Microbiology

Sheet

Slide

number

12

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We’ve discussed blue rectangles in last 3 lectures today we will talk about red rectangles.

We will concentrate on 5 terms:

-Differential characteristics

-Virulence factors

-Clinically

-Diagnosis

-Treatment and prevention
**Listeria monocytogenes:**

**Differential characteristics:**

Gram positive, catalase positive, rod in shape (bacilli), non-spore forming, motile (they can move), facultative anaerobic*, β-haemolytic in blood agar**.

Optimal growth temperature is 30˚C to 37˚C

However, it can grow in a wide range of 0.0˚C to 45˚C, so it can grow in fridge in contaminated food.

It has special pattern of motility called “Tumbling motility”, which means movement in random directions (forward and backward) at 22-25˚C.

https://www.youtube.com/watch?v=fjD_ruKmSfA

It doesn’t move tumbling motility at 37˚C.

Enhanced Beta-haemolysis in the area where *Listeria monocytogenes* meet *staphylococcus aureus* in blood agar (positive CAMP test).

**Virulence factors***:

1. Haemolysin (listeriolysin O): that allows it to escape the phagolysosomes of macrophages and avoid intracellular killing.
2. Phospholipase C
3. Internalin
4. Catalase

They help bacteria to enter the cell, the question is HOW?

This green rod resemble listeria. After internalization it becomes bounded with phagocytic vacuole, Haemolysin and Phospholipase C mediate the dissolution of vacuole membrane, so its released to the host cell cytoplasm, where bacterial growth occurs, the bacteria polymerize host cell actin at one pole, the result is tail like structure pushes the bacterium into an adjacent cell, where it again becomes bounded with a vacuole and the events are repeated in the newly invaded cell.

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* **facultative anaerobic**: makes ATP by aerobic respiration if oxygen is present, but can switch to anaerobic respiration if oxygen is absent.

** **β-haemolytic**: full haemolysis of blood

*** **Virulence factors**: https://en.wikipedia.org/wiki/Virulence_factor
Clinically:

It’s an important human pathogen (facultative intracellular organism).

It’s widely distributed in environment (soil, water, vegetation, sewage, animals and food chain e.g. coleslaw, soft cheese and milk), especially in cold things such as: yoghurt, salads and juices.

It is MOT (Motile via Flagella)

Asymptomatic carrier in ~ 5% (intestine & birth canal).

People at more risk of getting the infection (more susceptible to infection):

1. Pregnant women
2. Neonates
3. Elderly and immunocompromised patients
4. Those > 50 years and alcoholics

Infections are divided to:

1. Non-pregnancy associated:
   - mild flu or GI distress
   - Bacteremia (the presence of bacteria in the blood)
   - CNS infection (invasive*): meningitis, encephalitis, brain abscess
   - Infective endocarditis
   - sepsis
   - In immunosuppressed and older adults, and patient receiving chemotherapy cause invasive listeriosis (systemic infection).

2. Pregnancy associated:

   May be transmitted to fetus through placenta specially after the 4th month
   - Spontaneous abortion (2nd/3rd trimester) or stillbirth**.
   - Neonatal sepsis (after birth).

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* Invasive bacteria are pathogens that can invade parts of the body where bacteria are not normally present, such as the bloodstream, soft tissues like muscle or fat, and the meninges

**stillbirth: the birth of dead infant
Diagnosis:

1. **Take a sample from the site of infection** (blood, CSF “Cerebrospinal fluid”, amniotic fluid, placenta “in case of pregnancy associated infection”, urine).
2. **Culturing the bacteria**
3. **Tests:**
   a) Gram stain: its gram-positive rods or coccobacilli
   b) It grows well in blood agar and Chocolate agar for (1-2 days).
   c) Colonies are surrounded by narrow zone of β-hemolysis enhanced by S. aureus (discussed in the second page).
   d) Motility (using Listeria Motility Medium which is a semisolid medium Committee for testing motility of Listeria monocytogenes at 22-25 °C).

The following picture shows direct Gram Stain on CSF (seen in ~ 30% of cases)

These small rods are *Listeria monocytogenes* bacteria (they are found intracellularly)

**Treatment and prevention:**

- Ampicillin and Gentamicin for synergism*.
- Duration of treatment in CNS infection is 3 weeks
- Alternatives: e.g. in Penicillin allergic patients:
  1. Vancomycin
  2. Trimethoprim +sulfamethoxazole (septrin)

This is the last gram-positive bacteria in our discussion, now we’ll talk about gram negative bacteria.

