Bacterial infections of the respiratory system 3

By Ass. Nader Alaridah MD,PhD

dr. started by stating the following similarities, all mentioned under each backerium again.

lecture content:

-2 bacterical species (1 gram +ve and 1 gram -ve), grouped together because they share the same major virulence factor and same immunogenic factor which is their "TOXIN"

[Aquired by phage infection (lysogenic insertion)]

→ The phage (bacterial virus) inserts its genetic material by lysogenic mechanism

Are:
1. Corynebacterium diphtheriae
2. Bordetella.

- Pathogenesis and hallmark of both is toxin production

Incidence of both is now low because of vaccination; as their vaccine is part of the National vaccination program (jordan included); both their vaccines are in 1 (DTaP).

However the effect of the vaccine wanes with time; so do not provide like long immunity; AB level drops significantly after ex. 10 years and need booster doses.

In jordan vaccine is trivalent and given in 5 doses, (3 doses in the 1rst year; 4rth dose at 3 years old, 5tn dose at 5-6 yrs + booster dose (adult)

- Both do not have invasion of underlying tissue, not found in blood as well, however have systemic signs and symptoms, related to toxin production.

Toxin structure (A and B) (A - activating; B - binding unit)

1. CORYNEBACTERIUM DIPHTHERIAE

gram +ve bacilli

- *C. diphtheriae* causes diphtheria (Mainly disease of children)
  - Respiratory diseases
  - Cutaneous diseases
- Other Corynebacterium species (diphtheroids) are implicated in opportunistic infections.

Diphtheroids (diphtheroids are used to represent Corynebacterium that are non-pathogenic)
normal flora of upper respiratory tract ; in mucous membranes.

Corynebacterium diphtheriae is a diphtheroid

So under normal circumstances Corynebacterium diphtheriae is Avirulent, unless it gets infected by a phage and aquires the toxin genes ⟶ becomes virulent toxigenic bacterium.

Note that Both Corynebacterium and Clostridium are gram positive bacilli but you should be able to differentiate between them as
- Corynebacterium is non-motile and non-spore forming
- Clostridium is motile and is spore forming.

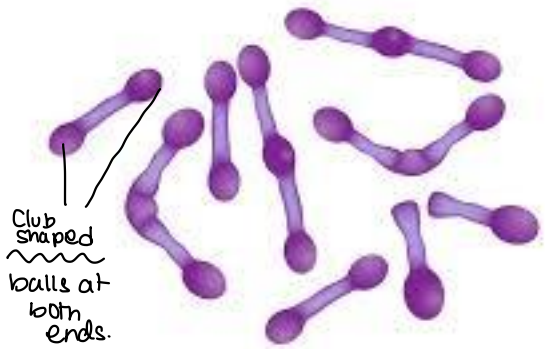
CORYNEBACTERIUM DIPHTHERIAE

3 descriptions for their arrangement:

greek for club shaped

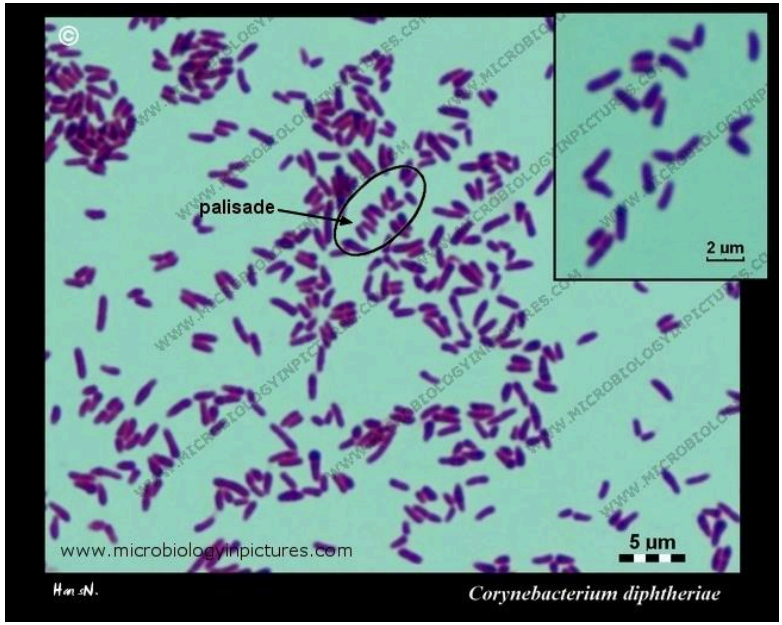
- Corynebacteria, club shaped (1) Gram positive rods (wider at one end) and are arranged in palisades (2) -parallel to each other- or (3) in V- or L-shaped formations (or chinse letters)
- The rods have a beaded appearance. The beads consist of granules of highly polymerized polyphosphate—a storage mechanism 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).

granules mainly concentrated close to the balls.



