SHEET NO. 4

Microbiology

Respiratory system

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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.

The underlined text is from the slides but not mentioned by the doctor

Corynebacterium diphtheria:

Before we start we should differentiate between diphtheroid and C.diphtheriae, so Corynebacterium diphtheriae (which we are interested in) causes diphtheria while other Corynebacterium species (diphtheroids) are implicated in opportunistic infections.

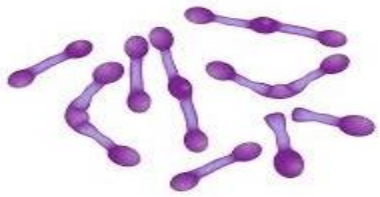
• It is not virulent (that it does not produce toxin) except when it is infected with bacteriophage, allowing them to produce the toxin (toxigenic).

• C. diphtheriae can be part of the normal flora in some people but those are asymptomatic because the bacteria is atoxigenic, once it possess the toxin it will cause the clinical picture of diphtheria.

• They cause cutaneous and respiratory infections *when they have the ability to produce the toxin.

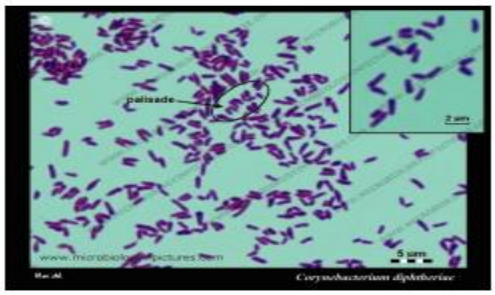
Morphology

• 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 , so it is pleomorphic.



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 or pink).

• Non-spore forming, non-motile, non-capsulated.

Transmission

• Humans are the only natural host of C. diphtheriae.

• Both toxigenic and nontoxigenic organisms (in the case of healthy Carriers) reside in the upper respiratory tract and are transmitted by airborne or droplets (like other respiratory pathogens).

• In the case of the cutaneous type of diphtheria , the organism can 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.

In both types of diphtheria there is 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 (note that this toxin has specificity to cardiac and neoronal cells). So, the systemic signs and symptoms are related to the toxin.

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 (A for activating and B for binding to the epidermal growth like factor), 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, the toxin is encoded on a gene trasmitted by transduction on a temperate phage (in a processc called lysogenic conversion).

Clinical Findings/complications

We have 2 types of diphtheria, classical and cutaneous:

1- classical diphtheria

• For respiratory infection, it starts by forming a thick pseudomembrane in the pharynx which could extend into the larynx and trachea which may cause airway obstruction (a tumor-like features) keep in mind to differentiate between strep. pyogenes throat and this pseudomembrane which is gray to white membrane and is dirtier looking,also removing off this membrane can cause bleeding so it is not dislodgeable.

This is how the pseudomembrane looks like.

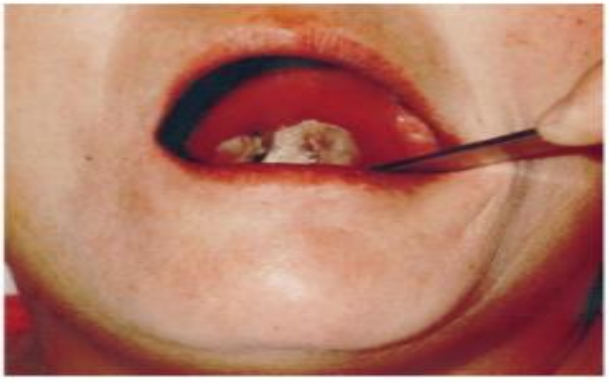


FIGURE 17–7 Diphtheria. Note whitish-gray pseudomembrane covering posterior pharynx and marked inflammation of palate and pharynx. Caused by diphtheria toxin, an exotoxin that inhibits protein synthesis by inhibiting elongation factor-2. (Courtesy of Dr. Peter Strebel.)

• The other aspects are nonspecific: fever, sore throat, and cervical adenopathy (bull's neck appearance).

• There are also 2 prominent systemic complications:

(1) Myocarditis accompanied by arrhythmias and circulatory collapse.

(2) Nerve weakness or paralysis, especially of the cranial nerves so there is difficulty in swallowing, speech and even vision, then peripheral neoropathy might occur.

•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 as we said.

2-cutaneous diphtheria

causes ulcerating skin lesions covered by a gray to white pseudomembrane, another thing to keep in mind that systemic symptoms rarely occur here so there will be pseudomembrane here but fortunately the absorption of the toxin through the skin to the blood is very slow, but still people who had this type of diphtheria are protected from the classical type which has a lot of systemic manifestation as we said.

