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Last lecture, we talked about gram positive rods, we discussed the first two types of *Clostridia* and now we will continue talking about the remaining two:

1. Clostridium Perfringens

It is one of the major causes of **gas gangrene**, it mostly happens in diabetic patients when they get deep wounds - normally surface wounds don't cause serious problems - especially those who have problem with blood supply (perfusion) to certain body areas, because as we mentioned earlier it is an obligate anaerobic bacteria and cannot grow with oxygen exposure. So the defect in blood supply provides the hypoxic environment for this bacterium to germinate, grow and produce its toxins.

It produces a wide range of exotoxins, around 12 toxins have been identified, and we care about 3 of them:

- α -toxin (phospholipase C): lipolysis
- Collagenase
- Hyaluronidase

Collectively, the toxins it produces cause necrosis of tissues especially the muscles (**myonecrosis**). If these tissues have good blood supply, the bacteria won't grow, it needs a hypoxic tissue. That's why when it infects a deep wound, it will initiate necrosis to the adjacent tissues via its toxins, stopping the blood supply and providing a suitable environment for its growth, and it continues spreading to the adjacent tissues by the same mechanism again. Remember, gangrene spread is relatively slow –as the bacteria need to cause necrosis and hypoxic areas in order to grow.

C. perfringens also causes **food poisoning** where its enterotoxins produces intense watery diarrhea.

Antibiotic treatment must be given, but alone, it is not enough because some of the bacteria will remain as spores (antibiotics don't have activity

against spores). The only treatment is surgical debridement or limb amputation with IV penicillin G which is highly effective against this bacterium.

****Note:** why is it called gas gangrene? Gangrene in Latin means black discoloration which is the color of necrotic tissues, because blood cannot return back to the circulation which means that it gets oxidized (dark color). "Gas" refers to some of the metabolites the bacteria produce, they have a distinct smell and can be noticed easily (very characteristic).

2. Clostridium Difficile

It is present as normal flora in many humans' intestine, but it is kept at low numbers by competition with other species of normal flora. So for example, if a patient takes a broad spectrum antibiotic that kills other competitive normal flora and *Clostridium Difficile* was resistant, it will grow in large numbers, producing certain toxins e.g. toxin A and toxin B that cause **GI colitis** (infection of the colon – its main site of growth), which is usually called **pseudo-membranous colitis** (superinfection) because it is accompanied by severe necrosis of the colon cells, which leads to sloughing of these cells and exiting the body as a pseudo-membrane. This usually leads to **severe diarrhea (sometimes with blood)**. Although it is a local GI infection, it's serious and could lead to death if not properly treated.

Treatment: first, you need to stop the antibiotic that killed the other normal flora. In theory, any broad spectrum antibiotic could cause the problem, most probably lincosamides e.g. clindamycin and lincomycin, and to lesser extent, broad spectrum penicillins and cephalosporins. You should prescribe oral metronidazole (commonly used) or oral vancomycin (more effective, but not available as oral preparation in many countries).

Now we will start talking about the third major group of pathogenic bacteria: gram negative cocci (spherical in shape) and coccobacilli (which are between the bacilli and cocci in shape). Coccobacilli are classified with gram negative cocci, because negative rods are already a large group.

We will specifically talk about five genera:

1. **Neisseria**: diplococci.
 2. **Moraxella**.
 3. **Haemophilus**: coccobacilli
 4. **Bordetella**: coccobacilli
 5. **Brucella**: coccobacilli
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1. **Neisseria**

In general, it is an intracellular, oxidase positive, diplococci bacteria, but it is not an obligate intracellular parasite, which means that it can grow within an artificial media on their own. But in the human host, they will live inside the cells. It is a fastidious bacteria i.e. it needs special growth requirements. It does not grow on routine lab media; they need blood/chocolate agar, 5-10% CO₂, 37 °C, and a certain level of humidity. They are highly susceptible for temperature change and dryness.

So based on these requirements, the bacteria cannot survive for a long time in the environment, and humans are their main reservoir. It has many non-pathogenic normal flora species, and two pathogenic species.

a. **Neisseria Gonorrhoea (also called Gonococcus)**

It has an attachment pilli, and proteases that break down IgA (the secretory immunoglobulin in mucus membranes – major defense line). So because of this, it is able to colonize mucosal membranes and strongly attach itself resisting the mechanical removal by flow of materials or fluids, and has the ability to degrade IgA.

It is present mainly in the urogenital tract and the anal canal in many females without causing any symptoms (asymptomatic carriers), these females are the major reservoir for this bacterium. It is transmitted via vertical route i.e. mother to newborn during delivery mainly, or horizontal routes i.e. by sexual intercourse mainly.

