

THE GENUS
STAPHYLOCOCCUS

The genus *Staphylococcus* contains about 50 species and subspecies today

- Only some of them are important as human pathogens:
 - *Staphylococcus aureus*
 - *Staphylococcus epidermidis*
 - *Staphylococcus hominis*
 - *Staphylococcus haemolyticus*
 - *Staphylococcus saprophyticus*
 - others

Staphylococci are Gram-positive cocci, 1 mikrometer in diameter, nonmotile, nonsporeforming, facultatively anaerobic.

The name *Staphylococcus* is derived from the Greek term „staphyle“, meaning „a bunch of grapes“. This name refers to the fact that the cells of these Gram-positive cocci grow in a pattern resembling a cluster of grapes. However, microorganisms in clinical material may also appear as single strain, pairs, or short chains.

Physiology and structure

- Capsule or polysaccharide slime layer
- Peptidoglycan layer
- Teichoic acid
- Protein A
- Cytoplasmatic membrane
- Clumping factor
- Cytoplasm

Capsule or polysaccharide slime layer

- A loose-fitting, polysaccharide layer (slime layer) is only occasionally found in staphylococci cultured in vitro, but is believed to be more commonly present in vivo.
- The capsule protects the bacteria by inhibiting the chemotaxis and phagocytosis of staphylococci by polymorphonuclear leukocytes.
- It also facilitates the adherence of bacteria to catheters and other synthetic material.

Peptidoglycan

- Half of the cell wall by weight is peptidoglycan, a feature common to Gram-positive bacteria.
- The subunits of peptidoglycan are N-acetylmuramic acid and N-acetylglucosamine.
- Unlike Gram-negative bacteria, the peptidoglycan layer in Gram-positive bacteria consists of many cross-linked layers, which makes the cell wall more rigid.

Teichoic acid

- Teichoic acid is species-specific, phosphate-containing polymer that is bound covalently to the peptidoglycan layer or through lipophilic linkage to the cytoplasmic membrane (lipoteichoic acid).
- Teichoic acid mediates the attachment of staphylococci to mucosal surfaces through its specific binding to fibronectin.

Protein A

- The surface of most *S. aureus* strains (but not the coagulase-negative staphylococci) is uniformly coated with protein A.
- This protein is covalently linked to the peptidoglycan layer and has a unique affinity for binding to the Fc receptor of immunoglobulin IgG.
- The presence of protein A has been exploited in some serological tests, in which protein A-coated *S. aureus* is used as a nonspecific carrier of antibodies directed against other antigens.
- Additionally, detection of protein A can be used as a specific identification test for *S. aureus*.

Coagulase and other surface proteins

- The outer surface of most strains of *S. aureus* contains clumping factor (also called bound coagulase).
- This protein binds fibrinogen, converts it to insoluble fibrin, causing the staphylococci to clump or aggregate.
- Detection of this protein is the primary test for identifying *S. aureus*.
- Other surface proteins that appear to be important for adherence to host tissues include:
 - collagen-binding protein
 - elastin-binding protein
 - fibronectin-binding protein

Cytoplasmic membrane

- The cytoplasmic membrane is made up of a complex of proteins, lipids, and small amount of carbohydrates.
- It serve as an osmotic barrier for the cell and provides an anchorage for the cellular biosynthetic and respiratory enzymes.

Staphylococcal toxins

- *S. aureus* produces many virulence factors, including at least five cytolytic or membrane-damaging toxins:
 - alpha toxin
 - beta toxin
 - delta toxin
 - gamma toxin
 - Panton-Valentin toxin
 - two exfoliative toxins
 - eight enterotoxins (A-E, G-I)
 - Toxic Shock Syndrome Toxin 1 (TSST-1)
- The enterotoxins and TSST-1 belong to a class of polypeptide known as superantigens.
- *Staphylococcus aureus* strains produce several other extracellular, biologically active substances, including proteases, phosphatases, lipases, lysozyme etc.

Exfoliative toxins

- Staphylococcal scalded skin syndrome (SSSS), a spectrum of diseases characterized by exfoliative dermatitis, is mediated by exfoliative toxins.
- The prevalence of toxin production in *S. aureus* strains varies geographically but is generally less than 5% to 10%.

Enterotoxins

- Eighth serologically distinct staphylococcal enterotoxins (A-E, G-I) and three subtypes of enterotoxin C have been identified.
- The enterotoxins are stable to heating at 100 °C for 30 minutes and are resistant to hydrolysis by gastric and jejunal enzymes.

Enterotoxins

- Thus, once a food product has been contaminated with enterotoxin-producing staphylococci and the toxin have been produced, neither reheating the food nor the digestive process will be protective.
- These toxins are produced by 30% to 50% of all *S. aureus* strains.
- Enterotoxin A is most commonly associated with disease. Enterotoxins C and D are found in contaminated milk products, and enterotoxin B causes staphylococcal pseudomembranous enterocolitis.

