LAB 9:

FAMILY: *PSEUDOMONADACEAE*

Genus: 1- *Pseudomonas*

Spp. A) Pseudomonas aeruginosa
B) Pseudomonas fluorescence
C) Pseudomonas putida
(Fluorescent group)
D) Pseudomonas pseudoalcaligene(opportunistic)
E) Pseudomonas mallei
F) Pseudomonas pseudomallei
G) Pseudomonas cepatia

(Pseudomallei group)

2- *Acinetobacter*

General characteristics :

G-ve, bacilli,motile with polar flagella(monotrichous or polytrichous)& some of them non motile,catalase +ve, oxidase +ve, non spore former, uncapsulated, strict aerobic but can utilize nitrate as a source for respiration.

They are found in soil, any moist areas like water(river and marine),they present in small no. of normal intestinal flora and skin, they are characterized by extracellular pigments, the colors of these pigments differ according to the spp.:

**P. aeruginosa* produce blue green pigment ⟶ pyocyanin

* ***P. fluorescence*** produces yellow to green pigment ⟶ fluorescen (pyoveridin)

Pyoveridin (composed from 2 pigments fluorescen A and fluorescen B).

Other produces red pigment → pyorubin

Some produce black pigment→ pyomelanin.

Pathogenicity:

P. aeruginosa is the most important species, it is invasive and toxigenic produce infection in patients with abnormal host defense and is an important nosocomial pathogen, they cause UTI, otitis media and septic shock, and the main infection of ***Pseudomonas*** is burn infection and wound infection.

They may found in antiseptic solution, eye drops, grows well in dettol,heating 55 C° kill ***Pseudomonas*** ,so it could survive in detergents, it shows also resistant to different and multiple antibiotics.

Enzymes and toxins:

They are extracellular include hemolysin, lipase,c ollagenase, protease,the most important toxin is exotoxin A which cause blockage of protein synthesis which leads to tissue necrosis.

Classification:

1- Biochemical test.
2- Serological (H-Ag, O-Ag, 110 serotype).
3- Pyocin typing ***Pseudomonas*** produce pyocin which is an antimicrobial agent.
4- Phage typing.
5- Sensitivity pattern antibiotics.

Drug of choice: Carbenicillin **(**Pyopen)
Specimens: skin lesion, pus, spinal fluid, sputum and urine.

Laboratory Diagnostic tests:

1- Gram stain: G -ve bacilli **.**
2- Milk agar (for pigmentation).
3- Blood agar (for hemolysis).
4- Kling A,King B (selective and differential)
5- MacConkey agar.
6- TSI
7- IMVIC
8- Motility
9- O/F (oxidation-fermentation) contain 1% glucose, bromothymol blue, K_2HPO_4 buffering, add paraffin on the slant to produce anaerobic condition,inoculation by stabing, the colour change to yellow.
10- Nitrate broth.
11- oxidase and catalase.

Test	*P. aeruginosa*	*P. fluorescence*
Indol	-	-
MR	-	-
VP	-	-
SC	+	+
TSI	K/K - -	K/K - -
Nitrate	+	+
Motility	+	+
Growth at 42 C°	+	-
Growth at 4 C°	-	+
King A	+ pyocianin	(- +) - pigment
King B	+ fluorescen	+ fluorescen
MacConkey	N.L.F. transparent,irregular	N.L.F. transparent,irregular
oxidase	+	+
catalase	+	+
OF medium	O (+)/F (-)	O (+)/F (-)

LAB 10:

FAMILY: *VIBRIONACEAE*

Genus: *Vibrio*

General characteristics

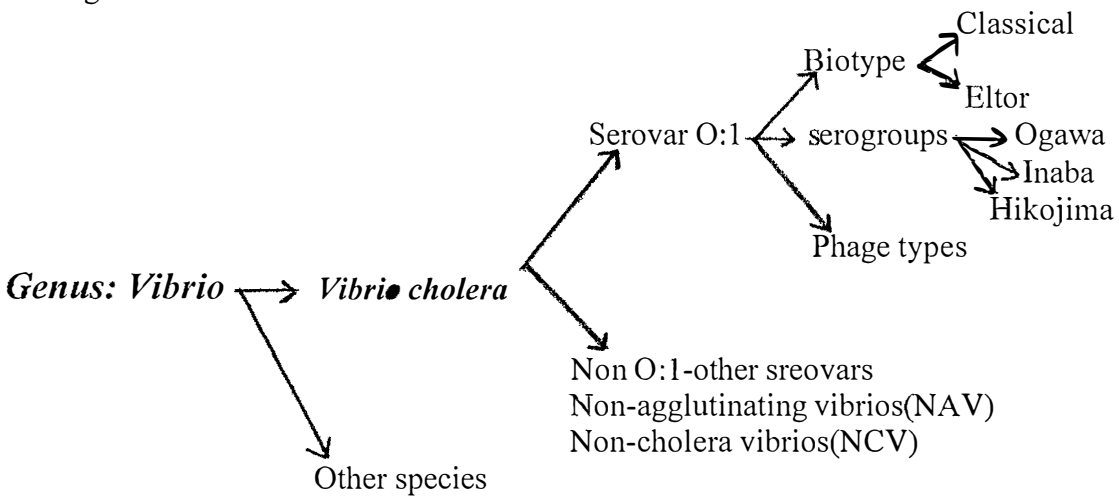
They are curved G -ve (comma shape) ,aerobic rods, motile with single polar flagellum, found in single or in cluster forming S shape, non spore former, on prolong cultivation ***Vibrio*** may become straight rods.
Vibrio found in nature mostly in water, fishes and food.

Culture:

Vibrio produce convex ,smooth round colonies, opaque and granular in transmitted light, they are oxidase +ve,most ***Vibrio*** grow well at 37 °C on media containing mineral salt and amino acids (Aspargin, Arginine, Lysine) as a source of carbon and nitrogen.
These organisms grow at alkaline pH(8.5-9.5) but they are rapidly killed by acid and heating at 55 °C for 15 min.,culture containing carbohydrates become sterile after few days.
Vibrio cholera grows well on TCBS (Thiosulfate citrate bile sucrose) media.

Serological classification

Vibrio has O and H Ag ***Vibrio cholera*** can be differentiated from other vibrio by O:1 Ag



Vibrio cholera (V.C.): O1,O139
Non vibrio cholera (N.V.C.):O2-O138

V. parahaemolyticus (food-asscciated diarrhea disease)
V. vulnificis(wound infection)
V. alginolyticus (otitis externa,wound infection)

Eltor	Classical
hemolytic	Non hemolytic
Polymixin B (resistant)	Polymixin B (sensitive)
Cause heme agglutination of sheep RBC	dose not
VP. +ve	VP. -ve

Laboratory Diagnostic tests:

1-Gram stain. (Weak)

* Motility under dark field (shooting star)

* Cholera immobilization test: bac. (Stool) + Ab ⟶ no movement (+ve)

2- TCBS (Na-citrate,Na-thiosulfate,bile salt,sucrose,bromothymolblue,pH:8)
3- Peptone water NaCl 7%, 0%
4- IMVIC
5- Motility
6- Nitrate reduction test.
7- Kligler iron agar (TSI without sucrose). +ve orange-red → pink
8- OF media.
9- Mannitol fermentation.
10- Catalase and oxidase test.
11 - String test : When an isolated colony (18-24 hour growth) of a suspected bacterium is emulsified in Sodium deoxycholate or Sodium taurocholate (commonly known as bile salt), it lyses the cell wall of the bacterium releasing the DN A .
Take a clean grease free slide and put a drop of 0.5% bile salt. Emulsify an isolated colony of the bacterium using an inoculating loop. Keep on rubbing the loop vigorously for 2-3 min. until it appears viscous. Gently, pull the loop upwards from the slide. Formation of a thread like mucoid string indicates a positive test.
12- Cholera red test.
Tryptophane + conc. H_2SO_4 → nitrosoindole (red color +ve)