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*synergism*: interaction between drugs in ways that enhance or magnify one or more effects of those drugs. Remember it as 1+1>2
Gram Negative Bacteria

Note: refer to the mind map in the first page to remember where we are now

**Neisseria**

Has two common species: *N. Gonorrhoea, N. Meningitides*

**Neisseria Gonorrhoea:**

N. gonorrhoeae, also known as *gonococcus*, causes the sexually transmitted disease gonorrhoea, which most commonly presents as a purulent infection of the mucous membrane of the urethra in men and the cervix uteri in women.

**Differential characteristics:**

- Gram negative, diplococcic “kidney shape” (two cocci meet at vertical line), oxidase positive*, non-capsulated

- Sensitive to dehydration and cool conditions, so it’s important to send the sample to the lab as fast as you can, otherwise gonococcus will die.

- Infects humans only but Not part of the normal flora (infection can be asymptomatic).

- *N. gonorrhoeae* is exclusively a human pathogen, it is never found as a normal commensals (so a person with gonorrhoeae must be treated), but a proportion of those infected, particularly women, may remain asymptomatic. These individuals may develop systemic or ascending infection at a later stage.

**Virulence factors:**

- **Pili**: The pili adhere to host cells, allowing the gonococcus to cause disease (for attachment).

- **IgA protease**: it is a highly specific enzyme that cleaves amino acid sequences of certain proteins. The natural substrate of IgA proteases is immunoglobulin A, hence its name, (simply it cleaves IgA antibodies).

- **Endotoxin (LOS: lipooligosaccharide)**

- **Outer membrane protein (OMP)**: it’s a type of protein found in the outer membrane of negative gram bacteria**, play a role in pathogenesis, act as passage tract.

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* oxidase positive: The oxidase test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain.

**refer to sheet number 2, page 7 and 8 to revise the structure of gram negative bacteria cell wall.
Gonococci infect mucosal surface such as urogenital area (urethra and vagina, cervix), rectum, pharynx and conjunctiva.

**Clinically:**

- Transmitted sexually amongst adults and can be transmitted to newborns during birth when they pass through birth canal (mainly affected their eyes).

- 95% of men are symptomatic and 40% of women are symptomatic, so usually they diagnose the woman according to her husband as it’s more probably for symptoms to appear on men.

- It has a short incubation period 2-5 day.

It causes Urogenital infection, but what we mean by Urogenital infection?

Simply, it’s the infections that affect urinary system or reproductive system, e.g. urethritis, UTIs (Urinary Tract Infections), infections in cervix and fallopian tube in females, these infections may cause sterility.

- Babies born to infected women may suffer ophthalmia neonatorum (infection in eyes), “remember that the get the infection during passage in birth canal”:
  
  - A severe purulent eye discharge with peri-orbital oedema occurs within a few days of birth.
  
  - If untreated, ophthalmia leads rapidly to blindness.
  
  - It may be prevented in areas of high prevalence by the instillation of 1% aqueous silver nitrate in the eyes of new born babies.
  
  - Alternatively, topical erythromycin antibiotic can be used; this has the advantage of being active against chlamydia* and less toxic.

Dr. Hamed illustrate that we have to know clinical aspect as mentioned in lecture Not more, since we take an introductory course not clinical on, later on in systems we will digging deeper: p in clinical aspect.

**Neisseria meningitis:**

**Differential characteristics:**

- It Shares many structural and virulence characteristics with N. gonorrhoea except two differences: it’s encapsulated* while gonorrhoeae are not, it can be part of normal flora while in gonorrhoeae can’t.

*Extra info: Chlamydia and G onorrhoea are sexually transmitted infections (STIs) caused by bacteria. Chlamydia is caused by the bacterium Chlamydia trachomatis and Gonorrhoea is caused by the bacterium Neisseria gonorrhoeae. Although caused by different kinds of bacteria, these infections spread the same way, may have similar symptoms and can both be cured with antibiotics, erythromycin is active against both.

*encapsulated means that it has a capsule.
- *Neisseria meningitis* affects meninges so it’s the major cause of meningitis, and it causes other systems infections.

- It has many serotypes

- Meningitis vaccine is one of the vaccines that Jordanian pilgrims have before they go to Hajj (they are given a vaccine for the most common serotype in Hajj).

**Neisseria Diagnosis:**

1-Sample:

- If there is a discharge take a sample from it, e.g. cerebrospinal fluid (CSF) or urethral discharge.

