

STAPHYLOCOCCI

Learning objectives

To know in detail about,

- Morphology, cultural and biochemical characteristics of Staphylococci
- Disease caused by Staphylococci species in domestic animals
- Clinical symptoms and laboratory findings to a possible diagnosis of a gangrenous mastitis, bumble foot and greasy pig disease
- The role of Mannitol salt agar in isolation of staphylococcus
- Double or hot-cold haemolysis, Coagulase test and Hotis test

SYSTEMATICS

- First discovered by Scottish surgeon Sir Alexander Ogston (1880) in infected tissues. He named it as staphylococcus (Greek staphyle, bunch of grapes; KOKKAS, berry).

Domain	Bacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Staphylococcaceae
Genus	Staphylococcus
Species	<i>S. intermedius</i> . <i>S. hyicus</i> .

HISTORY

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HABITAT

- Staphylococcus occurs worldwide in mammals although the spread of staphylococcal strains between different animal species is limited.
- They colonize the nasal cavity, skin and mucous membranes and can be transient in the intestinal tract. It is an opportunist type organism

MORPHOLOGY

- Spherical cells, 0.8 to 1 micrometer in diameter, on agar media the cocci are arranged in grape like clusters.
- In broth they occur as small groups, pairs, short chains of not more than four members.
- They are Gram positive, non-motile, non-acid fast, non-spore forming and have no flagella.

CULTURAL CHARACTERISTICS

- They are aerobic and facultatively anaerobic.
- They grow at an optimum temperature of 35-37°C.
- Staphylococcus grows in the presence of 7-10% Sodium chloride. Hence media- containing salts is selective for this organism (eg; Mannitol Salt agar) and it is highly inhibitory to many other bacteria particularly Gram-negative bacteria. ([Click here for visual](#))
- In Nutrient agar plate the colonies are round, smooth, glistening, opaque, low, convex, edge, entire end of a golden yellow or white colour.
- In nutrient broth a uniform turbidity is present with powdery sediment.

- Phenolphthalein diphosphate or tellurite agar selectively inhibits non-pathogenic strains.
- Haemolysis on blood agar. Capable of liberating α factor into the medium, which favours the growth of Haemophilus organism.
- Purple agar, containing bromocresol purple as a pH indicator and 1% maltose, is used to differentiate *S. aureus* and *S. intermedius*.
- Most strains form pigments, hence previously classified as per the colour of the pigment produced.
 - *S. aureus* – yellowish or golden orange pigment.
 - *S. albus* - white colonies.
 - *S. citreus* - lemon yellow colour pigment
- Later on it was found that pigment formation was variable. Hence such classification was no longer followed.
- In sheep or rabbit blood agar plate -double haemolysis! around colonies are formed with incubation at 37°C it produces an incomplete haemolysis, which develops into a complete haemolysis when held at 4°C. This is called as hot cold lysis phenomenon.

BIO-CHEMICAL PROPERTIES

- Staphylococcus aureus produces acid from glucose, maltose, mannitol, lactose, and sucrose and not from salicin, raffinose & inulin.
- The organism is indole negative, positive for NH₃, methyl red and Voges - Proskauer and catalase.
- Hydrolyses gelatin and coagulates serum. Negative for oxidase and H₂S production.

RESISTANCE

- Staphylococci are the most resistant of the cocci.
- Usually a temperature of 60°C for half an hour destroys all the cells.
- Death is accomplished in 1% phenol in 35 minutes or 10% formaldehyde in 10 minutes.

ANTIGENICITY

It consists of

Group antigen

- *Carbohydrates*
 - The cell wall of *S. aureus* contains ribitol teichoic acid.
 - *S. intermedius* contains glycerol teichoic acid.
- *Protein A* - Present only in *S. aureus* .

Type antigen

- Proteins other than protein A.

TOXINS AND VIRULENCE FACTORS

- It consists of capsule, Teichoic acid, which have antiphagocytic ability.