Corynebacterium diphtheriae

Note arrangement not branching or connected like nocardia (branching bacteria)



https://www.microbiologyinpictures.com/bacteria%20photos/corynebacterium%20diphtheriae%20photos/corynebacterium%20diphtheriae%20020.jpg

Transmission

- Humans are the only natural host of *C. diphtheriae* and reservoir
- Both toxigenic and nontoxigenic organisms reside in the upper respiratory tract and are transmitted by airborne droplets (similar to 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

- Mainly exotoxin mediated (similar to other G+ve rods), however, the bug must establish itself in the throat first (no invasiveness) prior to exotoxin production.
- 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.
- Similar to other toxins it is formed in an A- B fashion (active/binding)
- As mentioned, the toxin is encoded on a gene trasmitted by transduction on a temperate phage.

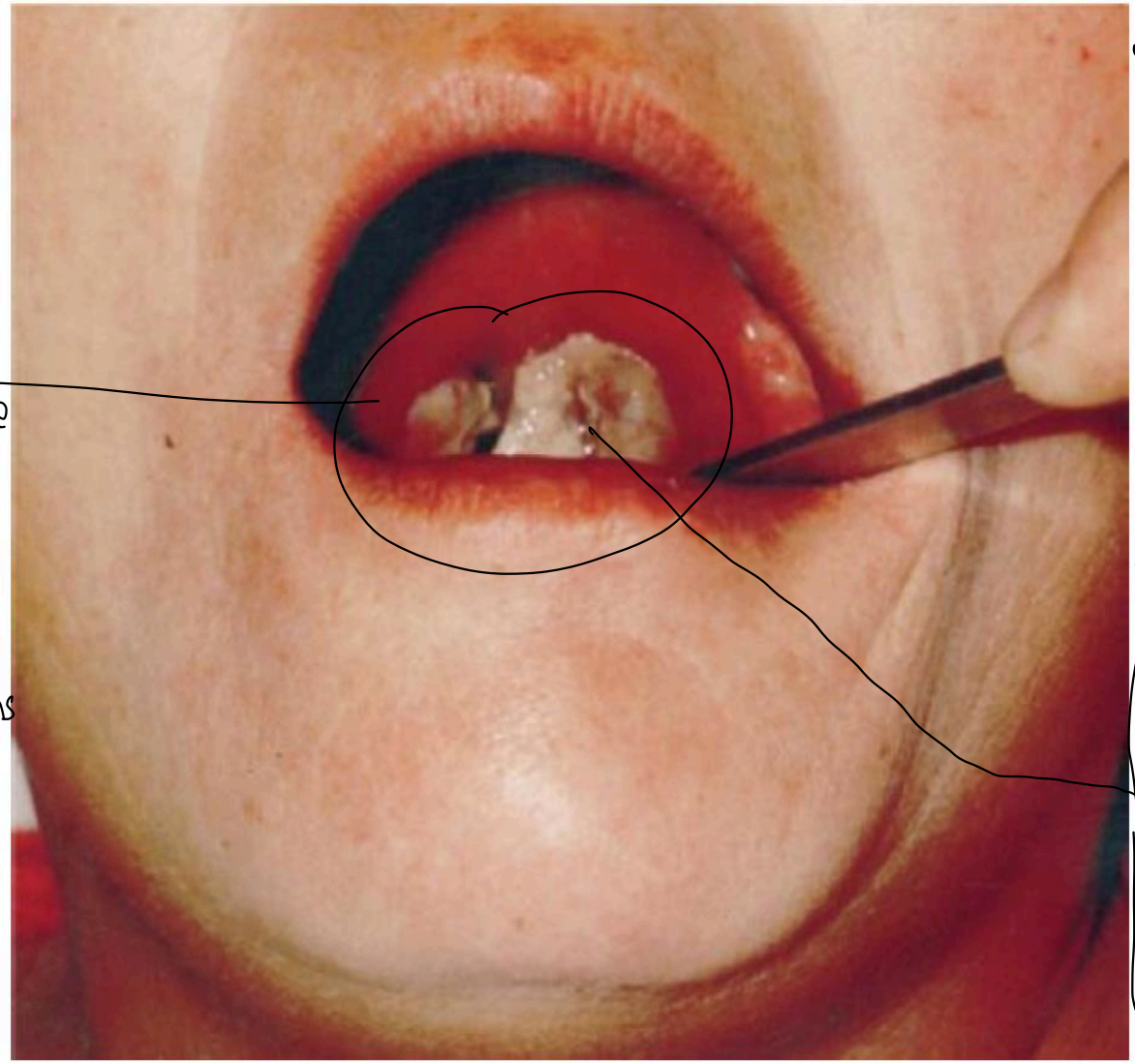
like how tetracyclines work on prokaryotes; by inhibiting protien synthesis; toxin work as antibiotic against human cells.

