وَقُل رَّبِّ زِدْنِي عِلْمًا

النادي الطبي

RESPIRATORY SYSTEM

HAYAT BATCH

SUBJECT : Microbiology

LEC NO. : 2

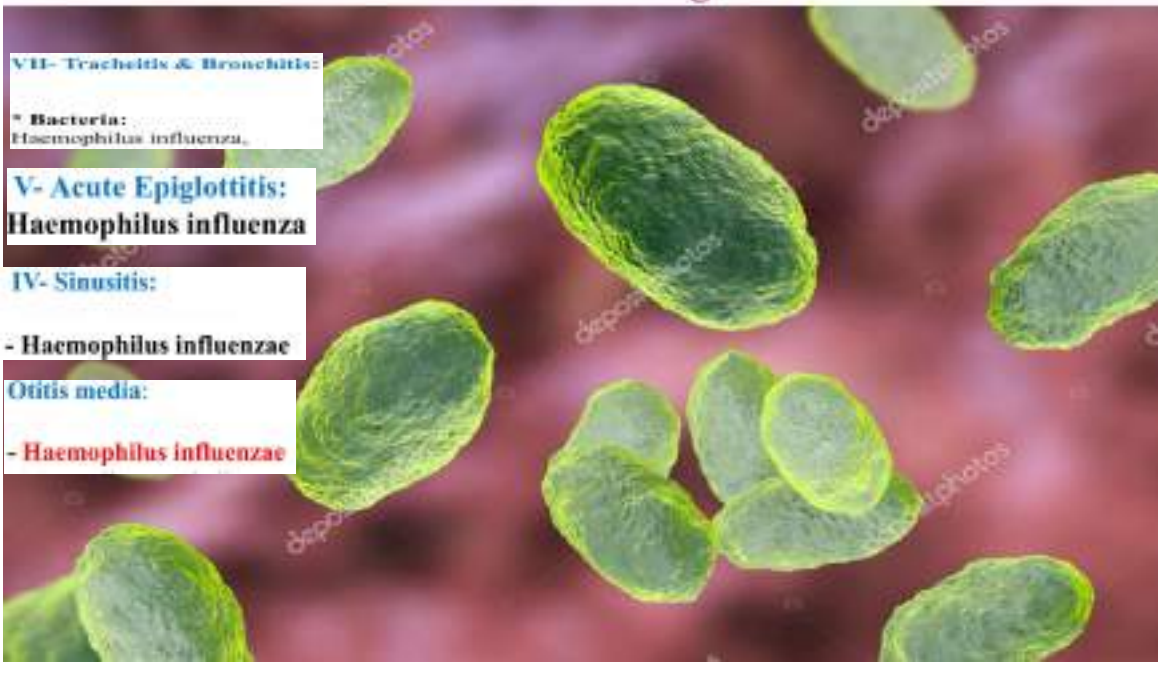
DONE BY : Ruba Almshaqba

Respiratory System

III- Infections of the ear: **Otitis Externa:** - **Pseudomonas aeruginosa.** - **Aspergillus niger** رح نأجلها لمحاضرة ال Mycology **Otitis media:** - **Strep. pneumoniae** Most common - **Haemophilus influenzae** - Moraxella catarrhalis - Streptococcus pyogenes - Staphylococcus aureus **IV- Sinusitis:** - **Strept. pneumoniae** - **Haemophilus influenzae** - Moraxella catarrhalis - Streptococcus pyogenes - Staphylococcus aureus	**V- Acute Epiglottitis:** **Haemophilus influenza** **VI- Laryngitis and croup: Mostly viral** **Parainfluenza**, Influenza, Adenovirus. **VII- Tracheitis & Bronchitis:** * **Mostly viral:** Parainfluenza, Influenza, Adenovirus and RSV. * **Bacteria: Bordetella pertussis,** Haemophilus influenza, Mycoplasma pneumonia, Chlamydia pneumonia and Streptococcus pneumonia. **VIII- Bronchiolitis:** **RSV, Parainfluenza virus**

HAEMOPHILUS INFLUENZA

"Blood Loving"



It was initially believed that this bacterium was the cause of influenza

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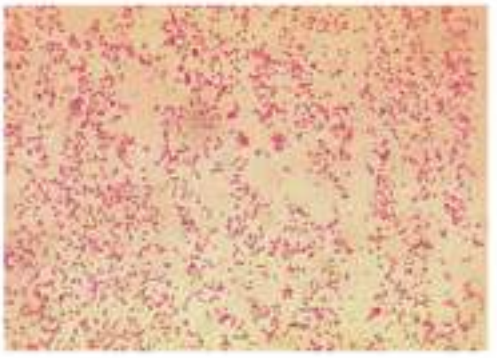
Morphology:

