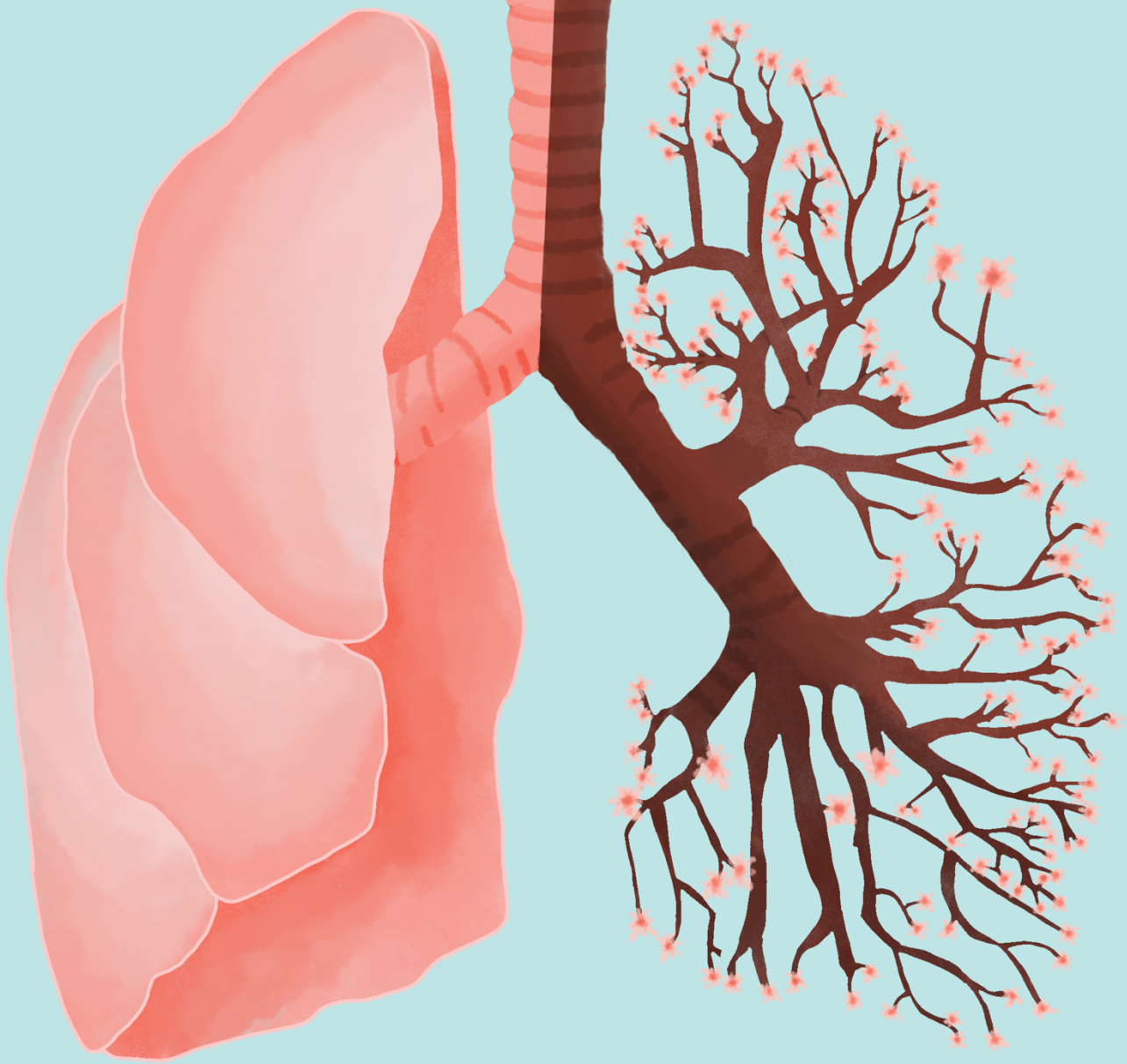
RESPIRATORY SYSTEM

MICROBIOLOGY



Title: Sheet 4 – C. diphtheria & B. pertussis

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بسم الله الرحمن الرحيم

In this lecture we will be talking about two rod shaped bacteria , one is Gram-positive and the other is Gram-negative. They are grouped together because of their ability to produce toxins.

