Chapter I
Pathogenic bacteria and fungi
1. Pathogenic bacteria and fungi

1.1 Bacteria

Staphylococcus

According to 1986 edition of Bergey's Manual of Systemic Bacteriology, the family Micrococcaceae includes three genera – Staphylococcus, Micrococcus and Stomatococcus. The Staphylococci are small Gram-positive and catalase-positive, whereas members of the family Streptococcaceae are catalase-negative.

Staphylococci are spherical, ovoid, Gram-positive cocci arranged in grape-like clusters. These are ubiquitous organisms and the primary natural habitat is mammalian body surfaces. Some are members of the normal flora of skin and mucous membrane of man and others are the commonest cause of suppuration. Pasteur 1880 isolated the organism from pus and produced abscesses in rabbit by inoculation of the organism. In the same year, Sir Alexander Ogston (1880) a surgeon from Scotland, established conclusively the pathogenic role of Staphylococci in abscesses and suppurative lesions. He gave the name Staphylococcus (staphyle, meaning bunch; kakkos, meaning a berry) to the organism. Micrococcus is strictly aerobic, from irregular clusters or tetrads and do not ferment glucose, whereas Staphylococcus is facultatively anaerobic and ferments glucose.
Species

The genus *Staphylococcus* contains at least 30 species. The medically important species are described as below:

**S. aureus**

The main pathogen causing pyrogenic infections in man.

**S. epidermidis** (*S. albus*)

A normal skin commensal.

**S. saprophyticus**

It occurs mainly on genitourinary mucus membrane and skin, similar to *S. epidermidis* but resistant to novobiocin. In the past, *Staphylococci* were differentiated into three types based on pigment production, *S. aureus*, *S. albus* and *S. citreus*. Since pigment production is not constant and an uncertain character, this classification is now obsolete.

Based on coagulase production, *Staphylococci* are classified into two groups - coagulase positive and coagulase negative. Since most of coagulase positive strains produce golden-yellow colonies, though some may be white or cream coloured, they are known as *S.aureus* (also known as *S. pyrogenes*). They produce toxins and are pathogenic. The coagulase negative strains are skin commensals that can cause opportunistic infections.

Because their hardy bodies, resistance to dry conditions, and high salt concentrations, *Staphylococci* are well suited to their ecological niche, which is the skin surface of humans and animals.
*Staphylococcus aureus*

**Morphology**

They are Gram-positive, nonsporing, nonmotile, usually non-capsulate, aerobic and normally facultative anaerobic cocci (1μm in diameter), characteristically arranged in clusters. Clusters formation results from sequential division of bacteria in three perpendicular planes with daughter cells tending to remain in close proximity. They may be arranged singly, in pairs.

A few strains of *S. aureus* possess capsules visible microscopically, particularly in young bacterial cultures.

**Culture**

They grow readily on ordinary culture media under aerobic or anaerobic conditions within a temperature range of 10-42°C, optimal temperature being 37°C.

**Nutrient agar**

After 24 hours incubation, the colonies large (2-4 mm diameter), round, smooth, shiny, opaque and are often pigmented. *Staphylococcal* pigments are believed to be carotenoid or its derivative located in cell membranes. Most strains from deep golden-yellow pigment some may produce creamy, orange or yellow pigments. Pigment production is best seen when grown aerobically at a temperature of 20-25°C. Pigment remains localized in the colony. Pigmentation is often poor after an incubation of 24 hours at 37°C and absent if grown anaerobically or in broth. The production of
pigments is enhanced by culturing the organisms on special media, e.g. 1% glycerol monoacetate or milk agar.

**Blood agar**
Colonies are almost similar to those on nutrient agar. They produce a beta type haemolysis, which is best seen with rabbit or sheep blood. Haemolysis is weak on horse blood agar. Human blood should be avoided because it may contain inhibitors or antibiotics.

**Liquid medium**
Uniform turbidity is produced in nutrient broth.

**Selective media**
Staphylococci can tolerate 5-10% concentration of sodium chloride, lithium chloride, tellurite and polymyxin. Salt containing agar and broth containing 9-10% NaCl (salt-milk agar, salt broth) are used for isolating *S. aureus* from samples containing large number of other bacteria such as faeces.

**MacConkey's agar**
The colonies are very small (pinhead size) and pink due to lactose fermentation.

**Staphylococcal toxin diseases**

**Staphylococcal scalded skin syndrome (SSSS)**
It is the cutaneous manifestation of infection with an exfoliatin-producing strain of *S. aureus*. Gotfried Ritter von Rittershain described in 1877- *Staphylococcal* bullous exfoliative dermatitis in 297 infants under 1 month, now called Ritter's disease or Staphylococcal scalded skin syndrome. Most cases are found in infants and children of 5 years of age.

1. Exfoliation leads to formation of numerous large blisters at sites away from the original lesion. Eventually, the blisters rupture exposing the dermis.

2. Milder clinical forms of SSSS are also observed. Bullous impetigo is characterized by formation of a new large, localized blisters that may rupture. Fluid from blisters when cultured, shows strains of toxin-producing *S. aureus*. *Staphylococcal* scarlet fever, similar to streptococcal scarlet fever, is characterized by a non-descamative erythematous rash.

**Staphylococcal food poisoning**

It is one of the most common food-borne illness. It is an intoxication rather than infection, caused by heat and proteases/stable enterotoxins.