Very short bacilli



- **Gram negative coccobacilli.**
- **Non - motile, non - spore** forming.
- Some types are **capsulated.**

Culture:

- Grows **aerobically**, requires **extra CO2** (5-10%).
- Requires certain **growth factors called X factor (hemin)** and **V factor (Coenzyme e.g. NAD).**

موجودين داخل ال RBCs

- **Grows on blood agar in the following conditions:**

1) On **blood agar supplemented with** e.g. **IsoVitalex.**

Best media for growth

2) **On heated blood agar (Chocolate agar)** where **V & X factors released from RBCs.**

RBCs rapture

3) **Close to colonies of Staph aureus (Satellitism).**

- **Produce NO hemolysis.**

لانها بتعمل b-hemolysis لهيك رح تعمل RBCs rapture

H. influenza ال

عملت colonies بس عند ال Staph aureus

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Respiratory System

Virulence factors:

1) Polysaccharide capsule: **The major virulence factor (antiphagocytic activity).**

- Capsulated strains can be classified into **6 types** (a-f).
- H. Influenzae **type b (Hib)** is the most pathogenic and its capsule composed of (**polyribitol phosphate**) (PRP).

2) Outer membrane: ↓↓ mucociliary clearance → colonization.

3) IgA protease: degrades secretory IgA, thus facilitating attachment to the respiratory mucosa.

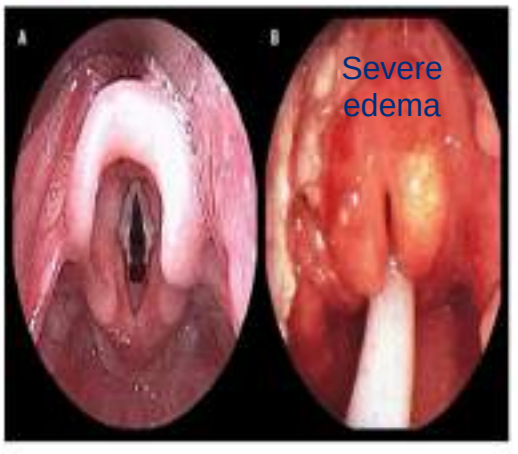
Pathogenicity:

Transmission: droplet infection.

Capsulated types exhibit greater virulence compared to non-capsulated types.

A. Capsulated types (invasive) particularly type b (Hib) cause:

1- Epiglottitis: This life-threatening disease of young children which can **obstruct the airway (medical emergency),** is caused almost exclusively by H. influenzae. A **swollen "cherry-red" epiglottis** is seen. **Tracheostomy or endotracheal intubation is life saving.**



2- Bacteraemia, Meningitis, Septic arthritis.

N.B. Asplenia (anatomical or functional) is important risk factor for infection with encapsulated organisms. Ex : Sickle cell anaemia

اهمهم H. influenza ال

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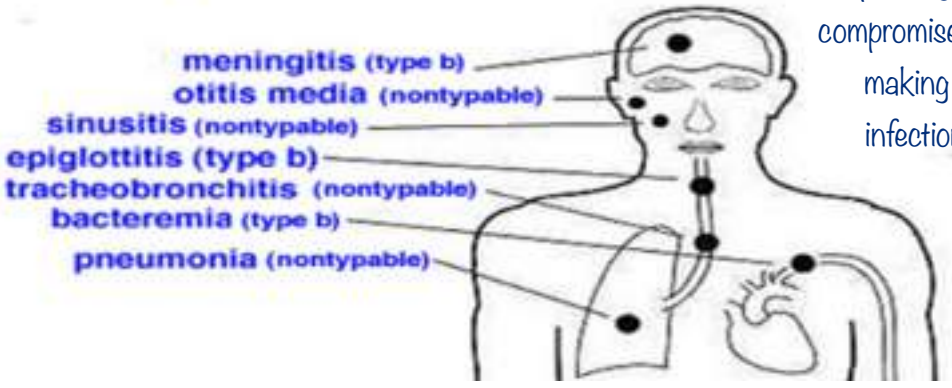
B. The non-capsulated (non-typable) (non-invasive) strains cause:

1- Otitis media and sinusitis: (**next to** *Streptococcus pneumoniae*).

2- Tracheobronchitis & Pneumonia: in adults and elderly, in **presence of predisposing factors** e.g. viral infections, malignancy COPD, cystic fibrosis..

These factors create an environment where the respiratory system may be compromised or weakened, making it easier for infections to occur.

