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NEISSERIAE

- Gram-negative cocci, kidney shaped and usually occurring in pairs (diplococcus).
- Normal inhabitants of the human and animal respiratory tracts, and are extracellular.

Media and Growth

- The organisms prefer well enriched media while the pathogenic ones are normally cultured on selective media. They prefer solid medium to liquid medium.
- *N. gonorrhoeae* and *N. meningitides* grow best on media containing complex organic substances such as blood or animal proteins, atmosphere of 10 percent CO₂.
- Although they grow well on chocolate agar, the popular selective medium used in the laboratories is the Thayer-Martins medium.
- Thayer-Martins medium contains sodium colistimethate and vancomycin to inhibit bacterial contaminants while Nystatin is also added to prevent the growth of fungal contaminants.

Species

- Aside the pathogenic species earlier mentioned, other non-pathogenic species include *N. flavescens*, *N. flava*, *N. sicca*, *N. pharyngis*, *N. canis*, *N. ovis*, and *N. lactamica*.
- The nonpathogenic species can grow at the low temperature of 22°C while the pathogenic species have optimum temperature of 35°C-36°C, minimum and maximum temperatures of 30°C and 38°C respectively.

Biochemical reactions

- Pathogenic species are scarcely saccharolytic, fermenting very limited carbohydrates with acid production but no gas.
- Biochemical tests on serum slope sugars, with 5 percent (human, rabbit or guinea pig serum) in addition to 1 percent carbohydrate, are preferred. Horse serum is usually not added because of its tendency to contain maltase, which splits maltose, resulting in false positive result.
- Non pathogenic species are biochemically more active. Such *Neisseria* species are oxidase and catalase positive, indole and methyl red (MR) negative.

CAMPYLOBACTER AND HELICOBACTER

Campylobacter:

- First isolated by Mcfadyean and Stockman in 1913 but were classified as vibrios because of their curved shape and rapid motility.
- Because of their association with infectious infertility and abortion in cattle and sheep, they were named *Vibrio fetus*.
- In 1963, Sebald and Veron proved that they were a different genus and hence, the name Campylobacter (Greek; meaning curved rod).
- nonsaccharolytic and microaerophilic and exhibit unique cork screw motility. In direct smears from clinical materials, they appear S-shaped or may have a “seagull” appearance.
- In cultures, they are longer and more variable. They may form spherical or coccoid bodies.

- There is variability in colony forms. On blood agar they are shiny, pale, grey, semi-translucent, flattened and nonhaemolytic.
- Biochemically they are relatively inactive. They do not ferment any carbohydrates but they are oxidase positive while some produce catalase.

Media:

- Media for isolation include blood agar, brucella medium and brain heart infusion broth and agar, the later is supplemented with 5 per cent blood.
- Basic selective media which suppress contaminants include Skirrow's or Butzler's medium. They are usually supplemented with antibacterial agents, such as, vancomycin (10µg/ml), polymycin B (2.5 i.u/ml), timethoprim (5 µg/ml), novobiocin (5µg/ml) and cephalothin (15 µg/ml). These media are commercially available.
- Because of their microaerophilic nature, they require atmospheric condition of 10 per cent CO₂, 5 percent O₂ and 85 per cent nitrogen. Incubation is at 37⁰ – 42⁰C and incubated cultures are examined daily up to 7 days.
- Thermophilic campylobacter sp. such as *C. jejuni* and *C. coli* grow at 37⁰C and 42⁰C and not at 25⁰C, whereas the non-thermophilic grow at 37⁰C and 25⁰C but fail to grow at 42⁰C.

Resistance:

- The organism is sensitive to acid pH. It is rapidly killed by HCl at pH 2.3, hence the gastric acid is an effective barrier against infection.
- It can survive for 2-5 weeks in bovine milk or water kept at 4⁰C.

Virulence Factors: Factors associated with virulence and infections include:

- (a) **Motility and chemotaxis:** associated with the flagella. Campylobacter move in response to chemotactic stimuli which direct motility and enhances the effectiveness of mucosal colonization.
- (b) **Adhesion:** Fimbriae have not been demonstrated in *C. jejuni* and *C. coli*.
- (c) **Enterotoxin:** heat labile enterotoxin has been demonstrated in *C. jejuni*. There is also some evidence that *C. jejuni* and *C. coli* secrete a cytotoxin which is toxic for mammalian cells, for example, bovine kidney and HeLa cells.
- (d) **Invasiveness:** The organism penetrates intestinal mucosa and proliferates in the lamina propria and mesenteric lymph nodes. This results in low grade damage of the affected tissues. The virulence factors may not be manifested by all strains of *C. jejuni* and this may explain variation in symptoms of Campylobacter enteritis.

Laboratory diagnosis:

- Isolation and identification of *C. jejuni* and *C. coli* seem more common. *C. jejuni* is the more important pathogen and frequently associated with *Campylobacter* enteritis.
- Direct field microscopy of stool specimens may be carried out for presumptive identification. Care must be taken not to misdiagnose the organism for *V.cholerae*. *Campylobacter* sp. exhibits corkscrew motility while *V.cholrtae* exhibits darting motility. The later is coma-shaped while the former is spiral or s-shpaed.
- Immunofluorescent technique can be used to detect *C. jejuni* in various specimens. Other tests which are promising are ELISA and bacteriophage typing using c-phages specific for *C. jejuni*.

Virulence factors:

- They are associated with the cell wall LPS.

Infection is acquired during coitus or by artificial insemination procedures. Bull to bull transmission may take place during mounting when many bulls are enclosed together. Bovine venereal campylobacteriosis is a chronic infection of the female genital tract, characterized by mild endometritis and transient infertility. The infection is confined primarily to the surfaces of the mucous membrane. Soon after infection the organism can be found in the vagina, the cervix, uterus and oviduct of susceptible cows. The infection is transient in the uterus but becomes established in the cervix and vagina. Abortion may be due to bacterial inflammatory placentitis or allergic response to endotoxin of the organism. The endotoxin has been shown to be abortifacient in pregnant cows.

The earliest antibodies to appear are IgM followed by IgG and IgA. IgG predominates in the uterine secretion of convalescing animals, and IgA is found in the cervico-vaginal secretions. IgA helps to immobilize the organism thereby limiting its entry to the uterus while IgG plays a role in opsonization during phagocytosis.

The organism persists in the vagina for up to 2 years. The persistence may be associated with antigenic variation resulting from phase conversion. Asymptomatic vaginal carrier may arise in animals which regained their fertility but continue to harbour the organism in the vagina or in convalescent animals which have become susceptible to reinfection due to decline or loss of immunity.

Campylobacter foetus

Laboratory diagnosis

(a) Bacteriological diagnosis in the bull is carried out by culture of the preputial materials.

From the cow, materials are obtained from the vagina and cervix by aspiration. In the case of abortion, specimens are obtained from the placenta, cotyledon and the aborted foetus including the stomach content. Cultures are made on selective media. Suspected colonies may be identified by the fluorescent antibody technique or biochemically.

(b) Serological test

(i) Vaginal mucus agglutination (VMA) is useful as a herd test but of little value in identifying individual infected animals.

(ii) Indirect haemagglutination (IHA) using tanned sheep red blood cells, sensitized with phenol-extracted antigen. False positive results may occur in about 1 percent of mucus samples from non-infected cattle.

(iii) Immunofluorescent technique. It is a useful rapid screening method as an adjunct to cultural examination. It cannot distinguish the two subspecies though it is specific for *C. fetus*.

Campylobacter fetus ss fetus. The organism has been isolated from gall bladder, intestinal tract and occasionally from the genital tract of healthy animals. The important infections in animals are abortion and enteritis.