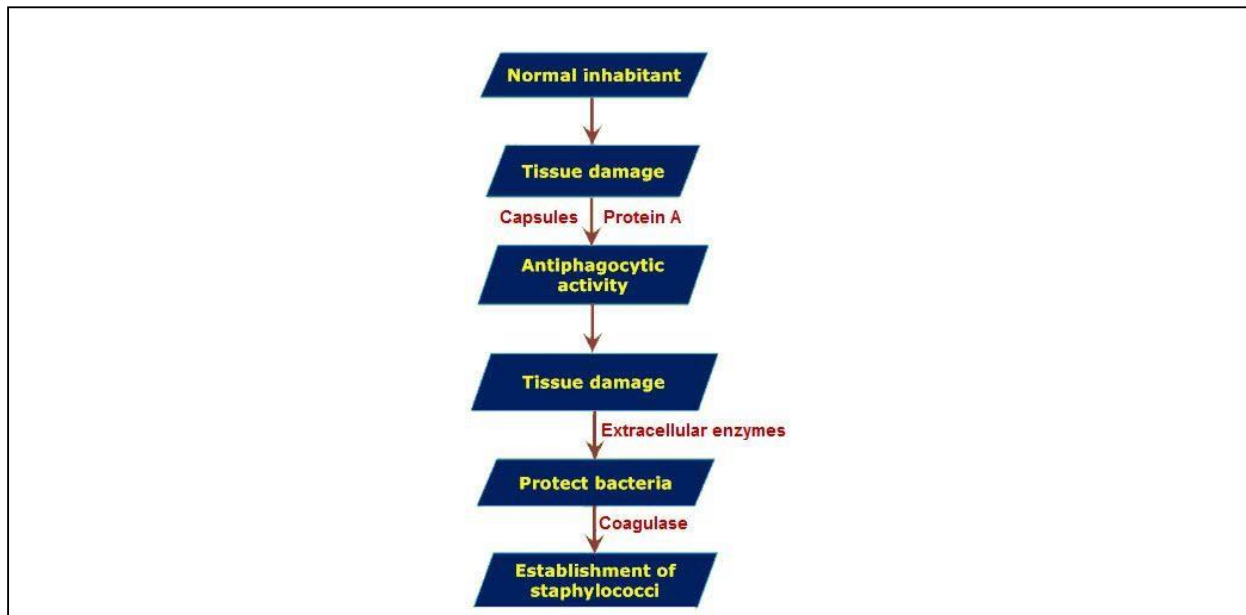
Exotoxins

- *Hemolysin*
 - Four antigenically distinct hemolysins causes hemolysis of erythrocytes. They are Alpha, Beta, delta and Epsilon toxin, which produce partial hemolysis (hot – cold hemolysis). The alpha toxin is the major toxin in gangrenous mastitis. It causes spasm of smooth muscle and is necrotizing and potentially lethal.
- *Leukotoxin*
 - It has the leukocidal activity and includes a and d toxins. By doing so, the organisms may spread more easily to other parts where they develop secondary lesions.
- *Enterotoxin*
 - It is seldom produced by animal strains. There are several antigenically distinct types of heat stable enterotoxins. They are not destroyed at 100°C for 80 minutes. They are responsible for food poisoning in man.
- *Exfoliative or Dermonecrototoxin*
 - This toxin causes necrosis of skin by exfoliation and intraepidermal separation.
- *Toxic shock syndrome toxin (TSST)*
 - Induce excessive lymphokine production and results in tissue damage. Bovine and human strains of *Sta. aureus* produce TSST.