Haemophilus influenzae infections



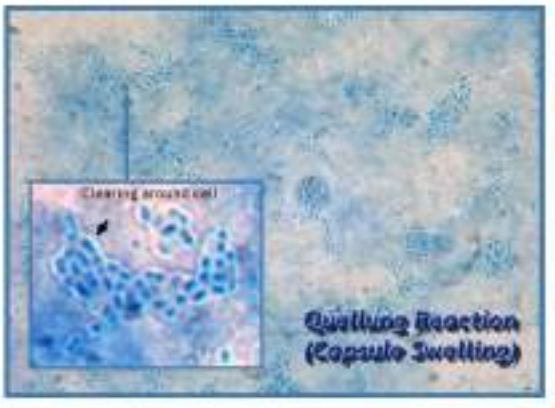
Laboratory diagnosis:

حسب مكان ال infection

A. Specimens: CSF, blood, sputum, ear swab,…

B. Microscopic examination:

Gram-negative coccobacilli.



=capsule swelling test

C. Detection and typing of capsule: **Quellung reaction.**

H.influenza appears as a halo or a surrounding ring around the bacterial cell when stained and viewed under the microscope.
the stained capsule appears enlarged or swollen

Best media

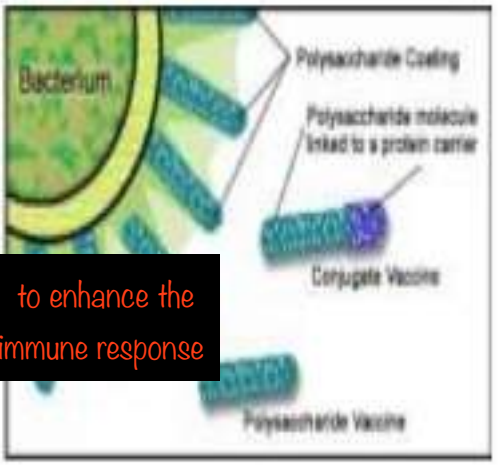
D. Cultivation: on **chocolate agar.**

"It only grows on X and V disc

E. X&V factor test: **(It requires both factors).**

Specific anti- serum بجيب العينة و بحط عليها
type B فمثلاً لو بدي أشوف
antibodies against type B capsule يحتوي serum بجيب
Hib لو هي methylene blue stain و بنستخدم صبغة
capsule ال و antibodies بين ال reaction رح يصير
capsule و نتيجة لذلك رح تنتفخ ال

Prophylaxis:



- **H. influenza type b vaccine (Hib vaccine):**

1- Polysaccharide vaccine. Prepared from polysaccharide capsule

2- **Conjugate** vaccine (capsule + carrier protein). to enhance the immune response

(given in 3 doses at 2, 3 and 4 months of age)

Succeeded in reducing cases to near zero level.

- **Rifampicin:** is used for chemoprophylaxis of unvaccinated close contacts of cases of Hib meningitis (decreases respiratory carriage of the organism).

BORDETELLAE PERTUSSIS

"The causative agent of Whooping cough (Pertussis)"

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Respiratory System

Morphology:

It is **Gram negative coccobacillus**.

Culture:

- It is a strict aerobe.
- It grows on complex enriched media e.g. **Bordet Gengou medium** or **charcoal-cephalexin blood agar.**
- Colonies are greyish white with shiny convex surface "**Mercury drop**" appearance.
- **It does NOT require X and V factors.**
- Virulent strains produce **haemolysis** on blood agar.

Charcoal-cephalexin blood agar

Bordet Gengou medium

Virulence factors:

- **Filamentous hemagglutinin (FHA):** Adherence factor

- Colonization factor that **promote attachment** of the organism to the cilia of the epithelial cells of respiratory mucosa.

- **Pertussis toxin (PTx):**

- Colonization factor.
- It has **adenyl cyclase activity** → ↑↑cAMP → **edema** of the respiratory mucosa.
- It suppress phagocytic activity (immune evasion).

this leads to increased secretion of fluids in the respiratory tract, causing edema of the respiratory mucosa.

- **Tracheal cytotoxin (TCT):**

- **Necrosis (cell death) of ciliated cells** of the respiratory mucosa.

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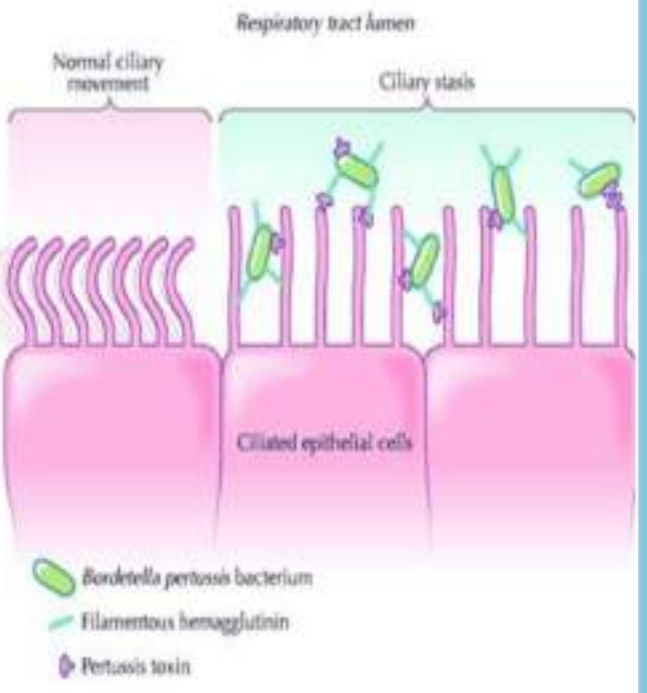
Respiratory System

