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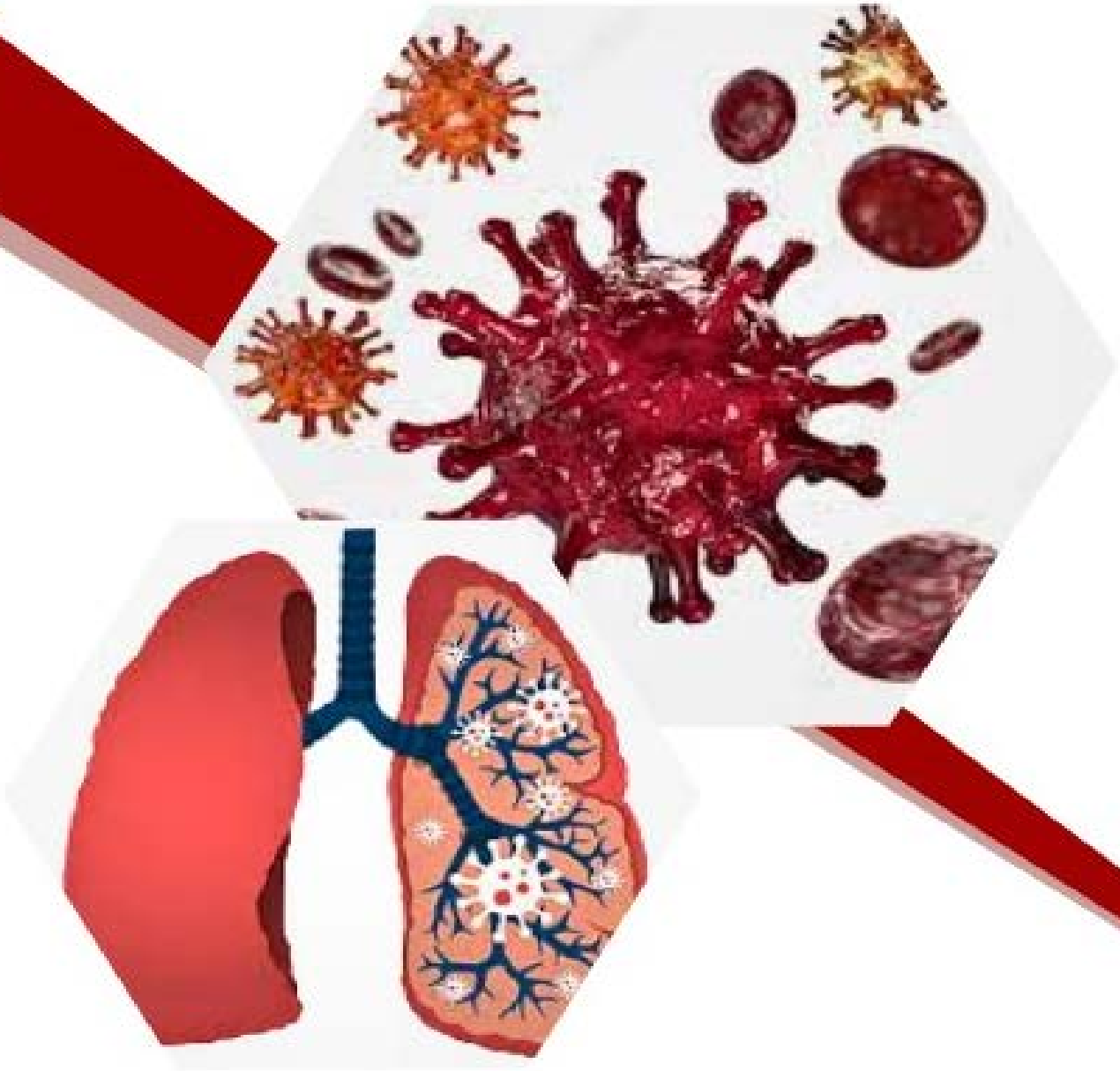

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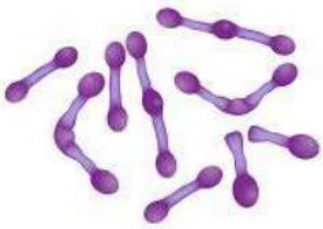
Bacterial infections of the respiratory system 3

