



Microbiology

Doctor 2018 | Medicine | JU

● Sheet

○ Slides

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DOCTOR

Anas

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

Pseudomonas, Legionella, Moraxella, Bordetella, haemophilus

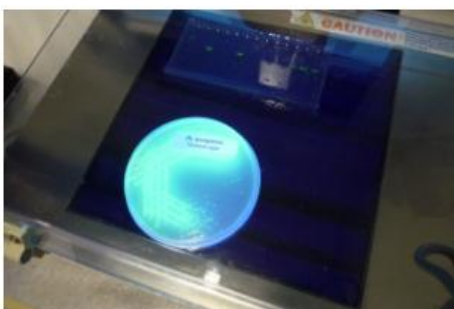
1) *pseudomonas*:

properties

- **Motile**, straight or slightly curved, **gram-negative rods** ($0.5 \text{ to } 1.0 \times 1.5 \text{ to } 5.0 \mu\text{m}$) typically arranged in **pairs “grape-like”**. 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 aeruginosa*, normal flora, is an **opportunistic pathogen**, especially with people who take immunosuppressive drugs. Also, it's a common pathogen for **wounds (disturbance of epithelial barrier)**.
 - **cytochrome oxidase**, found in *pseudomonas*, is used to differentiate between **Enterobacteriaceae** and **pseudomonas**. HOW?
- We add a drop of oxidase to the colony. The organism that produces cytochrome c will change the color.
- *Pseudomonas aeruginosa* colonies are distinguished by the production of **blue-green** pigment, **pyocyanin**.
 - they have a **fruity** 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.



fluorescence test for *p. aeruginosa* is called: **fluorescein**

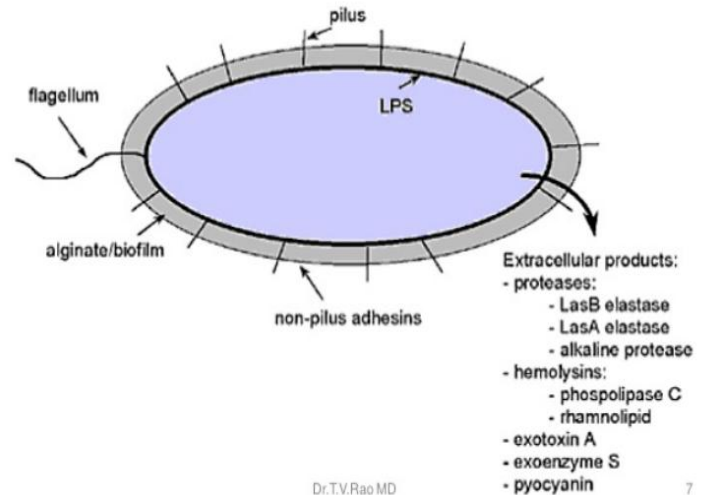
Pathogenesis and immunity

* it has virulence factors that helps in **adhesion**, movement, etc...

LPS, flagella, pili and alginate, which is a mucoid **exopolysaccharide** that forms a **capsule** around pseudomonas cell wall.

* it produces **EXOTOXIN A** that **disrupt protein synthesis** in eukaryotic cells, similar to diphtheria toxin, by blocking peptide chain elongation.

* pseudomonas expresses phospholipase c which is a heat-labile hemolysin that breaks down lipids and lecithin, facilitating tissue destruction.



p. aeruginosa is intrinsically resistant to many antibiotics. HOW?

By the:

- low rate of entrance of antibiotics through the outer membrane pores into the cell, it impedes the entrance of antibiotics.
- and the rapid efflux of antibiotics by the efflux pump.
- acquired and adaptive resistant.

