

Introduction to Microbiology



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Lecture 23

Overview

Bacterial genera that will be discussed this lecture are Gram negative bacilli and coccobacilli, that usually infect the respiratory tract:

Pseudomonas

Legionella

Moraxella

Bordetella

Haemophilus

Pseudomonas

Case Study and Questions

A 63-year-old man has been hospitalized for 21 days for the management of newly diagnosed leukemia. Three days after he entered the hospital, a urinary tract infection with *Escherichia coli* developed. He was treated for 14 days with broad-spectrum antibiotics. On day 21 of his hospital stay, the patient experienced fever and shaking chills. Within 24 hours he became hypotensive, and ecthymic skin lesions appeared. Despite aggressive therapy with antibiotics, the patient died. Multiple blood cultures were positive for *Pseudomonas aeruginosa*.

1. What factors put this man at increased risk for infection with *P. aeruginosa*?
2. What virulence factors possessed by the organism make it a particularly serious pathogen? What are the biological effects of these factors?

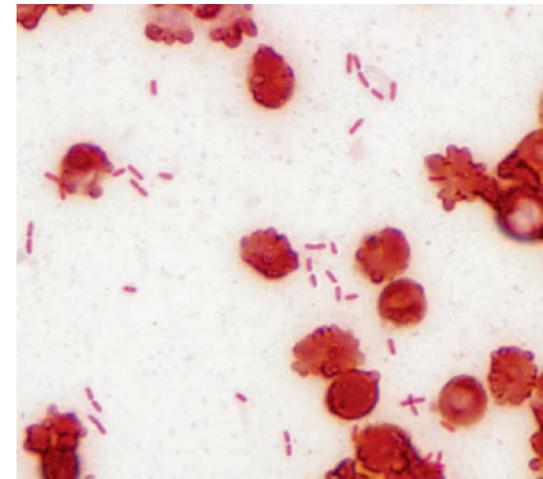


FIGURE 27-5 Colonial morphology of *Pseudomonas aeruginosa*. Note the green pigmentation that results from the production of two water-soluble dyes: blue pyocyanin and yellow fluorescein.

Pseudomonas

- Motile, straight or slightly curved, **gram-negative rods** (0.5 to 1.0 × 1.5 to 5.0 μm) typically **arranged in pairs**. Capable of using many organic compounds as sources of carbon and nitrogen, (**broad environmental distribution**)
- Members of the genus are found in soil, decaying organic matter, vegetation, and water. Also found throughout the **hospital environment**. And is **resistant to many antibiotics** and **disinfectants**.
- *Pseudomonas* infections are **primarily opportunistic**.
- The presence of **cytochrome oxidase** (detected in a rapid 5-minute test) is used to differentiate them from Enterobacteriaceae
- Production of the blue-green pigment pyocyanin with ***Pseudomonas aeruginosa*** (*most important pathogen of the group*)
- **Distinctive smell !**

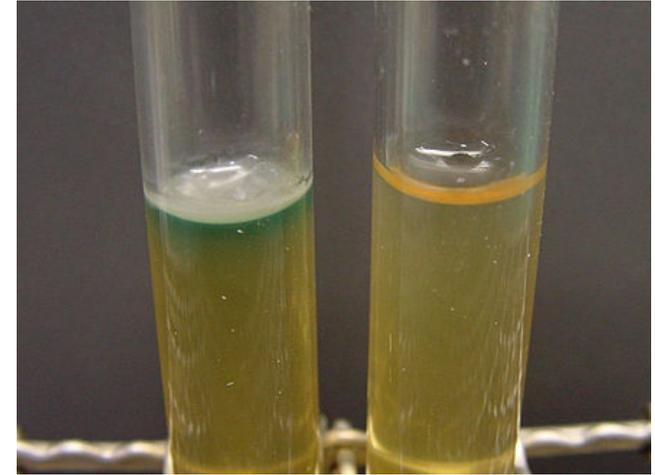
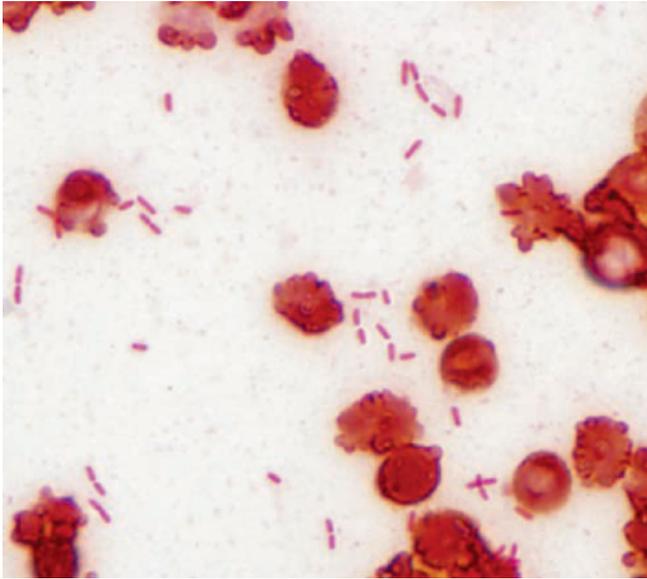
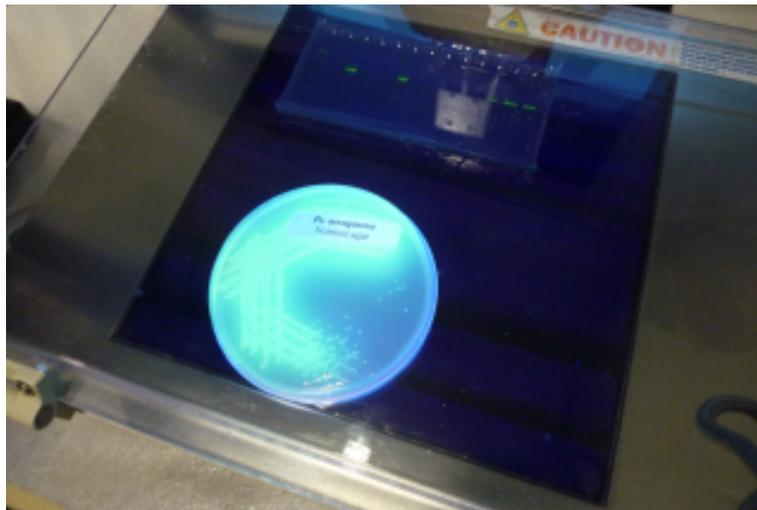


