Characteristics, Description and the Morphology of Major gram Negative Bacteria

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Summary: The major Genera gram negative bacteria are Enterobacteriaceae family (E. coli, Klebsiella, Salmonella, Shigella.), Genera Pasteurella and Mannheimia, Genus Haemophilus, Spirochetes, Genera Pseudomonas and Burkholderia, Bordetella, Actinobacillus, Genus Brucella, Genus Campylobacter and Genus Moraxella.
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1. Enterobacteriaceae:

Members of the family Enterobacteriacea are Gram negative medium sized rods (up to 3µm in length). Facultative anaerobes, fermentative, catalase positive, oxidase positive, nonspore forming and some have prominent capsules. Motile by peritrichous flagella except Shigella and Klebsiella which are non-motile. The microscopic morphology is similar among all species and different genera can't be differentiated by it. Therefore, direct microscopy is not helpful for diagnosis. The family Enterobacteriaceae contains more than 41 genera and over 80 species. The taxonomy of the Enterobacteriaceae has seen repeated changes in recent decades. It will probably continue to change in the future too. 1) Escherichia, 2) Shigella, 3) Salmonella, 4) Edwardsiella, 5) Citrobacter 6) Yersinia 7) Klebsiella 8) Enterobacter 9) Serratia and 10) Proteus. Coliforms that ferment lactose are called lactose fermenters such as Escherichia, Enterobacter, Klebsiella, Citrobacter and Serratia spp. Those which do not ferment lactose can be non-lactose fermenters such as Salmonella, Shigella, Proteus, and Yersinia spp. Habitat: soil, water and plant as well as intestines of animal and humans. They can be killed by sunlight, drying, pasteurization and the common disinfectants. Survive for many months in moist, shaded environments pastures, manure, litter and bedding. Many are susceptible to broad spectrum antimicrobial agents. The cell wall, cell membrane, and internal structures are morphologically similar for all Enterobacteriaceae (Albano *et al*., 20007).

Variability:

Variability among isolates of the same genus and species is due to differences in antigens of the following type: capsular (K), somatic (O), flagella (H). Additional source of variation is due to: the presence of genes residing on plasmids encoding certain phenotypic traits such as: resistance to antimicrobial agents, production of toxin and secretion of hemolysis.

Antigenic structures are:

i) Components of the cell wall and surface - are antigenic and form the basis of systems dividing species into serotypes, ii) The outer membrane lipopolysaccharide (LPS) - O antigen. Its antigenic specificity is determined by the composition of the sugars that form the long terminal polysaccharide side chains linked to the core polysaccharide and lipid A, iii) Capsule - K antigen (from the Danish Kapsel, capsule) and iv) Flagella - H antigen Present in motile strains; extends well beyond the cell wall Albano *et al*., 20007).

Selective and /or indicator media for the Enterobacteriaceae

Almost all Enterobacteriaceae will grow on **blood and MacConkey agars and these are used routinely for isolation.** The most commonly used selective media are: **1) MacConkey agar** (Fermentable sugars= lactose and Inhibitors of bile salts and crystal violet reaction). Differential mediums for lactose fermentation inhibits gram

positives and fastidious gram negative bacteria; MAC agar selective for gram-negatives. 2) Brilliant green agar (Fermentable sugars= lactose and sucrose and Inhibitors, brilliant green dye inhibits the growth of most enterobacteria except salmonella.

Identification of Enterobacteriacea groups

Gram staining for species identification is very difficult. So identification is based on:- Colony morphology, Grow readily at 35-37ºC except Yersinia (25ºc-30ºC). On blood agar they are large, almost non hemolytic, shiny, round and grayish in color. MacConkey - lactose fermented or not. All members of Enterobacteriacea are oxidase negative, facultative anaerobes while most other of gram negative bacteria is oxidase positive. Enterobacteriacea can be divided based on pathogenicity. **Major pathogens** are: E.coli, Salmonella and Yersinia species. **Opportunistic pathogens** are occasionally cause infection in animal and these are Klebsiella, Enterobacter, Morganella, Serratia, Edwardsiella, Citrobacter, Shigella and Proteus. All gram negative including members of the Enterobacteriacea have lipopolysaccharides in outer membrane of the cell wall are potent endotoxins which results fever, leucopenia, hyperglycemia and shock. Virulence factors associated with Enterobacteriaceae are: endotoxin, capsule, antigenic phase variation, sequestration of growth factors, and resistance to serum killing and antimicrobial resistance.

