# 4 Bacteria as Human Pathogens

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## Staphylococcus



■ Staphylococci are Gram-positive cocci occurring in clusters. They can be cultured on normal nutrient mediums both aerobically and anaerobically. The most important species from the viewpoint of human medicine is *S. aureus*. A number of extracellular enzymes and exotoxins such as coagulase, alphatoxin, leukocidin, exfoliatins, enterotoxins, and toxic shock toxin are responsible for the clinical symptoms of infections by this pathogen, which are observed in the three types invasive infections, pure toxicoses, and mixed forms. The antibiotics of choice for therapy of these infections are penicillinase-resistant penicillins. Laboratory diagnosis involves identification of the pathogen by means of microscopy and culturing. *S. aureus* is a frequent pathogen in nosocomial infections and limited outbreaks in hospitals. Hand washing by medical staff is the most important prophylactic measure in hospitals.

Coagulase-negative staphylococci are classic opportunists. *S. epidermidis* and other species are frequent agents in foreign body infections due to their ability to form biofilms on the surfaces of inert objects. *S. saprophyticus* is responsible for between 10 and 20 % of acute urinary tract infections in young women. ■

Staphylococci are small spherical cells (1 µm) found in grapelike clusters. Staphylococci are nonmotile, catalase-producing bacteria. The genus *Staphylococcus* includes over 30 species and subspecies. Table 4.**1** briefly summarizes the characteristics of those most important in the medical context. *S. aureus* (and *E. coli*) are among the most frequent causal organisms in human bacterial infections.

Table 4.1 Overview of the *Staphylococcus* Species That Affect Humans Most Frequently

| Species | Parameter |
|---|---|
| *S. aureus* | Coagulase-positive; colonies golden yellow. Local purulent infections: furuncles, carbuncles, bullous impetigo, wound infections, sinusitis, otitis media, mastitis puerperalis, ostitis, postinfluenza pneumonia, sepsis. Toxin-caused illnesses: food poisoning, dermatitis exfoliativa, toxic shock syndrome |
| *S. epidermidis* | Coagulase-negative; sensitive to novobiocin; most frequent CNS* pathogen; opportunist; infection requires host predisposition; foreign body infections with discrete clinical symptoms |
| *S. saprophyticus* | Coagulase-negative; resistant to novobiocin. Urinary tract infections in young women (10–20 %); occasional nonspecific urethritis in men |

* CNS: coagulase-negative staphylococci



## Staphylococcus Aureus

**Morphology and culturing.** Fig. 4.**1a** shows the appearance of Gram-stained *S. aureus*. This is a facultative anaerobe that is readily cultured on normal nutrient mediums at 37 °C. Colonies as in Fig. 4.**1b** develop after 24 hours of incubation. Hemolytic zones are frequently observed around the colonies.

**Fine structure.** The cell wall consists of a thick layer of murein. Linear teichoic acids and polysaccharides are covalently coupled to the murein polysaccharide (Fig. 3.**10**, p. 154). The lipoteichoic acids permeating the entire murein layer are anchored in the cell membrane. Teichoic and lipoteichoic acids can trigger activation of complement by the alternative pathway and stimulate macrophages to secrete cytokines. Cell wall-associated proteins are bound to the peptide components of the murein. Clumping factor, fibronectin-binding protein, and collagen-binding protein bind specifically to fibrinogen, fibronectin, and collagen, respectively, and are instrumental in adhesion to tissues and foreign bodies covered with the appropriate matrix protein. Protein A binds to the Fc portion of immunoglobulins (IgG). It is assumed that "false" binding of immunoglobulins by protein A prevents "correct" binding of opsonizing antibodies, thus hindering phagocytosis.

*Staphylococcus aureus*

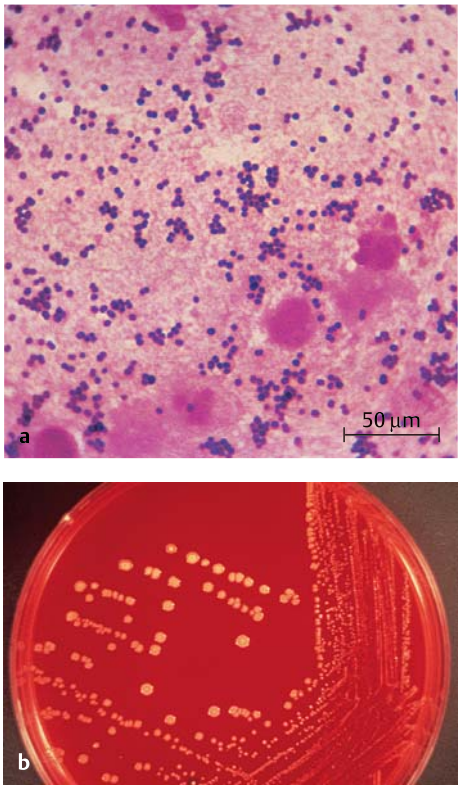



Fig. 4.1 **a** Gram staining of a pus preparation: Gram-positive cocci, some in grapelike clusters. Clinical diagnosis: furunculosis. **b** Culture on blood agar: convex colonies with yellowish pigment and porcelainlike surface.



**Extracellular toxins and enzymes.** *S. aureus* secretes numerous enzymes and toxins that determine, together with the fine structures described above, the pathogenesis of the attendant infections. The most important are:

- **Plasma coagulase** is an enzyme that functions like thrombin to convert fibrinogen into fibrin. Tissue microcolonies surrounded by fibrin walls are difficult to phagocytose.
- **α-toxin** can have lethal CNS effects, damages membranes (resulting in, among other things, hemolysis), and is responsible for a form of dermonecrosis.