Mechanism

After the bacterium adheres to and colonizes the ciliated epithelium of the respiratory tract, it secretes toxins that lead to the death of these epithelium cells, a ciliary stasis, edema of the mucus membrane and an accumulation of mucus and cell debris that triggers coughing.



Whooping cough (Pertussis)

- It is highly communicable disease that occurs primarily in **infants and young children**.
- Infection transmitted by **droplet** infection.
- Disease occurs in three stages:

Non specific manifestation

1- Catarrhal stage: (1-2 weeks): Fever, anorexia, malaise, rhinorrhea, sneezing.

specific

2- Paroxysmal stage: (2-4 weeks): **Repetitive cough with explosive character** followed by a high-pitched intake of breath that sounds like **"whoop"**. This may be associated with vomiting, cyanosis and convulsions.

3- Convalescent stage: Gradual recovery over weeks **(followed by long lasting immunity).**

- **Complications:** (pneumonia, subconjunctival or cerebral haemorrhage, encephalopathy, Rib Fracture).

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Laboratory diagnosis:

- **Specimen:** Nasopharyngeal swab.
- **Culture:** a-Direct plating on Bordet-Gengou medium
  b- The cough plate method. بدون swab مباشرة
- **Direct fluorescent antibody (FA) test.**
- **Serologic detection of antibodies**

Collection directly distance of 12-18" on cough plate

Treatment:

- **Supportive care:** (e.g., oxygen therapy and suction of mucus) during the paroxysmal stage is important, especially in infants.
- **Antibiotic** (Azithromycin): reduces the number of organisms in the throat and decreases the risk of secondary complications but has little effect on the course of the disease at the "prolonged cough" stage because the toxins have already damaged the respiratory mucosa.

Respiratory System

B- Active immunization (vaccine):
Diphtheriae toxoid (Toxin with removed toxicity but retained antigenicity)
Such toxoid is usually combined with tetanus toxoid and pertussis vaccine and given as follows:
DPT: Primary series: at the age of 2, 4 and 6 months followed by two boosters at 15-18 months and at 4-6 years.
Td: Boosters every 10 years are recommended. (Pertussis vaccine may cause encephalopathy if given after 6 years of age).

حكينا عنه مع Diphtheriae

Prophylaxis: Two types of vaccines:

A- Killed whole cell vaccine.

It is suspected of causing various side effects, including **post-vaccine encephalopathy** at a rate of 1 case/million doses. It is still in use in many countries other than the United States.

B- Acellular vaccine: (**fewer side effects than killed vaccine**), a combination of:

- Pertussis toxoid (genetically inactivated toxin).
- Filamentous hemagglutinin.
- Other virulence factors.

It is usually administered in combination with toxoid of diphtheria and tetanus as follow:

نفس طريقة يلي حكينا عنا في Diphtheriae

DPT or DTaP: Primary series: 2,4 and 6 months followed by two boosters at 15-18 months and at 4-6 years.

Td or Tdap: Boosters of every 10 years are recommended.

عادي هون نستخدم الثلاثي لانه
safe vaccine هو Acellular vaccine

PSEUDOMONAS AERUGINOSA

"One of the top antimicrobial resistance threats world-wide"
"One of the most important causes of nosocomial infections"

عدوى المستشفيات

"Aeruginosa" is meaning "copper rust" or "verdigris," and is often associated with the greenish color that can be observed in colonies of this bacterium.

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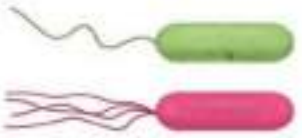
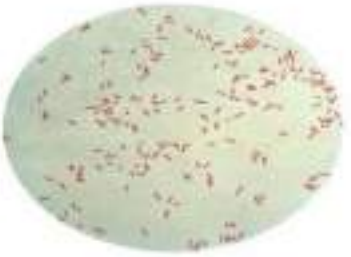
Respiratory System

Morphology:

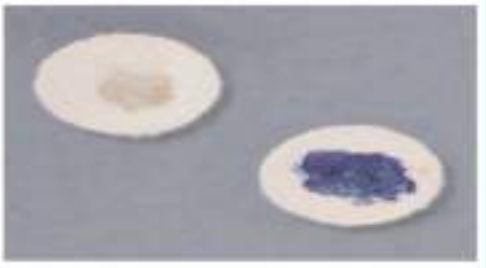




Gram negative bacilli.