Diagnosis

• For diphtheria we need to show the presence of the organism and production of the toxin (due to presence of atoxigenic strains) and this takes a lot of time (at least 3 days) so 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.

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

• 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) , CTBA (Cystine Tellurite Blood Agar) C.diphtheriae has cystinase and also can reduce tellurite and as a result of these 2 processes black colonies are seen in CTBA (important), and a blood agar plate.

• after culturing and identification of the bacteria, now we want to confirm that these colonies are toxin producing or no, we have several methods: 1-Elek test (filter paper with anti-toxin and shows a positive result when immune-precipitation lines are detected) 2-polymerase chain reaction (PCR) which is the most widely used now 3- antibody-based gel diffusion precipitin test 4- animal inoculation so if the bacteria is toxin producing the animal will die immediately.

• 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 (non specific), the finding of many tapered, pleomorphic Grampositive rods can be suggestive.

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

Treatment

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) supportive treatment with penicillin G or erythromycin is also recommended after the antitoxin but not as a substitute.

Antibiotics will reduce bacterial count and this toxin production, they will also reduce the chance of a carrier state so we cut the transmission cycle.

Prevention

•The vaccine (DTaP) is part of the national vaccination program worldwide and in Jordan which consists of inactivated diphtheria toxin (toxoid), tetanus toxoid (inactivated toxin) and inactivated pertussis toxin. The vaccine is given 5 times at 2, 4, 6 and 18 months of age, then between 4 to 6 years, and because immunity wanes, a booster every 10 years is recommended.

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

•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.

•<u>Immunization does not prevent nasopharyngeal carriage of the organism.</u>

Bordetella pertussis:

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

• It is still seen especially in infants under 2 months who <u>received no or little protection from mother, usually typical whooping cough is seen</u>.

• B. pertussis is a Gram-negative rod, also small coccobacillus shape, encapsulated but the capsule here is not the main virulence factor and not the basis of the vaccination.

•Unlike Corynebacterium diphtheria there is no systemic manifestations in the disease and no antitoxin in the treatment, but both of them infect only humans.

Epidemiology

It is mainly a disease of children, also can infect adults but manifests here as a chronic cough (the 1oo days cough) .

it is similar to other repiratory pathgens a highly infective disease, but it is more so than most, this is why this organism is one of the targeted organisms in scheduled vaccines, the vaccine was successful in reducing worldwide pertussis.

Pathogenesis

• 5 well studied virulence factors are involved in the pathogenesis:

(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 (non invasive bacteria). (no cilia= no more clearing of mucus) (antibodies against this protein are protective)

(2) **Pertussis toxin** (2 subunits, A for activating B for binding) stimulates (by enzymatic ADP ribosylation of inhibitory 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)

• No more clearing of mucus from (1) + a lot more mucus is being produced (2) both contribute to the 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)

(3) The organisms also synthesize and export **adenylate cyclase**. This enzyme, when taken up by phagocytic cells can inhibit their bactericidal activity so now it also evaded immune cell destruction, bacterial mutants that lack cyclase activity are avirulent.

(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.

(5) **pertactin**, this is an outer membrane protein.

Note: all these 5 virulence factors play a role in the pathogenesis but the main virulence factor is the pertussis toxin

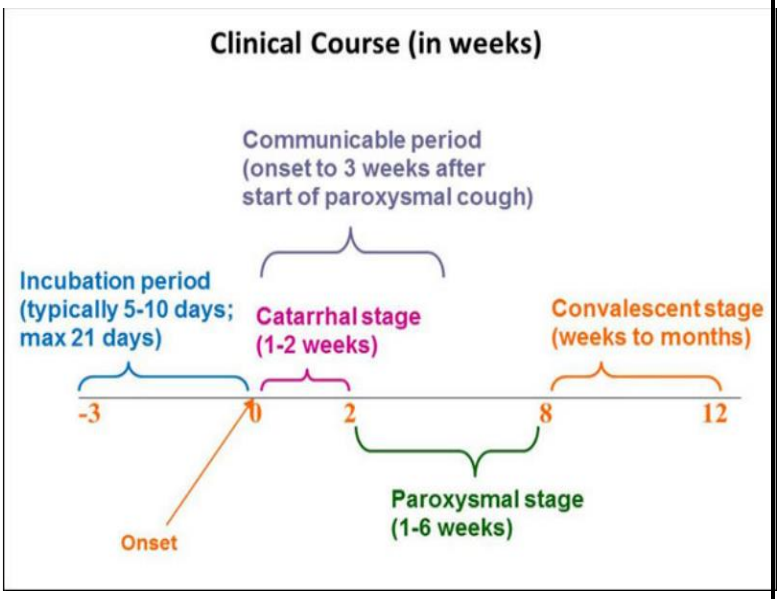
Note: patients might have 4 manifestations which are all related to the pertussis toxin action, these are:

1- Infection with this organism will cause leukocytosis with lymphocytosis (which is more commonly present within viral infections, 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.