Clinical Findings/complications

- Diphteria is rare now thanks to vaccines, however we should be aware of the thick throat psudomembrane
- The other aspects are nonspecific: fever, sore throat, and cervical adenopathy. 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.

Systemic effects of toxin : Mainly → heart and nerves
C. Diphtheria ; one of the causative agents for endocarditis and pancarditis

+ cranial nerves.



- bacteria only colonizes are that has the pseudomembrane and from there diphtheria toxin is elaborated; So bacteria doesn't invade blood or other organs

- Starts in throat, more dirty looking than strep throat; having a pseudomembrane composed of (Fibrin + WBC + dead bacteria + RBC + exudate)

The pseudomembrane is a thick membrane, unlike exudate seen in strep throat (strep pyogenes)

→ like a tumor mass

→ worst case scenario: ↓ block airways

→ Don't try to remove membrane as that leads to profuse bleeding

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.)

- 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.

2nd type of manifestation of C. diphtheria.

(common in tropics); Advantage of skin diphtheria; AB production; protective from formation of pseudo membrane in throat.

☆ It's diagnosis is Mainly Clinical, Labratory is just for confirmation as treatment (Antitoxin) should be started immediatly. (passive immunization)

Laboratory Diagnosis

- For diphtheria we need to show the presence of the **organism and production of the toxin** (due to presence of atoxigenic strains). Isolation of organism + ensure it makes toxin.
- 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. - Clinical hallmark: pseudomembrane
- A throat swab should be cultured on ① Loeffler's medium (cream colored colonies are shown in the slant) , a ② 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. broth fluid, solid. - Culture takes 48 hrs ~ you can't wait as toxin is Lethal... 2 cultures used: (selective for C. diphtherium)
- 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).

http://www.medical-labs.net/wp-content/uploads/2014/05/b350-3-Corynebacterium-diphtheriae-on-tellurite.jpg

- 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).

↑ alanine dyes for granules

Note that isolation and identification of C. diphtheria is NOT enough; you should confirm that its a toxigenic strain)

You use Elek's test (immunoprecipitation test)

use bacterial colonies that grew on tellerite agar and place next to filter paper containing Antitoxin; toxin diffuse from toxigenic strain and antitoxin diffuse from filter paper and when these meet; they create lines called immunoprecipitation lines.

or a faster method is using PCR, gene for diphtheria toxin.

Treatment

from horse or goat.

Cornerstone as their pathogenesis is is toxin mediated

- 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 (as the one that already bound already produced irreversible effect).
- 2) ANTIBIOTICS) Treatment with penicillin G or erythromycin is **also recommended** with antitoxin but not as a substitute.

drug of choice. for bortedella as well.

- Sensitive to penicillins and Macrolides.
- Antibiotics will reduce bacterial count and this toxin production, they will also reduce the chance of a carrier state ; shorten duration of illness, less viable bacteria to produce toxin.

Prevention

- Trivalent vaccine (contains diphtheria, tetanus and acellular pertussis) (DTaP)
- Diphtheria is now rare in the world due to its inclusion in the scheduled vaccine regiment (DTaP) with diphtheria toxoid. (inactivated toxin by formalin).
- **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 intact the antigenicity
- Immunization consists of three doses given at 2, 4, and 6 months of age, with boosters at 1 and 6 years of age. (five total)
- Because immunity wanes, a booster every 10 years is recommended. (not life long immunity.)
- Immunization does not prevent nasopharyngeal carriage of the organism.

DTaP (capital letters) → initial doses

dtap: (small letters) → booster doses.

* toxin mediated (reason why we grouped it with Corynebacterium)
* has vaccine

2. BORDETELLA

gram-ve bacilli. (so has endotoxin (LPS)) but pathogenesis mediated by bordetella exotoxin;

- Disease: Mainly affect children as well.
- *B. pertussis* is the cause of whooping cough (pertussis). (السعال الديكي)
- 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** (unlike corynebacterium which is not encapsulated)

capsule unlike that of strep pneumonia, not immunogenic; not virulent factor.

