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Characteristics, Description and the Morphology of Major gram Positive Bacteria

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Abstract: Gram positive rods can be divided in to **endospore forming** bacteria (bacillus and clostridium spp), **non-endospore forming. Non-endospore forming** again divided in to regular shape and staining properties (listeria and erysipelothrix) **and** irregular shape and staining properties (Non Acid fast (corynebacterium and propionibacterium), acid fast (mycobacterium spp) and filamentous branching cell (actinomyces and Nocardia).
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1. Introduction

Gram positive (bacilli) are Endospore forming gram positive rods. Endospore-forming bacteria produce a unique resting cell called an endospore. The most commonly studied endospore forming gram positive rod shaped bacteria are: Genus Bacillus and Genus Clostridium (Albano *et al*., 2007).

2. Bacillus species

Rods shaped, Gram positive or variable, endospore forming and large (up to 10.0 um in length) in size. Arrangement: In smear from tissue or cultures, cells occur singly, in pairs or in chains. They are catalase-positive, aerobic or facultative anaerobic, and **motile** with the **exception** of B. anthracis and B. mycoides. Capsule could be demonstrated during growth in infected animals.

Natural habitat

Bacillus species are widely distributed in the environment mainly because they produce highly resistant endospores. Most of them are **saprophytes** widely distributed in air, soil and water. Other species, including humans, are exposed via infected animals and animal products. Spores are formed in culture, dead animal's tissue but not in the blood of infected animals. Spores are oval and centrally located. The spores of B. anthracis are extremely resistant to heat, cold, desiccation and chemical disinfection. In soil, endospores of B. anthracis can survive for more than 50 years.

A vegetative phase may be required in which the organisms multiply to a density **sufficient to infect grazing** animals. In suitable limestone soils, with pH 6.0 and ambient temperature above 15.5°C, a spore vegetative cell-spore cycle may be maintained indefinitely and livestock grazing on such pastures may become infected. Cattle dying with anthrax commonly discharge large numbers of anthrax bacilli from the **nose, mouth and intestinal tract, thus returning organisms to the soil** (Albano *et al*., 2007).

Resistant mechanism

Vegetative cells in unopened carcasses may survive for up to 1–2 weeks whereas spores can persist for decades in a stable and dry environment. **Autoclaving** (132°C, 30‟) and dry heat kill spores, but not by boiling (100°C < 10‟). Heat fixation of smears does not kill spores. Not highly susceptible to phenolic, alcoholic and quaternary ammonium disinfectants. However, aldehydes, oxidizing and chlorinating disinfectants, beta propiolactone and ethylene oxide are more useful.

Pathogenicity and pathogenesis

The majority of the bacillus species have little or no pathogenic potential. Commonly found as contaminant of laboratory media. B. anthracis is major animal pathogen in the genus - anthrax in both, B. cereus (food poisoning in human but rare infection in animals) and B. licheniformis, causing abortions in cattle and sheep and B. mycoides diseases in fish (Albano *et al*., 2007).

Virulence Factors

B. anthracis has a plasmid (pXO1) is encoded (tripartite) protein toxin with protective (Protective antigen), lethal (Lethal toxin) and edema (Edema toxin) factors. The toxin is leukocidal, increases vascular permeability and produces capillary thrombosis causing shock. Proetctive factor is the binding fragment of the toxins required for activity of the other factors. It is also cytotoxic and also triggers **apoptosis** of macrophages. The polypeptide capsule is antiphagocytic. To be fully virulent, B. anthracis must produce both tripartite toxin and capsule (Al-Harbi and Uddin, 2004).

Laboratory diagnosis

Specimens: all procedures should be carried out in a biohazard safety cabinet. Stained smears, that have been heat fixed are potential dangerous as they may contain viable spores. Specimens for B. anthracis:of Sheep or cattle are blood smears from ear or tail veins. Equines, pigs: edematous fluid from localized sites. Blood or homogenized spleen can be used for culture. **Direct microscopy:** B. anthracis produce a capsule in vivo and either Giemsa or polychrome methylene blue stains are used to demonstrate the capsule. Gram stained smears: Made from clinical samples, show large gram positive bacilli in long chains, **bamboo** like appearance. Giemsa stained smears: Purple bacilli with halo/non-stained capsule.

Isolation

B. cereus group grow well on sheep or ox blood agar, aerobically at 37°C in 24-48 hrs. A selective medium for B. anthracis is a polymyxin-lysozyme-EDTA-thallous (PLET) acetate agar.

