VIBRIO

Vibrio:

- Organisms in this genus are Gram-negative, non-sporing, curved rods, with single polar flagella.
- They are oxidase and catalase positive. Generally they ferment glucose with acid production only.
- They are aerobic and facultatively anaerobic,
- some are proteolytic and liquefy gelatine.

The type species of this genus is *V. cholera* and was first isolated by Robert Koch in Egypt in 1883.

Vibrio cholerae:

- Gram-negative curved rod with single polar flagella.
- It is aerobic and facultative anaerobic.
- It ferments glucose with acid production only
- Grows well in alkaline pH with little or no growth in acidic pH. The optimum temperature of growth is 37^0C with a range of 15^0C to 42^0C.

Growth Media:

V. cholerae can be cultured on ordinary media such as nutrient agar, blood agar or nutrient broth. Selective and enrichment media may also be used when contamination is suspected. Alkaline peptone water (pH 8.4) is a good enrichment medium for the growth of this organism. Examples of selective media include, thiosulphate-citrate-bile slat sucrose (TCBS) agar and taurocholate tellurite gelatine agar (TTGA). The two media are popularly used in cholera laboratories.

Biotypes:

Two biotypes have been identified. They are EI Tor and classic biotypes.

Antigenic groups:

With reference to the use of the O antigen, three serotypes are known.

(a) Inaba with antigenic components A and C,

(b) Ogawa with components A and B and

(C) Hikojima with components A, B and C.

The first two are more common. Serotype Ogawa can change to serotype Inaba but the reverse does not occur.

Other Vibrios

V.parahaemolyticus.

- Halophilic marine *vibrio*, which was first recognize as a cause of food poisoning in Japan in the early 1950s.
- Shares some characteristics with *V.cholerae* but tolerates a high concentration of salt in contrast to *V. cholerae.*
- Causes two types of enteritis in humans

  (1) Watery diarrhea with abdominal cramp, nausea, vomiting and fever, and

  (2) Dysentary-like infection with a shorter incubation period, (2.5 hours or more) than the former (15 hours). In both cases the illness is usually self-limiting.
- Enteritis caused by this organism is mainly transmitted by food, particularly seafood. Infection is most common during the warmer months. This may reflect both enhanced opportunity for *V.parahaemolyticus* to multiply in unrefrigerated foods and increased prevalence of the organisms in the environment during the warmer months.

- Generation time of this organism is 9 minutes under normal conditions in food and can quickly reach the rather large infectious dose (ID50) of $10^5 – 10^7$. Growth is inhibited at temperatures below 15^0C and above 65^0C.

Other vibrios which have been associated with human infections include: *V. vulnificus, V. fluvialis* and *V .mimicus, V.anguillarum* and *V. ordalii.*

V.metchnikovii: It was oringinally isolated from the blood and gut contents of chickens dying of fowl cholera-like disease. It grows rapidly in peptone water and grows well on DCA. It is aerobic and facultative anaerobic. Temperature range of growth is 30^0-40^0C.

BRUCELLA

- Organisms in this genus are Gram-negative cocco-bacilli, which are non-sporing and non-motile.
- They are strict aerobes but some strains require 5-10 percent CO_2 for growth.
- Utilize various carbohydrates with negligible acid production.
- *Brucella* species are obligate intracellular organisms.

Media

- Include liver infusion agar, blood agar, chocolate agar, glycerol glucose agar and serum dextrose agar. The latter which is more popular in the isolation of this organism consists of 5 percent serum and 1 percent dextrose.
- Antibacterial and antifungal agents such as bacitracin, actidione, fungizone, polymyxin B, cycloheximide and vancomycin may be added to a medium to suppress contaminant for primary culture.
- CO_2 requirement.-

B.abortus and *B. ovis* require optimum 10 percent CO_2 for growth.

B. melitensis and B. *suis* would rather prefer addition of CO_2 for growth.

- Maximum temperature of growth is 37^0C and growth occurs in 2-4 days with small and transparent to translucent colonies.
- They are catalase positive and some species reduce nitrates to nitrites.
- Both *B. abortus* and *B. suis* will agglutinate monospecific antiserum to *B. abortus* while B. *melitensis* agglutinates only its monospecific homologous antiserum.

Antigens:

- Two antigens designated A and M are possed by smooth strains of *B. abortus, B. suis* and *B. melitensis*.

- Crossreactions occur with other Gram-negative bacteria such as *E. coli 0116* and 0157, *F.tularensis, Salmonella sp., Y. enterocolita* 09 and *V. cholerae* 01.

Bacteriophage typing:

- The bacteriophage, Tbilisi (Tb) is specific for smooth strains of *B. abortus* in routine test dilution (RTD). At 10,000 RTD, the phage will lyse *B. suis* but not *B. melitensis* . The phage is stable at 4^0C for a long period.

Virulence factors:

- Virulence of *Brucella* species is derived from their intracellular niche within the reticuloendothelial systems.
- Cell Wall - the cell wall lipopolysaccharide (LPS) of brucellae aid in its survival within macrophage.
- *Erythrito*l- a four-carbon alcohol, is one of several “allantoic fluid factors” found in the gravid uterus, and appears responsible for the preferential localization of brucellae to the

reproductive tract of the pregnant animals. “Allantoic fluid factors” stimulate the growth of brucellae.