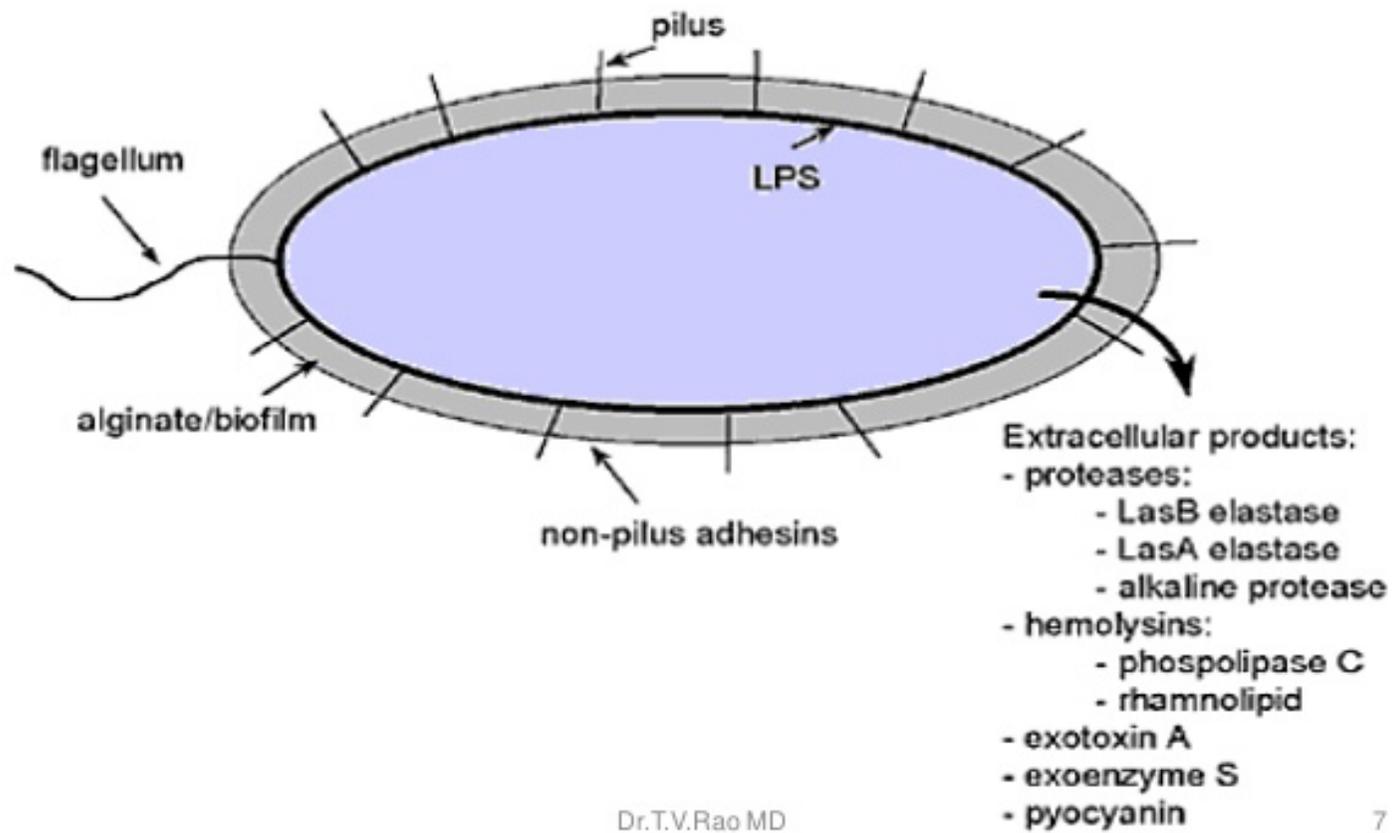
FIGURE 27-5 Colonial morphology of *Pseudomonas aeruginosa*; note the green pigmentation that results from the production of two water-soluble dyes: blue pyocyanin and yellow fluorescein.

Smells fruity, "grape-like" ,
"fresh-tortilla"



Pseudomonas/ Pathogenesis and Immunity

- **Adhesins** : (1) flagella, (2) pili, (3) lipopolysaccharide (LPS), and (4) **alginate** (a mucoid exopolysaccharide that forms a prominent **capsule**)
- **Toxins and Enzymes : Exotoxin A** (This toxin **disrupts protein synthesis** by **blocking peptide chain elongation** in eukaryotic cells).
- **Phospholipase C** is a heat-labile hemolysin that breaks down lipids and lecithin, facilitating tissue destruction
- *P. aeruginosa* is **intrinsically resistant to many antibiotics** (the low rate of movement of antibiotics through the outer membrane pores into the bacterial cell, combined with the rapid efflux of antibiotics with efflux pumps . Also **acquired and adaptive resistance**).