- It’s took from Exudates, swabs, CSF....

- *NEISSERIA* is intolerant of drying and temperature changes; it readily undergoes autolysis, so it should be sent immediately.

- Where there is likely to be any delay, transport media must be used to carry the material on swabs.

- **Gram stain:** gram negative intracellular (within neutrophils) organism

2-Culture:

- It’s cultured in special media, either Thayer-Martin medium or chocolate agar*.

- A rapidly positive oxidase test on any Gram-negative diplococci that are grown is strong evidence that the isolates are pathogenic Neisseria.

So, whenever you detect gram negative, oxidase positive, intracellular diplococci it’s Neisseria.

**Treatment and prevention:**

Treatment:

*N. Gonorrhea: Cefixime + Azithromycin orally*, cefixime works on Gonorrhea but we give Azithromycin with it, because usually a patient with Gonorrhea has another sexually transmitted disease, e.g. Chlamydia in most of cases are found with Gonorrhea.

*N. Meningitis: 3rd G cephalosporins (IV)*, its bactericidal.

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*chocolate agar* is an example of Enriched media (refer to sheet 5, page 5 for more information).
Prevention:

Meningitis: Vaccine

Gonorrhea:

- No vaccine / non-capsulated + antigenic variability
- Sexually transmitted
- Rapid diagnosis “It’s important to diagnose and treat it rapidly”.
- Use of effective antibiotics
- Tracing, examination and treatment of contacts

What we mean by tracing?

It means that you should trace the other partner, e.g. if you detect a man with Neisseria you must trace his wife.

- Neonates: erythromycin or silver nitrates (discussed in page 7)

**Haemophilus influenzae**

- Haemophilus means Blood loving bacteria, requires a blood containing medium for growth

**Differential characteristics:**

- It’s part of Respiratory normal flora
- It’s dividing according to the presence of capsule to encapsulated and non-encapsulated.
- The encapsulated bacteria (more dangerous) carried by 2-4% of population, while non-encapsulated bacteria (lower dangerous) carried by 80% of population.
- Gram negative small bacilli (old culture) or coccobacilli (young culture)
- It Requires Factors X and V in growth media, so it grows in chocolate agar.
- It’s Catalase and Oxidase positive

**Virulence factors:**

- Polysaccharides capsule (in 10% of H. influenzae): inhibits phagocytosis and complement activation
The polysaccharide capsule is the major virulence factor for Hib. When the organism invades the bloodstream, the capsule enables the organisms to evade phagocytosis and complement-mediated lysis in the non-immune host.

- A polysaccharide capsule composed of polyribitol ribose phosphate confers virulence: There are 6 types of capsules, designated a, b, c, d, e, and f (which can be identified by a polymerase chain reaction (PCR) method*). type b is commonly associated with invasive Haemophilus influenzae disease in children, such as meningitis, epiglottitis, and septic arthritis.

- The most important and common type is type b, a polymer of ribosyl ribitol phosphate.

- Fimbriae: which assist attachment to epithelial cells

Remember**: Fimbriae are structures for adhesion they are straight and shorter than flagella, we differentiate between fimbriae and pili based on the function.

- Immunoglobulin (Ig) A proteases, which are also involved in colonization (discussed earlier in page 6)

- Outer membrane proteins and lipopolysaccharide, which may contribute to invasion at several stages (discussed earlier in page 6).

**Clinically:**

- It’s an obligate human parasite that is transmitted through respiratory route.

- The common age of infection between 5 months and 5 years (by capsulated bacteria), and elderly (by non-capsulated bacteria)

The mother possesses antibodies against the b capsule which she has acquired in her lifetime. She passes these antibodies to the fetus transplacentally and in her breast milk. These "passively" acquired antibodies last for about 6 months. It takes 3-5 years of Haemophilus influenzae colonization and infection for children to develop their own antibodies. So, there is a window during which children are sitting ducks for the invasive Haemophilus influenzae. “Thank you, mama <3”

- It causes invasive infections and non-invasive infections

Invasive infections commonly caused by encapsulated bacteria mainly serotype b, examples on these infections:

- meningitis, epiglottitis, pneumonia and septic arthritis.

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*PCR reaction: Polymerase chain reaction (PCR) is a technique used in molecular biology to amplify a single copy or a few copies (used to amplify and detect bacterial DNA)

**refer to sheet 2 for more details.
Diagnosis:

-History and Examination

- Gram-stained smears of cerebrospinal fluid, pus, sputum or aspirates from joints, middle ears or sinuses can provide a rapid presumptive identification.

