Lecture 5

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Medical Microbiology

Systematic Bacteriology

Gram-Positive Cocci:

**GENUS : Staphylococcus :**

The general properties of *Staphylococcus* are Gram-Positive spherical cells usually they appear to be irregular clusters as a result of division in many planes during replication producing pigments that vary from white to deep yellow. Some are normal flora of the skin and mucous membranes of human; other cause suppuration, abscess, a variety of pathogenic infection and even fatal septicemia.

**Classification:**

There are three main species medically important in this groups on the basis of pigment production:

1. **Staphylococcus aureus** is responsible for most Staphylococcal infections. Nasal carriage occurs in 20 – 50% in human. Colonies golden-yellow in culture N. Agar in pathogenic species.
2. **Staph. epidermidis (Staph. Albus)** white colonies causes opportunistic infections in debilitated or immunocompromised patients.

**General characteristics:**

Microscopically appearance they are all gram positive cocci or spherical arranged in irregular clusters, non motile, non-sporing and non-capsulated. Each cell being approximately 1 μm in diameter. On nutrient agar they form colonies, white, yellow-golden in color. Their hemolytic capacity is variable. Pathogenic strains produce coagulase ferment sugar (glucose, lactose, manitol) with acid production. Liquefy gelatin and produce pus cells in lesion.

**Cultural Characteristics:**

Prefer aerobic conditions but may behave as facultative anaerobes. Grow on laboratory media like nutrient agar, colonies or 2-3 mm in diameter after 24 hrs., incubation at 37°C, circular, convex, opaque, smooth, moist with an entire edge and pigmented. Tolerate high conc. of NaCl (7.5-10%). Grow will on blood agar.

**Staphylococcus aureus:**

Morphology of *Staph. aureus* are spherical, or cocci non-motile, non-capsulated, non-sporing, stain with gram positive, they are arranged in cluster formation due to cell division occurring in three planes with daughter cell. β-haemolysis (clear zone) around colonies, haemolysis is marked on rabbit or sheep blood and weak on horse blood agar. On MacConky agar media they are small and pink color. Selective media add 8-10% NaCl.
Important characteristic of *Staph. aureus*:

1. Produce coagulase.
2. Produce golden-yellow pigment.
3. Ferment manitol.
4. Produce β-haemolysis.
5. Liquefy gelatin.
6. Produce phosphatase.
7. Produce catalase.

Biochemical reactions:

*Staph. aureous* fermented number of sugar producing acid and gas in (glucose, lactose, sucrose, maltose and manitol) they are catalase, and coagulase positive, liquefy gelatin, they are lipolytic egg yolk media.

Determinates of pathogenicity:

1. **Exotoxin**:
   - e.g., Pyrogenic Exotoxin.

2. Enterotoxin:
   - a. Enterotoxin A (responsible for food poisoning)
   - b. Enterotoxin B through F

3. Leukocidin (kills leukocytes and macrophage)

4. Exofoliatins.

5. Hemolysins (α, β, and Y) lyses RBC cells.

6. Protein A (binds with Fc of IgG)

7. Enzymes produce β-lactamase, penicillinase, DNase, phospholipase … etc.
Coagulase test:

Coagulase is an enzyme which coagulates human or rabbit plasma, it's distinguishes *Staph. aureus* (+ ve) there are two types of coagulase:

1. Coagulase bound demonstrated by the slide method (plasma clumping factor). Take one or two colonies in a drop of water on a clean slide, then add one loopful of undiluted plasma and mix gently. Result: Appear white clumping of fibrinogen around bacteria occurs within 5 - 20 sec. give positive. No dumping negative. This method term plasma clumping factor or bound coagulase. It is heat stable protein. It is not require coagulase reaction factor (CRF) and fibrinogen is not convert into fibrin.

2. Free coagulase demonstrated by the tub method: To 0.5 ml of 1/10 dilution of citrated plasma add 0.5 ml of an overnight broth culture of *Staphylococcus aureus* and incubate at 37°C examine at 1.4 and 6 hours, leave it at room temperature overnight and re-examine, Result clot formation (+ ve) No clot (-ve). Free Coagulase it is filtrated and heat labile enzyme produced in lag and early log phase of bacteria, clotting of human or rabbit plasma is brought about by coagulase present in plasma convert fibrinogen into fibrin.

Pathogenicity:

Pathogenicity cause the majority of acute Pyrogenic lesions in man. *Staphylococcus* lesions are characteristically localized and can be divided in cutaneous lesions and deep infection. Superficial infections, osteomyelitis, pneumonia, acute endocarditis, arthritis, bacteremia, septicemia, and deep organ (e.g., brain, kidney, lung), and Eczema.
Staphylococcal toxin disease (bulbous impetigo scalded skin syndrome, scarlet fever) food poisoning.

**Laboratory diagnosis:**

1. Microscopic examination (smear).
2. Culture (e.g., 7.5% sodium chloride or 40% bile salt) Manitol Salt Agar is selective.
3. Total leukocyte count.
5. Serological diagnosis.

**Treatment:**

Deep infection require chemotherapy penicillinase resistant penicillin (e.g., Methicillin, Oxacillin, Nafcillin) or first-generation Cephalosporins (e.g., Cephalexin). Vancomycin, Erythromycin, or Clindamycin.

**GENUS: Streptococcus:**

The *Streptococcus* are gram-positive spherical bacteria that general characteristically arranged form pairs or chains of varying length, during growth. The genus Streptococcus includes a large number of species some of them are pathogens, and others are normal flora of the Oropharynx and Gastrointestinal, Urinal tract.

Diseases associated with Streptococci range from dental plaque and trivial skin infections to life threatening complications such as:
Glossary

**Abscess**: A localized collection of pus within a tissue.

**Acid-fast stain**: Staining technique in which the organism resists decolorization with an acidic solution of alcohol after being stained with a basic dye.

**Agar**: A polysaccharide obtained from various species of seaweeds used to solidify microbial media.

**A virulent**: Lacking disease-causing attributes such as a capsule, for example.

**Catalase**: An enzyme which degrades hydrogen peroxide (H₂O₂) to oxygen and water.

**Coagulate**: An enzyme that clots plasma.

**Conjugation**: A mechanism of gene trans for in bacteria that involves cell-to-cell contact.

**Generation time**: The time required for one cell to divide into two cell.

**Log phase**: The stage of growth of a bacterial culture in which the cells are multiplying exponentially.

**Medium**: (Pl. media) Any material used for growing organisms.