Dr.T.V.Rao MD

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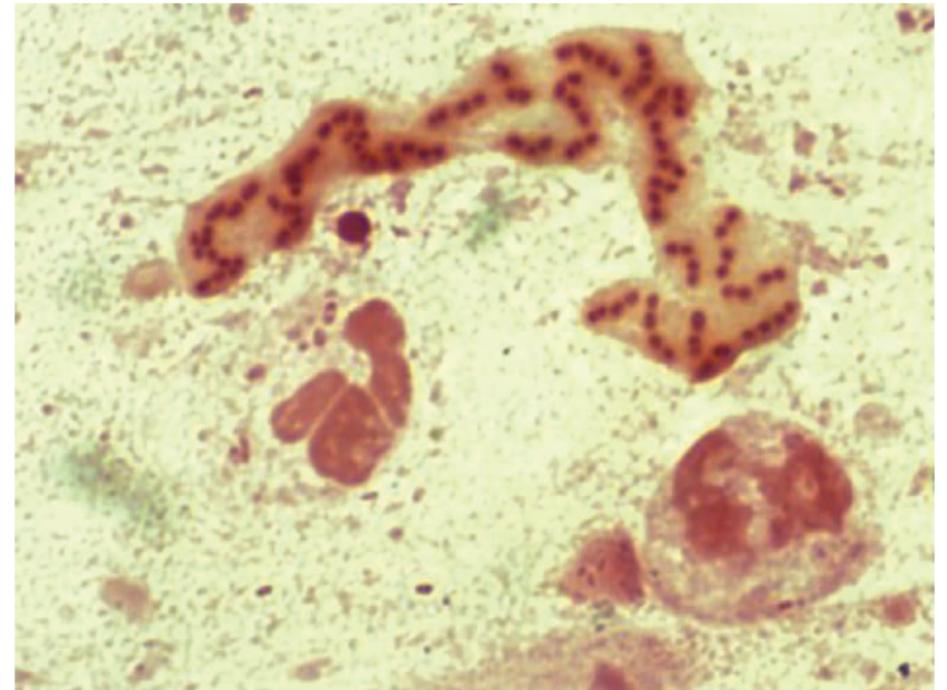


FIGURE 27-2 Gram stain of *Pseudomonas aeruginosa* surrounded by mucoid capsular material in cystic fibrosis patient.

Pseudomonas/ Clinical Diseases

- **Pulmonary Infections: asymptomatic colonization** (in patients with **Cystic Fibrosis** and other chronic lung diseases) or benign inflammation of the bronchials (**tracheobronchitis**) to severe **necrotizing bronchopneumonia**. Previous **therapy with broad-spectrum antibiotics** and use of **mechanical ventilation equipment** predispose to infection.
- **Skin and Soft-Tissue Infections:** The most recognized are infections of **burn wounds**. **Folliculitis** (associated with immersion in contaminated waters such as hot tubs).
- **Urinary Tract Infections:** seen primarily in patients with long-term **indwelling urinary catheters**.
- **Ear Infections: External otitis** is frequently caused by *P. aeruginosa*, with swimming an important risk factor (“**swimmer’s ear**”).
- **Eye Infections :** Occur after initial trauma to the cornea (e.g., abrasion from contact lens).
- **Bacteremia and Endocarditis:** mortality rate in affected patients is higher with *P. aeruginosa* bacteremia

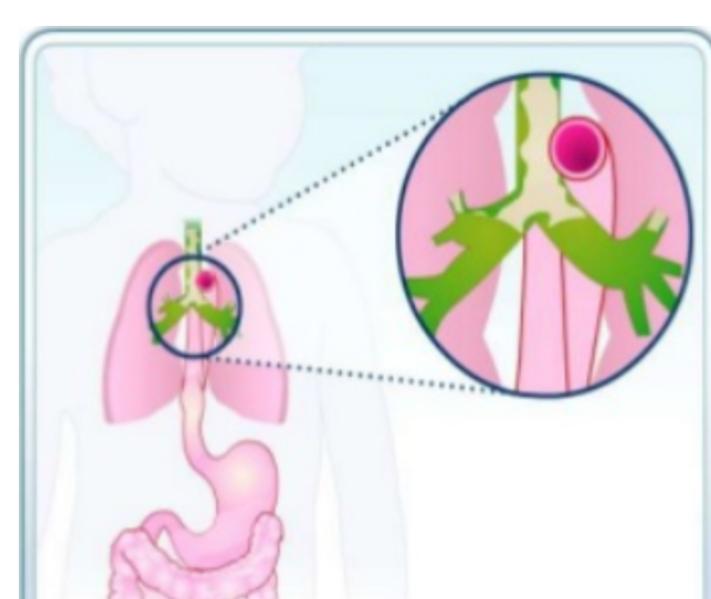
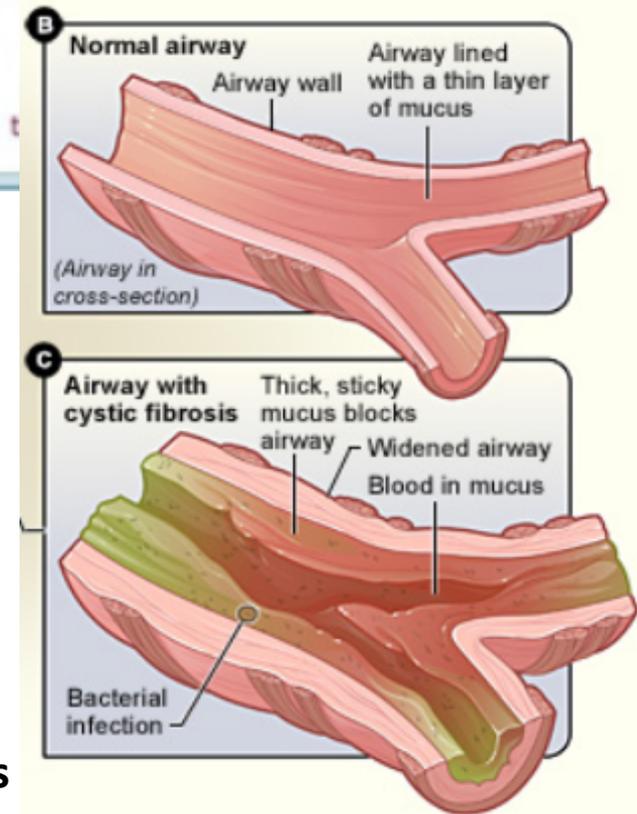