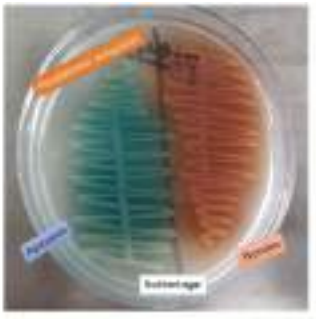
Motile with single or multiple polar flagella.

Biochemical Reaction:

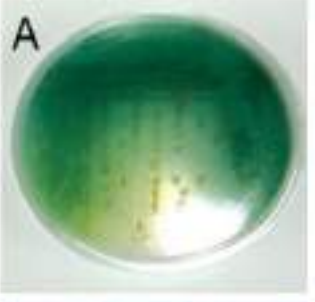


- It is **oxidase positive.**
- It does not ferment sugars (non-fermenters).

Culture:



- Obligate (strict) aerobe.
- Grow well between **37°C-42°C**, its growth at **42°C** differentiate it from other pseudomonads.
- Produce **a sweet or grape like odor** (fruity aroma).
- On MacConkey's → **non–lactose-fermenting (pale yellow)** colonies. non-fermenters



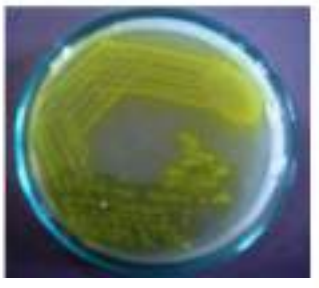
- Produce **exopigment (useful in clinical and laboratory diagnosis)**:

(1)Pyocyanin, blue-green pigment. Most common

(2)Pyoverdin, a yellow-green pigment (fluoresces under UV light).

(3)Pyorubin, a red pigment.

(4)Pyomelanin, a brownish black pigment.



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Respiratory System

Virulence factors:

1- Pili (fimbriae). مسؤول عن ال attachment

2-Endotoxin (Lipopolysaccharide): causes septic shock.

3- Exotoxin A: Inhibit protein synthesis and causes tissue necrosis.

4- Extracellular enzymes: e.g., elastases, facilitate invasion into the blood.

The mechanism by which elastases facilitate invasion into the bloodstream involves breaking down elastin and other host tissue components. This enzymatic degradation weakens the structural integrity of the host tissues, including blood vessel walls. As a result, the barriers that normally prevent bacteria from entering the bloodstream are compromised.

exopigment

5- Pyocyanin: damages the cilia and cause cell death.

6- Alginate (glycocalyx): **(Mucoid strains)** that forms adherent **Biofilm** protecting from antibodies, complement, and antibiotics.

This biofilm acts as a protective shield, providing a physical barrier against the host's immune response and antimicrobial agents.

7- Broad antibiotic resistance: (intrinsic and acquired).

Medical importance of P. aeruginosa:

- It flourishes in wet environments and can grow in **simple aqueous solutions** (only traces of nutrients) (e.g., tap water, swimming pool, spa and jacuzzi, sinks, contact lens solution, …).
- It has a remarkable ability to **withstand disinfectants**, it has been found growing in soap solutions, in antiseptics, and in detergents.
- All these factors favor their persistence in the hospital environment and hence, account for their role in **hospital-acquired (nosocomial) infections**.
- P. aeruginosa is an **opportunistic pathogen** that causes infections in :
  - In whom skin host defenses are destroyed (e.g., **extensive burns**).
  - In those with chronic respiratory disease (e.g., **cystic fibrosis**).
  - In those who are **immunosuppressed** and with neutropenia.
  - With **medical devices** e.g. catheters, ventilators, I.V line, ….

it typically causes infections in individuals with weakened immune systems or underlying health conditions

biofilm بكون عليهم

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Respiratory System

Clinical findings:

1- Respiratory infections:

Hospital-acquired pneumonia (especially **ventilator-associated pneumonia** and in **cystic fibrosis** patients).

Greenish colour of sputum

2- External ear infections:

عادي مش كل ال strains بكون discharge ملون

Malignant otitis externa (esp. in diabetics), **swimmer's ear.**

malignant otitis externa

3- Eye infections:

Corneal ulcer usually follow minor trauma to the cornea (frequently associated with contact lens use).

Corneal ulcer

4- Folliculitis (hot tub rash).

Folliculitis

5- Skin & wound infections:

(e.g. Ecthyma Gangrenosum, green nail syndrome).

Green nail syndrome

6- Urinary tract infections:

in those with indwelling catheters.

