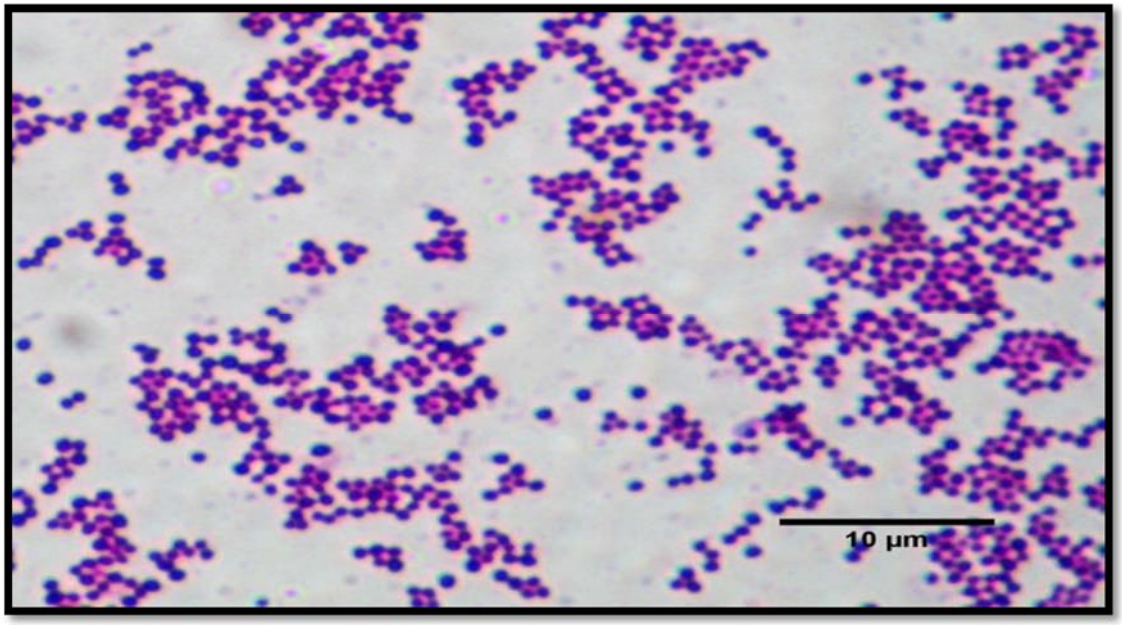
STAPHYLOCOCCACEAE



SPECIAL MICROBIOLOGY

Instructor

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STAPHYLOCOCCACEAE

Genus: *Staphylococcus*

Staphylococci genera, are Gram-positive cocci, approximately 1 µm in diameter, that tend to occur in irregular clusters resembling bunches of grapes. *Staphylococcus* species occur as commensals on skin and mucous membranes; some may act as opportunistic pathogens causing pyogenic infections.

Most staphylococci are facultative anaerobes and catalase-positive. They are non-motile and oxidase-negative and do not form spores. Two species, *S. aureus* subsp. *anaerobius* and *S. saccharolyticus*, are anaerobic and catalase-negative. Micrococcus spp. (packs of tetrads), are positive to modified oxidase test.

A total of 45 species of *Staphylococcus* have been described to date, seven of which are coagulase-positive or coagulase-variable species. The coagulase-positive *S. aureus* subsp. *aureus* (referred to as *S. aureus*), *S. pseudintermedius*, and the coagulase- variable *S. hyicus* are important pathogens of domestic animals. *Staphylococcus intermedius* it is now belong to the species *S. pseudintermedius.* Coagulase production correlates with pathogenicity.

Although coagulase- negative staphylococci are usually of low virulence, some occasionally cause disease in animals and humans like *S. epidermidis* .

Staphylococci - Natural Habitat

- Non-pathogenic staphylococci - normal flora of skin

- Pathogenic staphylococci - skin and mucous membranes (salt tolerant)
- Survive in environment
- Opportunistic infections:

Wounds / teat damage (mastitis) / invade tick bites / (tick pyaemia) / pyoderma / more serious life-threatening bacteraemia & septicaemia.

Differentiation of Gram Positive Cocci

Organism	Appearance in stained smears	Coagulase production	Catalase production	Oxidase production	O-F test[a]	Bacitracin disc (0.04 units)
Staphylococcus spp.	Irregular clusters	±	+	–	F	Resistant
Micrococcus spp.	Packets of four	–	+	+	O	Susceptible
Streptococcus and *Enterococcus* spp.	Chains	–	–	–	F	Resistant

Growth & Culture Characteristics

- Staphylococci grow readily on most bacteriologic media under aerobic or microaerophilic conditions at 37°C but form pigment best at room temperature (20–25°C).
- Colonies on solid media are round, smooth, raised, and glistening.
- *S. aureus* usually forms deep golden yellow colonies while *S epidermidis* colonies usually are gray to white on primary isolation; many colonies develop pigment only upon prolonged incubation.
- *S. aureus* isolated from human & cow produced golden-yellow discoloration, while those from dog were white.

- No pigment is produced anaerobically or in broth.
- Are relatively resistant to drying, heat (they withstand 50°C for 30 minutes), and 9% sodium chloride but are readily inhibited by certain chemicals (eg, 3% hexachlorophene).
- Tolerate bile up to 40%.
- Produce a wide range of Exoenzymes & Exotoxins, playing an important role in their virulence & pathogensity.
- Staphylococci are variably susceptible to many antimicrobial drugs.
- Resistance to several antimicrobial drugs like β-Lactamase production made them resistant to Penicillins.
- Methicillin-Resistant *S aureus* **(MRSA)** , Resistance to methicillin, nafcillin and to oxacillin. Most antimicrobial agents resistance properties carried via plasmid mainly by conjugation.