13- Oxidase test (4,5 tetramethyl paraphenyl diamine dihydro chloride),The oxidase test is based on the bacterial production of an oxidase enzyme.
Transfer 1 colony (not from blood agar) to filter paper by loop then add 1 drop of (4,5 tetramethyl paraphenyl diamine dihydro chloride)
+ve darkviolet
-ve no change in color

Test	*Vibrio cholera*	*Vibrio parahaemolyticus*
Catalase and oxidase	+	+
NO_2 reduction	+	+
Indol	+	+
MR	+ weak	-
VP	-	-
SC	+\ -	+\ -
Peptone water 7% NaCl	-	+
Peptone water 0% NaCl	+	-
TSI	A\A - -	K\A - -
Motility	+	+
Cholera red	+	-
Mannitol	+ weak	+ weak
String test	+	+
OF media	Oxidation and fermentation	oxidation

LAB 11:

FAMILY: *NEISSERIACEAE*

GENERA

1-***Moraxella (associated with UTI)***
2-*Neisseria*
3-***Branhamella***

ssp:
- *N. gonorrhea* (also called the gonococcus), which causes gonorrhea.
- *N. meningitidis* (also called the meningococcal), one of the most common causes of bacterial meningitis and the causative agent of meningococcal septicaemia.
- *N. lactamica*
- *Branhaemella catarrhalis (N. catarrhalis* -normal flora of U.R.T.)

General characteristics of *Neisseria*

The *Neisseria* cells appear in pairs, bean like opposite to each other, they are non motile, very fastidious organism, need either serum or heated blood with some supplement (antibiotics specially for the first isolation), it dose not grow well on blood agar because it lake enzymes which destroy (lysis) RBC, optimum condition for growth is present of CO_2 5-10% with moisture and 3 7C° for the pathogenic strain, they are oxidase, catalase positive & can reduce nitrate. Found associated with or inside polymorphnuclear leukocytes while other *Neisseria* are normally inhibiter of human respiratory tract and occur extracellulary. Gonocci and meningococci are closely related with 70% DNA homology & differentiated by few lab. test and specific characteristic.

***N. meningitides* capsulated, this ssp. Contain 4 serotype Ag, type A is responsible for about 95% of the cases isolated from CSF & from blood.

***N.gonorrhea* include 16 serotype, the Ag of pili is the base of seroclassification,gonococci characterized by ability to produce β–lactamase which inhibit penicillin antibiotic, isolated from urethral discharge and blood.

***Branhaemella catarrhalis*: non capsulated, penicillin sensitive & vancomycin resistant.

Laboratory Diagnostic tests:

1- Gram stain.
2- Oxidase test (+ve)
3- Carbohydrates fermentation (Muller-Hinton + bromothymol blue indicator + pH (6-7)+10% sugar: lactose , sucrose, glucose, maltose, fructose)
4- Streaking on chocolate agar (for colony morphology and pigmentation): the colonies appear very small, concave and greenish on choclate agar.
5- Blood agar
6- Nitrate and nitrite reduction test: inoculate 1-2 loop full of culture to nitrate broth(NO_3) → incubation 24 hr. at 37 C° → 2 ml of nitrate media add 5 drops of Sol. A (N,N-Dimethyl-α-naphthylamine) + sol. B (sulphanilic acid) (Reagents A and B should be protected from light and stored in the refrigerator) and after 30 sec.:

Red color ($NO_3 \longrightarrow NO_2$) +ve

No changes in color add trace of Zinc powder:

** No change in color ($NO_3 \longrightarrow N_2$) +ve

** Red color (NO_3 doesn't reduced) –ve

Test	*N. gonorrhea*	*N. meningitidis*	*B. catarrhalis*
Glucose	+	+	-
Maltose	-	+	-
Fructose	-	-	-
Sucrose	-	-	-
Lactose	-	-	-
Pigment	Grayish-white	Grayish-white	Opaque gray
CO_2 requirement	necessary	Not necessary	Not necessary
Growth at 35 C°	+	+	+
NO_2 reduction	-	+/-	+
Morphology	Smooth,non-capsulated	Transparent,flattened,mucoid if capsulated	Opaque,smooth,capsulated