FIGURE 27-3 *Pseudomonas* infection of burn wound.



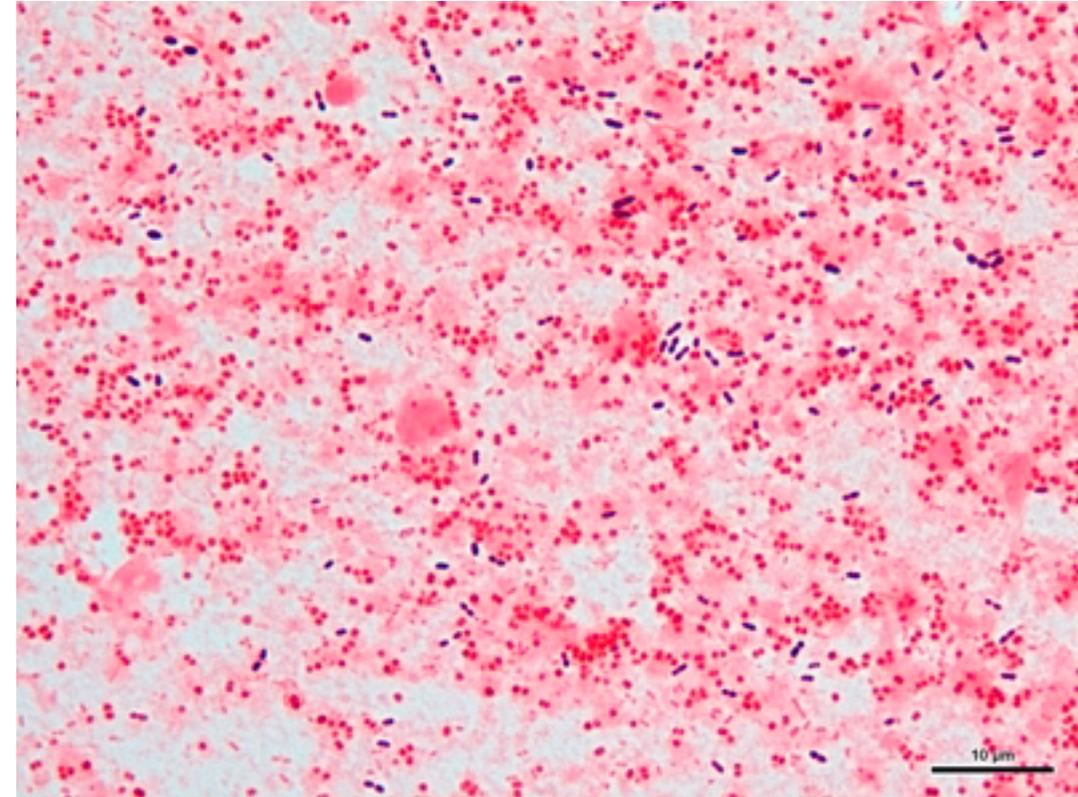
Cystic fibrosis

Pseudomonas/ Clinical Diseases

- The underlying conditions required for most infections are
 - (1) the presence of the organism in a **moist reservoir** and
 - (2) **compromised host defenses** (e.g., cutaneous trauma, elimination of normal microbial flora as a result of antibiotic usage, neutropenia)
- antimicrobial **therapy for *Pseudomonas* infections is frustrating** because the bacteria are typically resistant to most antibiotics and the infected patient has compromised immune defences
- A **combination of antibiotics** is generally required for therapy to be successful in patients with serious infections.