- **Leukocidin** damages microphages and macrophages by degranulation.
- **Exfoliatins** are responsible for a form of epidermolysis.
- Food poisoning symptoms can be caused by eight serologically differentiated **enterotoxins** (A-E, H, G, and I). These proteins (MW: 35 kDa) are not inactivated by heating to 100 °C for 15–30 minutes. *Staphylococcus* enterotoxins are superantigens (see p. 72).
- **Toxic shock syndrome toxin-1** (TSST-1) is produced by about 1% of *Staphylococcus* strains. TSST-1 is a superantigen that induces clonal expansion of many T lymphocyte types (about 10%), leading to massive production of cytokines, which then give rise to the clinical symptoms of toxic shock.



**Pathogenesis and clinical pictures.** The pathogenesis and symptoms of *S. aureus* infections take one of three distinct courses:

- **Invasive infections.** In this type of infection, the pathogens tend to remain in situ after penetrating through the derma or mucosa and to cause local infections characterized by purulence. Examples include furuncles (Fig. 4.**2**), carbuncles, wound infections, sinusitis, otitis media, and mastitis puerperalis.

Other kinds of invasive infection include postoperative or posttraumatic ostitis/osteomyelitis, endocarditis following heart surgery (especially valve replacement), postinfluenza pneumonia, and sepsis in immunocompromised patients. *S. aureus* and *E. coli* are responsible for approximately equal shares of nearly half of all cases of inpatient sepsis.

Inert foreign bodies (see p. 158 for examples) can be colonized by *S. aureus*. Colonization begins with specific binding of the staphylococci, by means of cell wall-associated adhesion proteins, to fibrinogen or fibronectin covering the foreign body, resulting in a biofilm that may function as a focus of infection.

**Multiple Furuncles**

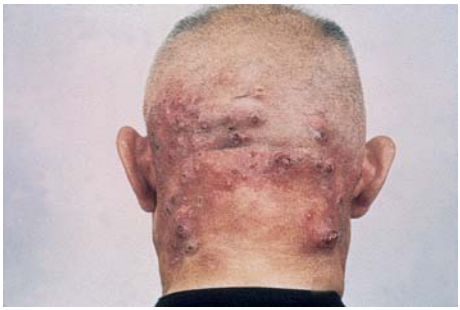


Fig. 4.**2** Furuncles in a patient with type 2 diabetes mellitus.

■ **Toxicoses.** Food poisoning results from ingestion of food contaminated with enterotoxins. The onset a few hours after ingestion takes the form of nausea, vomiting, and massive diarrhea.

■ **Mixed forms.** Dermatitis exfoliativa (staphylococcal scalded skin syndrome, Ritter disease), pemphigus neonatorum, and bullous impetigo are caused by exfoliatin-producing strains that infect the skin surface. Toxic shock syndrome (TSS) is caused by strains that produce TSST-1. These strains can cause invasive infections, but may also only colonize mucosa. The main symptoms are hypotension, fever, and a scarlatiniform rash.

**Diagnosis.** This requires microscopic and culture-based pathogen identification. Differentiating *S. aureus* from the coagulase-negative species is achieved by detection of the plasma coagulase and/or the clumping factor. The enterotoxins and TSST-1 can be detected by means of immunological and molecular biological methods (special laboratories).



**Plasma Coagulase and Clumping Factor Test**

■ To detect plasma coagulase, suspend several colonies in 0.5 ml of rabbit plasma, incubate the inoculated plasma for one, four, and 24 hours and record the levels of coagulation.

■ For the clumping factor test, suspend colony material in a drop of rabbit plasma on a slide. Macroscopically visible clumping confirms the presence of the factor.

**Therapy.** Aside from surgical measures, therapy is based on administration of antibiotics. The agents of choice for severe infections are penicillinase-resistant penicillins, since 70–80% of all strains produce penicillinase. These penicillins are, however, ineffective against methicillin-resistant strains, and this resistance applies to all betalactams.

**Epidemiology and prevention.** *S. aureus* is a frequent colonizer of skin and mucosa. High carrier rates (up to 80%) are the rules among hospital patients and staff. The principle localization of colonization in these persons is the anterior nasal mucosa area, from where the bacteria can spread to hands or with dust into the air and be transmitted to susceptible persons.

*S. aureus* is frequently the causal pathogen in nosocomial infections (see p. 343f.). Certain strains are known to cause hospital epidemics. Identification of the epidemic strain requires differentiation of relevant infection isolates from other ubiquitous strains. Lysotyping (see p. 186) can be used for this purpose, although use of molecular methods to identify genomic DNA "fingerprints" is now becoming more common.

The most important preventive measure in hospitals is washing the hands thoroughly before medical and nursing procedures. Intranasal application of antibiotics (mupirocin) is a method of reducing bacterial counts in carriers.

### Coagulase-Negative Staphylococci (CNS)

CNS are an element in the normal flora of human skin and mucosa. They are classic opportunists that only cause infections given a certain host disposition.

■ ***S. epidermidis.*** This is the pathogen most frequently encountered in CNS infections (70–80% of cases). CNS cause mainly foreign body infections. Examples of the foreign bodies involved are intravasal catheters, continuous ambulant peritoneal dialysis (CAPD) catheters, endoprostheses, metal plates and screws in osteosynthesis, cardiac pacemakers, artificial heart valves, and shunt valves. These infections frequently develop when foreign bodies in the macroorganism are covered by matrix proteins (e.g., fibrinogen, fibronectin) to which the staphylococci can bind using specific cell wall proteins. They then proliferate on the surface and produce a polymeric substance—the basis of the developing biofilm. The staphylococci within the biofilm are protected from antibiotics and the immune system to a great extent. Such biofilms can become infection foci from which the CNS enter the bloodstream and cause sepsislike illnesses. Removal of the foreign body is often necessary.