Toxic Shock Syndrome Toxin - 1

- TSST-1, formerly called pyrogenic exotoxin C and enterotoxin F, is a heat and proteolysis resistant, chromozomally mediated exotoxin.
- The ability of TSST-1 to penetrate mucosal barriers, even though the infection remains localized in the vagina or at the site of a wound, is responsible for the systemic effects of TSS.
- Death in patients with TSS is due to hypovolemic shock leading to multiorgan failure.

Staphylococcal enzymes

- Coagulase
- Catalase
- Hyaluronidase
- Fibrinolysin
- Lipases
- Nuclease
- Penicillinase

Coagulase

- *S. aureus* strains possess two forms of coagulase:
 - bound,
 - free.
- Coagulase bound to the staphylococcal cell wall can directly convert fibrinogen to insoluble fibrin and cause the staphylococci to clump.
- The cell-free coagulase accomplishes the same result by reacting with a globulin plasma factor.
- Coagulase may cause the formation of fibrin layer around a staphylococcal abscess, thus localizing the infection and protecting the organisms from phagocytosis.

Catalase

- All staphylococci produce catalase, which catalyzes the conversion of toxic hydrogen peroxide to water and oxygen.
- Hydrogen peroxide can accumulate during bacterial metabolism or after phagocytosis.

Hyaluronidase

- Hyaluronidase hydrolyzes hyaluronic acid, the acidic mucopolysaccharides present in the acellular matrix of connective tissue.
- This enzyme facilitates the spread of *S. aureus* in tissues.
- More than 90% of *S. aureus* strains produce this enzyme.

Fibrinolysin

- Fibrinolysin, also called staphylokinase, is produced by virtually all *S. aureus* strains and can dissolve fibrin clots.
- Staphylokinase is distinct from the fibrinolytic enzymes produced by streptococci.

Epidemiology

- Staphylococci are ubiquitous. All persons have coagulase-negative staphylococci on their skin, and transient colonization of moist skin folds with *S. aureus* is common.
- Colonization of the umbilical stump, skin and perineal area of neonates with *S. aureus* is common.
- *S. aureus* and coagulase-negative staphylococci are also found in the oropharynx, gastrointestinal and urogenital tract.
- Because staphylococci are found on the skin and in the nasopharynx, shedding of the bacteria is common and is responsible for many hospital-acquired infections.

The genus *Staphylococcus* can be divided into two subgroups (on the basis of its ability to clot blood plasma by enzyme coagulase):

- coagulase-positive
- coagulase-negative

- Subgroup of coagulase-positive species contains from human staphylococci only one species
 - *Staphylococcus aureus*
- Other coagulase-positive species are animal staphylococci
 - e.g. *Staphylococcus intermedius*

Subgroup of coagulase-negative species contains from human staphylococci:

- *Staphylococcus epidermidis*,
- *Staphylococcus hominis*,
- *Staphylococcus haemolyticus*,
- *Staphylococcus saprophyticus*,
- *Staphylococcus simulans*,
- *Staphylococcus warneri* and other.

The species *Staphylococcus aureus*

- *Morphology*

- Gram-positive, spherical cells, mostly arranged in irregular grape like clusters.
- The peptidoglycan layer is the major structural component of the cell wall. It is important in the pathogenesis of staphylococcal infections. Other important component of cell wall is teichoic acid.
- Protein A is the major protein component of the cell wall. It is located on the cell surface but is also released into the culture medium during the cell growth. A unique property of protein A is its ability to bind to the Fc part of all IgG molecules except IgG3. It is not an antigen-antibody specific reaction.

The species *Staphylococcus aureus* culture characteristics

- Colonies on solid media are round, regular, smooth, slightly convex and 2 to 3 mm in diameter after 24h incubation.
- Most strains show a β -hemolysis surrounding the colonies on blood agar.
- *S. aureus* cells produce cream, yellow or orange pigment.

The species *Staphylococcus aureus* resistance

- Like most of medical important non-spore-forming bacteria, *S. aureus* is rapidly killed by temperature above 60 °C.
- *S. aureus* is susceptible to disinfectants and antiseptics commonly used.
- *S. aureus* can survive and remain virulent long periods of drying especially in an environment with pus.

The species *Staphylococcus aureus* pathogenicity

- *S. aureus* is pathogenic for human as well as for all domestic and free-living warm-blooded animals.
- *S. aureus* causes disease through the production of toxin or through direct invasion and destruction of tissue.
- The clinical manifestations of some staphylococcal diseases are almost exclusively the result of toxin activity (e.g. staphylococcal food poisoning and TSS), whereas other diseases result from the proliferation of the staphylococci, leading to abscess formation and tissue destruction (e.g. cutaneous infection, endocarditis, pneumonia, empyema, osteomyelitis, septic arthritis).