2-hypoglycemia 3- hyperinsulinemia 4- extreme histamine sensitivity

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, note that infants don't receive IgA from the mother instead they receive IgG antibodies which won't protect him from colonization by such bacteria.

• It has four stages as you see in picture:

1- incubation period which lasts up to 3 weeks.

2- catarrhal stage which is characterized by flu like non-specific symptoms (sneezing, coughing, low grade fever) then develops into an acute tracheobronchitis followed by the third stage which is severe paroxysmal (sudden outbursts) cough.

3- paroxysmal stage (lasts for 2 to 6 weeks), the paroxysmal pattern is characterized by: a series of hacking coughs, production of large amounts of mucus (productive/wet), ended by **inspiratory whoops** (trying to catch their breath) , the characteristic noise is due to narrowing of the glottis.

- patients may experience as many as 50 paroxysms daily with like 100 coughs in each paroxysym.
- This heavy coughing might also cause vomiting so the patient might present with GI symptoms.
- Central nervous system anoxia and exhaustion can occur as a result of the severe coughing so they might have convulsions and seizures. However, death is mainly due to pneumonia.
- The classic picture of whooping cough described above occurs primarily in young children.

4- convalescent stage, the whooping sounds and the severity is much less here.

Note: the communicable period extends from onset to 3 weeks after start of paroxysmal cough (notice the picture)

Clinical findings in adults

- Adults have larger airways so they do not really develop the whooping cough characteristic as in children. Adults infected develop what is called a chronic *100-day cough*
- Recent studies have shown that 20% of chronic cough in adults is caused by this bacteria.

Laboratory Diagnosis

• The organism can be isolated from nasopharyngeal swabs taken during the paroxysmal (cough) stage, however the doctor said that in children nasal washings are preferred over nasopharyngeal swabs because the organism is found on the destructed cilia, so we may get a false negative if the sample is taken from the nasopharnx in children.

• The selective agar plate for Bordetella pertussis is 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.

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

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 for anoxia and suction of mucus) during the paroxysmal stage is important, especially in infants.

Note: no antitoxin is used here because there is no systemic manifestations (unlike diphtheria).

Prevention

• Vaccine based: either an acellular one (aP) or killed vaccine containing inactivated B. pertussis organisms but it has many side effects, so only the acellular one is used these days.

• Now for the acellular one, there are 2 versions which are used:

1. 5 purified antigen proteins are used, which are the inactivated pertussis toxin (pertussis toxoid), filamentous hemagglutinin, pertactin, and fimbriae types 2 and 3
2. 2 antigens are used, the inactivated pertussis toxin (pertussis toxoid) and one of the other 4 virulence factors, this version is the most widely used by the manufacturers.

• As we said before, it is taken as DTaP vaccine, 5 doses are required and because immunity wanes, a booster every 10 years is recommended. However, natural infection with B. pertussis provides a long lasting immunity.

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

The End

3 past paper questions:

1-Which of the following is false regarding B. pertussis?

a. It causes whooping cough

b. It's still endemic in Jordan

c. It's an encapsulated gram-negative rod

2- Mechanism of action of toxin for bacteria grow Bordet-Gengou medium is?

a) ADP ribosylation of GTP binding protein

b) ADP ribosyl of Gi

c) inhibition of acetylcholine

d) Inactivation of elongation factor 2

3- An 8-year-old boy, who recently arrived in the United States, develops a severe sore throat. On examination, a greyish exudate is seen over the tonsils and pharynx with oral membrane that bleeds profusely when touching it, he also has lymphadenopathy The cause of the boy's pharyngitis is most likely:

a) Gram negative aerobic non encapsulated bacteria

b) Gram positive anaerobic encapsulated bacteria

c) Gram negative anaerobic encapsulated bacteria

d) Gram positive aerobic non encapsulated bacteria

Ans:1)b 2)b 3)d

This is a summary from 019 sheet:

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