■ ***S. saprophyticus*** is responsible for 10–20% of acute urinary tract infections, in particular dysuria in young women, and for a small proportion of cases of nonspecific urethritis in sexually active men.

Antibiotic treatment of CNS infections is often problematic due to the multiple resistance often encountered in these staphylococci, especially *S. hemolyticus*.

## Streptococcus and Enterococcus

■ **Streptococci** are Gram-positive, **nonmotile**, catalase-negative, facultatively anaerobic cocci that occur in chains or pairs. They are classified based on their hemolytic capacity (α-, β-, γ-hemolysis) and the antigenicity of a carbohydrate occurring in their cell walls (Lancefield antigen).

β-hemolytic group A streptococci (*S. pyogenes*) cause infections of the upper respiratory tract and invasive infections of the skin and subcutaneous connective tissue. Depending on the status of the immune defenses and the genetic disposition, this may lead to scarlet fever and severe infections such as necrotizing fasciitis, sepsis, or septic shock. Sequelae such as acute rheumatic fever and glomerulonephritis have an autoimmune pathogenesis. The α-hemolytic pneumococci (*S. pneumoniae*) cause infections of the respiratory tract. Penicillins are the antibiotics of choice. Resistance to penicillins is

known among pneumococci, and is increasing. Laboratory diagnosis involves pathogen detection in the appropriate material. Persons at high risk can be protected from pneumococcal infections with an active prophylactic vaccine containing purified capsular polysaccharides. Certain oral streptococci are responsible for dental caries. Oral streptococci also cause half of all cases of endocarditis.

Although **enterococci** show only low levels of pathogenicity, they frequently cause nosocomial infections in immunocompromised patients (usually as elements of a mixed flora).



Streptococci are round to oval, Gram-positive, nonmotile, nonsporing bacteria that form winding chains (streptos [greek] = twisted) or diplococci. They do not produce catalase. Most are components of the normal flora of the mucosa. Some can cause infections in humans and animals.

**Classification.** The genera *Streptococcus* and *Enterococcus* comprise a large number of species. Table 4.**2** lists the most important.

### α-, β-, γ-hemolysis.

*α-hemolysis*. Colonies on blood agar are surrounded by a green zone. This "greening" is caused by $H_2O_2$, which converts hemoglobin into methemoglobin.

*β-hemolysis*. Colonies on blood agar are surrounded by a large, yellowish hemolytic zone in which no more intact erythrocytes are present and the hemoglobin is decomposed.

*γ-hemolysis*. This (illogical) term indicates the absence of macroscopically visible hemolytic zones.

**Lancefield groups.** Many streptococci and enterococci have a polymeric carbohydrate (C substance) in their cell walls called the Lancefield antigen. They are classified in Lancefield groups A-V based on variations in the antigenicity of this antigen.

Specific characteristics of enterococci that differentiate them from streptococci include their ability to proliferate in the presence of 6.5% NaCl, at 45 °C and at a pH level of 9.6.

Table 4.2 The Most Important Human Pathogen Streptococci and Enterococci

| Species | Hemolysis | Group antigen | Remarks |
|---|---|---|---|
| **Pyogenic, hemolytic streptococci** | | | |
| *Streptococcus pyogenes* (A streptococci) | β | A | Frequent pathogen in humans; invasive infections, sequelae |
| *S. agalactiae* (B streptococci) | β | B | Meningitis/sepsis in neonates; invasive infections in predisposed persons |
| C streptococci | β(α; γ) | C | Rare; purulent infections (similar to *S. pyogenes* infections) |
| G streptococci | β | G | Rare; purulent infections (similar to *S. pyogenes* infections) |
| *S. pneumoniae* | α | – | Pneumococci; respiratory tract infections; sepsis; meningitis |
| *S. bovis* | α; γ | D | Not enterococci, although in group D; rare sepsis pathogen; if isolated from blood work up for pathological colon processes |
| **Oral streptococci (selection)** | | | |
| *S. salivarius*<br>*S. sanguis*<br>*S. mutans*<br>*S. mitis*<br>*S. anginosus*<br>*S. constellatus*<br>*S. intermedius*<br>etc. | α; γ<br><br><br><br>*S. milleri* group | A, C, E, F, G, H, K occasionally detectable | Greening (viridans) streptococci; occur in oral cavity; endocarditis; caries (*S. mutans*, *S. sanguis*, *S. mitis*) Purulent abscesses |
| **Enterococci (Enterococcus)** | | | |
| *E. faecalis* | α; γ; β | D | Occur in human and animal intestines; low-level pathogenicity; endocarditis; nosocomial infections. Often component of mixed florae. |
| *E. faecium* | α | D | |

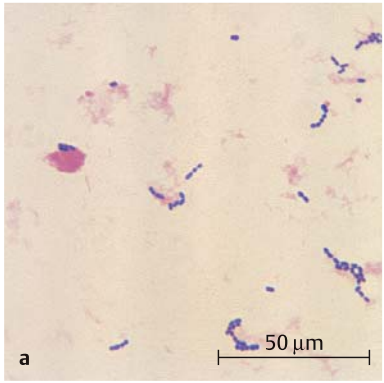
## Streptococcus pyogenes (A Streptococci)

**Morphology and culturing.** Gram-positive cocci with a diameter of 1 µm that form chains (Fig. 4.**3a**). Colonies on blood agar (Fig. 4.**3b**) show β-hemolysis caused by streptolysins (see below).