Clinical diseases

- Staphylococcal pyogenic infections:
 - folliculitis,
 - furuncle,
 - carbuncle,
 - bullous impetigo,
 - panaritium and paronychia,
 - wound infections,
 - mastitis,
 - osteomyelitis,
 - staphylococcal pneumonia and other.

Clinical diseases

- Staphylococcal intoxications:
 - staphylococcal food poisoning (enterotoxigenesis),
 - exfoliative intoxications (Ritter's disease or SSSS),
 - staphylococcal toxic shock syndrome (TSS).

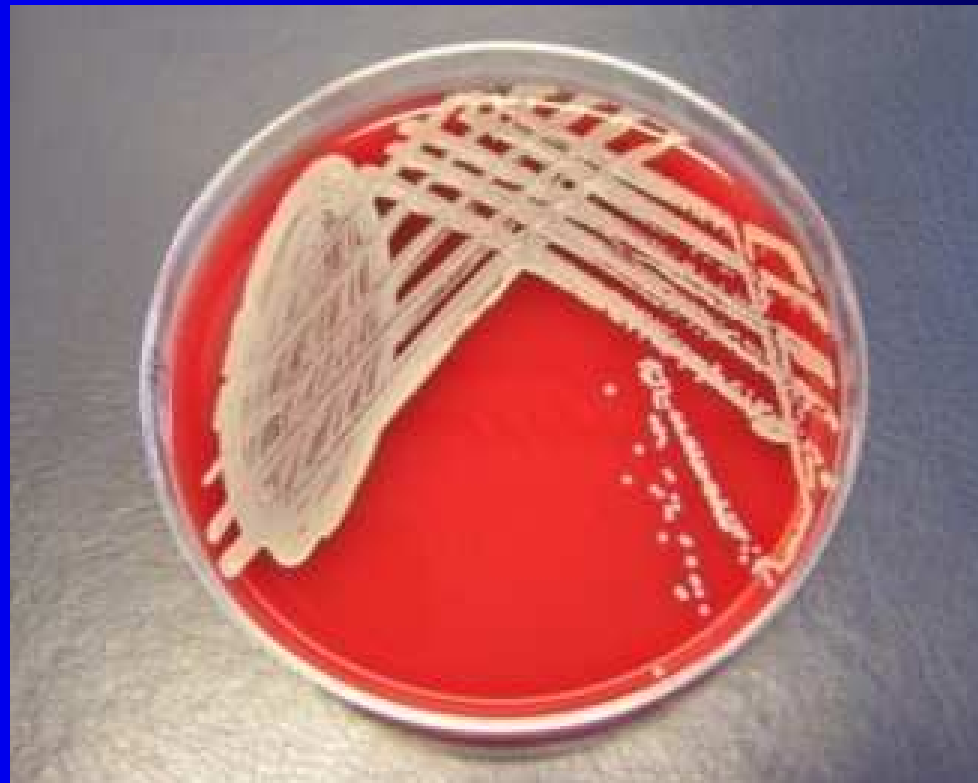
Treatment

- Antistaphylococcal antibiotics of the first choice:
 - oxacillin (methicillin)
 - cephalosporins of I. generation (cefazolin, cephalotin)
- Antistaphylococcal antibiotics of the second choice:
 - lincosamides (e.g. clindamycin)
 - glycopeptides (vancomycin, teicoplanin)
 - linezolid
 - tigecyklin
 - daptomycin
 - and others

Resistance of *S. aureus* to oxacillin (MRSA)

in University hospital Olomouc

5 - 6 %



Diagnosis

- Microscopy – smears of clinical materials are stained according to Gram.
- Cultivation on solid media (usually blood agar).
- Biochemical tests.
- Phage typing – susceptibility of *S. aureus* strains to various temperature phages.

Peptococcus species

- Members of the genus *Peptococcus* are strictly anaerobic cluster-forming Gram-positive cocci.
- These microorganisms grow only under anaerobic conditions.
- They are part of the normal microflora of the mouth, upper respiratory tract, bowel, and female genital tract.
- They often participate with many other bacterial species in mixed anaerobic infections in the abdomen, pelvis, lung, or brain.

Related microorganisms

- The genus *Micrococcus*
 - *Micrococcus luteus*
 - *Micrococcus lylae*
- Both species are found in nature and colonize humans, primarily on the surface of the skin.
- Although micrococci may be found in patients with opportunistic infections, their isolation in clinical specimen usually represents clinically insignificant contamination with skin flora.

Related microorganisms

- The genus *Stomatococcus*
 - *Stomatococcus mucilaginosus*, the only species in this genus, is a commensal microorganism that resides in the oropharynx and upper respiratory tract.
- In recent years, this microorganism has been reported to be the cause of an increasing number of opportunistic infections (endocarditis, septicemia, catheter-related infections) in immunocompromised patients.