

Bacterial Infections

For the means of this textbook, we will only focus on a selected spectrum of microorganisms. Nevertheless, we may mention certain microorganisms for completion without further going into deeper discussion.

Gram-positive cocci

Staphylococcus aureus

Clinical significance

- **Impetigo:** superficial infection of the face and limbs, with macules, vesicles, pustules, erythema
- **Folliculitis:** purulent infections of hair follicles
- **Stye :** infection of the glands found in eyelids
- **Furuncles:** spread of infection to sebaceous glands with a considerable collection of pus, accompanied by pain
- **Carbuncles:** spread of infection to several pilosebaceous glands and deep subcutaneous tissue
- **Hidradenitis:** localized infection of sweat glands within axillary, perineal, or genital regions
- **Mastitis:** mammary gland infection in women during lactation
- **Wound infection:** post-traumatic or post-surgical, with oedema and local erythema, pain and accumulation of pus
- **Bacteraemia and endocarditis:** 50% acquired in the hospital, after surgical procedures or use of a contaminated intravenous catheter; often associated with septic secondary spread usually into the endocardium
- **Pneumonia and pulmonary empyema:** frequent in the extreme ages of life (infants, elderly)
- **Osteomyelitis and septic arthritis:** results of septic secondary dissemination of staphylococcal infections at other sites, or post-traumatic
- **Suppuration of serosa:** pleurisy, peritonitis, periphrenic phlegmon
- **Septicaemia** with possibility of secondary localizations (pyaemia) reaching bone, kidney and lung
- **Infections associated with specific toxins:**
 - Food poisoning (**enterotoxin**), staphylococcal enterocolitis (now exceptional)
 - Generalized bullous lesions (**exfoliatin**): **Scalded skin syndrome** in young children, Ritter's disease
 - Staphylococcal **toxic shock syndrome** (TSST-1): fever with hypotension, desquamated erythroderma observed in epidemic form in women during menstruation due to use tampons with high absorption capacity
- **Nosocomial infections**
- **Indigenous microflora:** skin and nasal passage

Collection

S. aureus can be practically isolated from all clinical specimens, depending on the type of infection:

- Pus from abscesses, furuncles, whitlow, pustules
- Throat swab for angina tonsillaris
- Cutaneous mucosal lesions
- Effusion fluids
- Blood for haemocultures
- Urine

Microscopic examination

Microscopic examination of Gram-stained smears may be useful in the analysis of normally sterile specimens, such as cerebrospinal or synovial fluids, but also for non-sterile specimens. Microscopic examination provides an important indication by highlighting **Gram-positive cocci** grouped into **grape-like clusters**.

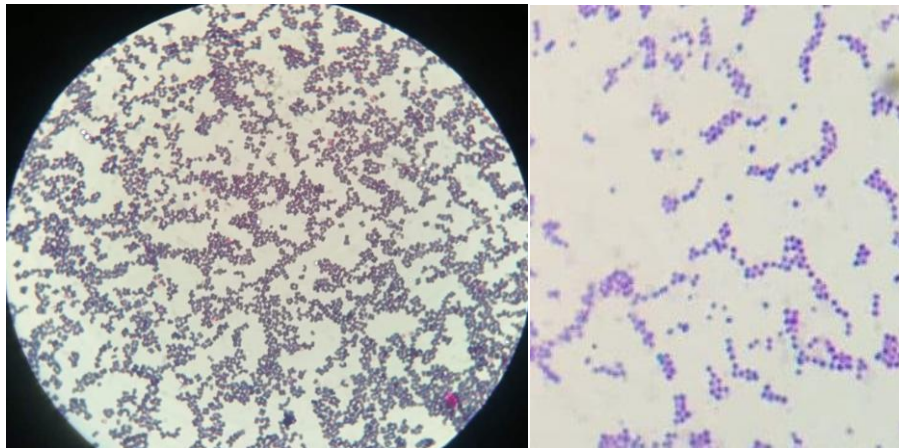


Figure 11. *Staphylococcus* sp.: Microscopic examination- Gram staining

Culture Media

Staphylococci grow well on common, simple media, as well as in most environments that promote the growth of Gram-positive bacteria.