7- Meningitis: following lumbar puncture.

Ecthyma Gangrenosum

Green drainage in diabetic foot

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Laboratory diagnosis:

1-Specimens: Sputum, ear discharge,….

2- Smear: Gram negative bacilli.

3- Culture: On different media. The organism identified by:

- **Its odor.**
- **Exopigment production.**
- **Ability to grow at 42°C.**
- **Oxidase-positive.**

Treatment:

- Because *P. aeruginosa* is **resistant to many antibiotics (MDR),** treatment must be tailored to the sensitivity of each isolate and monitored frequently; resistant strains can emerge during therapy.
- **Combinations** of active antibiotics generally required.

Respiratory System

مقارنة ممكن تساعدكم في المراجعة

Aspect	Haemophilus influenzae	Bordetella pertussis	Pseudomonas aeruginosa
Morphology	Gram-negative coccobacilli	Gram-negative coccobacillus	Gram-negative bacilli
Culture	Aerobic, requires extra CO2; growth factors X & V	Aerobic, doesn't require X & V factors	Obligate aerobe; sweet odor; doesn't ferment sugars
Virulence factors	Polysaccharide capsule, outer membrane, IgA protease	Filamentous hemagglutinin, pertussis toxin, tracheal cytotoxin	Pili, endotoxin, exotoxin A, extracellular enzymes, pyocyanin, alginate, broad antibiotic resistance
Pathogenicity	Capsulated types cause invasive diseases like epiglottitis, meningitis, etc.; non-capsulated cause otitis media, pneumonia, etc.	Causes whooping cough, transmitted by droplet infection	Causes various infections including respiratory, external ear, eye, skin, wound, urinary tract, and meningitis
Capsule	Present (some types)	Absent	Absent
Motility	Non-motile	Non-motile	Motile
Culture Medium	Chocolate agar, blood agar	Bordet-Gengou medium charcoal-cephalexin blood agar	Various media
Treatment	Hib vaccine, antibiotics	Supportive care, antibiotics	Combination of antibiotics
Prophylaxis	Hib vaccine, Rifampicin	Killed whole cell vaccine, acellular vaccine	No specific prophylaxis
Laboratory Diagnosis	Quellung reaction, culture	Culture, DFA test, serology	Gram stain, culture, odor, exopigment production

لينك Flash cards شاملين كل معلومات المحاضرة :

https://ankipro.net/shared_deck/tfw23wYp

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Respiratory System

Study Questions

Choose the ONE correct answer

13.1 Which of the following is true of *Haemophilus influenzae*?

A. Invasive infections are most commonly associated with encapsulated strains.
B. Most invasive infections occur in infants during the neonatal period.
C. Most human infections are acquired from domestic pets.
D. The organism can be readily cultured on sheep blood agar in an environment of elevated CO_2.
E. Older adults are rarely at risk for infection with this organism because they typically have a high level of immunity.

Correct answer = A. The capsule is antiphagocytic, and facilitates hematogenous dissemination of *Haemophilus influenzae*. Although *H. influenzae* is an important pathogen of infants and young children, passive transfer of maternal immunoglobulin G may afford neonates protection. Immunity begins to wane in older adults, increasing the risk of infection for this population. Humans are the only natural host for *H. influenzae*. *H. influenzae* requires both hemin, X factor, and nicotinamide adenine dinucleotide (NAD), V factor, which are not available in sheep blood agar. Heating the blood lyses the erythrocytes, releasing both X and V factors, and simultaneously inactivating an NAD-inactivating enzyme present in blood. Media made with such heated blood is termed "chocolate agar." The organism does prefer elevated CO_2.

13.3 Which of the following statements about *Bordetella pertussis* infection is true?

A. Infection causes a leukocytosis characterized primarily by a marked elevation in polymorphonuclear leukocytes.
B. Isolation of the organism from clinical specimens is greatest during the early stages of illness.
C. Clinical diagnosis of whooping cough can usually be made within a few days of onset of initial symptoms.
D. Children who receive a full series of immunizations with the pertussis vaccine generally develop solid, lifelong immunity to pertussis.
E. The organism can be cultured on standard laboratory media such as sheep blood agar.

Correct answer = B. *Bordetella pertussis* typically causes a lymphocytic leukocytosis. Initial symptoms of *Bordetella* infection are relatively nonspecific (rhinorrhea, etc.). The characteristic paroxysmal coughing begins somewhat later. Maintenance of solid immunity depends on repeated exposure to the organism, either through natural causes or by administration of booster shots. Growth of *Bordetella* requires a medium containing a substance such as charcoal to absorb or neutralize inhibitory substances and also antibiotics that inhibit the growth of normal flora.