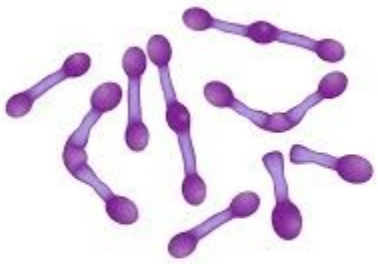
1 *Corynebacterium diphtheriae:*

Generally, diphtheroids are part of the normal flora of the upper respiratory tract, but we are interested in C. diphtheria which is implicated in opportunistic infections too.

- This is the G-positive rod/ bacilli.
- It is not virulent (that it does not produce toxin) except when it is infected with bacteriophage, allowing them to produce the toxin (toxigenic).
- They cause cutaneous and respiratory infections *when they have the ability to produce the toxin.

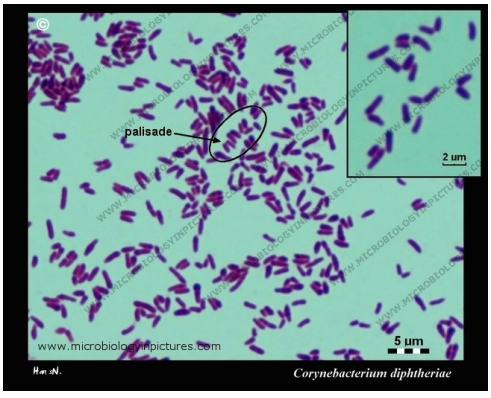
Morphology

Corynebacteria , club shaped Gram positive rods (wider at one end) and are arranged in palisades or in V- or L-shaped formations (or Chinese letters).



Corynebacterium diphtheriae

The rods have a beaded appearance. The beads consist of granules of highly polymerized polyphosphate—a storage mechanism for high-energy phosphate bonds.



The granules stain metachromatically (i.e., a dye that stains the rest of the cell blue will stain the granules red).

Non-spore forming, non-motile

Palades: parallel to each other

Transmission

- Humans are the only natural host of C. diphtheriae
- Both toxigenic and nontoxigenic organisms reside in the upper respiratory tract and are transmitted by airborne or droplets (like other respiratory pathogens).
- The organism can also infect the skin at the site of a preexisting skin lesion.
- This occurs primarily in the tropics but can occur worldwide in indigent persons with poor skin hygiene.

Pathogenesis

As we said before , they are not harmful, unless they take the phage for toxin production. The production of toxins is the cause of the disease.

No invasion into the blood, but how the systemic involvement is present in diphtheria disease? It is due to the produced toxin which will enter the blood. So, the systemic signs and symptoms are related to the toxin.

Here is what is written in the slides:

The pathogenisis:

- Mainly exotoxin mediated (similar to other G+ve rods), however, the bug(bacteria) must establish itself in the throat first (no invasiveness) prior to exotoxin production.

- Similar to other toxins it is formed in an A- B fashion (active/binding).

- Diphtheria toxin inhibits protein synthesis by ADP-ribosylation of elongation factor-2 (EF-2) used to maintain elongation of the peptide chain = no protein synthesis in eukaryotic cell.

- As mentioned, toxin is encoded on a gene transmitted by transduction on a temperate phage.

Clinical Findings/complications

For respiratory infection, it starts by forming a thick pseudomembrane in the pharynx which could extend into the larynx and may cause airway obstruction . (a tumor-like features 😊)keep in mind to differentiate between strep. throat and this pseudomembrane which is dirtier looking and consists of fibrin, WBCs, RBCs, bacteria and exudates, removing off this membrane can cause bleeding.

This is how the pseudomembrane looks like. Formed by the non-invasive bacteria.