On **blood agar**, the colonies are round, white or sometimes pigmented (golden-yellow), opaque, smooth, 1-3 mm in diameter, bulging, **with** various **haemolysis** (due to multiple, different haemolysins).

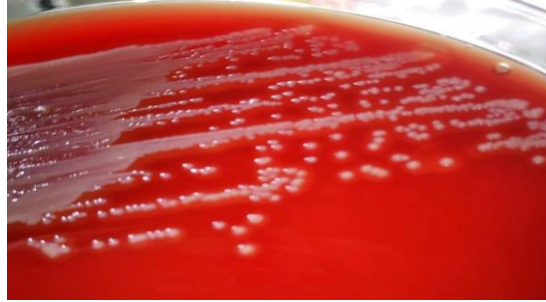


Figure 12. *S. aureus* on blood agar

Specimens that are highly contaminated with normal flora bacteria should be inoculated on a selective media - **Chapman's media**. On Chapman's media *S. aureus* ferments mannitol turning the colour of the medium from red to yellow, where *S. aureus* is growing.



Figure 13. *S. aureus* on Chapman's culture media

Pathogenicity and Biochemical tests

S. aureus has two types of enzymes, **catalase** and **coagulase**, which can aid in the diagnosis.

- **Catalase test**

- **Principle:** catalase catalyses a reaction that leads to the formation of **water** and **oxygen** by decomposing hydrogen peroxide (H_2O_2): $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$
- **Procedure:** 2-3 drops of hydrogen peroxide are placed on a glass slide on which the colony to be studied has been smeared on.
- **Result:** if **gas bubbles** are formed when the hydrogen peroxide drops are added on the colony, the test is considered **positive**. A negative test indicates absent catalase, so that the before mentioned reaction does not take place, i.e. no gas bubbles.
- **Use:** catalase is found in **all Staphylococcal** species and thus, with the Catalase test, we can differentiate between **Staphylococci** (**positive** reaction) and **Streptococci** (**negative** reaction).

- **Slide coagulase test**

- **Principle:** bound coagulase, also termed “clumping factor”, catalyses a reaction that converts fibrinogen into fibrin, leading to the formation of clots
- **Procedure:** 2 drops of saline solution are added onto 2 circles drawn on a glass slide. Then the colony to be studied is emulsified into each of the two circles. After that, 1 drop of plasma (rabbit plasma with EDTA) is placed in one of the circle, whereas on the other circle 1 drop of water is added (control). The slide is then moved back and forth.
- **Result:** If **clumping** (white precipitate) can be observed within 10 seconds, the test is considered to be **positive**. The control circle should remain smooth.
- **Use:** bound coagulase or “clumping factor” is found on the surface of *S. aureus*. Thus, a positive test indicates the presence of *S. aureus* that differentiates it from other *Staphylococci*.



Figure 14. Coagulase test

Antimicrobial susceptibility testing (Antibiogram)

S. aureus is a microorganism that easily (nearly all strains) becomes resistant to penicillin by producing **penicillinase** (or beta-lactamase). In the 1950s and 1960s, the excessive use of penicillin led to the effect of favouring resistant strains to penicillin. Due to the emergence of penicillin resistant strains, clinicians had to treat infections caused by *S. aureus* with other antibiotics. Because of the high incidence of *S. aureus* infections, certain strains became multidrug resistant. *S. aureus* with a multidrug resistance are termed MRSA (**M**ethicillin **R**esistant *Staphylococcus aureus*) – meaning that these are resistant to a multitude of antibiotics. Methicillin resistance indicates a resistance to **all beta-lactam antibiotics** (penicillins, cephalosporins – except fourth generations, monobactams and carbapenems). MRSA strains are treated with vancomycin or linezolid.