1.1. Escherichia coli

Natural inhabitant of the large intestine and lower small intestine of all mammals. E. coli is excreted in faces and can survive in fecal particles, dust and water for weeks or months. The presence of E. coli in water samples, being tested for portability is taken as evidence of fecal pollution. Motiles with peritrichous flagella. No H_2S or phenylalanine deaminizes. Produce metallic sheen colonies on EMB (Eosin Methylene blue) agar. On macConkey agar are : pink colonies, dry, pink (**lactose positive) colony** with surrounding pink area and ferments glucose, lactose, trehalose and xylose Albano *et al*., 20007).

Types of pathogenic E.coli

Nonpathogenic strains of E. coli can cause opportunistic infection in mammary gland (mastitis) and uterus (metritis). E. coli strains that cause enteritis/ diarrhea have been classified as: - **I) Entero toxigenic E .coli (ETEC).** Present in large numbers in the small intestine but insufficient merely to isolate it. The production of colonization factors correlates with enterotoxin production. Cause the majority of cases of neonatal colibacillosis. It causes diarrhea in human, porcine and bovine diarrhea. **II) Entero pathogenic E. coli (EPEC)**. Do not produce enterotoxins but they can cause enteritis and diarrhea by other mechanisms .These strains could be recovered from neonates with diarrhea. **III) Enterhemorragic E. coli (EHEC):** Found in the intestines of healthy cattle, goats, deer and sheep and causes a severe intestinal infection in humans. It differs from other E.coli pathotypes since it produces a potent Shiga like toxin. The toxin damages the lining of the intestinal wall, causing bloody diarrhea. Raw meat, contaminated vegetables and water including swimming ponds could be source of infection. Generally it is responsible for hemorrhagic colitis and hemolytic-uremic syndrome. **IV) Enteroaggregative E. coli (EAEC):** heterogeneous category of an emerging enteric pathogen associated with cases of acute or persistent watery diarrhea (Al-Harbi, and Uddin, 2004).

Isolation

In blood agar often hemolytic. In MacConkey agar: they are strong lactose fermenter; colonies are bright pink. In Eosin methylene blue (EMB) agar: the E. coli colonies have a unique and characteristic metallic sheen indole plus citrate is a quick presumptive method of identifying E. coli.

1.2. Salmonella

Contains O (somatic), H (flagellar), and K (capsular) antigens. Most of the salmonellae that are significant animal pathogens have been named according to clinical consideration. The majority of salmonellae of veterinary importance belong to S. enterica sub species enterica. The sub species are further qualified by the serotype to give a final designation such as S. enterica sub species enterica serotype Typhimurium (S. Typhimurium).

Salmonella serotypes adapted to man are S. Typhi and S. Paratyphi (Al-Harbi, and Uddin, 2004).

Natural Habitat

Reservoirs are the intestinal tract of warm blooded and cold-blooded animals. That is the habitat of the genus Salmonella seems to be limited to the digestive tract of humans and animals. Thus, the presence of Salmonella in other habitats (water, food, natural environment) is explained by fecal contamination. Infected animals majorly becomes subclinical excretes. In moist soil, water, fecal particles, animal feeds (blood, bone, fish meals) Salmonellae can survive for 9 months.