C. Identification (colony morphology after 48 hours of incubation): B. anthracis almost always **non-hemolytic.** Colonies: about 5 mm in diameter, flat, dry, grayish with a granular ´ground-glass appearance (Al-Harbi and Uddin, 2004).

3. Genus Clostridium

The species are:- large, Gram positive, generally anaerobic, endospore forming rods and the spores usually bulge mother cell. Fermentative, oxidase negative, catalase negative and the majority are motile (except C. perfringens) by peritrichous flagella. Don' t produce capsule (except C. perfringens). All the pathogenic spp are straight rods (except C. spiroforme) which are curved or spiral. The strictness of anaerobic requirements varies among the species but they all prefer an atmosphere containing between 2 and 10% CO2. Most clostridium requires enrich media that include amino-acids, CHOs, vitamins and blood or serum. Optimum growth of the pathogenic clostridia occurs at 37°C. There are over 80 species of which 11 are veterinary importance. Most of the pathogenic species produce one or more exotoxins of varying potency (Al-Harbi and Uddin, 2004).

Natural habitant

Clostridia species are truly ubiquitous. Pathogenic clostridia including C. botulinum and C. tetani can be isolated from nearly every aerobic soil sample and dust. The reason that both are still important pathogens despite available immunization against C. tetani. When animals die, C. botulinum spores, which are common in gut and tissues, germinate and generate toxin. C. tetani is widely distributed in soil and is often a transient in the intestine. Spores can be introduced into wounds (Al-Harbi and Uddin, 2004).

Pathogenicity

The potency of the exotoxines produced and the invasive ability of the clostridia vary.

The three broad types of pathogenesis:

1) Histotoxic group: tissue infections: cellulilitis on tissues, myonecrosis, gas gangrene. Example, C. chauvoei, C. perfringens type A and C, septicum, 2) Enterotoxigenic group: gastrointestinal disease (C. perfringens type A), necrotizing enteritis (beta toxin-producing and C.perfringens type and human intestinal infection (C. difficile), 3) Tetanus

(exogenously acquired) by C. tetani neurotoxin. Generalized (most common), cephalic (primary infection in head, comnnonly ear) and 4) Botulism (exogenously acquired) by C. botulinum neurotoxin, botulism intoxication in both human and animals.

Laboratory diagnosis

Specimens: Specimens should be taken from recently dead animals. For isolation: blocks of affected tissue or fluids in air free containers should be collected. Swabs in oxygen free gas and placed in Cary-Blair transport medium. In some Clostridial diseases, such as Enterotoxaemia, the toxins required for diagnosis. (The content of small intestine collected as soon as possible, as the toxin is labile).

Direct microscopy: gram stained smear from affected tissues reveals, large Gram positive rods, which decolorize easily when sporing. C. spiroforme is curved or helical. C. tetani is drumstick form and fluorescent Antibody techniques: used commonly for C. chauvoei, C. septicum, C. novyi and C. sordellii as flourecent lebelled antisera can be obtained commercially.

Growth condition

Clostridium grows at 37°C and prefers 2-10% Co2 that can be produced in a jar in which the O2 is reduced catalytically by H2, generated along with Co2 from a commercially available packet. Anaerobic jar with a catalyst, anaerobic indicator (methylene blue) and an envelope delving H2 + Co2 is satisfactory.

Incubation on blood agar

C. tetanus is incubation at 37°C for 3-4 days, C. botulinum is incubation at 35°C for up to 5 days, C. chauvoei is incubation at 37°C for up to 2-4 days and Clostridial cultures typically emit putrefied odors due to products of peptide catabolism.

Growth characteristics: Several clostridia swarm across moist agar media without forming colonies. Most clostridia produce haemolysis.

Pathogenesis

The gas-gangrene disease: vary from simple wound infection, anaerobic cellulites to severe and fatal gas-gangrene. The infection is endogenous or exogenous in origin. Endogenous infections often caused by C. chauvoei are black leg in calves. Endospore ingested intestine and lymphatic blood streams muscle mass plus cardiac muscle. Spores cause tissue necrosis supply food and anaerobic environment. Vegetative germinates and produce toxins. In exogenous infections, spores are introduced into wounds germinate in anaerobic environment and produce toxins.

4. Corynebacterium spps

Most coryneform are catalase positive, oxidase negative, non-spore forming and facultative anaerobes. They need enriched media for growth (fastidious), mostly blood agar or chocolate agar in some spp. Pathogenic corynebacteria is **non-motile**. Common species are: C. Kutscheri, C. Pseudotuberculosis, C. Renale and C. pilosum.