Moraxella

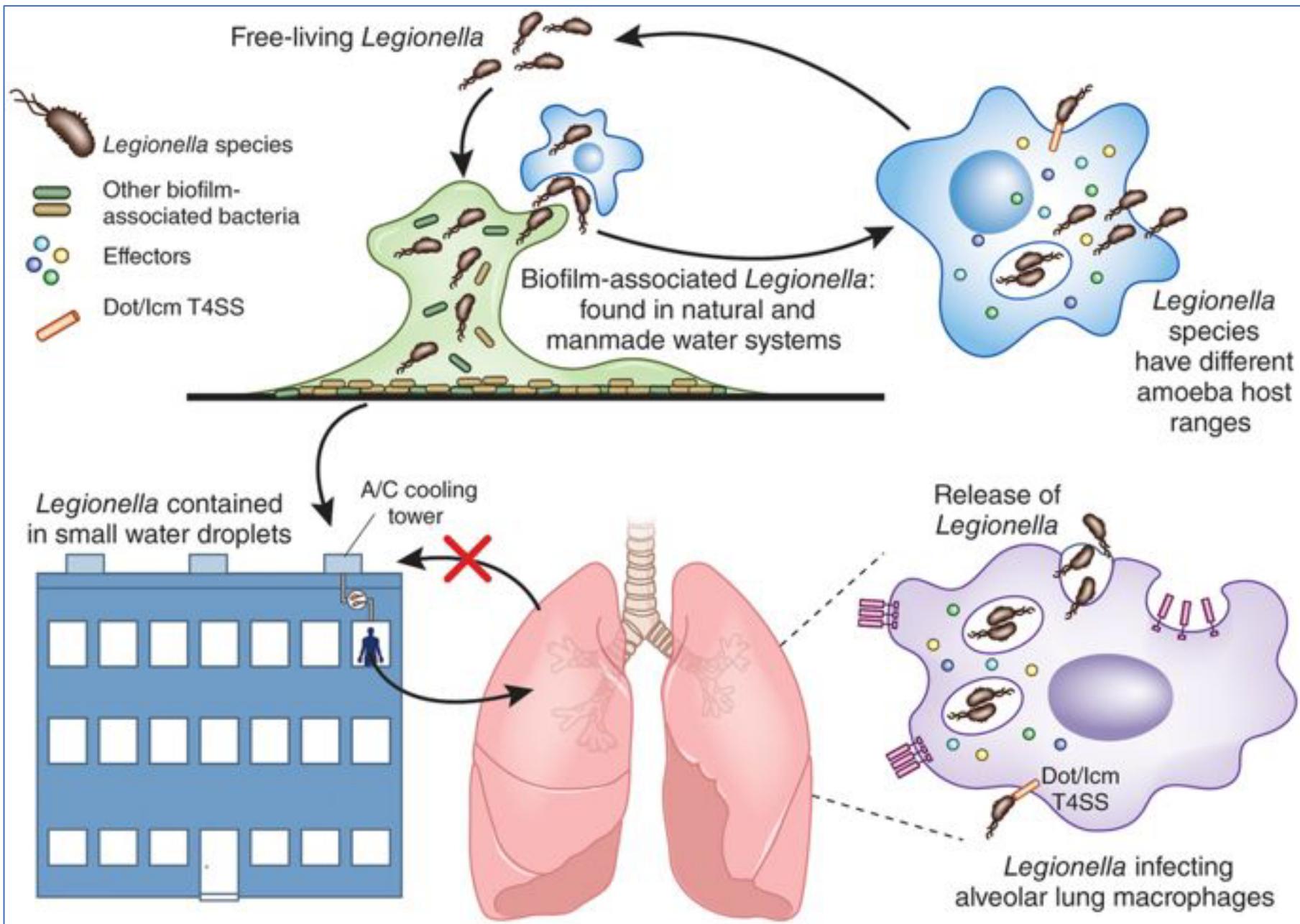
- *M. catarrhalis* is a strictly aerobic, oxidase-positive, gram-negative diplococci
- The peak rate of colonisation by *M. catarrhalis* appears to occur around 2 years of age, with a **striking difference in colonization rates between children and adults** (very high to very low).
- Over the last 20 to 30 years, the bacterium has emerged as a genuine pathogen and is now considered **an important cause of upper respiratory tract infections** in otherwise healthy children and elderly people.
- *M. catarrhalis* is an important cause of lower respiratory tract infections, particularly in adults with chronic obstructive pulmonary disease (COPD)
- *M. catarrhalis* is considered a nosocomial pathogen.



|| Numerous gram negative diplococci ||
Numerous gram positive diplococci || Few
small gram negative rods

Legionella

- **Legionella** acquired its name after an outbreak of a then-unknown "mystery disease" sickened 221 persons, causing 34 deaths, at a convention of the American **Legion**.
- **Slender, pleomorphic, gram-negative rods**
- Legionellae are obligatively aerobic and nutritionally fastidious (They require media supplemented with **cysteine**).
- Human infections are most commonly associated with **exposure to contaminated aerosols** (e.g., air conditioning cooling towers, whirlpool spas, showerheads, water misters). The organisms can survive in moist environments **for a long time**.
- Legionellae are facultative **intracellular bacteria** (infect and replicate in **macrophages and amoeba**). Cytokines released by the infected macrophages stimulate a **robust inflammatory response** that is characteristic of infections with *Legionella*.



Legionella

- ***L. pneumophila*** is the cause of 90% of all Legionella infections, affecting **the lungs** and present in one of two forms :
 - (1) an influenza-like illness (referred to as **Pontiac fever** (self-limited, febrile illness)) and
 - (2) a severe form of pneumonia (i.e., **legionnaires disease**).
- **Legionnaires disease** is characteristically more severe and, if untreated, promptly causes considerable morbidity.
- The medium most commonly used for the isolation of legionellae is **buffered charcoal yeast extract (BCYE) agar**