- Chocolate agar is a good, general purpose, culture medium and can be used without further supplementation for specimens obtained from sites that would normally be expected to be sterile, because it contains X and V factors

-Blood for culture in invasive conditions and in epiglottitis

-Blood cultures are usually positive in epiglottitis.

-Obtaining throat swabs from patients with suspected acute epiglottitis should not be carried out, as attempts to obtain the sample may precipitate complete airway obstruction.

-The viability of *H. influenzae* in clinical specimens declines with time, immediate transfer

1. Antigen detection

-The detection of type b polysaccharide antigen in body fluids or pus is useful, particularly in patients who received antibiotics before specimens were obtained.

-A rapid latex agglutination test with rabbit antibody to type b polysaccharide Capsular antigen is used most commonly.

-In the absence of confirmatory cultures, the results should be regarded with caution as some serotypes of *Streptococcus pneumoniae* and *E. coli* may share similar antigens.

2. Culturing on chocolate agar

• Gram stain (discussed earlier)
• Catalase oxidase tests (discussed earlier)
• Antibiotic sensitivity: based on determination of the antibiotic susceptibility of *H. influenzae*.
• Capsular serotyping

Treatment and prevention:

-Untreated invasive infection: Mortality rate of 90%
- Among cephalosporins, compounds such as cefuroxime, cefotaxime and ceftriaxone are highly active.

- Ceftriaxone (or a related cephalosporin such as cefotaxime) is the antibiotic of first choice for the treatment of meningitis and acute epiglottitis. It is bactericidal for H. influenzae, achieves good concentrations in the meninges and cerebral tissues, and it is highly effective.

**Conjugate* vaccines for type b:**

- Polysaccharide capsule is covalently coupled to proteins such as a non-toxic variant of diphtheria toxin or *Neisseria meningitidis* outer membrane protein

- 3 doses separated by a month 2, 3, 4 months age and a booster at 12 months of age

- Immunization of infants significantly reduces pharyngeal carriage of Hib, but has no effect on the carriage of other capsular types or non-capsulate strains.

Remember: capsule b = bad

**Bordetella**

**Differential characteristics:**

- *Bordetella organisms are small, gram-negative bacilli/coccobacilli*
- capsulated, non-motile
- *strict aerobes.*
- *The most important human pathogen in this genus is B. pertussis, the organism which causes whooping cough.* A man pathogen (which means it infects humans only).
- spread via the respiratory route and the organism is non-invasive (non-invasive means that Bordetella pertussis does not actually invade the body, it attaches to ciliated epithelial cells of the bronchi and then releases its damaging exotoxins).

**Virulence factors:**

1. **Filamentous hemagglutinin (FHA):** a pili rod extending from its surface, is involved in adhesion of Bordetella pertussis to ciliated epithelial cells).

2. **Pertussis toxin:** for adhesion

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*Conjugate vaccine:* more than one vaccine given with each other such as DTaP (is a combined vaccine against diphtheria, tetanus, and pertussis, in which the component with lower case ‘a’ is acellular*.

*Extra info:* **Acellular vaccine** A vaccine that may contain cellular material but does not contain complete cells.
3- **Tracheal toxin:** cytotoxin cause paralysis of the respiratory cilia. This toxin destroys the ciliated epithelial cells, resulting in impaired clearance of bacteria, mucus, and inflammatory exudate. This toxin is probably responsible for the violent cough, during these paroxysms of coughing the patient can become hypoxemic and cyanotic (blue from low oxygen), the tongue may protrude, eyes bulge, and neck veins engorge.

**Clinically:**

- whooping cough (non-invasive infection of the respiratory mucosa),

**Diagnosis:**

1- Culturing: it has the highest specificity of the test available (Charcol cephalexin medium).
2- Serology*
3- PCR (discussed earlier).

**Treatment and prevention:**

- Treatment: azithromycin
- Prevention: DTaP (HBsAg and IPV added nowadays)

The abbreviation stands for: (Diphtheria Tetanus [acellular] Pertussis)

**HBsAg:** Hepatitis vaccine

**IPV:** Polio vaccine

Sorry for any mistake:‘)

Ask me in case of misunderstanding

And tell me about any mistake

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*Serology:* the scientific study or diagnostic examination of blood serum, especially about the response of the immune system to pathogens or introduced substances