic acid testing is not currently available

Haemophilus
Lecture Objectives:

• Identify the unique structural and culture characteristics of *Haemophilus* species
• Distinguish the pathogenic and epidemiologic significance of encapsulated versus non-encapsulated *Haemophilus influenzae* strains

---

*Haemophilus*

• Small, pleomorphic gram-negative coccobacilli
• Many have polysaccharide capsule
  – Antigenic serotypes a-f
• Pili
• LPS (endotoxin)

---

*Haemophilus*

• Culture and growth:
  – oxidase and catalase +
  – chocolate agar with growth factors
    • “X” (hemin)
    • “V” (nicotinamide adenine dinucleotide)
  – some require CO₂
Haemophilus species

- H. influenzae
  - Encapsulated (6 serotypes a-f) and non-encapsulated strains
- H. parainfluenzae – bacteremia, endocarditis
- H. (Aggregatibacter) aphrophilus - endocarditis
- H. ducryi – Chancroid
- H. aegyptius - conjunctivitis

Lecture Objectives:

- Identify the primary mode of transmission of *Haemophilus influenzae*
- Identify the factor responsible for the dramatic decline in incidence of *Haemophilus influenza* type B disease in the U.S.
- Differentiate between the clinical manifestations of invasive and non-invasive disease caused by *Haemophilus influenza*
**H. influenzae**: pathogenesis and virulence

- Transmission is via respiratory droplets
- Colonization of throat and nasopharynx
- Adherence to mucosal epithelium with pili, adhesins, and other outer membrane proteins
  - Followed by endocytosis and tissue invasion
- For type B, polysaccharide capsule is major virulence factor and promotes bloodstream invasion and more severe infection

**H. influenzae**: epidemiology

- *H. influenzae* type b was responsible for >95% of all invasive infections up to 1990
- With Hib vaccine, 94% decline
- More than half of all disease is now caused by non-encapsulated (non-typeable) strains
- Peak incidence: 6 mo – 2 years

**H. influenzae**: clinical manifestations

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Invasive disease</th>
<th>Non-invasive disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. influenzae</em></td>
<td>type b</td>
<td>Non-encapsulated strains</td>
</tr>
<tr>
<td>Rarely, non-encapsulated strains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical diseases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meningitis</td>
<td>Otitis media</td>
<td></td>
</tr>
<tr>
<td>Epiglottitis</td>
<td>Sinusitis</td>
<td></td>
</tr>
<tr>
<td>Septic arthritis</td>
<td>Acute exacerbations of chronic obstructive lung disease (COPD) in adults</td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>Conjunctivitis</td>
<td></td>
</tr>
<tr>
<td>Bacteremia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catarrhalis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>6 mos – 2 years</td>
<td>Children and adults</td>
</tr>
<tr>
<td>Vaccine</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
**H. influenzae**: diagnosis and treatment

- Gram stain and culture – CSF, blood, lower resp tract, joint fluid
  - Requires chocolate agar and supplementation
- NAAT
  - PCR testing from clinical specimens exists but may not be widely available
- Severe, invasive disease
  - 2nd and 3rd generation cephalosporins
- Less severe, non-invasive disease
  - amoxicillin/clavulanate (20-30% produce a beta-lactamase enzyme),
  - macrolides, fluoroquinolones, trimethoprim/sulfamethoxazole

---

**Epiglottitis**

**Cellulitis**
**H. Influenzae type B: vaccination**

- Conjugate vaccine: polysaccharide capsule antigens + protein
- Universal vaccination all children
  - Two or three dose primary series: 2, 4, (6) months
  - Booster dose between 12-15 mos
- Prophylaxis for household contacts: rifampin

---

A 65-year-old woman with a history of long-term smoking, COPD, and diabetes presented to her physician with 2-3 days of low grade fever, cough productive of thick yellow sputum, and fatigue. Wheezes are heard diffusely on auscultation of her lungs. The physician suspects a COPD exacerbation. A chest X-ray shows a faint infiltrate in her L upper lung. Gram stain of induced sputum shows many gram-negative coccobacilli that grow well on chocolate agar.

Which of the following is the most likely cause of her pneumonia?