Clinical diseases

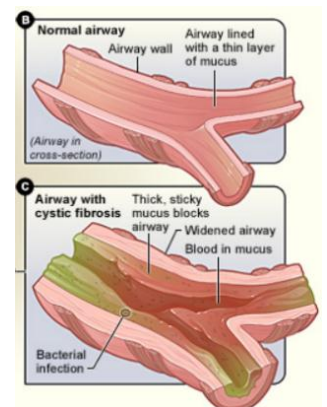
It affects many parts in our body

* **pulmonary infections:**

it could be presented as **asymptomatic colonization** especially with **immunocompromised patients**, (**cystic fibroses** which is a common disease that affects lung and GIT and impair our immune system) and people with **chronic lung diseases**.

It can also be presented as benign inflammation of the bronchioles (tracheobronchitis) to severe necrotizing bronchopneumonia.

- the use of **mechanical ventilation equipment** and broad-spectrum antibiotics predispose to **p. aeruginosa infections**.



* **eye infections:** Occur after initial trauma to the cornea or by contaminated lens.



* **Bacteremia and Endocarditis:** *p. aeruginosa* can cause bacteremia with a high mortality rate.

* **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 in a contaminated water.

* **Skin and Soft-Tissue Infections:** pseudomonas is the most common pathogen of **burn wounds (slows the healing process)**. Also, immersion in contaminated water causes **folliculitis**, inflammation in *hair follicles*.



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

In general: *p. aeruginosa* is the most common pathogen in pseudomonas genus.

((((Conditions required for *p. aeruginosa* infections are:

- **compromised host defense** (e.g., cutaneous trauma, elimination of normal microbial flora as a result of antibiotic usage, neutropenia)

- the presence of the organism in a **moist reservoir**. e.g folliculitis and ear infections.)))))

* the **antimicrobial therapy for pseudomonas is usually frustrating “disappointing”** as It infects immunocompromised patients and it is typically resistant to most antibiotics.

A **combination of antibiotics** is generally required for therapy to be successful in patients with **serious** infections.

Now read this case and try to answer its questions.

1st answer: **He is immunocompromised (leukemia).**

2nd: **Exotoxin A, LPS, Etc..**

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?

2) *Moraxella*

* *M. catarrhalis* is a strictly aerobic, oxidase-positive, **gram-negative diplococci**, *two joined cells*.

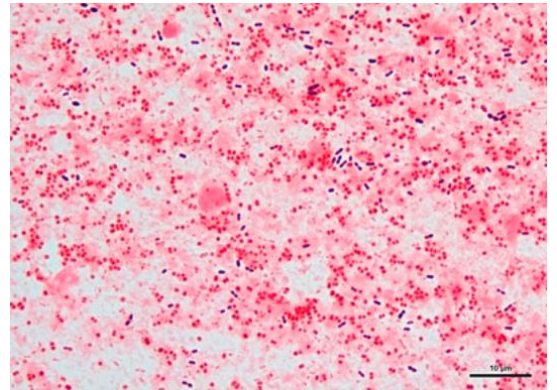
M. catarrhalis is a normal flora in the lung. So, it is opportunistic pathogen for patients of Chronic Pulmonary Obstructed Disease (**COPD**).

In this picture you can see many gram +ve diplococci “blue” as well as gram -ve diplococci “pink”.

e.g: G+ve diplococci: *Strep. pneumoniae*

G-ve diplococci such as *Neisseria*.

Moraxella is considered **coccobacilli** bacteria, which means that it has features of both *bacilli* and *cocci*. (this what the doctor said!)



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

Moraxella is a genus of Gram-negative bacteria. The organisms are short rods, coccobacilli, or as in the case of *Moraxella catarrhalis*, diplococci in morphology. “Wikipedia”

- The peak rate of colonization 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)**.

