

## Lab-15 Gram Negative Bacteria

### Neisseria:

The genus *Neisseria* consists of gram-negative, catalase –ve, oxidase +ve, non motile, diplococci. Grows well at aerobic conditions and some spp. are facultative anaerobes. 5% CO<sub>2</sub> enhance the growth of *N.*, some spp. are capsulated and others are not. *N.* spp. are very fastidious in their growth.

Two *Neisseria* species are pathogenic for humans:

1. *Neisseria gonorrhoeae* (Gonococcus) causes “Gonorrhoea”.
2. *Neisseria meningitidis* (Meningococcus) causes “Meningitis”.

Both spp. frequently observed inside polymorphonuclear leukocytes (Neutrophils) of clinical samples obtained from infected patients, but cannot be distinguished from each other under the microscope. However, they can be differentiated in the lab by sugar use patterns, and the sites of their primary infections. Both bacteria are classified as pyogenic cocci because infections by these organisms are also characterized by the production of purulent (pus-like) material comprised largely of white blood cells.

***Neisseria gonorrhoeae*:** is usually transmitted during sexual contact or, more rarely, during the passage of a baby through an infected birth canal. It does not survive long outside the human body because it is highly sensitive to dehydration.

### Lab. Diagnosis:

1. **Specimens:** Include material from genital sources (for both ♂ and ♀) like urethral, vaginal discharge [creamy yellowish color] and extra-genital specimens in disseminated gonococcal infections eg. skin, joint fluid and blood.
2. **Direct stain:** Gram stain is made to the specimens above and will show G –ve, diplococci, non-capsulated by (capsular stain).
3. **Growth conditions for cell culture:** *N. gonorrhoeae* grows best under aerobic conditions in the presence of CO<sub>2</sub>, cannot grow at temp. below 22°C, the colonies are non hemolytic, gray to white in color. *N. gonorrhoeae* ferments glucose but not maltose, lactose, or sucrose. [Note: *N. meningitidis* ferments glucose and maltose]. All members of the genus are oxidase-positive. [Note: The oxidase test is used to identify neisseriae, but does not distinguish between gonococci, meningococci, and non-pathogenic neisseriae.]

**4. Culture:** *N.* is fastidious grows on enriched media like chocolate agar and the selective one is **Thayer Martin medium** which is chocolate agar supplemented with several antibiotics that suppress the growth of nonpathogenic neisseriae and other normal and abnormal flora) is typically used to isolate gonococci. The use of this medium is important for cultures that are typically obtained from sites such as the genitourinary tract or rectum, where there is normally an abundance of flora. On nonselective media, the normal flora overgrows the gonococci.

### **Lab. Diagnosis of *Neisseria meningitidis*:**

*N. meningitidis* is one of the most frequent causes of meningitis. It also can take the form of a fulminant meningococemia.

**1. Specimens:** CSF: is the main specimen (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.-meningitis).

- The normal CSF is clear, bacterial infection makes the CSF turbid with elevated protein, decreased glucose (partly resulting from its consumption as a bacterial nutrient), and many neutrophils, whereas in case of viral meningitis the CSF is clear, except *H. simplex* 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 (traumatic).

**2. Gram staining:** Resemble to Gonococcus with one exception. Meningococcus is encapsulated (Gonococcus → no capsule).

**3. Culture:** In contrast to Gonococcus, meningococcus strains are able to grow on plain blood agar and chocolate agar as it usually cultured from CSF or blood, which are normally sterile; therefore a selective medium is not required. Although both of them grow better when CO<sub>2</sub> increased (the colonies of Meningococci larger than Gonococci) 5% CO<sub>2</sub>, 37°C, 24-48 hr.

**4. Serology:** Nine serotype of *N. meningitidis* according to the capsular polysaccharides (A, B, C, D, X, Y, Z, E29, W135). 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 aeruginosa* which is invasive and toxigenic, produce infection in patient with abnormal host defense (immunocompromised) such as disruption of skin and mucous membrane and intravenous or urinary instrumentation. *P. aeruginosa* is an important nosocomial pathogen.

*P. aeruginosa* may cause: wound infection, burn, UTI, meningitis, pneumonia, bacteremia and invasive otitis externa in diabetic patients.

### **General characteristics and morphology:**

G <sup>-ve</sup> rods, motile with monotrichous flagella, non fermentive bacilli, obligate aerobic, catalase and oxidase (+ve). The most important features of *P. aeruginosa* is the production of water soluble exopigments.

### **Culture:**

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

*P. aeruginosa* produce round smooth colonies with fluorescent greenish color.

*P. aeruginosa* 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 doesn't ferment CHO but utilize over 80 different organic compounds.

### **Specimens:**

Specimens from skin lesions, pus, urine, blood, spinal fluid, sputum, and other material should be obtained as indicated by the type of infection.

### **Smears:**

Gram-negative rods are often seen in smears. There are no specific morphologic characteristics that differentiate pseudomonads in specimens from enteric or other gram-negative rods.

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

1. Pyocyanin → blue-green pigment.
2. Pyoverdinin → green.
3. Pyorubin → red.
4. Pyomelanin → black.

#### **Sensitivity to antimicrobial and antibiotics agents:**

Clinically significant infections with *P. aeruginosa* should not be treated with single-drug therapy, because the success rate is low with such therapy and because the bacteria can rapidly develop resistance when single drugs are employed, and susceptibility tests should be done before the selection of antimicrobial therapy.

*P. aeruginosa* is resistant to many disinfectants 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 ferments 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, 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.

#### **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. *V. cholerae* grows well on **thiosulfate-citrate-bile-sucrose (TCBS)** agar. 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).

#### **- Biochemical test:**

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

- Isolation of organism from the stool as the following:

Patient stool (sewer 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).

#### **- Classification of *V. cholerae*:**

*V. cholerae* has 2 biotypes, Eltor and Classical.

Characteristics:	Classical	Eltor
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, does not ferment sucrose and lactose, VP (–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 stomach, *H. pylori* grow well on blood agar, chocolate agar and sheep blood agar giving small colonies that are grey and translucent 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).