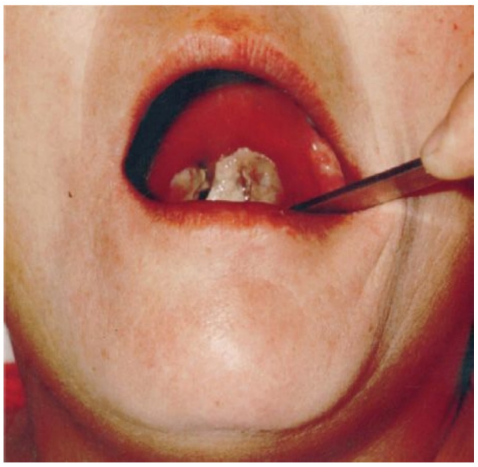


FIGURE 17–7 Diphtheria. Note whitish-gray pseudomembrane covering posterior pharynx and marked inflammation of palate and pharynx. Caused by diphtheria toxin, an exotoxin that inhibits protein synthesis by inhibiting elongation factor-2. (Courtesy of Dr. Peter Strebel.)

• There are three prominent complications:

(1) Extension of the membrane into the larynx and trachea, causing airway obstruction.

(2) Myocarditis accompanied by arrhythmias and circulatory collapse.

(3) Nerve weakness or paralysis, especially of the cranial nerves.

• The other aspects are nonspecific: fever, sore throat, and cervical adenopathy.

-The systemic effects resulted from the toxin are mainly associated with cardiac and neural symptoms:

Cardiac: endocarditis, pancarditis (inflammation of endocardium, myocardium and pericardium)

- Paralysis of the muscles of the soft palate and pharynx can lead to regurgitation of fluids through the nose.
- Peripheral neuritis affecting the muscles of the extremities also occurs.
- Cutaneous diphtheria causes ulcerating skin lesions covered by a gray membrane.
- These lesions are often indolent and often do not invade surrounding tissue. Systemic symptoms rarely occur.

Diagnosis

-diagnosis is mainly by clinical suspicion and the treatment is by giving anti-toxin immediately without waiting for laboratory results.

*note: people with previous cutaneous form of diphtheria(skin diphtheria) have anti-bodies against the toxin, so they can be protected against the pseudomembrane formation in the throat.

Laboratory Diagnosis

strong clinical suspicion (throat pseudomembrane) with systemic effects >> immediate treatment with antitoxin.

•For diphtheria the presence of the organism is not enough, we need to find the toxin, because there is atoxigenic strains.

•Due to the quick nature of toxin mediated disease, the decision to treat with an antitoxin should be clinical and not wait for lab confirmation.

Culture results take up to 48 hours

2 types of media for culture which are selective for C. diphtheria:

• A throat swab should be cultured on **Loeffler's medium (**cream colored colonies are shown in the slant) , a **tellurite plate** (black colonies seen a tellurium salt that is reduced to elemental tellurium within the organism thus black colored colonies), and a blood agar plate.

•The typical gray-black color of tellurium in the colony is a telltale diagnostic criterion.

•If C. diphtheriae is recovered from the cultures then we can confirm toxin (either animal inoculation, antibody-based gel diffusion precipitin test or PCR test for the presence of the gene).

•Smears of the throat swab should be stained with both Gram stain and methylene blue.

•Although the diagnosis of diphtheria cannot be made by examination of the smear, the finding of many tapered, pleomorphic Gram-positive rods can be suggestive.

•The methylene blue stain is excellent for revealing the typical metachromatic granules (the club shape is due to these granules).

-Again, culturing of C. diphtheria is not enough, we need to know if it is toxigenic or not.

The gold standard for the detection of diphtheria toxin is the immuno-precipitation test (Elek test). An alternative method is the detection of the exotoxin gene using a polymerase chain reaction (PCR) which is a faster approach. Basically, Elek test shows a positive result when immune-precipitation lines are detected. This is a YouTube video about Elek test: https://youtu.be/-HHSC9Q9314.

Treatment

-remember the non-invasive colonization and the circulating toxin.

1(**ANTITOXIN**) The treatment of choice is **antitoxin**, which should be given immediately on the basis of clinical impression (not on lab confirmation, this takes while to get both isolation of organism and detection of toxin).

•The need for immediate treatment with antitoxin is due to the toxin's RAPID and IRREVERSIBLE action on cells, thus antitoxin will work on unbound toxin in the blood only

2(**ANTIBIOTICS**) Treatment with **penicillin G** or **erythromycin** is also recommended with antitoxin but not as a substitute.

•Antibiotics will reduce bacterial count and toxin production, they will also reduce the chance of a carrier state.