- **Now, it's considered an important cause of upper respiratory tract infections** in otherwise healthy children and elderly people. **Also, *M. catarrhalis* is considered a nosocomial infection.**

3) *Legionella*:

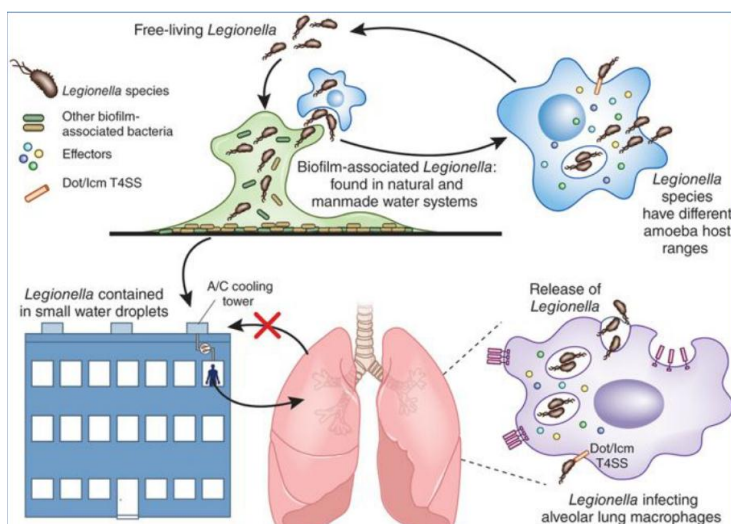
Properties:- Slender, pleomorphic, gram-negative rods.

- **obligative aerobes** and **nutritionally fastidious**, require media supplemented with **cysteine**.

- usually associated with contaminated aerosols (رذاذ) e.g; **air conditioning cooling towers** as you will see in the next picture.

- **can survive in moist environments for a long time**. Such as *pseudomonas*.

- **facultative intracellular** bacteria (infect and **replicate** in **alveolar macrophages** and **amoeba**).



Pathogenesis

- Once it reaches lungs by inhalation, it infects and replicates in **Alveolar Macrophages**.

- Cytokines released by the infected **macrophages** stimulate a **strong “robust” inflammatory response** that is characteristic of infections with *Legionella*.

- 90% of legionella infections are caused by *L. pneumophila*. It affects the lung and present in one of two forms:

1- influenza like illness called **Pontiac fever** which is an acute self-limited disease.

2- **severe** form of **pneumonia** (i.e., **legionnaires disease**).

- **Legionnaires disease** is more **severe** and, if **untreated**, promptly causes considerable **morbidity**.

- legionellae is isolated by a medium called **buffered charcoal yeast extract (BCYE) agar**.



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.

4- *Bordetella*:

Properties:

- *Bordetella* is an extremely small, fastidious, **strictly aerobic, gram-negative coccobacillus**. Even under ideal conditions, recovery of *B. pertussis* in culture is difficult.

Disease:

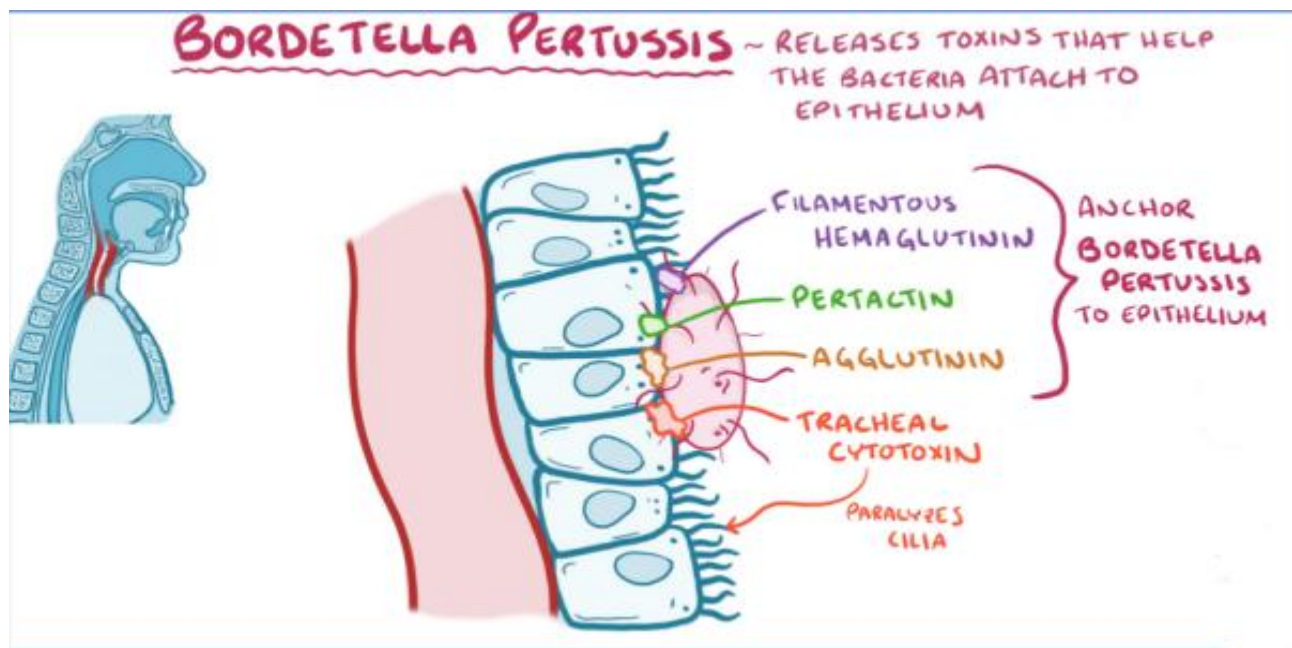
- *Bordetella pertussis*, the agent responsible for pertussis or whooping cough. Transmitted mainly by **respiratory** droplets.

