Microbiology

◉ Sheet ○ Slides

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Subject: Gram-negative coccobacilli and cocci

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Gram Positive Bacilli (continuation)

Clostridium difficile

- Special type of spore forming bacteria, found normally in the intestines of humans (in few numbers) with no clinical disease.
- Prevalence in newborns is (approximately 30%) and in hospitalized patients (hospital acquired). C. difficile is normally present in intestines but doesn't compete well with flora of the intestine, and patients in hospitals are usually taking antibiotics which gives C. difficile the advantage to grow and cause infection. The spores can be easily transferred if there is not proper cleaning in the hospital.
- Any change in the flora of the intestine (like E.coli) with antimicrobials (cephalosporins and tetracycline or any other wide spectrum antimicrobial drug) favors the growth of

  C. difficile because it is resistant to this antibiotic and can grow in its presence.

  C. difficile's most common feature is the secretion of **enterotoxins** . Therefore, when there's an increase in its number there's also an increase in the enterotoxins produced (Toxin A and toxin B).

  toxin A and toxin B can be released together or separately and can also associated with a form of necrotizing colitis .

  *enterotoxins are any toxins that released during the multiplication of the organism within the intestine .

  * if the toxin released outside the body such as in food articles it consider as exotoxin .

<u>Pathogenesis</u>

- Toxins produced are associated with a form of necrotizing **colitis** which is the damage in the mucosa of the large colon and blood vessels resulted in a form of bloody diarrhea which can be so severe and causing death .

- Depends on the age, in infants you can only recognize watery diarrhea, in older patients often the clinical feature starts with bloody diarrhea meaning it has developed perinatal not in the hospital. In such a case, patient must report because it might cause progressive damage in colon and developing fatal pseudomembranous colitis **PSM** especially in patients with immunocompromised or severe underlying disease condition
- In short, infection can start with something mild (diarrhea) and then develop later severe infection (colitis).

Diagnosis

- Rapid test by using specific antiserum we take the stool sample and look for presence on these toxin.
- Or we can culture the stool and recover the organism under anaerobic condition and then test if it's toxigenic or not because not all of C.difficile are toxigenic. 40-50% of C. difficile release toxins.
- No vaccine available against this type of infection .

Haemophilusgroup

- Haemophilus means that this group love to be associated with blood .
- Gram negative, coccobacillary* in shape.
- * coccabacillary means that under the stain it can appear as small cocci or small bacilli , and it need experience to be recognized .
- They are part of the **respiratory tract flora**
- Highly invasive especially in children with decreased immunity between the ages of 6 months to 5 yrs.
- We have non-pathogenic and pathogenic Haemophilus . in the respiratory tract in most of the population, the non-pathogenic being more prevalent. One of the most pathogenic species of haemophilus group is encapsulated Hemophilusinfluenzae type b (Hib) which is of clinical importance ,which can cause sepsis, conjunctiva, sinusitis, **meningitis** and mainly respiratory tract infections more than anything. Presence of the capsule in type b is a factor of virulence.

Pathogenicity:

1) Related to the secretion of endotoxins (lipopolysaccharide which is found in cell wall of Gram –ve bacteria)
2) Presence of capsule composed of polysaccharides and other factors.
3) Other factors

Diagnosis:

- Initial symptom is **sore throat** and it may progress to lower respiratory tract infection. If it reaches the blood it can go to the cerebrospinal fluid and cause **meningitis**. Since group A also causes sore throat, clinical features are not enough and clinical diagnosis must be done. If you take a sample from the pharynx for example, you won't be able to indicate any infection by H. influenza because the bacteria normally reside there. Therefore, the sample must be taken from a place where the bacteria normally is not there like the blood and cerebrospinal fluid. H. influenzae isolated from cerebrospinal fluid or blood would indicate H. influenzae infection. recognize filaments produced by it. You can notice the elongation of the bacteria and filaments produced associated with high number of protic proteins.
- Lab diagnosis is especially important in meningitis to select the proper antibiotic because Strep. Pnemounea and GBS (group B streptococci) also cause meningitis.

Culture:

- Haemophilusinfluenzae require two factors for proper growth
  1)Hemin or **factor X** (a protein with iron)
  2) NAD+ or **factor V**
- We have factorX which is hemoglobin in RBCs, this explains the name haemophilus, blood loving. So, if you want to culture this bacteria either use chocolate agar(lysed blood cells) or blood agar with addition of S.aureus, a beta-hemolytic bacteria, which will lyse the RBCs and cause release of these factors. You will notice the growth of H. influenzae only in the hemolytic zone of S. aureus due to the lack of nutrients such as factor V.

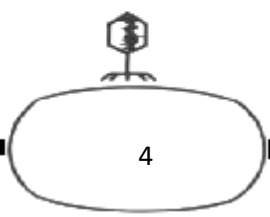
- By this you can tell that H. influenzae is an obligate parasite, it needs other organisms to grow (the addition of S.Aureus because it produces NAD which H. influenzae needs). This phenomenon is known as **satelliting**.

Vaccine

- Before the introduction of vaccine it was one of the most causative agent of meningitis in children. Vaccinated children are immune against meningitis but can still develop sore throat however.