Essential to remember:

- Gram-positive cocci
- Arranged in grape-like clusters (“*staphylé*” greek for grape)
- Aureus = Latin for golden (Blood agar - sometimes, Chapman medium)
- Coagulase positive

- Catalase positive
- Gained multidrug resistance (MRSA)

Streptococcus pyogenes (Group A *Streptococcus*)

Clinical Significance

- **Streptococcal pharyngitis** (strep throat)
- **Other upper respiratory infections:** sinusitis and acute otitis media
- **Scarlet fever** (strep throat accompanied by rash due to toxin secretion, followed by desquamation). *Streptococcus* remains localized in the tonsils or pharynx and the general manifestations are due to the spread of erythrogenic toxin and haematogenously transported enzymes (**notifiable disease**)
- **Impetigo** (may be streptococcal or staphylococcal)
- **Superinfections** of wounds, burns, eczema or ulcers
- **Erysipelas** (a recurrent acute infection of the upper dermis and lymph vessels of the skin)
- **Cellulitis** and **necrotizing fasciitis:** a cutaneous and subcutaneous infection, with a rapid extension - streptococcal tissue necrosis
- **Pyoderma**, closed (paronychia, arthritis) or open (cutaneous ulcers) suppuration that can lead to sepsis
- Salpingitis and puerperal infections
- Post-streptococcal diseases (**sequelae**): **acute rheumatic fever** (ARF) - following a pharyngeal infection; **Rheumatic heart disease** (with inflammation of the cardiac valves); **Acute glomerulonephritis** (GNA) following cutaneous or pharyngeal infection; **Chorea minor** (Sydenham); **erythema nodosum**. All these are the result of incomplete or incorrect treatment of streptococcal pharyngitis, scarlet fever, etc. with persistence of group A *Streptococcus* (GAS) in the patient's organism. This chronic presence of GAS will also trigger autoimmune phenomena i.e. the immune system of the patient starts producing antibodies which mistakenly start destroying own healthy cells (in the joints, heart, kidneys, etc.) along with the bacteria
- **Indigenous microflora:** upper respiratory tract

Bacteriological diagnosis

Collection

- Throat swab
- Mucocutaneous lesions
- Effusion fluids
- Blood for haemocultures

Microscopic examination

Gram-positive cocci arranged in **chains**. The observation of streptococci from throat swabs is inconclusive, since commensal streptococci are numerous.

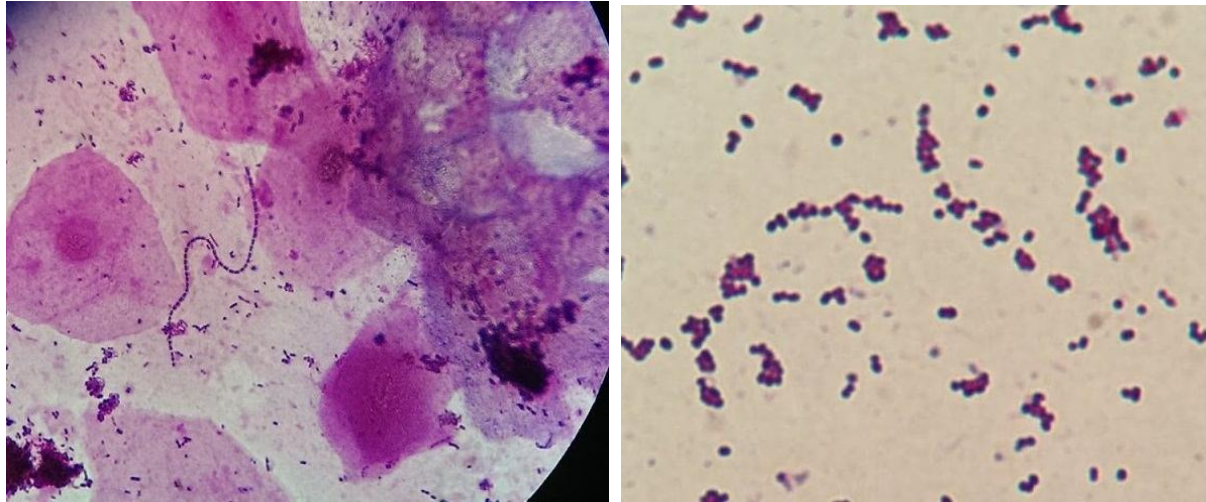


Figure 15. *Streptococcus sp.* : Microscopic examination (a) biological product, (b) culture

Culture media and Identification

Isolation is performed on **blood agar**.