Legionella



Clinical Case 29-6 Outbreak of Legionnaires Disease

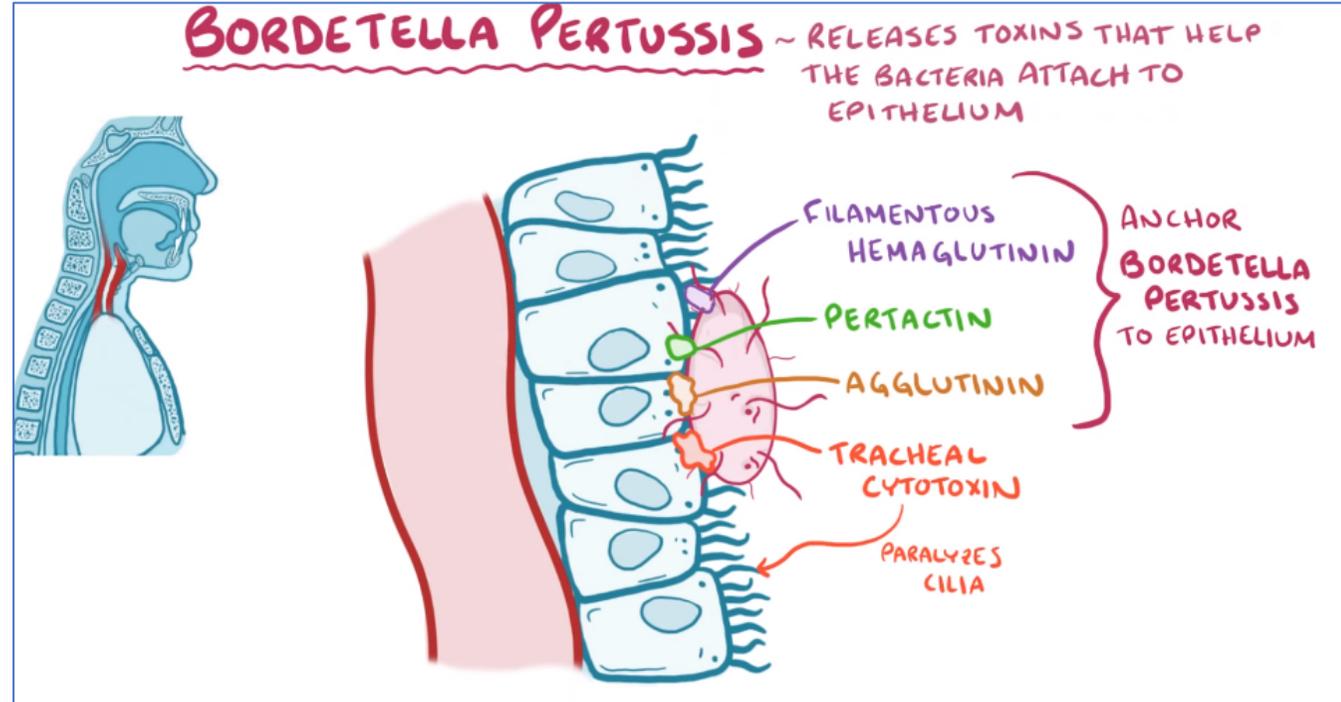
Kirrage and associates (*Respir Med* 101:1639–1644, 2007) described an outbreak of legionnaires disease (LD) that occurred in Hereford, England. On October 24, 2003, the public health agency was notified that an elderly man had died of LD. Three days later, the agency was notified that an elderly woman had also died of LD. As part of an active surveillance investigation, two additional patients with positive *Legionella* urine antigen tests were identified in a local hospital. Further investigations revealed 28 epidemiologically linked patients with the onset of disease from October 8 to November 20. All patients had positive urine antigen tests, four had high antibody titers, and two were culture positive. The implicated source of the outbreak was a cooling tower that had recently been restarted after a period of inactivity. After the tower was closed and recleaned, the epidemic was terminated. This outbreak illustrates the difficulty of recognizing the problem when the individuals infected may present to different hospitals. This is particularly a problem when the source is located in a hotel or vacation place.

Bordetella

- *Bordetella* is an extremely **small, fastidious, strictly aerobic, gram-negative coccobacillus**. Even under ideal conditions, recovery of *B. pertussis* in **culture is difficult**.
- ***Bordetella pertussis***, the agent responsible for **pertussis** or whooping cough. Transmitted mainly by respiratory droplets.
- **Pertussis** is a **human disease** with no other recognized animal or environmental reservoir
- Since widespread **use of the vaccine** began, incidence has decreased more than 75% compared with the pre-vaccine era. vaccines contain inactivated **pertussis toxin, filamentous hemagglutinin, and pertactin**

Bordetella

- Pertussis is primarily a **toxin-mediated disease**. **Pertussis toxin** (A-B toxin) inactivates the protein that controls adenylate cyclase activity, leading to an increase in cyclic adenosine monophosphate (cAMP) levels and a subsequent **increase in respiratory secretions and mucus production**.
- The bacteria attach to the **cilia of the respiratory epithelial cells**, produce toxins **that paralyze the cilia**, and cause inflammation of the respiratory tract, which **interferes with the clearing of pulmonary secretions**.



	Incubation	Catarrhal	Paroxysmal	Convalescent
Duration	7-10 days	1-2 weeks	2-4 weeks	3-4 weeks (or longer)
Symptoms	None	Rhinorrhea, malaise, fever, sneezing, anorexia	Repetitive cough with whoops, vomiting, leukocytosis	Diminished paroxysmal cough, development of secondary complications (pneumonia, seizures, encephalopathy)
Bacterial culture				



classic whooping cough paroxysms

FIGURE 29-3 Clinical presentation of *Bordetella pertussis* disease.

Haemophilus

Case Study and Questions

A 78-year-old man confined to a nursing home awoke with a severe headache and stiff neck. Because he had a high fever and signs of meningitis, the nursing home staff took him to a local emergency department. The cerebrospinal fluid (CSF) specimen was cloudy. Analysis revealed 400 white blood cells per mm³ (95% polymorphonuclear neutrophils), a protein concentration of 75 mg/dl, and a glucose concentration of 20 mg/dl. Small gram-negative rods were seen on Gram stain of the CSF, and cultures of CSF and blood were positive for *Haemophilus influenzae*.

