Lab. 3

Neisseria:

The genus N. is G –ve diplcocci, non motile, oxidase +ve, grow well at aerobic conditions and some spp. are facultative anaerobes. 5% CO_2 enhance the growth of N., catalase –ve, some spp. are capsulated and others are not. N. spp. are very fastidious in their growth, the pathogenic N. of 2 main spp.:

1. *Neisseria gonorrhoeae* (Gonococcus) causes "gonorrhoea".
2. *Neisseria meningitidis* (Meningicoccus) causes "Meningitis".

Both of them associated with or inside poly morphonuclear cells (Neutrophils).

Lab. Diagnosis:

1. Specimens: Include material from genital sources like uretheral, vaginal discharge [creamy yellowish color] and extra genital eg. skin, blood.
2. Direct stain: Gram stain is made to the specimens above and will show G –ve, diplococcic, non capsulated by (capsular stain).
3. Culture: N. is fastidious grows on enriched media like chocolate agar and the selective one is Thayer Martin medium which comprises of the following:
   - Chocolate agar (heated blood agar).
   - Antibiotics:
     1. Vancomycin inhibit the growth of G +ve.
     2. Colistin inhibit the growth of G –ve.
     3. Nystatin yeast inhibit the growth of yeasts.
     4. Trimethoprim may be added to Thayer Martin medium to inhibit the swarming of Proteus. This is called "modified Thayer Martin", incubation at 35-37°C for 24-48 hr., 5% CO_2 will enhance the growth. Growth culture can not grow at temp. below 22°C, the colonies are non hemolytic, gray to white in color.

Lab. Diagnosis of *Neisseria meningitidis*:

1. Specimens: Meningitis is the infection of meninges which may be due to bacteria *N. meningitidis* (*Streptococcus pneumonia* and *H. influenzae*) or viral (polio, *Herpes simplex*) or may be fungal (candida).

- CSF: Cerebro spinal fluid is the main specimens (blood is the alternative but rarely used), bacterial infection makes CSF either:
  1. Purulent (pyogenic) eg. *N. meningitidis*.
  2. Non purulent (aseptic) *Mycobacterium tuberculosis* (in case of T.B., meningitidis).
- The normal CSF is clear in case of bacterial infection makes the CSF turbid, whereas in case of viral meningitidis the CSF is clear except *H. simplx* viruses, where it is haemorrhagic CSF.
- CSF should be collected under aseptic condition and before the commencement of any therapy. CSF collected in screw capped bottles and should not be refrigerated, if CSF is delayed, a transport medium must be used (glucose broth 1%), then CSF must be centrifuged at room temp. 1500 rpm for (10-15) min. supernatant is removed in sterile tube for serology and the sediment used for direct staining and cultivation.
- CSF may contains RBCs in case of (error procedure transmatic).

2. Gram staining: Resemble to Gonococcus with one exception. Meningococcus is encapsulated (Gonocossus → no, capsule).
3. Culture: In contrast to Gonococcus, meningococcus strains are able to grow on blood agar as well as on chocolate agar. Although both of them grow better when CO_2 increased (the colonies of Meningococci larger than Gonococci) 5% CO_2, 37°C, 24-48 hr.
4. Serology: Nine serotype of *N. meningitidis* according to the capsular polysaccharides (A, B, C, D, X, Y, Z, E_{29}, W_{135}). Rapid diagnosis of meningitis is by detection of capsular polysaccharides antigens in body fluid (CSF supernatant) (ELISA).

Pseudomonas:

An opportunistic pathogen, the major human pathogen is *Pseudomonas aeroginosa* which is invasive and toxigenic, produce infection in patient with abnormal host defense (immunecompromised) such as disruption of skin and mucous membrane and intravenous or urinary instrumentation.

P. aeroginosa is an important nosocomial pathogen.

P. aeroginosa may cause: wound infection, burn, UTF, meningitis, pneumonia, bacterimia, invasive malignant otitis externa in diabetic patients and erythrom gangrenosum.