The identification is based on:

- **Colonial characters:**
 - Small, pinpoint, 0.5µm in diameter, transparent colonies
 - **Beta haemolysis**
- **Group identification:**
 - **Bacitracin sensitivity** test: group A streptococci are bacitracin sensitive, other streptococci resistant
 - Lancefield grouping: precipitation with group-specific antisera

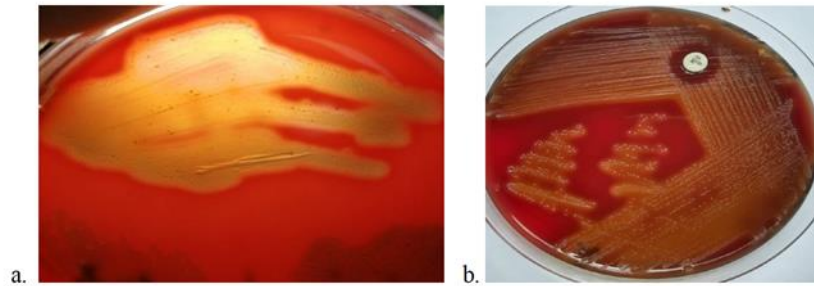


Figure 16. Group Identification (a) Pure culture of *S. pyogenes* on blood agar, beta hemolysis (b) Bacitracin sensitivity test

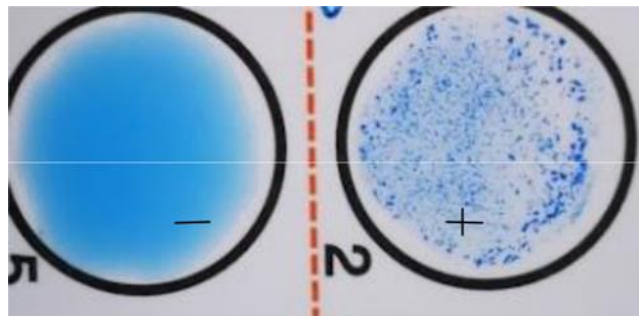


Figure 17. Group Identification

Antimicrobial susceptibility testing (Antibiogram)

For clinical purposes, antimicrobial susceptibility testing is not performed on group A streptococcus, as *Streptococcus pyogenes* is **sensitive to penicillin**. In people with penicillin allergy, **erythromycin** is used.

Serological diagnosis

Used in case of late complications, when germs are difficult to detect through culture, e.g. **in post-streptococcal sequelae**. Through a serological diagnosis, serum antibodies against bacterial products (streptolysin O – toxin responsible for haemolysis and streptokinase – enzyme responsible for fibrinolysis) and components (M antigen and M antigen associated protein) are detected. A serological test frequently used is the **Antistreptolysin O (ASLO)** test, which detects **anti-streptolysin O antibodies** within the patient's serum.

As the initial streptococcal infection may have been overlooked or neglected, antibody titres should be elevated, reaching or exceeding a value of 800 IU, which in the normal state would not exceed 200 IU. A significant variation of the titre between two samples taken at 15-21 days apart is of great diagnostic value.

Essential to remember:

- Equivalent to the term **Group A streptococcus** (*Streptococcus pyogenes* is the only group A streptococcus)
- Gram-positive coccus, arranged in chains
- Beta haemolytic
- Catalase negative (remember the Catalase test and what it is used to differentiate)
- Bacitracin sensitive

Streptococcus agalactiae (Group B *Streptococcus*)

Clinical significance

Severe infections:

- Newborns:
 - **pneumonia** acquired during childbirth, due to passage through the birth canal
 - **meningitis** as a hospital acquired infection
- Immune compromised adults:
 - Meningitis, myocarditis, respiratory infections, sepsis
- **Indigenous microflora:** intestine, vagina, male urethra, upper airways

Bacteriological diagnosis

Collection of specimens – depending on the site of infection:

- Vaginal secretion
- Cervical secretion
- Cerebrospinal fluid

Microscopic examination

Gram-positive cocci arranged in **chains**. Microscopic examination does not have a particular diagnostic value.