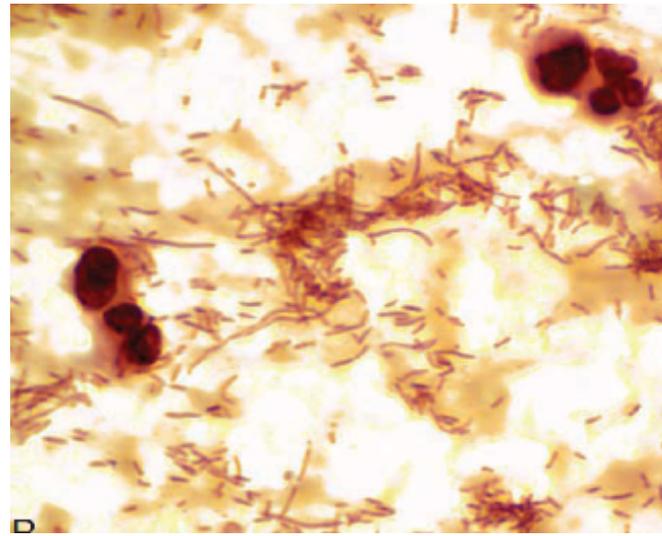
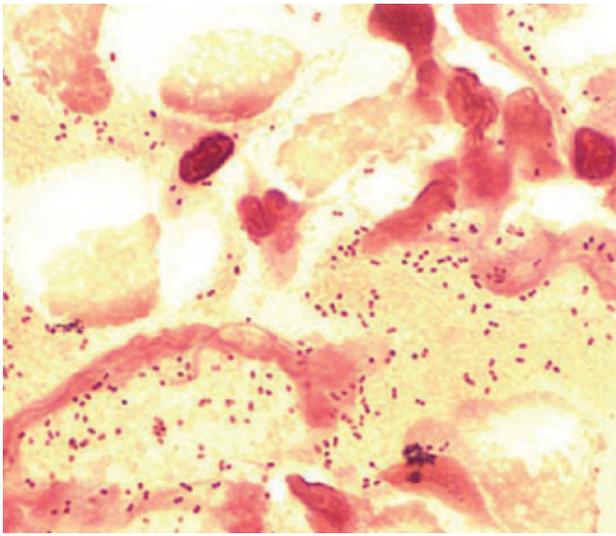
1. *Discuss the epidemiology of H. influenzae meningitis, and compare it with the epidemiology of meningitis caused by Streptococcus pneumoniae and Neisseria meningitidis.*
2. *Compare the biology of the H. influenzae strain that is likely to be the cause of this patient's disease with that of the strains that historically caused pediatric diseases (before vaccination).*

Haemophilus

Haemophilus

haemo, blood; *hilos*, lover (“blood lover”;
requires blood for growth on agar media)

- Haemophilae are small, sometimes pleomorphic, gram-negative rods present on the mucous membranes of humans.
- ***Haemophilus influenzae*** is the species most commonly associated with disease, also ***Haemophilus aegyptius***, and ***Haemophilus ducreyi***.
- *Haemophilus* requires supplementation of media with one or both of the following growth-stimulating factors: (1) **hemin** (also called **X factor** for “unknown factor”) and (2) **nicotinamide adenine dinucleotide (NAD; also called V factor** for “vitamin”)
- *Haemophilus* species are present in almost all individuals, primarily **colonizing the mucosal membranes of the respiratory tract**.
- The surface of many, but not all, strains of *H. influenzae* is covered with a **polysaccharide capsule**, and six antigenic **serotypes (a through f)**



Heated blood (chocolate) agar. Named for the color and contains no actual chocolate !



FIGURE 24-3 Satellite phenomenon. *Staphylococcus aureus* excretes nicotinamide adenine dinucleotide (NAD, or V factor) into the medium, providing a growth factor required for *Haemophilus influenzae* (small colonies surrounding *S. aureus* colonies [arrow]).

Haemophilus influenzae

- The major virulence factor in *H. influenzae* type b is the **antiphagocytic polysaccharide capsule**, which contains ribose, ribitol, and phosphate (commonly referred to as **polyribitol phosphate [PRP]**).
- Antibodies directed against the capsule greatly stimulate bacterial phagocytosis and complement-mediated bactericidal activity. These antibodies develop because of **natural infection, vaccination** with purified PRP, or the passive **transfer of maternal antibodies**.
- When vaccines containing purified PRP antigens conjugated to protein carriers (i.e., diphtheria toxoid, tetanus toxoid, meningococcal outer membrane protein) were introduced in December 1987, a protective antibody response in infants aged 2 months and older was produced, and systemic disease in children younger than age 5 was virtually eliminated in the United States.
- Most of the *H. influenzae* type b infections now occur in **children who are not immune** (because of incomplete vaccination or a poor response to the vaccine) and in **elderly** adults with waning immunity.
- *H. influenzae* serotype b was responsible for more than 95% of all invasive *Haemophilus* infections. **After introduction of the vaccine**, more than half of all invasive disease is now caused by nonencapsulated (**nontypeable**) strains

Haemophilus influenzae

- Adhesins mediate colonization of the oropharynx with *H. influenzae*. Cell wall components of the bacteria damage the respiratory epithelium. Then translocation across both epithelial and endothelial cells occurs and the bacteria can enter the blood.
- In the absence of specific opsonic antibodies directed against the polysaccharide capsule, high-grade bacteremia can develop.