Methicillin-resistant staphylococcal infections in animals

Infection with MRSA has been a major problem in human hospitals for many years and have become a major problem in veterinary medicine and animal production in the last decade.

Transmission of infection between pets and humans, including veterinary personnel, and between horses and humans has been reported. Dogs & horses are widely infected with MRSA like: wound infections, surgical site infections, pyoderma, otitis and urinary tract infections.

Virulence factors: Including Exoenzymes & Exotoxins, of *Staphylococcus aureus* and their pathogenic effects

Virulence factor	Pathogenic effects
Coagulase	Conversion of fibrinogen to fibrin. Fibrin deposition may shield staphylococci from phagocytic cells
Clumping Factor	A cell wall bound, is responsible for adherence of the organisms to fibrinogen and fibrin. When mixed with plasma, S aureus forms clumps.
Lipase, esterases, elastase, staphylokinase, deoxyribonuclease, hyaluronidase, phospholipase	Enzymes which contribute to tissue destruction and virulence
Catalase	Converts hydrogen peroxide into water and oxygen. The staphylococci, are positive, whereas the streptococci, are negative.
Protein A	Surface component which binds Fc portion of IgG and inhibits opsonization
Leukocidin Panton-Valentine L (PVL)	Cytolytic destruction of phagocytes of some animal species Composed of two components S & F
Alpha-toxin (alpha-haemolysin)	The major toxin in gangrenous mastitis. It causes spasm of smooth muscle and is necrotizing and potentially lethal
Beta-toxin (beta-haemolysin)	A sphingomyelinase which damages cell membranes
Exfoliative toxins	Proteases which contribute to skin lesion development in humans, dogs and pigs
Staphylokinase	Cause fibrinolysis
Enterotoxins	Heat-stable toxins associated with staphylococcal food poisoning in humans
T oxic shock syndrome toxins (T SST)	Induce excessive lymphokine production, resulting in tissue damage. Bovine and human strains of S. aureus produce T SST -1. Sheep and goat strains produce a variant of this toxin. T SST -1 has superantigen activity

Antigenic Structure

- Peptidoglycan , a polysaccharide polymer containing linked subunits, provides the rigid exoskeleton of the cell wall. Peptidoglycan is destroyed by strong acid or exposure to lysozyme.

It is important in the pathogenesis of infection: **1-** It induces production of interleukin-1 (endogenous pyrogen) ; **2-** Opsonic antibodies by monocytes; **3-** A chemoattractant for polymorphonuclear leukocytes; **4-** Have endotoxin-like activity and **5-** Activate complement.

- Teichoic acids , which are polymers of polyribitol–phosphate, are cross-linked to the peptidoglycan and can be antigenic.

- Protein A , is a cell wall component of *S. aureus* strains and is a bacterial surface protein that has been characterized among a group of adhesins.Protein A binds to the Fc portion of IgG molecules except IgG3.

Diseases Caused by Staphylococci Infection

Species	Hosts	Clinical conditions
Staphylococcus aureus	Cattle	Mastitis, udder impetigo
	Sheep	Mastitis Tick pyaemia (lambs) Benign folliculitis (lambs) Dermatitis
	Goats	Mastitis Dermatitis
	Pigs	Botryomycosis of mammary glands Impetigo on mammary glands
	Horses	Scirrhous cord (botryomycosis of the spermatic cord), mastitis
	Dogs, cats	Suppurative conditions similar to those caused by *S. intermedius*
	Poultry	Arthritis and septicaemia in turkeys Bumblefoot Omphalitis in chicks
S. intermedius	Dogs	Pyoderma, endometritis, cystitis, otitis externa, and other suppurative conditions
	Cats	Various pyogenic conditions

Diagnosis

- **Specimens:** Pus, blood, urine & exudates divided into two parts, one to be stained by Gram's stain & the other part cultured on nutrient agar, ox or sheep blood agar along with MacConkey agar.

- Selective media used for cultivation like Staph 110, Mannitol Salt Agar (MSA), Baird-Parker Agar with Egg Yolk or with Tellurite from food. Azide blood agar or Clositin-Naldixic Acid Blood Agar to isolate Stapylocooi from feces & sewage.
- Colonial morphology & pigmentation. Milk agar used for enhancement of pigment production with addition of Glycerol.
- Haemolysis on blood agar (α, β,γ & δ). *S. aureus* and *S. pseudintermedius* usually produce both alpha-haemolysin and beta-haemolysin with a characteristic double zone of haemolysis; 1st narrow zone Alfa & 2nd wide of Beta. Isolates of *S. hyicus* are non-haemolytic.
- Coagulase production clot pasma, done by:

1- Slide Coagulase: For detection of both bound coagulase & clumping factor, but mostly gave false positive.

2- Tube Coagulase: For detection of bound coagulase & used as confirmatory to slide coagulase.

Rabbit plasma mixed with isolates used with both tests.

- **Biochemical Profile:** Catalase, Coagulase, Dnase, Protein A, Phosphatase, CHO fermentation and others. Many manufacturers developed ready to use kits called biochemical identification system like: API STAP & RapID STAPH.

Purple Agar: Contains 1% maltose. The indicator is Bromocresol purple for differentiation of *S. aureus* from *S. pseudintermedius* & *S. intermedius*

- Antimicrobial susceptibility tests.
- **Molecular typing:** PCR-based techniques used widely recently for detection & genotyping of Staphylococci with gene sequencing for genetic barcoding of a defined target gene.
- **Phage Typing:** Used for many years in epidemiological investigations such as those relating to outbreaks of staphylococcal food poisoning in humans and animals. Specific type for each species result in plaques formation.