**Fine structure.** The murein layer of the cell wall is followed by the serogroup A carbohydrate layer, which consists of C substance and is covalently bound to the murein. Long, twisted protein threads that extend outward are anchored in the cell wall murein: the M protein. A streptococci are classified in serovars with characteristic M protein chemistry. Like the hyaluronic acid capsules seen in some strains, the M protein has an antiphagocytic effect.



*Streptococcus Pyogenes*



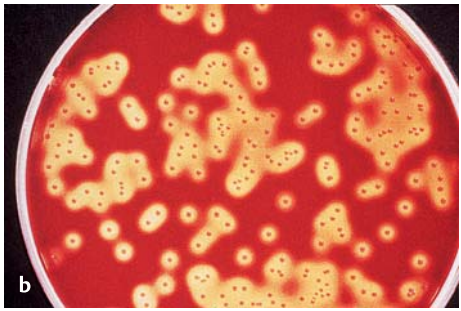




Fig. 4.**3** **a** Gram staining of pleural puncture biopsy material: gram-positive cocci in twisted chains. **b** Culture on blood agar: small, whitish-gray colonies surrounded by large β-hemolysis zones; a 5% $CO_2$ atmosphere provides optimum conditions for β-hemolysis.

**Extracellular toxins and enzymes.** The most important in the context of pathogenicity are:

- **Streptolysin O, streptolysin S.** Destroy the membranes of erythrocytes and other cells. Streptolysin O acts as an antigen. Past infections can be detected by measuring the antibodies to this toxin (antistreptolysin titer).
- **Pyrogenic streptococcal exotoxins** (PSE) A, B, C. Responsible for fever, scarlet fever exanthem and enanthem, sepsis, and septic shock. The pyrogenic exotoxins are superantigens and therefore induce production of large amounts of cytokines (p. 77).
- **Streptokinase.** Dissolves fibrin; facilitates spread of streptococci in tissues.
- **Hyaluronidase.** Breaks down a substance that cements tissues together.
- **DNases.** Breakdown of DNA, producing runny pus.

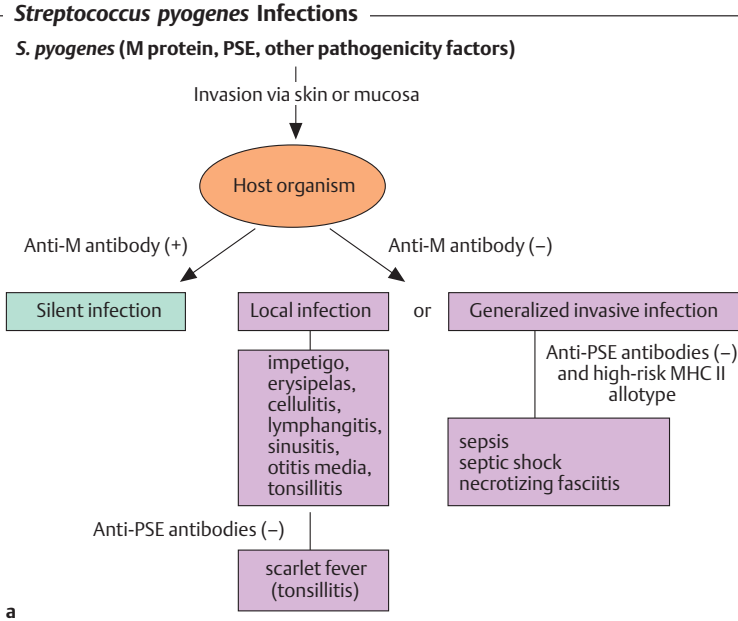


**Pathogenesis and clinical pictures.** Streptococcal diseases can be classified as either acute, invasive infections or sequelae to them.

- **Invasive infections.** The pathogens enter through traumas or microtraumas in the skin or mucosa and cause invasive local or generalized infections (Fig. 4.**4**). The rare cases of severe septic infection and necrotizing fasciitis occur in persons with a high-risk MHC II allotype. In these patients, the PSE superantigens (especially PSEA) induce large amounts of cytokine by binding at the same time to the MHC II complex and the β chain of the T cell receptor. The excess cytokines thus produced are the cause of the symptoms.
- **Sequelae.** Glomerulonephritis is an immune complex disease (p. 113) and acute rheumatic fever may be a type II immune disease (p. 109).

**Diagnosis.** What is involved in diagnosis is detection of the pathogen by means of microscopy and culturing. Group A antigen can be detected using particles coated with antibodies that precipitate agglutination (latex agglutination, coagglutination). Using these methods, direct detection of A streptococci in tonsillitis is feasible in the medical practice. However, this direct detection method is not as sensitive as the culture. Differentiation of A streptococci from other β-hemolytic streptococci can be realized in the laboratory with the bacitracin disk test, because A streptococci are more sensitive to bacitracin than the other types.

**Therapy.** The agents of choice are penicillin G or V. Resistance is unknown. Alternatives are oral cephalosporins or macrolide antibiotics, although resistance to the latter can be expected. In treatment of septic shock, a polyvalent immunoglobulin is used to inactivate the PSE.



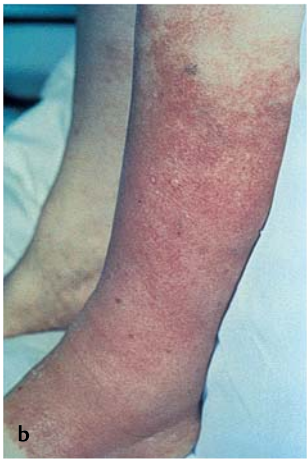




Fig. 4.4 **a** Pathogenesis and clinical pictures of *S. pyogenes* infections (simplified scheme). **b** Erysipelas caused by *S. pyogenes*.