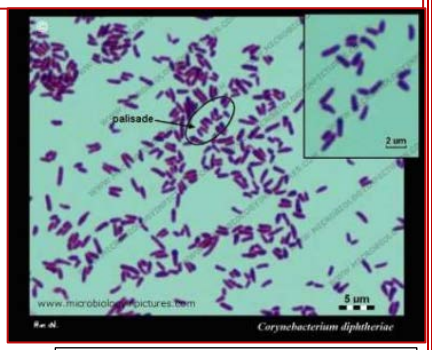
- In this lecture we're going to talk about Corynebacterium Diphtheriae (gram +ve, the causative agent of diphtheria) and Bordetella pertussis (gram-ve, the causative agent of whooping cough or pertussis).
- Both cause toxin mediated disease, have vaccines (toxoids mainly).
- Remember: GAS, strep. pneumoniae, H. Influenzae, Corynebacterium and bordetella are almost exclusively human pathogens.

CORYNEBACTERIUM DIPHTHERIAE

- C. Diphtheriae causes diphtheria, possess the toxin gene, so it can produce the toxin.
  - Diphtheria has two types: respiratory and cutaneous disease.
- Other Corynebacterium species (diphtheroids) are implicated in opportunistic infections.
  - They are non-pathogenic and mainly have animal reservoirs.
  - They can cause cutaneous diphtheria if they possess the toxin gene.
- 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) or parallel rays.
- 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 or pink).
- Non spore forming, non-motile, aerobes.



Corynebacterium diphtheriae



https://www.microbiologyinpictures.com/bacteria%20photos/corynebacterium%20diphtheriae%20photos/corynebacterium%20diphtheriae%2002

Transmission

- Humans are the only natural host of C. Diphtheriae, Dephtheroids also have animal reservoir.
- Both toxigenic and nontoxigenic organisms reside in the upper respiratory tract and are transmitted by airborne droplets (aerosols and micro aerosols; similar to other respiratory pathogens).
- However, the carriage rate (as normal flora) only constitutes a small percent (3-4%), similar to the typeable influenza (less than 1%, although non-typeable can reach up to 80%) and B. pertussis (less than 3%, although it is highly contagious). Unlike Strep pneumoniae (which can reach 50%) and Dephtheroids that may reach up to 80%.
  - This is mainly due to vaccines.
- The organism (C. Diphtheriae and Diphtheroids) can also infect the skin at the site of a pre-existing skin Lesion.
  - It prevents wound healing and characterized by pseudo-membranes formation.
  - Unlike respiratory diphtheria (which affects the myocardium-myocarditis- and neural cells -peripheral myoneuropathies or neuritis), the systemic manifestations of the cutaneous toxins are negligible.
  - However, cutaneous disease causes antibodies production, which boost the host's immunity.
- 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 (tetanus and pertussis) it is formed in an A-B fashion (A; activating, B; binding).
  - B-subunit binds heparin epidermal-like growth factor precursors on the host cells, causing their internalization, which will activate the A subunit that functions by ADP-

ribosylation of Elongation factor-2 irreversibly, inhibiting protein synthesis in eukaryotic cell.
    - This toxin can affect respiratory tract epithelial cells, myocardial and neural cells.
- As mentioned, the toxin is acquired by lysogeny because it is encoded on a gene transmitted by transduction on a temperate bacteriophage. So, always make sure that the bacteria are pathogenic (able to produce the toxin) not normal flora with no acquired toxin gene.
  P.S. lysogeny is characterized integration of the bacteriophage genome into the host bacterium's chromosome and replicates in concert with it.

Clinical Findings/complications

- Has **systemic** manifestations caused by the toxin (unlike B. Pertussis which is mainly localized).
- Diphtheria is rare now thanks to vaccines, however, we should be aware of the thick throat *pseudo-membrane* (characteristic).
- The other aspects are nonspecific: fever, sore throat, and cervical adenopathy (bull-neck; in young children).
- 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 causing dysphagia, odynophagia, difficulties in speech, then difficulties with vision.
- 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 which will delay wound healing.
- These lesions are often indolent and often do not invade surrounding tissue. Systemic symptoms rarely occur.
- The pseudo membrane is composed from: C. Diphtheriae, necrotic epithelial cells, fibrin and platelets. It appears as dirty looking, grey to white membrane

- Similar to epiglottitis, it is contraindicated to manipulate the membrane because it can cause laryngo-spasm, causing complete airway obstruction. Also, if you tried to remove the membrane it will be ruptured causing bleeding, moreover, it will stress the bacteria to produce more toxins, so you should never manipulate it even if it seems intriguing ;).