- *Pertussis* (السعال الديكي) is a human disease with no other recognized animal or environmental reservoir.

Vaccine:


- Since widespread use of the vaccine began, incidence has decreased more than 75% compared with the pre-vaccine era.

Pertussis vaccine contain inactivated *pertussis toxin (toxoid)*, filamentous hemagglutinin, and pertactin.



Pathogenesis:

- 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**. (Remember: Enterotoxigenic E. coli produces two classes of enterotoxin that increases cAMP and cGMP that causes hypersecretion of fluids into the lumen and causing diarrhea).
- 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** and there we have **Pertussis**.

	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				

- An incubation period takes place for 7-10 days, permitting the production of larger number of bacteria.
- After two weeks, we notice a repetitive cough with whoops that might progress to vomiting.
- (whoops: شهقة مثل صياح الديك لمحاولة أخذ نفس)
- Then, paroxysmal cough diminishes, usually due to exhaustion.
- It is a serious infection that can develop secondary complications (pneumonia, seizures, encephalopathy).

SOME NOTES:

1. Pertussis happens usually to children (usually) or to adults who weren't vaccinated.
2. You should not miss the diagnosis of pertussis because the cough is Distinctive.

5- *Haemophilus*:

- 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 (**fastidious bacteria**) 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")

- We use chocolate agar (heated blood agar) for *Haemophilus* bacteria.
- *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) depending on the sugar found on the capsule.

Thus, there is a vaccine, called (**Hib**), directed against the capsule of serotype b, because it is the most important type of them.

Why they are called *Haemophilus*?

<i>Haemophilus</i>	<i>haemo</i> , blood; <i>hilos</i> , lover ("blood lover"; requires blood for growth on agar media)
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What this tells us?

- 1- We said that many strains of *H. influenzae* is covered with a polysaccharide capsule, thus they can survive in the blood (**bacteremia**) and reach BBB and meninges causing **MENINGITIS**. (**S. pneumonia* and *Neisseria* also have capsule and can survive in the blood and cause meningitis and what one will cause meningitis depends on several factors such as age...etc).
- 2- It is fastidious, can be grown on blood agar or on heated blood agar (chocolate agar).



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]).

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- 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, or they infected before vaccination (because of incomplete vaccination or a poor response to the vaccine) and in elderly adults with waning immunity.
- *H. influenzae* normally colonize the upper respiratory system (in immunocompetent individuals).
- *H. influenzae* serotype b was responsible for more than 95% of all invasive Haemophilus infections. After introduction of the vaccine (Hib vaccine), more than half of all invasive disease is now caused by non-encapsulated (non-typeable) strains.
- 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

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

- 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.
- Non-encapsulated (nontypeable) 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 **localized and chronic** otitis and sinusitis.
- 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 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

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).