General characteristics and morphology:

G –ve rods, motile with monotrichious flagella, non fermentive bacilli, obligate arerobic, catalase and oxidase (+ve). The most important features of *P. aeroginosa* is the production of water soluble exopigments.

Culture:

P. aeroginosa grow readily on many types of culture media, obligate aerobs (Nutrient agar, blood agar and MacConkey agar), sometimes producing a sweet or grape like odor, some strains and β-hemolytic on blood agar.

P. aeroginosa produce round smooth colonies with fluorescent greenish color.

P. aeroginosa grows well at (37-42°C), its growth at 42°C helps to differentiate it from other pseudomonas spp. pH of about 4.5, it doen't ferment CHO but utilize over 80 different organic compounds.

Pigment production: There are 4 types of exopigment produced by *P. aeroginosa*:

1. Pyocyanin → blue-green pigment.
2. Pyoveridin → green.
3. Puorubin → red.
4. Puomelanin → black.

Sensitivity to antimicrobial agent of antibiotics:

P. aeroginosa is resistant to many disinfectant like detol cetrimide which have been used in preparing selective media for this organism.

Vibrios:

Are short, often curved, G –ve that are motile by single polar flagellum, oxidase and catalase (+ve), non spore forming, it ferment range of carbohydrate, most vibrio spp. are halophilic can grow in media containing 6-10% NaCl, optimum concentration 3% NaCl, but *V. cholerae* is non halophilic, the most important 2 spp. are:

1. *V. cholerae*:

Cause cholera, it G –ve curved or comma shaped, these show vigorous darting, motility due to single polar flagellum, catalase and oxidase (+ve), non spore forming.

Culture:

It produce convex, smooth, round colonies, facultative anaerobic. They grow well on ordinary media, such as nutrient agar, pepton water and blood agar, most strains grow well on MacConkey agar giving pale

colonies, the optimum temp. for growth 37°C, range 10-40°C, sensitive to acid media and prefer to grow on alkaline media (pH 8.5-9.5). the sucrose ferment produce yellowish colonies and turn the medium to yellow, other spp. don't ferment sucrose appear greenish colonies and the medium remain green.

- Biochemical test:

Indole +ve, VP +ve, gelatinase +ve, urease –ve.

- Isolation of organism from the stool as the following:

Patient stool (sever rice water) → alkaline pepton water broth (pH 8.5-9.5) incubated for 6 hr. at 37°C → T.C.B.S (24 hr. at 37°C).

Results → Yellowish colonies due to ferment of sucrose *V. cholerae* (yellow medium).

Or → Other spp. of V. can't ferment sucrose produce green colonies (green medium).

- Clssification of *V. cholerae*:

V. cholerae has 2 biotypes, Eitor and Classical.

Characteristics	Classical	Eitor
1. Hemolysis	–	+
2. Resistant to polymyxin B	–	+
2. VP	–	+

2. *V. parahaemolyticum*:

Causes gastroenteritis and associated with sea food, resemble to *V. cholerae* in many aspects, but differ in that tolerant to grow in NaCl 8% and unable to grow in medium without NaCl, it grow on T.C.B.S and produce green colonies, on MacConkey agar the colony is pale, is not ferment sucrose and lactose, VPC (–ve), gelatinase (+ve).

Helicobacter:

G –ve, motile, catalase and oxidase (+ve), microaerophilic.

Genus: *H. pylori*

Is the leading cause of peptic ulcer, curved found under the layer of mucous on the epithelium of the gastic, oxidase and catalase (+ve), *H. pylori* grow well on blood agar, chocolate agar and sheep blood agar giving small colonies that are grey and transluscent in 3-7 days at 37°C, it is also strong urease (+ve) (2-4 hr.) or helical shape.

Specimens:

Biopsy of mucous from gastric taken by endoscopy.

Culture:

H. pylori grows in 3-6 days when incubated at 37°C in microphilic environment, chocolate medium and other selective media with antibiotics (eg. Vancomycin, nalidixic acid, amphotericin). The colonies are translucent and 1-2 mm in diameter.

Biochemical test:

Oxidase +ve, catalase +ve, urease +ve (very strong).