3) A 10-month-old male child presents with episodes of repetitive coughing with intermittent large gasps of air as well as some vomiting. Parents indicate that the child has been suffering from this condition for about 1 week. Incidentally, the previous week he was reported to have a coldlike illness with a fever and sneezing. A white blood cell count shows 65% lymphocytes and 30% neutrophils. An oxidase-positive, Gram-negative coccobacillus is grown from a nasopharyngeal swab plated on Regan-Lowe charcoal agar. Which one of the following organisms is most likely responsible for this disease?

(A)Bordetella Pertussis
(B)Corynebacterium Diphtheria
(C)Haemophilus Influenza
(D) Mycoplasma pneumoniae

Correct answer =A

Bordetella pertussis. The case fits the description of whooping cough or pertussis. This disease is characterized by repetitive bouts of unrelenting coughing punctuated by gasps of air and often end in vomiting. A whooping sound is often made when patients gasp for air. Lymphocytosis, at times as high as 70% of the peripheral white blood cell count, is typical for this disease. The causative agent is Bordetella pertussis, a fastidious organism that can be cultured on Regan-Lowe charcoal agar. While the other organisms listed cause respiratory disease, they are not associated with the disease described or microbiologic characteristics of the organism causing this case.

4) A 48-year-old man had a long history of alcoholism (including alcoholic hepatitis and hallucinations) and was admitted to the ICU with hypotension and GI bleeding. He was given IV fluid and transfused with packed RBCs. He remained intubated and ventilator dependent for several weeks. He developed a high fever and was treated with broad-spectrum antibiotics. Culture of his tracheal aspirate initially grew S. aureus. After more antibiotic treatment, Gram stain of his aspirate showed PMNs and gram-negative rods. A chest x-ray demonstrated an infiltrate with possible small abscesses. Tracheal aspirate then yielded a heavy growth of a gram-negative, nonfermenting rod that produced a greenish hue in the culture plates.
Which of the following is the most likely organism causing this patient's problems ?
(A) H.influenza
(B)L. Pneumophilia
(C) M.Pneumonia
(D) P. aeruginosa

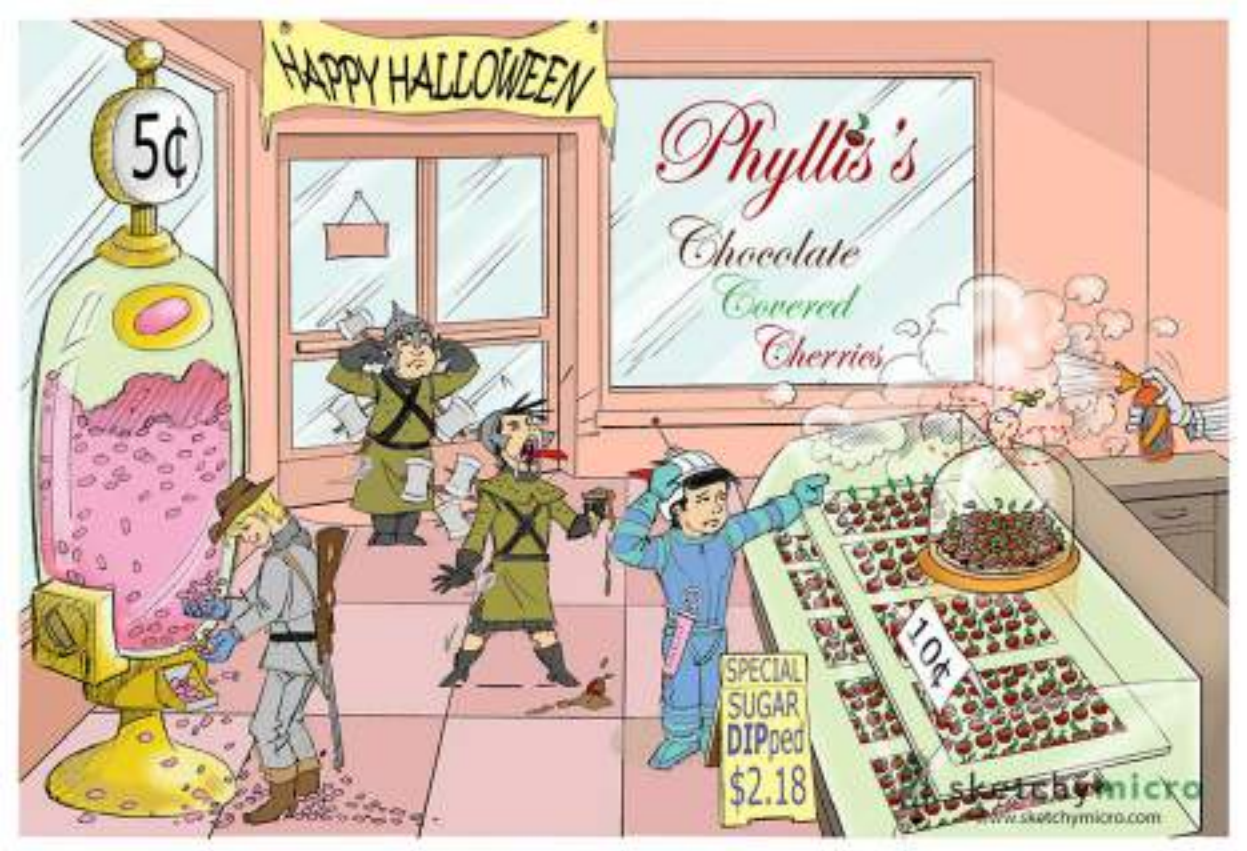
D) The pseudomonads are gram-negative, motile, aerobic rods that produce water-soluble pigments. They occur widely in soil, water, plants, and animals. P. aeruginosa is frequently present in small numbers in the normal intestinal tract and on the skin of humans. It is also commonly present in moist environments in hospitals. While a saprophyte on normal immune-competent humans, it is a most efficient opportunist in people with deficient host defenses.