Prevention

The vaccine (DTaP) is part of the national vaccination program worldwide and in Jordan. Which consists of inactivated diphtheria toxin, tetanus toxoid (inactivated toxin) and inactivated pertussis toxin. The vaccine is given 3 times at the first age after birth, then at the second year, and then between 4 to 6 years. But immunity for diphtheria and pertussis are not lifelong, they stay for 10 years, so adults receive a booster dose every 10 years. The adult's booster dose is abbreviated by small letters (dtap).

• Diphtheria is now rare in the world due to its inclusion in the scheduled vaccine regiment (DTaP) with diphtheria toxoid.

• In warzones or areas with lapse in immunization, reemergence (and atypical symptoms) are on the rise.

• formaldehyde treatment of the toxin, destroys the toxin but leaves the antigenicity intact.

• Immunization consists of three doses given at 2, 4, and 6 months of age, with boosters at 1 and 6 years of age.

• Because immunity wanes, a booster every 10 years is recommended.

• Immunization does not prevent nasopharyngeal carriage of the organism.

❷ Bordetella pertussis:

bacilli= rod-shaped

•*B. pertussis* is the cause of whooping cough (pertussis).

• It is still seen especially in infants under 2 months (received no or little protection from mother, usually typical whooping cough is seen)

• Important Properties:

• *B. pertussis* is a Gram-negative rod, also small coccobacillus shape, **encapsulated.**

Pathogenesis & Epidemiology

Although it is a gram negative and has LPS, the main virulence factor is the production of exotoxins . 5 well studied virulence factors are involved in the pathogenesis.

First of all, the bacteria is non-invasive, so it needs a factor that will help to attach and colonize the pharynx to establish the disease.

{1} Filamentous hemagglutinin, is the protein that the bacterium uses to attach itself to the cilia of the epithelial cells, damages these cells as well. (no cilia= no more clearing of mucus) (antibodies against this protein are protective). ** no mucus clearance

{2}Pertussis toxin stimulates (by enzymatic ADP ribosylation of G-proteins) the intracellular cAMP, once cAMP rises (similar to the diarrhea mechanism by cholera) it increases extracellular secretions (now a lot more respiratory secretions are being produced).** over production of mucus

- No more clearing of mucus + a lot more mucus is being produced => Both contribute to the PROLONGED severe cough of pertussis.

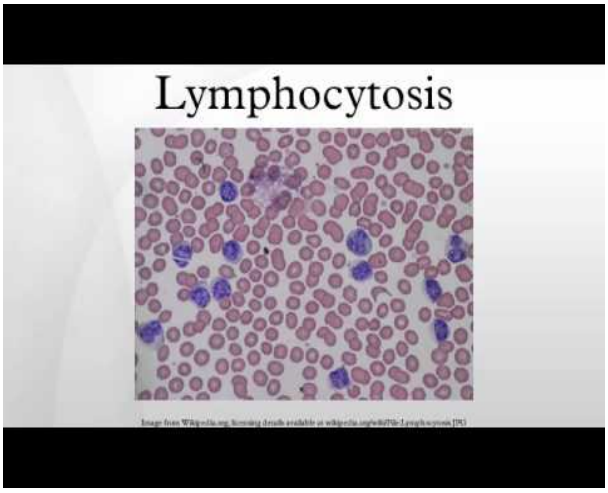
(the only mechanism left to clear airways is to forcefully cough it out)

-pertussis toxin is the main factor in the vaccine concerning B. pertussis. The pertussis toxin is part of the DTaP vaccine (all three components of this vaccines are A-B configuration toxins). The vaccine mainly contains 3 of the 5 factors, 2 of which are filamentous hemagglutinin and pertussis toxin, the third one is any of the remaining.