**Epidemiology and prophylaxis.** Infection frequency varies according to geographical area, season, and age. Humans are the only pathogen reservoir for *S. pyogenes*. Transmission is by direct contact (smear infection) or droplets. The incubation period is one to three days. The incidence of carriers among children is 10–20%, but can be much higher depending on the epidemiological situation. Carriers and infected persons are no longer contagious 24 hours after the start of antibiotic therapy. Microbiological follow-up checks of patients and first-degree contacts are not necessary (exception: rheumatic history).

In persons with recurring infections or with acute rheumatic fever in their medical histories, continuous penicillin prophylaxis with a long-term penicillin is appropriate (e.g., 1.2 million IU benzathine penicillin per month).



## Streptococcus pneumoniae (Pneumococci)

**Morphology and culturing.** Pneumococci are Gram-positive, oval to lancet-shaped cocci that usually occur in pairs or short chains (Fig. 4.**5a**). The cells are surrounded by a thick capsule.

When cultured on blood agar, *S. pneumoniae* develop $\alpha$-hemolytic colonies with a mucoid (smooth, shiny) appearance (hence "S" form, Fig. 4.**5b**). Mutants without capsules produce colonies with a rough surface ("R" form).

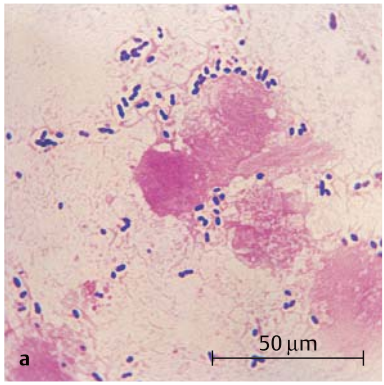
**Antigen structure.** Pneumococci are classified in 90 different serovars based on the fine chemical structure of the capsule polysaccharides acting as antigens. This capsule antigen can be identified using specific antisera in a reaction known as capsular swelling.

**Pathogenesis and clinical pictures.** The capsule protects the pathogens from phagocytosis and is the most important determinant of pneumococcal virulence. Unencapsulated variants are not capable of causing disease. Other potential virulence factors include pneumolysin with its effects on membranes and an $IgA_1$ protease.

The natural habitat of pneumococci is provided by the mucosa of the upper respiratory tract. About 40–70% of healthy adults are carriers. Pneumococcal infections usually arise from this normal flora (endogenous infections). Predisposing factors include primary cardiopulmonary diseases, previous infections (e.g., influenza), and extirpation of the spleen or complement system defects.

The most important pneumococcal infections are **lobar pneumonia** and **bronchopneumonia**. Other infections include acute exacerbation of chronic bronchitis, otitis media, sinusitis, meningitis, and corneal ulcer. Severe pneumococcal infections frequently involve sepsis.

***Streptococcus pneumoniae***



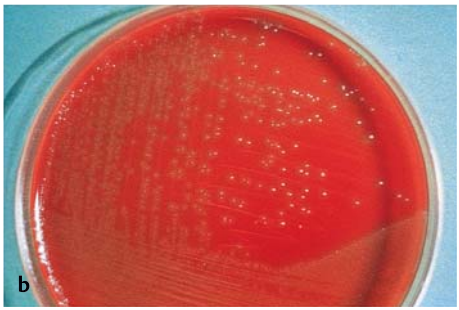




Fig. 4.5 **a** Gram staining of a preparation of middle ear secretion: gram-positive, round-oval, encapsulated cocci; clinical diagnosis: otitis media.
**b** Culture on blood agar: gray colonies showing little intrinsic color, often mucoid (due to capsules); a zone of greening is often observed around the colonies, caused by α-hemolysis; the shiny appearance of the colonies is caused by light reflections from their mucoid surface.



**Diagnosis.** The laboratory diagnosis includes detection of the pathogen in appropriate test samples by means of microscopy and culturing. Pneumococci can be differentiated from other α-hemolytic streptococci based on their greater sensitivity to optochin (ethyl hydrocuprein hydrochloride) in the disk test or their bile solubility. Bile salts increase autolysis in pneumococci.

**Therapy.** Penicillin is still the antibiotic of choice. There have been reports of high-frequency occurrence of strains resistant to penicillin (South Africa, Spain, Hungary, USA). These strains are still relatively rare in Germany, Switzerland, and Austria (5–10%). Macrolide antibiotics are an alternative to penicillins, but resistance to them is also possible.

Penicillin resistance is not due to penicillinase, but rather to modified penicillin-binding proteins (PBPs) to which penicillins have a lower level of affinity. PBPs are required for murein biosynthesis. Biochemically, penicillin re-

sistance extends to cephalosporins as well. However, certain cephalosporins (e.g., ceftriaxone) can be used against penicillin-resistant pneumococci due to their higher levels of activity.

**Epidemiology and prophylaxis.** Pneumococcal infections are endemic and occur in all seasons, more frequently in the elderly. Humans are the natural pathogen reservoir.

The vaccine product Pneumovax® is available for immunization purposes. It contains 25 mg of the purified capsule polysaccharides of each of 23 of the most frequent serovars. Eighty to ninety percent of all isolated pneumococci have antigens contained in this vaccine, which is primarily indicated in persons with predisposing primary diseases. There is also a seven-valent conjugate vaccine that is effective in children under two years of age (p. 33). Exposure prophylaxis is not necessary.



## Streptococcus agalactiae (B Streptococci)

B streptococci occasionally cause infections of the skin and connective tissues, sepsis, urinary tract infections, pneumonia, and peritonitis in immunocompromised individuals. About one in 1000 neonates suffers from a sepsis with or without meningitis. These infections manifest in the first days of life (early onset type) or in the first weeks of life (late onset type). In the early onset form, the infection is caused intra partum by B streptococci colonizing the vagina. Potential predisposing factors include birth complications, premature birth, and a lack of antibodies to the capsule in mother and neonate.