Extracellular enzymes

- *Coagulase*
 - On their ability to coagulate plasma they are classified as coagulase positive staph. (CPS) and coagulase negative staph. CPS are considered to be significant pathogens. They are resistant to heat. The coagulation of plasma produces a fibrin film on the surface of the organisms, which allows multiplying.
- *Hyaluronidase*
 - It hydrolyses hyaluronic acid, the mucoid ground substance of connective tissue.
- *Nucleases*
 - Deoxyribonucleases (DNAase) hydrolyze DNA and Ribonucleases hydrolyze RNA.
- *Fibrinolysin*
 - Fibrinolysin is commonly referred to as staphylokinase, is an activator of the plasma system leading to the breakdown of fibrin.
- *Lipases and esterases* - They hydrolyze lipids.
- *Lysozyme* - Hydrolyses the peptidoglycan in the cell wall of many bacteria

PATHOGENESIS



PATHOGENICITY

- **Horse:** Botryomycosis: Infrequent chronic granulomatous lesions involving the udder of the mare, cow and sow and the spermatic cord of horses.
- **Cattle:** Mastitis: Staphylococcal bovine mastitis may be chronic, acute and peracute. Gangrenous mastitis due to a toxin is seen in postparturient cows.
- **Sheep:** Tick pyemia in lambs occurs in 2-5 week old lambs, which is heavily infected with *Ixodes ricinus*.
- Periorbital eczema is an infection due to abrasions, Staphylococcal dermatitis due to scratches from vegetation.
- **Poultry**
 - Bumble foot: A pyogranulomatous process of subcutaneous tissue of foot that can involve the joints.
 - Staphylococcal arthritis and septicemia in turkeys, omphalitis – yolk sac infection, wing rot or gangrenous dermatitis infection in poultry
- **Pig:** Exudative epidermitis (greasy pig disease) is an acute generalized infection of suckling and weaned pigs caused by *S. hyicus*. This disease is characterized by excess sebaceous secretion, exfoliation and exudation.
- **Dogs and Cats:** Pyoderma is one of the most common skin diseases of dogs.
- In addition to this, Otitis externa and other suppurative conditions are caused by *S. intermedius*.
- Staphylococcal antigens produce intense inflammatory reaction and promote persistence of the bacteria.
- **Other Staphylococcal organisms**
 - *S. aureus* sub *sp. anaerobius* causes caseous lymphadenitis. They are anaerobic and catalase negative.
 - *S. caprae* in goat's milk.
 - *S. gallinarum* and *S. arlettae* - skin of chickens
 - *S. lentus* in skin of sheep and goats.
 - *S. equorum* in skin of horses
 - *S. simulans* and *S. felis* - clinical specimens in cats
 - *S. delphini* in skin of dolphins
 - *S. aureus* in Staphylococcal scalded skin syndrome (SSSS) and Toxic shock syndrome (TSS) in humans.
 - MRSA in Methicillin resistant *Staphylococcus aureus*

DIAGNOSIS

- Cultural characters in selective media.
- In case of food poisoning identification of the enterotoxin is of diagnostic use.
- CPS produces typical reaction in the Hotis test of milk samples.
- These organisms produced green or greenish brown colonies with white centres after the milk samples are incubated for 16-20 hours.
- DNA test and protein A tests are also employed.

Pathogenicity test

- *Coagulase test*: When culture added to Rabbit plasma, fibrinogen is converted to fibrin by coagulase enzymes.
- In this test, a suspension of staphylococci is mixed with rabbit plasma either on a slide or in a small tube.
- The slide test detects the presence of a bound coagulase or clumping factor on the bacterial surface.
- A positive reaction is indicated by clumping of bacteria within 1 to 2 min.
- The tube test detects the free coagulase or staphylocoagulase, which is secreted by bacteria into the plasma.
- It is the definitive test for coagulase production and positive reaction is indicated by clot formation in the tube following incubation at 37°C for 24 hrs. ([Click here for visual](#))

Phage typing

- Phage typing is carried out for epidemiological purposes, particularly for *S. aureus* strains of bovine mastitis.

TREATMENT

- Penicillin is the drug of choice if strains are susceptible.
- New synthetic penicillins such as methicillin, oxacillins are effective.
- Tetracyclines, bacitracin, nitrofurans, Trimethoprim, Cephalosporins, enrofloxacin are effective.