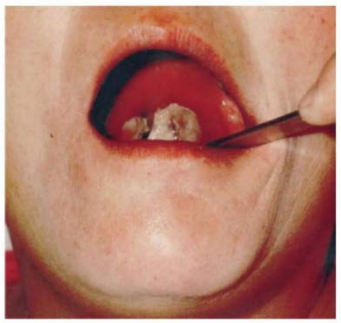


FIGURE 17–7 Diphtheria. Note whitish-gray pseudomembrane covering posterior pharynx and marked inflammation of palate and pharynx. Caused by diphtheria toxin, an exotoxin that inhibits protein synthesis by inhibiting elongation factor-2. (Courtesy of Dr. Peter Strebel.)

Laboratory Diagnosis

- For diphtheria we need to show the presence of the organism and production of the toxin (due to presence of 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.
- A throat swab should be cultured on Loeffler's medium (cream-colored colonies are shown in the slant), or a CTBA agar (highly selective, used more often) which contains cysteine, 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.
  - These bacteria have cysteine reductase activity (reduce cysteine) and can reduce tellurite.
  - The typical gray-black color of tellurium in the colony is a telltale diagnostic criterion.

http://www.medical-labs.net/wp-content/uploads/2014/05/b350-3-

- If C. Diphtheriae is recovered from the culture then we can confirm toxin (either animal inoculation - mainly in guinea pig - antibody-based gel diffusion precipitin test or PCR test for the presence of the gene if you have the gene props-).
  - Inoculation of the sample in the animal -if positive- will cause animal death within 10 min.

- Another test that can be used is the Elek test. It is done by placing a filter paper with the antitoxin on the agar plate with toxin-producing colonies, thus, the toxin and the antitoxin will diffuse towards each other causing precipitin lines.
- 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).
- Unlike H. influenzae and strep. Pneumonia, blood cultures of C. diphtheriae and B. Pertussis are negative because it is a toxin mediated disease.

Treatment

- 1) **ANTITOXIN** (mainstay of treatment) 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 (in penicillin-resistant patients, macrolides) is also recommended with antitoxin but not as a substitute; mainly supplementary)
  - Antibiotics will reduce bacterial count and this toxin production, they will also reduce the chance of a carrier state (although it is debatable since the antibiotic would stress the bacteria causing the production of toxins, however, if the patient is supervised in the hospital, you can give him the antibiotics).

Prevention

- Diphtheria is now rare in the world due to its inclusion in the scheduled Vaccine regiment (DTaP) with diphtheria toxoid.
- Here (Jordan), it is given as a part of the Hexaxim vaccine, which contains diphtheria, acellular pertussis and tetanus.

- **In warzones or areas with lapse in immunization, re-emergence (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.
- Because immunity wanes, a booster every 10 years is recommended.
- Immunization does not prevent nasopharyngeal carriage of the organism.
- Again, cutaneous Diphteria can boost the immunity.

BORDETELLA

- It's a gram-negative, aerobic, non-motile and non-spore forming bacteria.

- It 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).
- B. pertussis is a Gram-negative rod, also small coccobacillus shape, encapsulated (The capsule doesn't play a role in the pathogenesis or the immunity against bordetella pertussis as the toxin is the main virulence factor in the pathogenesis and the toxin is the basis of the vaccine).

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).
- This causes a **localized** infection (and manifestations) rather than systemic one.
- Mainly affects children and young adults, it is similar to other respiratory pathogens **a highly infective (contagious) disease** (when a child is diagnosed with pertussis, the physician must be careful of his contacts), 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.

Pathogenesis, due to 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)***

- No invasion, as in coronybacterium.

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 (mucus) 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 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).
   - Patients with pertussis exhibit a high number of lymphocytes in their blood ***lymphocytosis** -that contradicts with what normally happens with bacterial infections 'neutrophilia', 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.
   - Pertussis toxin also causes hyperinsulinemia that leads to hypoglycemia; it also causes histamine sensitivity.