Identification of Salmonella

Xylose fermentation (except Salmonella serotype Paratyphi A) acidifies agar activating lysine decarboxylase. With xylose depletion fermentation ceases and colonies of Salmonella (except S. Paratyphi A) alkalinize the agar due to amines from lysine decarboxylation. Xylose fermentation provides H^+ for H_2S production (Al-Harbi, and Uddin, 2004).

1.3. Shigella

Characteristics are: Non-motile, do not produce gas from glucose, do not hydrolyze urea, do not produce H_2S on TSI, Lysine decarboxylase negative, fragile organisms and Possess O and some have K antigens (Bairagi *et al*., 2002).

2. Gram Negative Rods of the Respiratory Tract

The genera includes: Pasteurella, Mannheimia, Actinobacillus and Haemophilus.

2.1. Pasteurella:

The Pasteurellaceae comprise a large family of Gram negative bacteria. Most members live as commensals on mucosal surfaces of birds and mammals, especially in the upper respiratory tract and lower genital. Pasteurellaceae are typically rod-shaped and are a notable group of facultative anaerobes. They can be distinguished from the related Enterobacteriaceae by the presence of oxidase and from most other similar bacteria by the absence of flagella (Bairagi *et al*., 2002).

Pasteurella species and Mannheimia haemolytica

Small gram negative rods or coccobacilli, non- motile, nonspore forming, facultative anaerobic, **oxidative catalase positive except P. cabali, fermentative except P. anatipestifer, Bipolar staining with Giemsa or methylene blue.** They grow best on media supplemented with serum or blood. Major pathogenic Pasteurella and Mannheimia species include: P. multocida, M. haemolytica (P. haemolytica biotype A) (Bairagi *et al*., 2002).

Natural habitat:

They are worldwide in distribution with a wide spectrum of host. Most are commensals on the mucous membranes of upper respiratory and intestinal tracts of animals.

Direct microscopy:

Specimens from live animals include tracheo-bronchial aspirates, nasal swabs, Tissue or blood smears from septicemia cases stained by Giemsa or Leishman methods reveal large number of bipolar-staining organisms.

Isolation:

A. Growth an enriched media supports their growth. Sheep or ox blood agar or serum, they grow best and routine medium. The specimen should be cultured on blood agar and MacConkey agar and incubated aerobically at 37ºC for 24 to 48 hours. Blood agar supplemented with neomycin, bacitracin, and acetidione, can be used for isolation of P. multocida from heavily contaminated specimens. Differentiation of Pasteurella and Mannheimia species via colonial characteristics: **P. multocida are non** haemolytic, No-growth on macConkey, Colonies have characteristic of sweetish odour and M. haemolytica is β-haemolysis and odourless.

Clinical infection: Hemorrhagic septicaemia in cattle caused by P. multocida. Bovine pneumonic pasteurellosis (shipping fever) caused by M. haemolytica and P. multocida causes Pasteurellosis in **sheep**. Ovine pneumonic pasteurellosis caused by M. haemolytica and P. multocida (sporadic case). Septicaemic pasteurellosis in Lambs less than 3 month caused by M. haemolytica (Bairagi *et al*., 2002).

2.2. Genus Haemophilus

Small gram negative rods, coccobacilliary and may occasionally form pleomorphic: short filaments (pairs, chains**).** Motile, facultative anaerobes and variable in catalase and oxidase test. Do not grow on MacConkey agar. **The main pathogens in the genus are:** H. influenza and H. Para influenza- in humans and H. somnus – in cattle and sheep. **Usual habitat:** They are commensals on the mucous membranes of the upper respiratory tract (Saha *et al.*, 2006).

2.3. Genus Actinobacillus:

General characteristics: Slow growing, gram negative rods or coccobacilli, non-motile. Require CO2 for growth on chocolate or blood agar. Ferment carbohydrates producing **acid but not gas.** Most species are **urease and oxidase positive.** Exhibit some host specificity and are mainly pathogens for animals and A. lignieresii, A. equuli and A. suis grow on macconkey agar.