From its name; it causes gonorrhoea (السيلان) which is one of the most common STDs. Gonorrhoea affects the urogenital tract causing mainly acute **urethritis** in both sexes. *N. gonorrhoea* can also affect other tissues such as joints; causing **arthritis**, sometimes **skin** and rarely other body parts e.g. **eyes**. These bacteria are transmitted by direct contact.

In neonates, its major site of action is the eyes, causing conjunctivitis (**Ophthalmia Neonatorum**: neonatal conjunctivitis caused by vertical transmission of *N. gonorrhoea*)

Major symptoms of urethritis: dysuria, pain or discomfort during urination and purulent discharge – a characteristic of gonorrhoea that's usually used for diagnosis. In males, it can ascend to affect other parts of the urogenital tract e.g. prostate gland (prostatitis), epididymis (epididymitis) and testes (orchitis). Because of their ability to cause inflammation in these tissues, they may cause infertility.

In females, urethritis accompanied by cervicitis that can ascend to the fallopian tubes (salpingitis) which can lead to sterility (infertility) in some women. Not all women are carriers only; some of them have the symptomatic disease.

Treatment: penicillins and fluoroquinolones are mainly used. Usually, these are given in combination with tetracyclines or macrolides to cover *Chlamydia*, because gonorrhoea co-infects with chlamydia.

**Notes:

- Vertically transmitted diseases are not considered STDs.
- *N. gonorrhoea* is one of the major infections that cause infertility in both sexes.
- Penicillins don't cover *Chlamydia*.

b. Neisseria Meningitis (also called meningococcus)

It is an upper respiratory tract normal flora in some people. Usually, the first symptom of its infection is strep throat (pharyngitis), and then it gains access to blood in some people travelling all the way to the meninges causing **bacterial meningitis**. It is a medical emergency because meningococcal is one of the fastest killing infections i.e. rapidly fatal disease, and its treatment should start immediately with IV antibiotics without waiting for the lab culture and antibiotics susceptibility result. All bacterial meningitis cases are medical emergencies and meningococcal infection is the most dangerous. It is a notifiable disease i.e. it must be reported to the appropriate authorities, not reporting the case has legal consequences. Not all bacterial meningitis cases are reportable, only meningococcal infection.

The infection is highly contagious and extremely serious. In addition to typical meningitis symptoms, it causes more severe complications such as **hemorrhage**, **DIC** (disseminated intravascular coagulation) and **hematoma** (i.e. petechiae). Why is DIC associated with hemorrhage, even though it is a coagulation? Because it depletes the coagulation (clotting) factors, so some vessels become totally closed and other vessels become hemorrhagic.

It also causes septicemia (**meningococemia**), this is also a major cause of its fatality. Remember, it is a gram negative bacteria i.e. it has endotoxins which make it even more virulent than other gram positive bacterial meningitis.

****Scenario (important):** a patient shows up to you with typical symptoms of meningitis, how to know what caused it – bacterial or viral? By taking sample of the CSF – lumbar region. Unlike bacterial meningitis, viral meningitis is self limiting. We analyze polymorphs (PMNs), glucose and protein level of the CSF:

- High number of polymorphs, high concentration of proteins and low glucose level: bacterial.
- Low polymorphs, slight increase in protein level, and normal glucose level: viral.

This is a relatively quick test - the results are shown in the same day, and we can start empirical treatment if it is a bacterial infection. But we need to know whether it is meningococcal or not by culturing and doing the gram staining. Gram negative, oxidase positive diplococci: meningococcal.

Treatment: IV penicillin G or IV third generation cephalosporins e.g. cefttriaxone (more used in Jordan) or cefotaxime.

Because of its seriousness, even the close-contacts must receive prophylactic treatment e.g. oral rifampin. Remember that this bacterium is present in the upper respiratory tract and it is highly contagious.

N. meningitis has many serotypes (subspecies): A, B, C are the most pathogenic. A and C serotypes have vaccines, while B serotype does not. This is important because the serotypes are distributed geographically:

- USA: serotypes A and C are the most common.
- Europe: Serotype B is the most common.

****Notes:**

- Rifampin is used mainly against *Mycobacteria*.

- *Moraxella catarrhalis* also a gram negative, oxidase positive diplococci, but it does not cause meningitis (not present in the CSF).
 - In other bacterial meningitis, close contacts are not treated with antibiotics also the patient must not be isolated.
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2. Moraxella

Not all moraxella species are pathogenic, many of them are coccobacilli. However, we care about the pathogenic *Moraxella catarrhalis* which is diplococci, gram negative and oxidase positive. It is present as an upper respiratory tract normal flora. It is an opportunistic bacteria, and can cause upper respiratory tract infections e.g. **sinusitis** and **otitis media**, and to much lesser extent, it can cause **pneumonia – this is very common in COPD patients** (Chronic obstructive pulmonary disease).