Bordetella Pertussis

- Gram negative, facultative anaerobe but prefers aerobic environment.
- Causes infection of the **upper respiratory tract** (larynx and bronchi) and intensive **cough**.
- Causative agent of whooping cough. Listen to the sound of whooping cough: https://www.soundsofpertussis.com/
- It is mostly a childhood disease because of decreased immunity, age of 10 and less. It rarely causes infection in the older group which could be due to the fact that with time they get in contact with other organism like **Bordetella parapertussis** which doesn't produce toxin but is associated with inducing certain immunity especially in relation to the cell wall of bacteria. Therefore, any contact with para pertussis can provide some sort of immunity against the classical type(pertussis).
- **Bordetella Para Pertussis**: causes a disease similar to but milder than pertussis because it doesn't produce pertussis toxin.
- At first, the symptoms are similar to common cold: **fever, small cough, runny nose** but then as it replicates and increases the damage in the mucosa it causes **extreme reoccurring coughs** that are absolutely exhausting lasting for a minute or more and are followed by vomiting. Also, lack of oxygen to the limbs resulting in blue color of the skin, this is called **cyanosis.**
- In Gram stain it looks exactly like H. influenzae and cannot distinguish them from each other. What is more difficult is to isolate them in culture media. During



growth in mucosa produces potent toxin responsible for severe inflammatory reaction in the larynx and associated with the cough.

Pathogenicity

- During growth in mucosal tract release toxins.
- **Pertussis toxin** which is responsible for the severe inflammatory reaction in the larynx and bronchi and associated with whooping cough.

Diagnosis

- Easily recognized by the **whooping cough**.
- Not easily diagnosed in laboratory, it requires a lot of experience and is also dangerous to isolate. Protection is needed when isolating it because a person can get infected by a large number and cause disease even if they have immunity. Diagnosis is important to begin with the treatment because any delay might result in complication and death.
- Most pediatrics have no experience with pertussis because they have not seen a case in 30 years. They might misdiagnose cases of para pertussis (less pathogenic) which is similar to pertussis.

Vaccine

The vaccine is given to children with diphtheria toxoid and tetanus toxoid.

- Infection of adults can be fatal and not easily cured.

Additional information on why the cough happens:

The bacterial attaches to the cilia of epithelial cells and secretes toxins (tracheal cytotoxin and pertussis toxin) causing immobilization and death of the cells. This produces an epithelium with no ciliary blanket which is needed to move foreign matter away. Persistent coughing is the way to remove them now.

Neisseria

Gram negative diplococci resembling kidney shape and part of respiratory tract flora and alimentary tract.

We have pathogenic and non-pathogenic Neisseria.

- **Pathogenic:** N. meningitidis which causes meningitis and N. gonorrhoeae which causes gonorrhea.
- **Non-Pathogenic**: N.sicca, N.flava and more. Part of respiratory tract flora and are important to prevent access of other pathogens. Rarely cause clinical infection but can sometimes cause localized infection of sinusitis, conjunctivitis, and other non-invasive infections but nothing more serious.

Like H. influenzae especially type B, they're highly susceptible to environmental factors meaning they have **autolytic enzyme** in their cell wall and these begin to be active at room temperature and killed rapidly at cold temperature. Samples collected shouldn't be kept for more than 30 minutes at room temperature. and also shouldn't be kept in the refrigerator. The sample should be cultured without delay, otherwise we cannot detect the presence of these pathogens.

Neisseria Gonorrhoae

Inflammatory discharge from urethra or vagina (this what gonorrhea means).

- One of the most common **sexually transmitted disease** resulting of the death of millions of people. During sexual contact, N. gonorrhea attaches rapidly to mucosa of urethra and vagina and produces inflammatory reactions due to the release of IgA proteases, lipopolysaccharides(part of the cell wall), and other factors.
- Symptomatic in men; generally asymptomatic in women.
- Highly invasive for urinary tract mucosa associated with **urethral discharge** meaning there will be release of epithelial cell with fluid, inflammatory cells, and the organism.
- In men, infection is typically in the **urethra**. In women infection is in the **vagina**.

- It can be easily recognized in men because during flow of this discharge men feel presence of burning sensation ,painful urination. While in women ,there'll be increased vaginal discharge and urinary frequency .Vaginal discharge might reach, urethra, uterus and fallopian tube is less associated with burning sensation, that's why it can go un-noticed for years. On the long run, however, this can cause fertility problems, ectopic pregnancies and abortion because of damage to the fallopian tube.
- Most men have **acute infections**, rarely subacute or chronic because it's detected early(burning sensation), some rare complications may result in the prostate and epididymis affecting fertility. While women are associated with **subacute** and **asymptomatic infection**.
- It is a serious disease and must be detected early to start treatment.

Diagnosis

- We cannot always rely on clinical features like the burning sensation or urethral discharge. Lab diagnosis must be done. This highly important because you cannot label a person with N. gonorrhea because it is a sexually transmitted disease and it means a lot especially in our community.
- Preparation with Gram stain shows diplococci inside WBC not outside from a smear taken from vagina or urethra. If it's outside WBC then this not N. gonorrhea but another bacteria which is also Gram negative diplococci in vagina or urethra. If presence of N. gonorrhea inside WBCs couldn't be proven then isolation and culture growth must be carried out.
- Lack of treatment can cause a pregnant woman to transfer the infection to her new born during delivery causing conjunctivitis which, if not treated, the baby can suffer from **blindness**.

Treatment:

Penicillin was drug used but now 50% of the bacteria is considered resistant.

A third generation of cephalosporin is often used.

Neisseria Meningiditis

Causative agent of epidemic meningitis (outbreak). If we see a case of N.meningiditis we should expect the presence of more cases in the population.

THE END