Usual habitat:

Commensals on mucus membrane of animal particularly in the upper respiratory tract and oral cavity. **Species of veterinary importance are:** A. lignieresii, A. pleuropneumonial, A. equuli, A. suis.

Clinical infection:

Actinobacilli can cause a variety of infection in farm animal including:- (wooden tongue) in cattle caused by A. lignieresii, pneumonia in pigs caused by A. Pleuropneumoniae and Systemic disease in foals and piglets caused by A. equuli and A. suis respectively (Saha *et al.*, 2006).

3. The Pseudomonads

Pseudomonas aeruginosa: P. aeruginosa, B. mallei and B. pseudomallei are:- Gram negative rods, **obligate aerobes, oxidative (do not ferment carbohydrates), oxidase and catalase positive.** Resistant to multiple drugs. Motile by one or more polar flagella and grow on MacConkey agar as pale colonies.

Clinical infection:

Glanders in equine, Human and carnivores are susceptible. Affect a wide range of species.

Diagnostic procedure, Specimen, depends on clinical signs and site of lesion. The specimen is inoculated on blood and MacConkey agar and incubated aerobically at 36 ºC for 24 to 48 hrs.

Identification criteria:

Colonial characteristics, Pale colonies on MacConkey agar and Oxidase positive.

4. Aerobic / microaerophilic rods and cocci

4.1. Genus Brucella:

They are rods or coccobacilli gram negative, non-capsulated, non-motile, non-spore forming. Typically arranged singly but also occurs in pairs or clusters. Partially acid-fast in that they are not decolorized by 0.5% acetic acid in the Modified Ziehl-Neelsen (MZN) stain. They are aerobic and capnophilic (carboxyphiliic) that is B. ovis and B. abortus requires 5-10% CO2. They are urease positive (except B. ovis). Do not grow on MacConkey agar and require media enriched with blood or serum, fastidious. They are obligate intracellular parasite.

Genus Brucella contains **six species:- B. abortus, B. mellitensis, B. suis, B. ovis, B. canis and B. neotomae** (Saha *et al.*, 2006).

Natural habitat

Predilection sites: reticuloendothelial system and genital tract (placenta, fetal fluids and tests of bulls, rams, boars and dogs). **Allantoic fluids** factors in the gravid uterus stimulate the growth of Brucella, **Erythritol is considered to be one of the growth stimulating factors in the male reproductive tract.** B. abortus is excreted in bovine milk and can remain viable.

Laboratory diagnosis

Specimens: Abortion – full fetus if feasible, Alternatively fetal stomach contents, fetal lesion, cotyledons, uterine discharges, semen, tissue from epididymis or tests. **Direct examination:** MZN stain reveals small, red staining coccobacilli in **clumps because of their intracellular growth.**

Isolation

Growth on blood agar (5 -10%) – supports growth of Brucella spp, Serum dextrose, tryptose and brucella (Albimi) agars can also be

used. Selective media is required to avoid overgrowth by contaminating bacteria. Blood agar base plus 5% sterile equine or bovine serum and an antibiotic supplement [actidione (30mg/L). **Skirrow agar is satisfactory medium for Brucellae.** Incubation is under 5 -10% Co_2 at 37°C for up to 15 days, but in some cases (highly suspicious cases) incubation for up to 21 days is required growth can occur between 20°C and 40°C.

Clinical infection

Brucellosis is essentially a disease of the sexually mature animal. Pathogenic Brucella species can cause a**bortion in female animals by colonization of placental trophoblasts, and sterility in male animal.** Species are host specific: Bovine brucellosis are B. abortus, Caprine and ovine brucellosis are B. mellitensis, Ovine epididymitis are B. ovis, Porcine brucellosis are B. suis, Canine brucellosis are B. canis and **Brucellosis in humans are B. abortus, B. suis, B. mellitensis and rarely B. canis and** the manifestations (Human) are an undulating pyrexia, malaise, fatigue, night sweats, muscle and joint pains, but not abortion (Saha *et al*., 2006).

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