## Oral Streptococci

Most of the oral streptococci of the type often known as the viridans group have no group antigen. They usually cause $\alpha$-hemolysis, some $\gamma$-hemolysis as well.

Oral streptococci are responsible for 50–70% of all cases of bacterial **endocarditis**, overall incidence of which is one to two cases per 100 000 annually. The origins of endocarditis lie in invasion of the vascular system through lesions in the oral mucosa. A transitory bacteremia results. The heart valves are colonized and a biofilm is formed by the organism. Predisposing factors include congenital heart defects, acute rheumatic fever, cardiac surgery, and scarred heart valves. Laboratory diagnosis of endocarditis involves isolation of the pathogen from blood cultures. Drug therapy of endocarditis is carried out with either penicillin G alone or combined with an aminoglycoside (mostly gentamicin). Bactericidal activity is the decisive parameter.

**Pronounced Dental Caries**

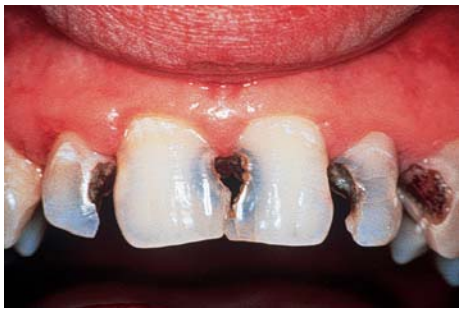


Fig. 4.**6** Certain oral streptococci (*S. mutans*) are the main culprits in tooth decay.



*S. mutans*, *S. sanguis*, and *S. mitis* are, besides *Actinomyces viscosus* and *A. naeslundii*, responsible for **dental caries** (Fig. 4.**6**). These streptococci can attach to the proteins covering the tooth enamel, where they then convert sucrose into extracellular polysaccharides (mutan, dextran, levan). These sticky substances, in which the original bacterial layer along with secondary bacterial colonizers are embedded, form dental plaque. The final metabolites of the numerous plaque bacteria are organic acids that breach the enamel, allowing the different caries bacteria to begin destroying the dentin.

## Enterococcus (Enterococci)

Enterococci are a widespread bacterial genus (p. 220) normally found in the intestines of humans and other animals. They are nonmotile, catalase-negative, and characterized by group antigen D. They are able to proliferate at 45 °C, in the presence of 6.5 % NaCl and at pH 9, qualities that differentiate them from streptococci. As classic opportunists, enterococci show only low levels of pathogenicity. However, they are frequently isolated as components of a mixed flora in nosocomial infections (p. 343). Ninety percent of such isolates are identified as *E. faecalis*, 5–10 % as *E. faecium*. Among the most dangerous enterococcal infections is endocarditis, which must be treated with a combination of an aminopenicillin and streptomycin or gentamicin. Therapeutic success depends on the bactericidal efficacy of the combination used. The efficacy level will be insufficient in the presence of high levels of resistance to either streptomycin (MIC $>1000$ mg/l) or gentamicin (MIC $>500$ mg/l) or resistance to the aminopenicillin. Enterococci frequently develop resistance to antibiotics. Strains manifesting multiple resistance are found mainly in hospitals, in keeping with the classic opportunistic

character of these pathogens. Recently observed epidemics on intensive care wards involved strains that were resistant to all standard anti-infective agents including the glycopeptides vancomycin and teicoplanin.

## Gram-Positive, Anaerobic Cocci

Gram-positive, strictly anaerobic cocci are included in the genera *Peptococcus* and *Peptostreptococcus*. The only species in the first genus is *Peptococcus niger*, whereas the latter comprises a number of species. The anaerobic cocci are commonly observed in normal human flora. In a pathogenic context they are usually only encountered as components of mixed florae together with other anaerobes or facultative anaerobes. These bacteria invade tissues through dermal or mucosal injuries and cause subacute purulent infections. Such infections are either localized in the head area (cerebral abscess, otitis media, mastoiditis, sinusitis) or lower respiratory tract (necrotizing pneumonia, pulmonary abscess, empyema). They are also known to occur in the abdomen (appendicitis, peritonitis, hepatic abscess) and female genitals (salpingitis, endometriosis, tubo-ovarian abscess). Gram-positive anaerobic cocci may also contribute to soft-tissue infections and postoperative wound infections. See p. 317ff. for clinical details of anaerobe infections.



## Bacillus

■ The natural habitat of *Bacillus anthracis*, a Gram-positive, sporing, obligate aerobic rod bacterium, is the soil. The organism causes **anthrax** infections in animals. Human infections result from contact with sick animals or animal products contaminated with the spores. Infections are classified according to the portal of entry as dermal anthrax (95% of cases), primary inhalational anthrax, and intestinal anthrax. Sepsis can develop from the primary infection focus. Laboratory diagnosis includes microscopic and cultural detection of the pathogen in relevant materials and blood cultures. The therapeutic agent of choice is penicillin G. ■

The genera *Bacillus* and *Clostridium* belong to the *Bacillaceae* family of sporing bacteria. There are numerous species in the genus *Bacillus* (e.g., *B. cereus*, *B. subtilis*, etc.) that normally live in the soil. The organism in the group that is of veterinary and human medical interest is *Bacillus anthracis*.

## Bacillus anthracis (Anthrax)

**Occurrence.** Anthrax occurs primarily in animals, especially herbivores. The pathogens are ingested with feed and cause a severe clinical sepsis that is often lethal.