Culture media and Identification

Isolation is performed on blood agar.

The identification is based on:

- Colonial characters:
 - grey-white colonies, slightly larger than group A streptococci
 - **Beta haemolysis** (less pronounced and narrower than for group A streptococci)
- Group identification:
 - **Bacitracin sensitivity test:** group B streptococci are bacitracin resistant (group A streptococcus is sensitive)
- **CAMP test:** *Streptococcus agalactiae* produces an extracellular protein termed the **CAMP factor** that acts synergistically with the haemolysis produced by *Staphylococcus aureus*, resulting in a region of **enhanced haemolysis** where the two cultures meet. The enhanced area of haemolysis where the two cultures meet = **positive** test result.

Antimicrobial susceptibility testing (Antibiogram)

As opposed to group A streptococcus, antimicrobial susceptibility testing is mandatory in infections with *Streptococcus agalactiae*, because strains resistant to penicillin have been isolated.

Essential to remember:

- Pertain to group B streptococci
- Gram-positive coccus, arranged in chains
- Beta haemolytic (less prominent and narrower than in group A streptococci)
- Catalase negative (remember the Catalase test and what it is used to differentiate)
- Bacitracin **resistant**
- **CAMP test positive**
- Major causative agents for **septicaemia** and **meningitis** in newborns

Streptococcus pneumoniae (pneumococcus)

Clinical Significance

- Pneumonia (most common causative agent)
- Broncho-pneumonia
- Meningitis
- ENT infections

- **Indigenous microflora:** upper respiratory tract

Bacteriological diagnosis

Collection

- Sputum
- Pus from otitis
- CSF
- Blood for haemocultures

Microscopic examination

For *Streptococcus pneumoniae* microscopic examination is an essential diagnostic tool, because of their characteristic morphology. Gram-stained smears will reveal **Gram-positive cocci arranged in diplo** (in pairs; diplococci) or short chains. Recall from theory that *Streptococcus pneumoniae* are encapsulated microorganisms – giving them the characteristic appearance of “two candle flames” ◀▶ touching with their bases.

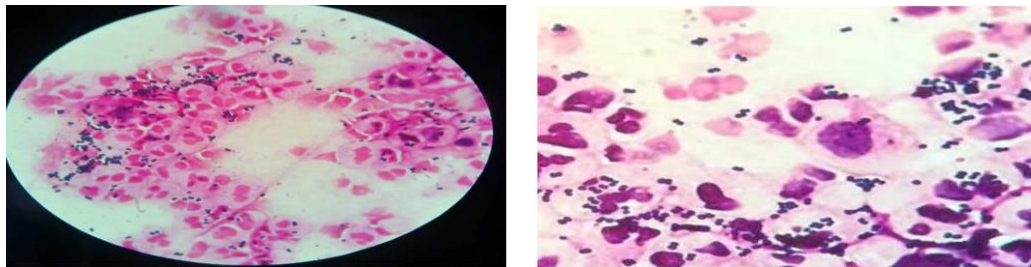


Figure 18. *S. pneumoniae*- microscopic examination of biological specimen

Culture media and Identification

Isolation is performed on **blood agar**

The identification is based on:

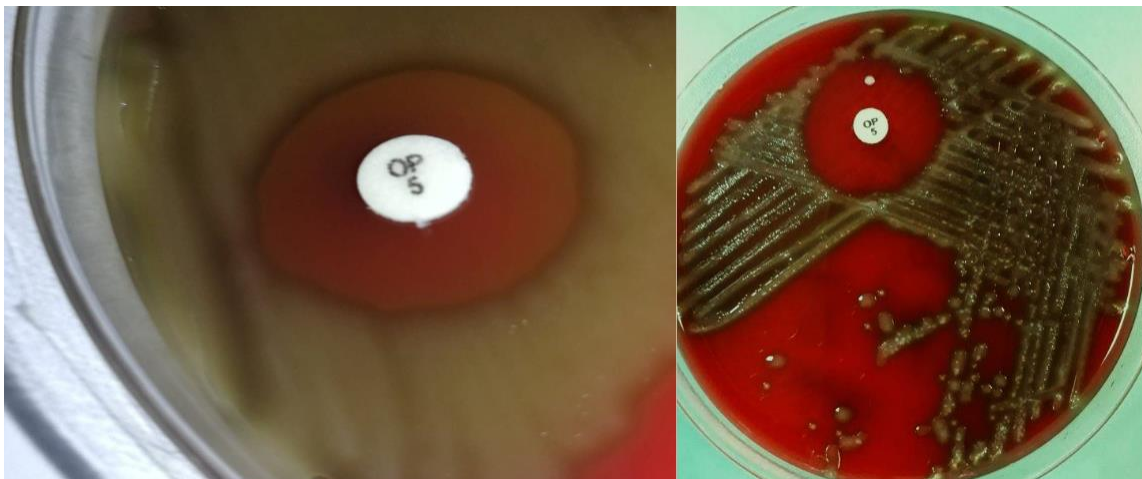
- **Colonial characters:**
 - Small, smooth colonies with (sometimes) depressed centres (RBC appearance)
 - **Alpha haemolysis**
 - **Autolysis** of colonies in time
- **Optochin sensitivity test** (P-disk test): In order to differentiate *Streptococcus pneumoniae* from other **alpha-haemolytic** streptococci (viridans streptococci) the optochin sensitivity test is

performed. Paper disks impregnated with the antimicrobial substance optochin are placed on a blood culture medium that has been inoculated with the microorganisms to be tested. After overnight incubation at 37°C, because *Streptococcus pneumoniae* is sensitive to optochin, growth will be inhibited around the disk, while in case of viridans streptococci growth is visible around and over the optochin disk.

Figure 19. Optochin test on blood agar, α -lysis

Antimicrobial susceptibility testing (Antibiogram)

Antimicrobial susceptibility testing is performed to determine sensitivity to beta-lactams, aminoglycosides, macrolides, lincosamides, glycopeptides, and fluoroquinolones.



Clinical Significance

- Constitute the commensal microflora of the oral cavity, intestines, skin and genital tract

- Viridans group streptococci include: *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus anginosus* and more
- *Streptococcus mutans*: involved in the aetiology of **dental caries**
- Other viridans streptococci: involved in other mouth or gingival infections e.g. periodontal disease
- If introduced into the bloodstream, for example after tooth extraction, patients with prosthetic heart valves are susceptible to developing **subacute bacterial endocarditis**

Bacteriological diagnosis

Collection

Depending on the site/type of infection:

- Fluid/pus from the periodontal pockets
- Pus from dental abscesses
- Blood for haemocultures

Microscopic examination

Gram-positive cocci arranged in **chains**. Microscopic examination does not have a particular diagnostic value.

Culture media

Isolation is performed on **blood agar**

The identification is based on:

- **Colonial characters:**
 - **Alpha haemolysis**
 - **Domed** appearance
- **Optochin sensitivity test** (P-disk test): negative – meaning that they are resistant and growth occurs.

Antimicrobial susceptibility testing (Antibiogram)

Viridans streptococci are sensitive to penicillin G and macrolide antibiotics and most of them have a low level of resistance to aminoglycosides: aminoglycosides act synergistically with penicillin.

Essential to remember:

- Viridans streptococci include a multitude of species
- Gram-positive cocci, arranged in **chains**
- **Alpha haemolytic** with **domed** appearance (as opposed to autolytic and disc shapes pneumococci)
- Catalase negative (remember the Catalase test and what it is used to differentiate)

- **Optochin resistant**
- Involved in the aetiology of **dental caries**, oral infections and **subacute bacterial endocarditis** (for example after tooth extraction, in patients with pre-existing cardiac valve diseases i.e. prosthetic valves or valves affected by rheumatic heart disease as a part of chronic GAS infection – see above: *Streptococcus pyogenes*)