Pathogenesis & Epidemiology

- *B. pertussis* infects only humans (this is a recurring pattern in many URT pathogens) and is transmitted by respiratory droplets from infected individuals (usually through coughing).
- Once it finds its way to the epithelium of the upper respiratory tract, it attaches itself (without invading the tissue) and causes reduction (and eventually death of) the ciliated epithelial cells (= no more clearing of mucus).
- Mainly affects children and young adults, it is similar to other repiratory pathgens a highly infective disease, but it is more so than most.
- This is why this is organism is one of the targeted organisms in scheduled vaccines, the vaccine was successful in reducing worldwide pertussis
- Lapse in vaccination due to wars or trends, but also due to waning(reduced overtime) immunity of the vaccine has caused outbreaks of pertussis during the years 2005, 2010, and 2012 , has raised concerns and is pushing forward for additional vaccine boosters.

Note: Bordatella has no invasion, local and systemic manifestations are toxin mediated. So All these toxins have both systemic and local effects.

- Pathogenesis, due to virulence factors: 5 virulence factors:
- (1) Filamentous hemagglutinin, is the protein the bacterium uses to attach itself to the cilia of the epithelial cells , damages these cells as well (antibodies against this protein are protective). (no cilia= no more clearing of mucus) Secreted factor for attachment and colonization of naso/oropharynx; (upper respiratory tract ciliated epithelial cells).
- (2) Pertussis toxin stimulates increase (by enzymatic ADP ribosylation of G proteins) of the intracellular cAMP, once cAMP rises it causes (similar to the diarrhea mechanism by cholera) increase extracellular secretions (now a lot more respiratory secretions are being produced).
- No more clearing of mucus from (1) + a lot more mucus is being produced (2) Both contribute to the severe PROLONGED severe cough of pertussis (the only mechanism left to clear airways is to forcefully cough it out)
- The pertussis toxin is part of the DTaP vaccine (all three components of this vaccines are A-B configuration toxins)

Main → factor in vaccine

Vaccine contains Atleast 2 antigens
① Pertussis toxin
② filamentous hemagglutinin
these 2 Must be present in vaccin
+
one of the other 3 (Choice depends on manufacturer.)

UNLIKE all other bacterial infections; you don't find granulocytosis; you find lymphocytosis (which is typically a viral finding) → has to do with toxins effect.

- 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.
- (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(bug stops cilia, causes extra secretions and now also evaded immune cell destruction).