Usual habitat

Many species normal flora (commensals) of skin, mucous membranes. Corynebacterium pseudotuberculosis (formerly C. ovis) can survive for months in the environment. Most species are **non-pathogenic** (referred to collectively as "**diphtheroids**"; but do not produce toxin unlike diphtheria).

Isolation:

Sheep or ox blood agar is for routine isolation - incubation at 37°C for 24hrs. MacConkey agar to detect any Gram-negative contaminants.

Colonial morphology: C. bovis – small, white, dry, non-hemolytic colonies - C. pseudotuberculosis – small, white and dry.

Identification

Gram stain morphology: Club-shaped and beaded with irregularly staining granules, pleomorphic, palisading (Chinese letters) gram positive rods. Methylene blue stain shows metachromatic granules (inclusion bodies, which are composed of inorganic polyphosphates (volutin) that serve as energy reserves (Bairagi *et al*., 2002).

Rhodococcus equi (Formerly, Corynebacterium equi): Gram positive, coccus or rod, Coccobacilli, Capsulated and weakly acid-fast species.

Main host Diseases are: R. equi foals (2-4 months old), Suppurative bronchopneumonia, older foals: Abscesses and Cattle Cervical lymphadenitis (Bairagi *et al*., 2002).

5. Genus Listeria

Medium sized, Gram positive, rods, motile, non-spore forming, facultative anaerobes (growth enhanced by 10% CO2), catalase positive ,oxidase negative, hydrolyze esculin tolerate 10% NaCl. Although L. monocytogenes is actively motile by means of peritrichous flagella at room temperature (20−25 °C), Species: 7 species with 2 distinct groups based on hemolytic and pathogenicity nature - L. murrayi and L. grayi is non-hemolytic; nonpathogenic, L. monocytogenes, L. innocua and L. ivanovi is hemolytic and pathogenic for animals.

L. monocytogenes

It is the most important pathogenic spp and it has been implicated worldwide in diseases of many animal species and humans. It is naturally found in: Soil and vegetation (wet and dry), freshly harvested grass, grass with higher moisture content, Fecal material (human and animal), Isolated in healthy livestock (2-16%), wild animals, human sewage, Polluted water and animal feed (silage and straw). Growth at -1 to 45°C, optimally at 30 to 37°C. Psychrophilic (refrigeration temperature) and mesophilic (room to body temperature) (Bairagi *et al*., 2002).

Natural habitat

Listeria are widely distributed in the environment and can be isolated from soil, plants, decaying vegetation & silage (pH 5.5-9.6) in which the bacteria can multiply & cause outbreak in cattle & sheep. L. monocytogenes can be excreted in bovine milk. Isolation of Listeria Spp. On blood agar, small transparent colonies with smooth borders appear in 24 hours. Commercial Listeria selective agar & indicator media are available. Because of the characteristic small zone of complete hemolysis expresses (a beta hemolysin) can be observed around and under colonies. Isolation can be enhanced if the tissue is kept at 4°C for some days, since intracellular, before inoculation into bacteriologic media.

Pathogenesis and pathogenicity

Spreads via lymph and blood to various tissues. In pregnant animals via transplacental transmission**.** The organism can invade through breaks in the oral or nasal mucosa and migrate to cranial nerves leading to **neural listeriosis.** L. monocytogenes has the ability to invade both phagocytic and non-phagocytic cells, to survive and replicate **intracellularly and to transfer from cell to cell without exposure to humoral defence** (Bairagi *et al*., 2002).

Clinical infections and Transmission

Listeriosis in ruminants caused by L. monocytogenes, L. ivanovi and L. innocu. L. monocytogenes infects both human and animals causing meningitis, sepsis and abortion. L. ivanovii and (L. innocua not clearly known) is restricted to sheep and cattle, in which it causes septicemic disease, neonatal sepsis and abortion, but no brain infection. The other species are generally considered nonpathogenic. Possible ways of transmission. Ingestion of vegetation and silages encephalitis and enteritis septicemia mastitis and abortion listeria in milk and meat human listeriosis (Saha *et al*., 2006).