Staphylococcal food poisoning is characterized by acute onset of nausea and vomiting, sometimes followed by diarrhoea. Symptoms appear within six hours of ingestion of contaminated food. Rapid onset of illness is due to preformed toxin in food.
**Pseudomonas sp.**

*Pseudomonas:* is a large group of aerobic, Gram-negative, non-fermentative bacilli containing over 200 species that were originally contained within the genus *Pseudomonas*.

**Pseudomonas aeruginosa**

Human carriage of the *P. aeruginosa* is uncommon as part of the normal microbial flora unless the person is hospitalized or an immunocompromised host. In these persons, the most frequent site of colonization is the gastrointestinal tract followed by other moist body sites, including throat, nasal mucosal, axilae and perineum.

**Morphology**

It is a slender, 1.5–3 μm × 0.5 μm, non-sporing, non-capsulated, gram-negative motile bacillus. Its motility is due to the presence of one polar flagellum. Occasional strains have two or three flagella. Most strains are fimbriate. It is non-capsulated, but some strains produced an alginate capsule.

**Culture**

*P. aeruginosa* is primarily aerobic but can grow anaerobically. It grows on standard laboratory media such as nutrient agar, 5% sheep blood or chocolate agar, and MacConkey agar media. Isolation of the organism from specimens with a mixed flora is facilitated by the use of selective media. For isolation of most *Pseudomonas* species, MacConkey agar is a useful selective medium. Selective agents such as cetrimide,
acetamide, and nitrofurantoin is be added to culture medium to isolate *P. aeruginosa* from clinical and environmental samples.

**Biochemical reaction**

Clinical isolates are oxidase and catalase positive. They do not ferment lactose but many strains oxidise glucose with acid production only. Since acid production by *P. aeruginosa* is weak and gets neutralized by alkali produced from peptone, an ammonium salt sugar medium should be used instead of peptone water sugars. All strains utilize citrate. Indole, MR, VP, and H₂S tests are negative.

**Resistance**

*P. aeruginosa* is fairly heat resistant and killed at 55°C in one hour. They are resistant to most of the routinely used antiseptics and disinfectants such as chloroxylenol and hexaclorophane. Sometimes *P. aeruginosa* grow in antiseptic lotion kept for hospital use. It is resistant to most commonly used antimicrobials. It is sensitive to acid, beta-glutaraldehyde, strong phenolic disinfectants and silver salts.

**Pathogenesis**

**Habitat**

The organism is widely distributed in nature, water, sewage, soil and air, and also on human skin. *P. aeruginosa* acts as opportunistic pathogens when body resistance is lowered and tissue is damaged.
Mode of infection

The infection occurs either from the environment or by cross infection. Cross-infection usually takes place contaminated hospital equipments, e.g., syringes, lumbar puncture needles, cystoscope, and catheters. The wound infection in the hospital usually consists of a mixed infection of *P. aeruginosa* and pyrogenic cocci.
Fig. 1. Colonies of *Pseudomonas aeruginosa*.

Fig. 2. Colonies of *Staphylococcus aureus*.
1.2 Fungi
Some saprophytic fungi of environment that usually do not produce diseases may cause infection under special condition such as in immunologically compromised patient and in terminal stages of chronic disease. As these fungi take advantage of the debilitated state of the individual to become pathogenic, they are referred to as opportunistic fungi. The incidences of these fungal infections has increased in AIDS and with wide use of antibiotics, steroids and immunosuppressive drugs.

Maintenance of culture

Comparative culture studies were made for the identification and differentiation of the species of pathogenic fungi on different media, viz., czapek dox agar (sucrose 30gm, sodium nitrate 2gm, dipotassium phosphate 1gm, magnesium sulphate 0.50gm, potassium chloride 0.50 gm, ferrous sulphate 0.01 gm), potato dextrose agar (250gm potato were peeled and cut into small pieces, dextrose 20 gm, agar 20 gm, distilled water 1000ml). The cultures of fungi were inoculated at 28± 2°C and were observed every 4-6 days.

Aspergillus
Aspergillus fumigatus is the main opportunistic pathogen. Other species, A. niger, and A. flavus are less frequently associated with infection.
Epidemiology

Aspergillus has a worldwide distribution. Organism belonging to the genus is ubiquitous and spores of Aspergillus are extremely common in the environment, such as soil, food, paint, air vents, and even disinfectant.

Clinical syndrome

Aspergillosis is acquired from exogenous sources. There are three clinical forms of systemic aspergillosis in man.

Respiratory disease

Aspergillus asthma: It is a hypersensitivity state to aspergilli, may occur in atopic individuals following inhalation to spores of aspergilli.

Bronchopulmonary aspergillosis: This fungus grows in the lumen of bronchioles and produces plugs of mycelium and mucus that occlude the lumen.

Aspergilloma: It is often called "fungus ball" in which the fungus colonises in the pre-existing cavities.

Disseminated (invasive) aspergillosis

The fungus first establishes pneumonia and then disseminates involving brain, kidney, heart and other organs particularly in immunocompromised hosts.

Superficial infection: A. fumigatus colonise in paranasal sinuses (sinusitis), external ear (otomycosis) and sometimes in eye (mycotic keratitis).
Fig. 3. Colonies of *Aspergillus niger*.

Fig. 4. Colonies of *Aspergillus fumigatus*. 