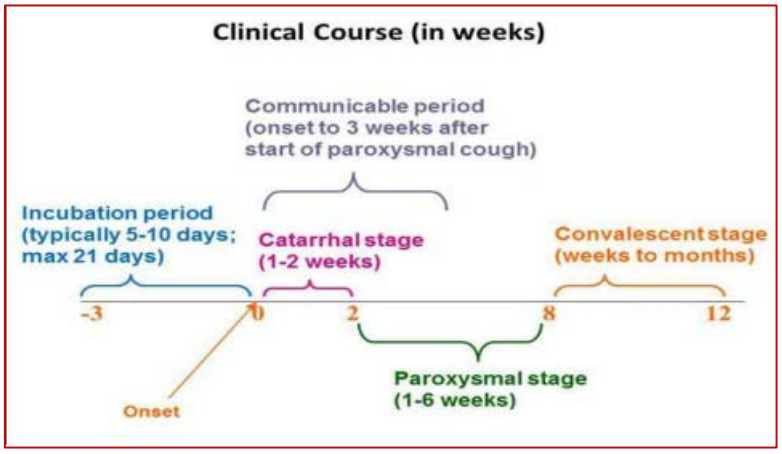
3) The organisms also synthesize and export adenylate cyclase. This enzyme, when taken up by phagocytic cells can inhibit their bactericidal activity (gives it more chance to produce the pertussis toxin). Bacterial mutants that lack cyclase activity are avirulent (bug

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

4) Tracheal cytotoxin and is a fragment of the bacterial peptidoglycan, this toxin, acts alongside with endotoxin to induce nitric oxide, which kills the ciliated epithelial cells.
5) Pertactin, which is pore forming protein, is present on the outer membrane of Bordetella.
6) Fimbria type 2 and type 3 act as virulence factors.

Clinical Findings

- Bordetella pertussis patients go through 4 stages that are classically seen in children, mainly infants who are less than 2 months old- Notice the following figure.



- There is nothing specific in the catarrhal stage (sneezing, coughing and low- grade fever).
- The paroxysmal stage represents the phase of cough attacks (50 attack a day, each attack is about 100 cough and each time it ends with the whooping sound).
- The recovery begins in the convalescent stage and the symptoms begin to diminish.
- The patient is excessively infectious for about 6 weeks (during the catarrhal and paroxysmal stages).
- Whooping cough begins with mild symptoms (sneezing, coughing, low grade fever) then develops into 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 closure of the glottis is what causes the whooping sound, as a doctor, you should certainly be able to recognize it.
- 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.**
- They might also have encephalitis.
- **The classic picture of whooping cough described above occurs primarily in young children.**

Clinical findings in adults

- Adults don't present with the same whooping sound as children because they have relatively larger airways.
- 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.
- It is one of the most common causes of chronic cough in adults (constituting 20% of the whole population)- chronic cough is the cough that lasts more than a month in children and more than 8 weeks in adults.

Laboratory Diagnosis

- The organism can be isolated from nasopharyngeal swabs taken during the paroxysmal (cough) stage.
  - Actually, the most preferrable specimen in these patients is **nasal washing**; we put a fluid inside the nose of the susceptible patient and then we take it back because they lost their cilia so when we take a swab we won't find the cilia, however, when you take a wash, it contains cilia and Bordetella.

- Bordet-Gengou medium used (Selective agar) 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) or even directly from the sample 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).
  - Once the patient has reached the paroxysmal stage then azithromycin is most probably useless.
- Supportive care (e.g., oxygen therapy and suction of mucus) during the paroxysmal stage is important, especially in infants.
- *No* antitoxin indication.

Prevention

- The whole cell vaccine is not used anymore because it might result in encephalitis, especially in children.
- 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 (basically, the virulence factors), any combination of the toxin and any other antigen is accepted by WHO as an acellular vaccine.
- The acellular vaccine has fewer side effects than the killed vaccine but has a shorter duration of immunity.

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V2

- Page 3: B-subunit binds heparin epidermal-like growth factor precursors on the host cells, causing their internalization, which will activate the A subunit that functions by ADP-ribosylation of Elongation factor-2 irreversibly, inhibiting protein synthesis in eukaryotic cell.
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