Adenylate cyclase toxin

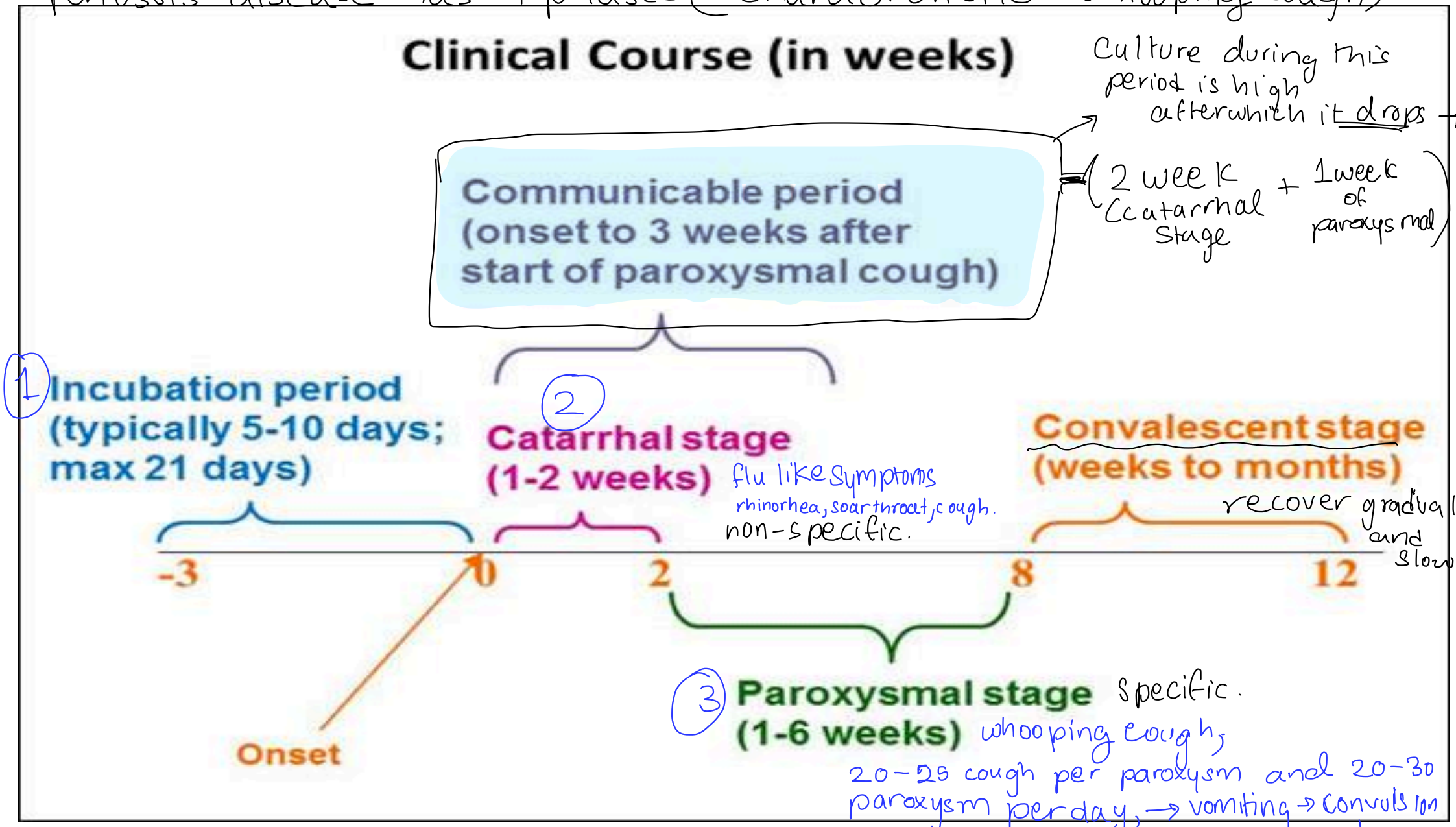
- (4) Tracheal cytotoxin is a fragment of the bacterial peptidoglycan, this toxin, acts alongside with endotoxin to induce nitric oxide, which kills the ciliated epithelial cells.

Clinical Findings

- Whooping cough begins with mild symptoms (sneezing, coughing, low grade fever) then develops into an an acute tracheobronchitis followed by a severe paroxysmal (sudden outbursts) cough, which lasts from 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 due mainly to pneumonia**.
- The classic picture of whooping cough described above occurs primarily in young children.

we worry about infants being infected (< 1yrs)
until age of 6 months children are protected by maternal IgG, then are unprotected
Occurance of disease is very low ; may be seen, specially unvaccinated ones

Pertussis disease has 4 phases (Characteristic whooping cough)



Culture during this period is high afterwhich it drops → -ve results.

(2 week Catarrhal Stage + 1 week of paroxysmal)

flu like Symptoms rhinorrhea, soarthroat, cough. non-specific.

recover gradually and slowly.

Specific.

whooping cough, 20-25 cough per paroxysm and 20-30 paroxysm perday, → vomiting → Convulsion → hypoxic and cyanotic need hospitalization.

https://www.cdc.gov/pertussis/clinical/features.html

Clinical findings in adults

- *B. pertussis* infection often manifests as a paroxysmal cough of varying severity lasting weeks.
- The characteristic whoop is often absent, leading to difficulty in recognizing the cough as caused by this organism (larger airways).
- In the correct clinical setting, adults with a cough lasting several weeks (often called the 100-day cough) should be evaluated for infection with *B. pertussis*

thats why no characteristic whooping cough? ↓ called

↓ Chronic non productive cough.

Usually if Adults dont manifest with the 100-day cough the disease is subclinical.

Remember in both toxin mediated diseases caused by these bacteria in this slide; you don't wait for Lab results; if you diagnose (Strong clinical suspicion) treatment started immediately.

Laboratory Diagnosis

→ (needs 1 week; more than C. diphtheria (48 hrs))
→ PCR faster.
→ important to diagnose ASAP to start treatment).

- 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.
  - 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.

for easier memorisation; drug of choice is Erythromycin, like C. diphtheria

• penicillin is effective as well.

Prevention

we are talking about the same DTaP used in diphtheria

part of the trivalent vaccine (DTaP) (a-acellular)

- 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

- No life long immunity as vaccine wanes over time.

The End