**Morphology and culturing.** The rods are 1 μm wide and 2–4 μm long, nonflagellated, with a capsule made of a glutamic acid polypeptide. The bacterium is readily grown in an aerobic milieu.

**Pathogenesis and clinical picture.** The pathogenicity of *B. anthracis* results from its antiphagocytic capsule as well as from a toxin that causes edemas and tissue necrosis. Human infections are contracted from diseased animals or contaminated animal products. Anthrax is recognized as an occupational disease.



**Dermal, primary inhalational, and intestinal anthrax** are differentiated based on the pathogen's portal of entry. In dermal anthrax, which accounts for 90–95% of human *B. anthracis* infections) the pathogens enter through injuries in the skin. A local infection focus similar to a carbuncle develops within two to three days. A sepsis with a foudroyant (highly acute) course may then develop from this primary focus. Inhalational anthrax (bioterrorist anthrax), with its unfavorable prognosis, results from inhalation of dust containing the pathogen. Ingestion of contaminated foods can result in intestinal anthrax with vomiting and bloody diarrheas.

**Diagnosis.** The diagnostic procedure involves detection of the pathogen in dermal lesions, sputum, and/or blood cultures using microscopic and culturing methods.

**Therapy.** The antimicrobial agent of choice is penicillin G. Doxycycline (a tetracycline) or ciprofloxacin (a fluoroquinolone) are possible alternatives. Surgery is contraindicated in cases of dermal anthrax.

**Epidemiology and prophylaxis.** Anthrax occurs mainly in southern Europe and South America, where economic damage due to farm animal infections is considerable. Humans catch the disease from infected animals or contaminated animal products. Anthrax is a classic zoonosis.

Prophylaxis involves mainly exposure prevention measures such as avoiding contact with diseased animals and disinfection of contaminated products. A cell-free vaccine obtained from a culture filtrate can be used for vaccine prophylaxis in high-risk persons.

## Clostridium

Clostridia are 3–8 μm long, thick, Gram-positive, sporing rod bacteria that can only be cultured anaerobically. Their natural habitat is the soil. The pathogenicity of the disease-causing species in this genus is due to production of exotoxins and/or exoenzymes. The most frequent causative organism in **anaerobic cellulitis** and **gas gangrene** (clostridial myonecrosis) is *C. perfringens*. **Tetanus** is caused by *C. tetani*. This pathogen produces the exotoxin tetanospasmin, which blocks transmission of inhibitory CNS impulses to motor neurons. **Botulism** is a type of food poisoning caused by the neurotoxins of *C. botulinum*. These substances inhibit stimulus transmission to the motor end plates. **Pseudomembranous colitis** is caused by *C. difficile*, which produces an enterotoxin (A) and a cytotoxin (B). Diagnosis of clostridial infections requires identification of the pathogen (gas gangrene) and/or the toxins (tetanus, botulism, colitis). All clostridia are readily sensitive to penicillin G. Antitoxins are used in therapy of tetanus and botulism and hyperbaric $O_2$ is used to treat gas gangrene. The most important preventive measure against tetanus is active vaccination with tetanus toxoid.



**Occurrence.** Clostridia are sporing bacteria that naturally inhabit the soil and the intestinal tracts of humans and animals. Many species are apathogenic saprophytes. Under certain conditions, several species cause gas gangrene, tetanus, botulism, and pseudomembranous colitis.

**Morphology and culturing.** All clostridia are large, Gram-positive rod bacteria about 1 μm thick and 3–8 μm in length (Fig. 4.**7**). Many cells in older cultures show a Gram-negative reaction. With the exception of *C. perfringens*, clostridia are flagellated. Clostridia sporulate. They are best cultured in an anaerobic atmosphere at 37 °C. *C. perfringens* colonies are convex, smooth, and surrounded by a hemolytic zone. Colonies of motile clostridia have an irregular, ragged edge.

### The Pathogens That Cause Gas Gangrene (Clostridial Myonecrosis) and Anaerobic Cellulitis

**Pathogen spectrum.** The pathogens that cause these clinical pictures include *Clostridium perfringens*, *C. novyi*, *C. septicum*, and *C. histolyticum*. Species observed less frequently include *C. sporogenes*, *C. sordellii*, and *C. bifermentans*. The most frequent causative pathogen in gas gangrene is *C. perfringens*.

**Toxins, enzymes.** The toxins produced by invasive clostridia show necrotizing, hemolytic, and/or lethal activity. They also produce collagenases,

***Clostridium perfringens* and *sporogenes***

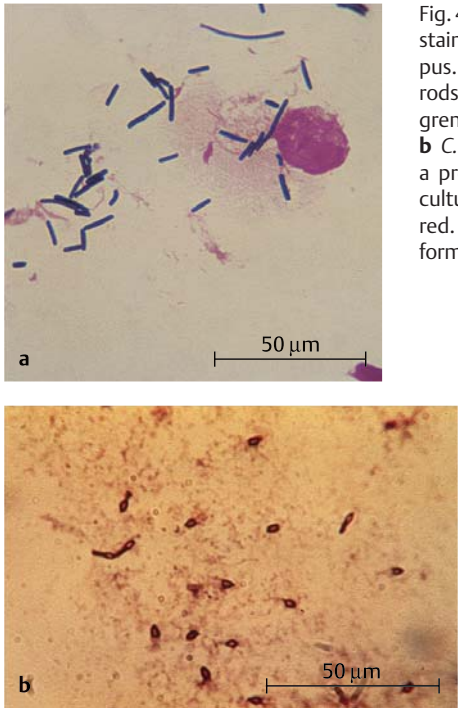



Fig. 4.7 **a** *C. perfringens*: gram staining of a preparation of wound pus. Large, thick, gram-positive rods. Clinical diagnosis: gas gangrene in a gunshot wound.
**b** *C. sporogenes*: Spore staining of a preparation from an aged broth culture. Thick-walled spores stained red. Occasionally "tennis racquet" forms.



proteinases, DNases, lecithinases, and hyaluronidase, all of which destroy tissue structures, resulting in accumulations of toxic metabolites.