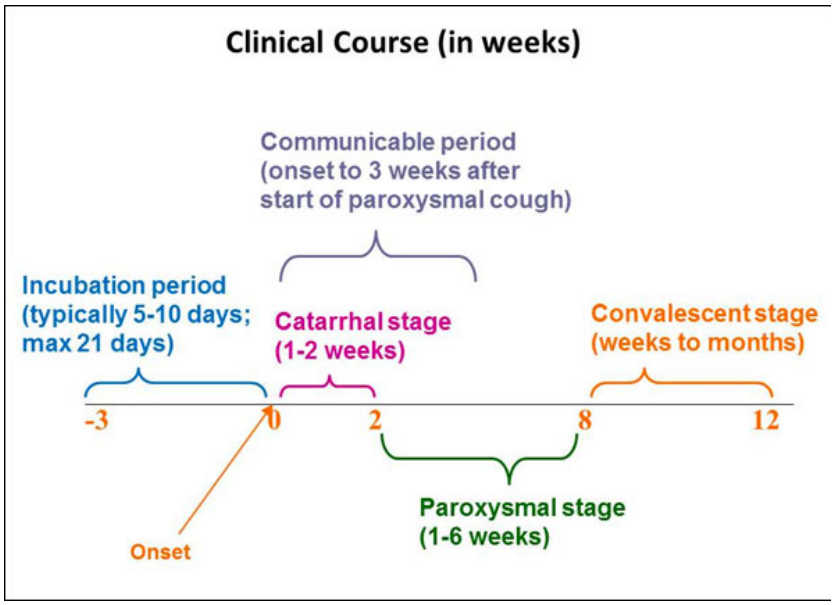
{3} The organisms also synthesize and export **adenylate cyclase**. This enzyme, when taken up by phagocytic cells can inhibit their bactericidal activity. Bacterial mutants that lack cyclase activity are avirulent.** evaded immune cell destruction.

{4} Tracheal cytotoxin is a fragment of the bacterial peptidoglycan, this toxin, acts alongside with endotoxin to induce nitric oxide, which kills the ciliated epithelial cells.

Note: infection with this organism will cause leukocytosis with lymphocytosis (which is more commonly present within viral infections). Patients with pertussis exhibit a high number of lymphocytes in their blood *lymphocytosis), this is due to Pertussis toxin inhibition of signal transduction (by ribosylation with ADP on G proteins) of chemokines, which in turn causes an inhibition of lymphocytes entering the lymph tissue and remaining in the blood.



Clinical Findings



-Whooping cough mainly affects children after the age of 6 months because the acquired maternal antibodies (IgG) will be depleted and not significant.
-It is of 4 stages:

• Whooping cough begins with mild symptoms (sore throat, rhinorrhea, sneezing, coughing, (low grade fever) then develops into an acute tracheobronchitis followed by a severe paroxysmal (sudden outbursts) cough, which lasts for 1 to 4 weeks.

• The paroxysmal pattern is characterized by: a series of hacking coughs, production of large amounts of mucus (productive/wet), ended by inspiratory (trying to catch their breath) whoops , the characteristic noise is due to narrowing of the glottis.

• The organism is restricted to the respiratory tract and blood cultures are negative, but with pronounced leukocytosis with up to 70% lymphocytes.

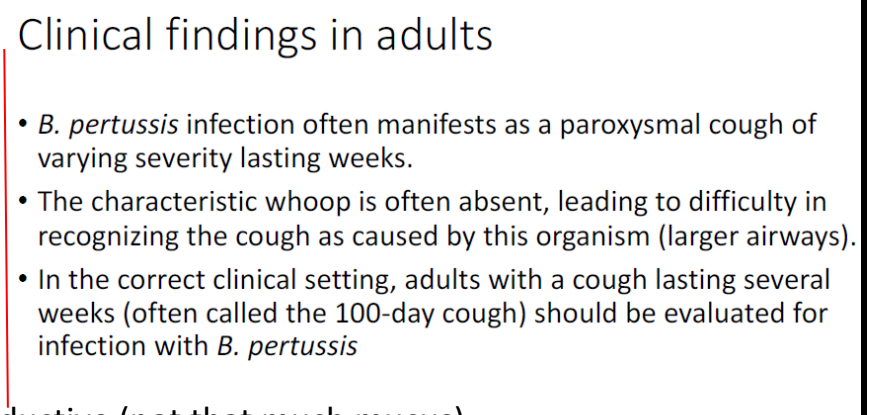
• Although central nervous system anoxia and exhaustion can occur as a result of the severe coughing, **death is mainly due to pneumonia**.

• The classic picture of whooping cough described above occurs primarily in young children.