5) An injured firefighter developed a wound infection, and culture of the site indicates a gram-negative rod that is oxidase-positive and produces a bluish-green pigment. The organism was relatively resistant to antibiotics, but susceptible to ticarcillin and tobramycin. The organism is most likely to be identified as which of the following?
(A) E. coli
(B) Klebsiella pneumonia
(C)p.mirabilis
(D) p.aeruginosa

P. aeruginosa is a gram-negative, oxidasepositive, aerobic rod that produces a greenblue pigment called pyocyanin. This microorganism has been associated frequently with wound infections in burn patients, and it is the second leading cause of burn infections after S. aureus. P. aeruginosa tends to develop resistance to various antibiotics.

Bacteria – Gram Negative Rods

Respiratory



Haemophilus Influenza - "Phyllis's Chocolate Covered Cherries"

1. Red Hues - Gram Neg
2. Shape of the candy machine and candy on top of the machine - Coccobacillary Shape
3. Chocolate sign – Grown in chocolate agar
4. 10 cent sign – Needs Factor 10 “Hemodin”
5. 5 cent sign - Grown on chocolate agar needs factor 5 (NAD, nicotinamide) and factor 10 (Hemodin) "hemoTEN"
6. Child Coughing and aerosol spray - Infection primarily moved by aerosol transmission leading to droplets going to respiratory track calling pneumonia
7. Child sticking out the red tongue screaming - Disease Epiglottitis - symptoms Drooling, inflamed epiglottis, strider, drooling
8. Cherries - "cherry red epiglottis"
9. Child plugging his ears - Otitis Media
10. Meningitis helmet and Bee flying around - Meningitides - only caused by type B capsular form.
11. Sickles attached to belts - Sepsis and Septic arthritis in patients without a spleen, hemophilic infections, especially sickle cell disease
12. Syringe and Capsule with the Bee flying around it - Vaccine for only the type B capsule is conjugated with diphtheria toxoid and haemophilus type B capsule
13. **Dip**ped for 2.18 - Vaccinate between 6 weeks - 18 months (bound to diphtheria) Dip=Diphtheria
14. Three Axes -Treatment Ceftriaxone
15. Rifle - Treatment for close contacts is rifampin

Bacteria – Gram Negative Rods

Respiratory

Bordetella Pertussis – Board and Care

1. Streamers to represent pili - Respiratory droplets are very infective using Pilus called filamentous hemagglutinin
2. Bow tie - Pertussis Toxin - Ribosylates Gi disabling it
3. GI uniform - Toxic inhibits GI, Disabled Gi (G inhibitor Protein)
4. Military Camp - Leads to a rise in cAMP
5. Popcorn, overabundance of white kernels - ADP Disables Chemokine receptors for lymphocytes leading to an overabundance of white blood cells in the blood stream, lymphocytosis
6. EF Shield - Adenylate cyclase toxin acts like the anthracis toxin edema factor, increases cAMP, Edema Factor, Most Virulent
7. Tractor on the middle road cutting the grass- Tracheal toxin damages ciliated cells in the epithelium, tractor cuts long cilia grass
8. Vet coughing vigorously - Catarrhal phase, limited symptoms nonspecific, most bugs, most contagious. 1-2 weeks
9. Whooping Horn - Paroxysmal - characteristic cough "Whoop"
10. 100 days war banner - Convalescence stage - final stage lasting 3 months with a cough, 100 day cough, most susceptible to secondary infections
11. Crow - Treatment Macrolides
12. Syringe with cell phone - DTaP - **acellular** vaccine using purified antigens
13. Red Hues - Gram Neg
14. Aerobic
15. Non motile