Treatment: usually treated with broad spectrum penicillins e.g. amoxicillin. *Haemophilus influenzae* along with *Moraxella catarrhalis* are the major causes of otitis media and sinusitis, amoxicillin covers them both. However, new strains started producing β -lactamases, a combination of **amoxicillin and clavulanate** is used. **Macrolides** are also used (e.g. erythromycin or azithromycin). Fluoroquinolones are used sometimes.

3. Haemophilus

Its entire species are upper respiratory tract normal flora. It is a fastidious gram negative coccobacilli that grows only in enriched media (i.e. blood agar containing X- and V-factors), only one is pathogenic: *H. influenzae*, not all serotypes of *H. influenzae* are pathogenic. Type B is the most virulent, it can cause a wide range of infections including **upper and lower respiratory tract infections (pneumonia), and meningitis**. It was known to be the first cause of these infections, but it is not the major cause anymore because of the widely used vaccination *H. influenzae B* Vaccination (i.e. Hib vaccine). It is an obligatory vaccine in many countries including Jordan, it is part of the pentavalent vaccine (المطعوم الخماسي) which includes DTP, Hib and Hepatitis B vaccinations (given at 2,3,4 months).

It is still a common cause for some URT infections including **otitis media, sinusitis, epiglottitis** and sometimes pharyngitis. URTi in general are considered minor except for epiglottitis (التهاب لسان المزمار), which is a medical emergency because it can interfere with breathing process as there will be swelling because of

inflammation therefore causing permanent closure of trachea . This has a high risk on children and elderly mainly who are the most recommended to get the vaccines.

Treatment: similar to moraxella, we use broad spectrum penicillins and clavulanate (as they produce B-lactamases), we could also use macrolides or fluoroquinolones e.g. levofloxacin. For meningitis and epiglottitis (emergencies): IV second or third generation cephalosporins e.g. cefotaxime.

4. Bordetella

Most important is *Bordetella Pertussis*. It causes **pertussis i.e. whooping cough disease** (السعال الديكي). It infects both upper and lower respiratory tract at the same time. It secretes exotoxins including pertussis toxin and tracheal cytotoxin, in addition to its own endotoxins (remember it is gram negative coccobacilli). These toxins collectively cause necrosis of ciliated cells in the lower respiratory tract, these ciliated cells along with the mucus make the mucus ciliated escalator: the major clearance mechanism of trapped particles including microorganisms.

****Note:** the lower respiratory tract should not contain any type of microorganism including normal flora.

So after the disease symptoms have declined, the patient is left susceptible for secondary infections (both viral and bacterial) because microorganisms will reach the lungs easily, usually full recovery of those ciliated cells needs weeks or even months.

Whooping cough disease is divided into stages:

Incubation period → catarrhal stage → paroxysmal stage → convalescent phase

- **Catarrhal stage:** still URT infection, very similar to strep throat.
- **Paroxysmal stage:** most severe, very characteristic, violent cough, it could last for several minutes, and after it's done, the patient takes a huge breath which causes the whooping sound.
- **Convalescent stage:** recovery stage (the symptoms decline), the patient is highly susceptible for secondary infections.

The paroxysmal cough is a huge concern because of its powerful mechanical stress on the body, especially the upper part of it. It is very common that it causes rapture in eyes and brain vessels (brain hemorrhage).

******(I recommend that you watch a video of the cough)

Treatment: vaccine is available; part of DTP (or DTaP). Usually treated with macrolides (e.g. erythromycin, clarithromycin or azithromycin) or sulfonamides (e.g. sulfamethoxazole with trimethoprim). Treatment is also given for close contacts because it is contagious.

5. Brucella

Gram negative coccobacilli that is mainly present in animals. Three pathogenic species are known, most important is *Brucella melitensis* which is mainly present in goats and sheep, and it is very common in the Middle East. From its name, it causes **brucellosis** (الحمى المالطية).

Brucella causes chronic infections that could last for the rest of the patient's life. It affects many parts including blood, lymphatics, liver, skin, etc. it is considered as a highly serious infection. It is transmitted to humans via milk and meat, proper cooking is enough for killing it, but it can survive in improper cooked meat or unpasteurized milk or dairy products. Also, it can be transmitted by the animal's secretions (direct contact).

Treatment: antibiotic combination, rifampin and tetracyclines. Sometimes, streptomycin is used in long term therapy.