A. Neisseria meningitidis  
B. Haemophilus influenzae type B  
C. Haemophilus parainfluenzae  
D. Non-encapsulated (non-typeable) Haemophilus influenzae

---

**H. influenzae: clinical manifestations**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Invasive disease</th>
<th>Non-invasive disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>H. influenzae type b</td>
<td>Non-encapsulated strains</td>
</tr>
<tr>
<td>Clinical diseases</td>
<td>Meningitis, epiglottitis, septic arthritis</td>
<td>Otitis media, sinusitis</td>
</tr>
<tr>
<td></td>
<td>Pneumonia, bacteremia, cellulitis, osteomyelitis</td>
<td>Acute exacerbations of chronic obstructive lung disease (COPD) in adults, conjunctivitis</td>
</tr>
<tr>
<td>Age</td>
<td>6 mos – 2 years</td>
<td>Children and adults</td>
</tr>
<tr>
<td>Vaccine</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

www.cdc.gov/vaccines
Bordetella

Learning Objectives:

• Identify at least two toxins important for the pathogenesis of *Bordetella pertussis* infection and their mechanisms of action

• Identify the primary mode of transmission of *Bordetella pertussis*

Bordetella

• Small, gram-negative coccobacilli

<table>
<thead>
<tr>
<th>Species name</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. pertussis</em></td>
<td>Pertussis</td>
</tr>
<tr>
<td><em>B. parapertussis</em></td>
<td>Milder form of pertussis. Uncommon</td>
</tr>
<tr>
<td><em>B. bronchoseptica</em></td>
<td>Respiratory disease in animals. Rare in humans</td>
</tr>
<tr>
<td><em>B. holmsei</em></td>
<td>Rare cause of sepsis</td>
</tr>
</tbody>
</table>
**Bordetella pertussis**

- Culture and growth:
  - Strict aerobe, non-motile, non-spore forming
  - Catalase +
  - Difficult to grow:
    - Sensitive to drying
    - Susceptible to toxic substances, metabolites
    - Agar supplemented with charcoal, starch, blood, or albumin

**B. pertussis**: pathogenesis and virulence

- NO polysaccharide capsule
- Filamentous hemagglutinin
- LPS / LOS (endotoxin)
- Major important toxins:
  - Pertussis toxin (PT)
  - Tracheal cytotoxin
  - Adenylate cyclase toxin

**B. pertussis: epidemiology**

- Spread via highly infectious respiratory droplets
- Human disease – no other reservoir
- Most common and severe in infants birth-2 years
- Adults can have asymptomatic infection and spread to others
- Decreased incidence with vaccination
  - endemic worldwide, ~16 million infections and 200,000 deaths each year, primarily in unvaccinated children
Learning Objectives

• Recognize the unique separate clinical stages and manifestations of infection with *Bordetella pertussis*
• Identify the methods of diagnosis of *Bordetella pertussis* infection
• Given an immunologic history and patient’s age, determine if vaccination with pertussis vaccine is recommended

**B. pertussis: clinical manifestations**

<table>
<thead>
<tr>
<th>Infection</th>
<th>Catarrhal</th>
<th>Paroxysmal</th>
<th>Convalescent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration</td>
<td>7-10 days</td>
<td>1-2 weeks</td>
<td>3-4 weeks (or longer)</td>
</tr>
<tr>
<td>Symptoms</td>
<td>None</td>
<td>Rhinorrhea, malaise, fever, cough, anorexia</td>
<td>Paroxysmal cough, with or without development of <em>B. pertussis</em> infection; cyanosis, seizures, encephalopathy</td>
</tr>
</tbody>
</table>

"100 day cough"
B. pertussis: diagnosis and treatment

- Lymphocytosis common
- Culture ‘pernasal’ (nasopharyngeal) swab
- NAAT - PCR
  - Higher sensitivity, specificity
  - Can be done at LUMC and state public health dept labs
- Macrolide treatment only effective in catarrhal stage

B. pertussis: prevention

- Acellular vaccines - contain inactivated pertussis toxin with other antigens
- DTaP
  - All infants receive 5-dose series starting at 2 mos
- Tdap
  - Adults receive this in place of one Td booster given every 10 years
  - Especially important for people in regular contact with infants

An 8 month old infant developed acute onset of a mild cough, watery nasal discharge, poor feeding, and a mildly elevated temperature to 100.1 F. Symptoms lasted about a week and were followed by episodes of severe, repetitive coughing with a characteristic whooping sound followed by vomiting. The infant required hospitalization for a week due to dehydration and respiratory distress, and a nasopharyngeal swab PCR was positive for B. pertussis. Now, the infant is back home with a residual cough but less severe in intensity.

Which stage of infection is the infant currently displaying?
A. Paroxysmal
B. Catarrhal
C. Convalescent
D. Incubation