-up to three weeks of incubation period >> Catarrhal stage (flu like symptoms) >>paroxysmal stage (patients may experience as many as 20-30 paroxysm with 20-25 coughs continuously daily which may cause vomiting ,convulsions and cyanosis.

*this is the stage in which patients seek hospitalization and improvement.

Clinical findings in adults



Adults have larger airways so they may not really develop the whooping cough characteristic as in children. Adults infected develop what is called a chronic *100-day cough* which is non-productive (not that much mucus).

Laboratory Diagnosis

Test results take up to 1 week so faster approaches are needed such as difluorescence antigen testing , PCR

Diagnosis should be done as early as possible to start treatment with antibiotics.

• The organism can be isolated from nasopharyngeal swabs taken during the paroxysmal (cough) stage.

•Bordet-Gengou medium used for this purpose contains a high percentage of blood (20%–30%) to inactivate inhibitors in the agar.

•The organism is then identified (from the above growth medium) by detecting its antigens (either by agglutination or by fluorescent antibody stains).

•The reason for depending on antigen detection is due to the slow nature of growth for this organism, rapid diagnosis is mandated and thus direct fluorescent-antibody staining of the nasopharyngeal specimens can be used for diagnosis.

•Polymerase chain reaction–based tests are highly specific and sensitive and should be used if available.

Treatment

Azithromycin (macrolide) is the drug of choice.
Basically, **erythromycin** is the drug of choice for both C. diphtheria and B. pertussis.

•It is essential to treat early, Azithromycin will reduce the bacterial load and reduce the change of complications, otherwise it will have little effect on progression of the disease once it has reached further stages (the toxin already caused damage to the mucosa.(

•**Supportive care** (e.g., oxygen therapy and suction of mucus) during the paroxysmal stage is important, especially in infants.

Prevention

Vaccine based: either an acellular one (contains 5 purified antigen proteins, no cells, this is the most used vaccine) or killed vaccine containing inactivated *B. pertussis* organisms.

• The main immunogen in acellular vaccine is the inactivated pertussis toxin.

(pertussis toxoid) the toxoid in the vaccine is pertussis toxin that has been inactivated genetically by introducing two amino acid changes, which eliminates its ADP-ribosylating activity but retains its antigenicity.

• It is the first vaccine to contain a genetically inactivated toxoid.

• The other antigens in the acellular vaccine are filamentous hemagglutinin, pertactin, and fimbriae types 2 and 3.

• The acellular vaccine has fewer side effects than the killed vaccine but has a shorter duration of immunity.

Corynebacterium diphtheriae — *Bordetella pertussis*

*causes Diphtheria

*causes pertussis (whooping cough)

Corynebacterium diphtheriae	*Bordetella pertussis*
- G-positive rod, not capsulated - not virulent, unless(bacteriophage> phage> bacteria> toxigenic) - club shaped - granules stain metachromatically - Non-spore forming, non-motile - humans are the only natural host and reservoir. -transmitted by airborne droplets. -not invasive. Systemic effects are produced by toxins. -pseudomembrane formation > which leads to airway obstruction. -cardiac and neural complications -diagnosis is based on clinical suspicion, laboratory findings for conformation -culture in tellurite plate> black dots -Elek test: for toxin detection -toxin> antitoxin Bacteria> erythromycin and penicillin G -DTaP containing diphtheria toxoid	- G-negative rod, encapsulated -5 virulence factors (filamentous hemagglutinin, pertussis toxin…) -not invasive -the cause of whooping cough -increases mucus production -lymphocytosis -mainly children -4 stages -adults form is called: chronic 100-day cough -death is mainly due to pneumonia -patients diagnosed with whooping cough> antibiotics -culture lasts for 1 week -Bordet-Gengou medium -azithromycin(macrolide) and erythromycin -acellular vaccine (proteins only). The main immunogen in acellular vaccine is the inactivated pertussis toxin
Things in common	
Both are rod-shaped, the major virulence factor is the toxin produced which is the main cause of systemic signs and symptoms, non-invasive bacteria, same mode of transmission which is air-borne droplets and both are present in the DTaP vaccine. This vaccine is given in doses and there is a booster dose every10 years because the vaccine does not give a life long immunity	

Good luck