- Outer Membrane Proteins- Porin proteins in the outer membrane are thought to stimulate delayed-type hypersensitivity and account for the varying susceptibility to dyes observed for the different species.
- Miscellaneous Products-

  i. Production of adenine and guanine monophosphate by Brucella inhibit phagolysome fusion and activation of the myeloperoxidase-halide system. *Brucella* are able to inhibit apoptosis in infected macrophages, thereby preventing host cell elimination.

  ii. Soluble protein products inhibit tumour necrosis factor alpha (TNF-α) production. iii. The Vir (for virulence) operon encodes a Type IV secretion system, which appear to be involved with intramacrophage survival.

Laboratory diagnosis:

Diagnosis in animals is based on microscopy, culture and serology.

(a) **Microscopy:** The foetal stomach content of infected animals is a good source of *Brucella* and can be examined for *B. abortus* by staining preferably by the modified Ziehl-Neelsen method. The same method is applicable to *B. melitensis* or any other *Brucella* species. *Brucella* organisms stain pink and are coccobacillary. They are usually present in large numbers.

In the absence of the foetus, smears are made from other foetal-maternal tissues interface such as cotyledon or placental materials. However, care must be taken to exclude other that may be bacteria present. *Brucella* organisms normally stain readily by the modified acid-fast staining technique.

(b) **Culture:** Samples like content of the foetal stomach, the placental materials or ground cotyledon is streaked on serum dextrose agar plates or on any other suitable media, with

or without antibacterial and antifungal agents. For primary cultures, antimicrobials like antibacterial and antifungal agents need to be added to suppress contaminants. The plates are incubated in 5-10 percent CO_2-enriched atmosphere at 37^0C for 4 days. If growth is absent after 4 days, the plates are incubated further for another 4 days before plates are discarded. If there is growth, slide agglutination with monospecific serum is then carried out for the identification of the *Brucella* species.

(c) Serology

(1) Rose Bengal Plate Test (RBPT) is very useful screening tool in the field. *Brucella* organisms are stained with rose Bengal stain at pH 3.5. The stained antigen preparations may be obtained commercially or from Reference Laboratories.

A drop of the serum from sampled blood is mixed with a drop of the stained antigen preparation. The suspension is rocked for 2-3 minutes to produce homogenous mixture. Occurrence of agglutination within 2-4 minutes certifies the sample positive. On the other hand, serum sample may be diluted up to 1:8 with phenol saline and spot agglutination carried out with each dilution. This test is specific, sensitive and useful in screening and survey work.

(2) Serum agglutination test (SAT): This test reliable and specific in bovine, caprine and ovine brucellosis. Its reliability in swine brucellosis diagnosis is in doubt. The RBPT is more reliable for swine brucellosis test. The SAT is sufficiently standardized regardless of the modification in the test in some countries, that the results can be reported in international units (i.u.). Standard serum can be obtained from the International Reference Centre for Brucellosis, Weybridge, England.

(2) Milk ring test (MRT). It is generally carried out on individual animals or on bulk milk sample. For MRT, 1ml of the milk in a 1ml – tube is mixed with a drop of stained suspension of the organism and shaken. The mixture is incubated in a water bath at 37^0C for 30 minutes or 1 hour. A positive reaction occurs when a blue ring is formed at the top due to antigen – antibody reaction. The cream is white or colourless when the test is negative. False positive results may occur in mastitis cases, particularly in goats. If the milk contains little cream, sterile cream is added to the milk to aid diagnosis.

(3) Other tests include Whey agglutination test, Enzyme linked inmmuosorbent assay (ELISA), etc.

MORAXELLA AND ACINETOBACTER

Moraxella: (formerly referred to as Diplobacillus Morax-Axenfeld)

- Organisms of the genus are bacilli or coccobacilli, usually in pairs.
- They can be pleomorphic and grow on simple media containing blood.
- They do not ferment carbohydrates.
- They are oxidase and catalase positive. Different species may liquefy gelatin and coagulate serum.

Important species are:

Species	**Host**	**Disease**
M. lacunata	Human	Conjunctivitis
M. liquefaciens	Human	Corneal ulceration
M. bovis	Cattle, goat	Kerato conjunctivitis ("pink eye")

M. anatipestifer (formerly *Pasteurella)*	Duck	Septicaemia and serositis
M. catarrhalis	Human, Cattle, sheep & dog	Bronchitis, Pneumonia Commensal
M. osloensis	Human	Commensal of genital tract

M. bovis:

- It is the most important animal species affecting cattle and to a less extent goats. It is opportunistic and was first isolated by Jones and Little in 1923 from cattle suffering from keratitis and conjunctivitis.
- It is Gram-negative plump coccobacillus often in pairs (diplobacillus) and exhibits Pleomorphism cultures.
- Fresh isolates from lesions are capsulated.
- It is an obligate parasite of the eye of cattle transmitted directly or via flies from carriers to other animals.
- It is the cause of infectious bovine kerato conjunctivitis(IBK), “pink eye”, a highly contagious and an important disease of beef cattle.

Virulence factors:

- Virulence is associated with the following; fimbriae or pili, haemolysin, fibrinolysin an dermonecrotic factors.

Laboratory diagnosis:

- Eye swab is taken and cultured on blood agar aerobically at 37^0C.
- Virulent *M. bovis* usually show β-haemolysis and may pit the agar.