6. Mycobacteria spp (Acid fast bacteria)

Thin rods of varying lengths (irregular shaped groups). Sometimes branching filamentous forms occur but these easily fragment into rods. Non-motile, non-sporing, aerobic and oxidative. Cytochemically: they are Gram-positive but **do not take up the dyes of the Gram-stain since cell walls are rich in lipids- mycolic acid** is the dominant type. The lipids, especially in their walls, account for acid fastness, pathogenic and immunologic properties.

Mycolic acids are linked to the innermost peptidoglycan layer by way of arabinogalactans. Arabinogalactan is biopolymer consisting **arabinose and galactose monosaccharaides** in furanose configuration.

Surface mycosides is mostly glycolipids and peptidoglycolipids. Determine colonial characteristics, serologic specificities, and **bacteriophage** susceptibilities, for survival within macrophages. Tuberculins are purified protein derivative or bacterial peptides secreted into culture media during growth.

The mycobacteria include diverse species ranging from environmental saprophytes and opportunistic invaders to obligate pathogens. Tuberculosis is a chronic granulomatous disease affecting man, many other mammals, birds, fish and reptiles (Saha *et al*., 2006).

Differentiation of pathogen mycobacteria

The definitive diagnosis of tuberculosis is based on the detection of **acid-fast bacilli in clinical specimens by microscopy or cultural techniques.** The ZN staining method is used to differentiate Mycobacteria from other bacteria. Mycobacteria appear as slender, red staining rods against blue black ground (if methylene blue is the counter stain). Safety precautions including the use of **biohazard** cabinet must be implemented when working with material containing Mycobacteria.

Bacterial isolation

A positive culture usually grows in 2 to 4 weeks. A selective liquid medium with a radio labeled carbon substrate allows automated detection of growth several days sooner than with conventional culture. Tubercle bacilli are able to grow on a wide range of enriched culture media, but Löwenstein-Jensen (LJ) medium is the most widely used in clinical practice. The egg based Lowenstein Jensen and Stone brink‟s media are most commonly used in veterinary bacteriology. **Malachite green** dye (0.025g/100ml) is commonly used as the selective agent (Saha *et al*., 2006).

7

. Actinomyces, Nocardia and Dermatophilus

Natural Habitat: Actinomyces is present on mucous membranes of the host animal, often in the oral cavity or nasopharynx. Nocardia - are soil microorganisms. Dermatophilus **(D. congolensis) is survives in skin scab for periods of 3 years.**

7.1. The Actinomycetes (family): A heterogenous group of bacteria. Form gram positive, branching filaments rods is less than 1µm in diameter. Anaerobes/capnophilic (carboxyphilic), often beaded due to uneven staining, cost readily demonstrable in pathogenic specimens and in culture, **diphtheroid forms predominate, like coryneforms.**

Direct microscopy: Specimens: Pus or exudate, sulphur/yellowish granules is the best specimens for direct examination. Placing the sample in a Petri dish, it should be washed carefully with water to expose the granules: A. bovis - yellowish sulphur granules, A. viscosus - softer greyish-white granules. **Gram staining of smears from granules is Gram positive, branching filaments** are seen short filaments or pleomorphic forms may be seen.

Isolation

Sheep or ox blood agar is supports growth of Actinomyces species, A. bovis is requires anaerobic conditions with 5 -10% CO2 (H2+CO2 commercial envelope). A. bovis is non-hemolytic, white, rough or smooth. Adhere strongly to solid medium. Grows in thioglycollate medium as a glistering diffuse growth in 7-10 days. Actinomyces species is MZN negative (Saha *et al*., 2006).

7.2. Nocardia spp

Direct microscopy: Gram stained smears is gram positive branching filaments that show some fragmentation into coccobacilli. Stain red on MZN staining

Isolation

Blood agar is support growth of Nocardia. Incubation is aerobically at 37°C for up to 7 days. Sabouraud dextrose agar cultures are incubated at 37°C for up to 10 days. **Colonial morphology:** Blood agar – colonies are **vivid white** and powdery if aerial filaments and spores are formed.

7.3. Dermatophilus congolensis

Are aerobe/ capnophili/ capnophiles requiring CO2 for maximum growth of bacteria. **Direct microscopy:** Shaved scab is softened in few drops of distilled water on a slide and a smear is made and stained with **Giemsa or Gram stains. Gram stain: filamentous and branching. Isolation:** grows well on **blood agar**. Incubation: **at 37°C** for up to 5 days. **Colony morphology**: At 24-48 hours, small greyish yellow, hemolytic colonies firmly adhered to the medium and after 3-4 days: colonies are rough, wrinkled, and golden-yellow in color (Saha *et al*., 2006).

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