Haemophilus influenzae

- A common cause of **disease in unvaccinated children** (i.e., meningitis, epiglottitis [obstructive laryngitis], cellulitis). *H. influenzae* type b remains the most significant pediatric pathogen in many countries of the world.
- **Nonencapsulated** strains of *H. influenzae* are opportunistic pathogens that can cause infections of the upper and lower airways. Most studies have shown that *H. influenzae* and *Streptococcus pneumoniae* are the **two most common causes of acute and chronic otitis and sinusitis**

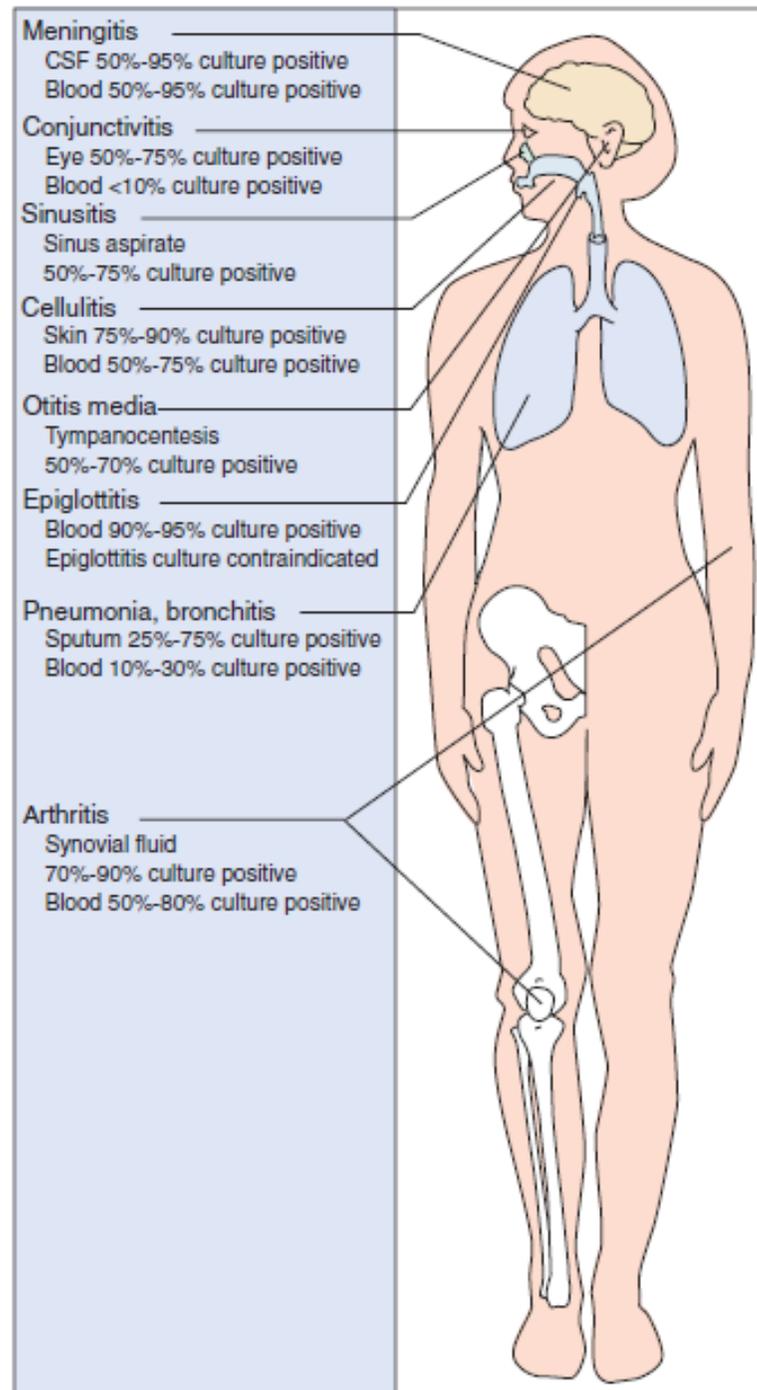


FIGURE 24-2 Infections caused by *Haemophilus influenzae*. With the advent of the conjugated vaccine, most infections in adults involve areas contiguous with the oropharynx (i.e., lower respiratory tract, sinuses, ears). Serious systemic infections (e.g., meningitis, epiglottitis) can occur in nonimmune patients. CSF, Cerebrospinal fluid.

Haemophilus influenzae

Haemophilus influenzae

Meningitis: a disease primarily of unimmunized children characterized by fever, severe headache, and systemic signs

Epiglottitis: a disease primarily of unimmunized children characterized by initial pharyngitis, fever, and difficulty breathing, and progressing to cellulitis and swelling of the supraglottic tissues, with obstruction of the airways possible

Pneumonia: inflammation and consolidation of the lungs observed primarily in the elderly with underlying chronic pulmonary disease; typically caused by nontypeable strains

- Patients with systemic *H. influenzae* infections require **prompt antimicrobial therapy** because the mortality rate in patients with untreated meningitis or epiglottitis approaches 100%.
- A **presumptive identification** of *H. influenzae* can be made by the **Gram stain morphology** and demonstration of a **requirement for both X and V factors**.
- The **immunologic detection** of *H. influenzae* antigen, specifically the **PRP capsular antigen**, is a rapid and sensitive way to diagnose *H. influenzae* type b disease.

Haemophilus

- ***Haemophilus aegyptius***, also called the **Koch-Weeks bacillus**, causes an acute purulent **conjunctivitis**
- ***Haemophilus ducreyi*** can cause chancroids, a sexually transmitted disease that is most commonly diagnosed in men. Approximately 5 to 7 days after exposure, a tender papule with an erythematous base develops on the genitalia or perianal area.

Species	Primary Diseases	Frequency
<i>H. influenzae</i>	Pneumonia, sinusitis, otitis, meningitis, epiglottitis, cellulitis, bacteremia	Common worldwide; uncommon in United States
<i>H. aegyptius</i>	Conjunctivitis	Uncommon
<i>H. ducreyi</i>	Chancroid	Uncommon in United States
<i>H. parainfluenzae</i>	Bacteremia, endocarditis, opportunistic infections	Rare

Further reading:

- Murray - Medical Microbiology 8th Edition
Section 4: Bacteriology
Chapter 27:
Chapter 29: