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Lecture. 11
Second Stage

College of Health & Medical Techniques
Department of Medical Laboratories Techniques

BORDETELLA & BRUCELLA

Bordetella pertussis

Is **mesophilic coccobacillus** causes **whooping cough** (pertussis). It is obligate pathogens of humans colonizing the ciliary epithelial cells of the respiratory tract. *B. pertussis* is a fastidious, slow-growing organism.

- **The cells of *B. pertussis* are Gram-negative minute coccobacilli** ranging in size between 0.2-0.5 µm × 0.5-2.0 µm.
- The cells are occasionally **filamentous** that can elongate several µm in length, usually observed in clinical samples.
- *B. pertussis* is **non-motile** with **no flagella**.
- The cells are either **encapsulated** or surrounded by a sheath of **slime**. The capsule is usually observed in freshly isolated species whereas the slime formation occurs in vitro in the form of **biofilm**.
- Both the capsule and slime sheath are composed of **polysaccharides.**
- The surface of the cell consists of fine filamentous appendages.
- The lipopolysaccharide of *B. pertussis* is different from that of other **Gram-negative bacteria** with different **phosphate composition** than the lipid A in other bacteria.
- The lipopolysaccharide of *B. pertussis* acts as **endotoxins**.

The organisms are minute gram-negative coccobacilli resembling *H influenzae.* With toluidine blue stain, bipolar metachromatic granules can be demonstrated. A capsule is present.

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Culture

Primary isolation of *B pertussis* requires **enriched media**. **Bordet-Gengou medium** (potato-blood-glycerol agar) that contains **penicillin G**, 0.5 g/mL, can be used; however, a **charcoal-containing medium**. The plates are incubated at 35–37 °C for 3–7 days in a moist environment (eg, a sealed plastic bag). The small, faintly staining gram-negative rods are identified by immunofluorescence staining. *B pertussis* is non-motile. The organism is a strict aerobe and forms acid but not gas from glucose and lactose. It does not require X and V factors on subculture. Hemolysis of blood-containing medium is associated with virulent *B pertussis.*

- The medium of **choice** for the selective isolation of *B. pertussis* is the Bordet-Gengou medium with **glycerol**. The medium is composed of a potato-extract medium without peptone containing **50**% blood.
- The **charcoal horse blood** agar is a better medium for the selective cultivation of *B. pertussis* as it has a longer shelf-life and is superior in its ability to support *B. pertussis* growth.
- Commercial media for *B. pertussis* include **Stainer-Scholte broth** and **cyclodextrin solid** medium.
- *B. pertussis* is an **obligate** aerobe with the most efficient growth at the temperature range of 30-37°C.

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- The metabolism is mostly based on the oxidation of **amino acids** as these bacteria do not usually utilize **carbohydrates**.
- The growth of *B. pertussis* on artificial media is difficult due to the susceptibility of the bacteria to various compounds like unsaturated **fatty** acids, **colloidal sulfur**, and **sulfides**.

The Virulence factors

1- **Adhesins such as filamentous hemagglutinin, fimbriae.**
2- **Pertussis toxin.**
3- **Adenylate cyclase,**
4- **Tracheal cytotoxin.**

Pathogenesis & Epidemiology

Bordetella pertussis, a pathogen **only for humans,** is transmitted by **airborne droplets** produced during the severe **coughing episodes.** The organisms attach to the ciliated epithelium of the upper respiratory tract but do not invade the underlying tissue. Decreased cilia activity and subsequent death of the ciliated epithelial cells are important aspects of pathogenesis.

Clinical Findings

Whooping cough is an acute trachea-bronchitis that begins with mild upper respiratory tract symptoms followed by a severe paroxysmal cough, **which lasts from 1 to 4 weeks**. The paroxysmal pattern is characterized by a series of hacking coughs, accompanied by production of copious amounts of mucus, that end with an inspiratory “whoop” as air rushes past the narrowed glottis.

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Laboratory Diagnosis

The organism can be isolated from nasopharyngeal swabs taken during the paroxysmal stage. Bordet-Gengou1 medium used for this purpose contains a high percentage of blood (20%–30%) to inactivate inhibitors in the agar.

Treatment

Azithromycin is the drug of choice.

Prevention

There are two types of vaccines: an acellular vaccine containing purified proteins from the organism and a killed vaccine containing inactivated *B. pertussis* organisms.

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Brucella

Brucella species cause brucellosis (**undulant** fever الحمى المتموجة, **Malta** Fever حمى مالطا).

Important Properties

Brucellae are **small**, **aerobic, Gram-negative** "but often stain irregularly" rods without a **capsule,** In young culture thaey varies from **cocci** to **rods** 1.2µm in **length**, with short cocco-bacillary forms predominating and they are, non-motile, and non-spore-forming.

There are three major human pathogens:

- *Brucella melitensis* (**goats** and **sheep**).
- *Brucella abortus* (**cattle**),
- *Brucella suis* (**pigs**).

Growth Characteristics

Fresh specimens from animal or human sources are usually inoculated on **trypticase-soy agar or blood culture media**. *Brucella* colonies are small, convex, smooth colonies appear on enriched media in 2–5 days. *B. abortus* requires 5–10% CO_2 for growth, whereas the other **three** species grow in **air**.

Brucellae **utilize** carbohydrates but produce neither **acid** nor **gas** in amounts sufficient for classification. **Catalase** and **oxidase** are produced by the species. **Hydrogen sulfide** is produced by many strains, and **nitrates** are reduced to **nitrites**.

Virulence factors

lipopolysaccharide (LPS), T4SS secretion system and BvrR/BvrS system, which allow interaction with host cell surface

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Pathogenesis

The common routes of infection in humans are the intestinal tract (ingestion of infected milk), mucous membranes (droplets), and skin (contact with infected tissues of animals). Cheese made from unpasteurized goats' milk is a particularly common vehicle They localize in the **reticuloendothelial system,** namely, the lymph nodes, liver, spleen, and bone marrow. Many organisms are killed by macrophages, **but some survive within these cells, where they are protected from antibody.** The host response is granulomatous, with lymphocytes and epithelioid giant cells, which can progress to form focal abscesses.

Clinical Findings

After an incubation period of 1 to 3 weeks, nonspecific symptoms such as fever, chills, fatigue, malaise, anorexia, and weight loss occur. The onset can be acute or gradual. The undulating (rising-and-falling) fever pattern that gives the disease its name occurs in a minority of patients

Enlarged lymph nodes, liver, and spleen are frequently found. Pancytopenia occurs.

Note:- *Brucella melitensis* infections tend to be more severe and prolonged, whereas those caused by *B. abortus* are more self-limited.

Treatment

The treatment of choice is tetracycline plus rifampin.

Prevention

Prevention of brucellosis involves pasteurization of milk, immunization of animals, and slaughtering of infected animals. There is no human vaccine.