**Pathogenesis and clinical picture.** Due to the ubiquitous presence of clostridia, they frequently contaminate open wounds, often together with other microorganisms. Detection of clostridia in a wound is therefore no indication of a clostridial infection. These infections develop when a low tissue redox potential makes anaerobe reproduction possible, resulting in tissue necrosis. Two such infections of differing severity are described below:

- **Anaerobic cellulitis.** Infection restricted to the fascial spaces that does not affect musculature. Gas formation in tissues causes a cracking, popping sensation under the skin known as crepitus. There is no toxemia.

**Gas gangrene (clostridial myonecrosis).** An aggressive infection of the musculature with myonecrosis and toxemia. The incubation period varies from hours to a few days.

**Diagnosis.** The diagnostic procedure includes identification of the pathogens in relevant materials by means of microscopy and culturing. Identification of anaerobically grown cultures is based on morphological and physiological characteristics.

**Therapy.** Primary treatment is surgical, accompanied by antibiosis (penicillins, cephalosporins). Treatment with hyperbaric $O_2$ in special centers has proved effective: patients breathe pure $O_2$ through a tube or mask in a pressure chamber (3 atm = 303 kPa) several times during two-hour periods.



**Epidemiology and prevention.** True gas gangrene is now a rare condition. Timely operation of contaminated wounds is the main preventive measure.

## Clostridium tetani (Tetanus)

Tetanus (lockjaw) is an acute clostridial disease, its clinical manifestations do not result directly from the invasive infection, but are rather caused by a strong neurotoxin.

**Toxin.** Tetanospasmin (an AB toxin, p. 16) consists of two polypeptide chains linked by a disulfide bridge. The heavy chain binds specifically to neuron receptors. The light chain is a zinc-metalloprotease that is responsible for proteolysis of components of the neuroexocytosis apparatus in the synapses of the anterior horns of the spinal cord. This stops transmission of inhibitory efferent impulses from the cerebellum to the motor end plates.

**Pathogenesis and clinical picture.** These ubiquitous pathogens invade tissues following injuries (Fig. 4.**8a**). Given anaerobic conditions, they proliferate and produce the toxin (see above), which reaches the anterior horns of the spinal cord or brain stem via retrograde axonal transport. The clinical picture resulting from the effects of the toxin is characterized by increased muscle tone and spasms induced by visual or acoustic stimuli. The cramps often begin in the facial musculature (risus sardonicus, Fig. 4.**8b**), then spread to neck and back muscles (opisthotonus). The patient remains lucid.

**Diagnosis.** The preferred method is toxin detection in wound material in an animal test (mouse) based either on neutralization or detection of the toxin gene with PCR. The pathogen is difficult to culture.

**Therapy.** Antitoxic therapy with immune sera is applied following a meticulous wound cleaning. The patient's musculature must also be relaxed with curare or similar agents.

**Tetanus**

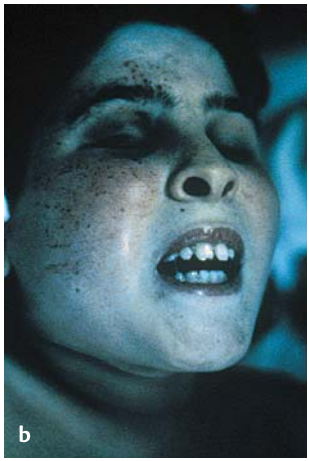




Fig. 4.**8** **a** Open lower-leg fracture following a traffic accident; the portal of entry of *C. tetani*.
**b** Risus sardonicus: fully manifest case of tetanus in a patient with lower-leg fracture. Patient was not vaccinated.



**Epidemiology and prophylaxis.** Tetanus is now rare in developed countries due to widespread vaccination practice with incidence rates of approximately one case per million inhabitants per year. The frequency of occurrence is much higher in developing or underdeveloped countries. Worldwide, about 300 000 persons contract tetanus every year, with a lethality rate of approximately 50%. Thus, the importance of the active vaccination as a protective measure can hardly be overstated (see p. 33 for vaccination schedule). A dose of Td should be administered once every 10 years to sustain protection (p. 33). A booster shot is also required in case of severe injury if the patient's last inoculation was administered longer than five years before, and in case of minor injury longer than 10 years. Human tetanus immunoglobulin (250 IU)

must be administered to severely injured persons with insufficient vaccination protection or if the basic immunization history is uncertain.

## Clostridium botulinum (Botulism)

Foodborne botulism is not an infection, but rather an intoxication, that is, the toxin is ingested with food. Infant botulism involves ingestion of spores and wound botulism results from infection of a wound.

**Toxin.** The very strong botulinum neurotoxin is a heat-labile protein. Seven toxigenic types are differentiated, each of which produces an immunologically distinct form of botulinum toxin. Types A, B, and E cause poisoning in humans. The toxin is a metalloprotease that catalyzes the proteolysis of components of the neuroexocytosis apparatus in the motor end plates, resulting in flaccid paralysis of the musculature.



**Pathogenesis and clinical picture.** Classic botulism results from eating spoiled foods in which the toxin has been produced under anaerobic conditions by *C. botulinum*. The toxin is absorbed in the gastrointestinal tract, and then transported to the peripheral nervous system in the bloodstream.

Within a matter of hours or days paralysis symptoms occur, especially in the nerves of the head. Frequent symptoms include seeing double, difficulty swallowing and speaking, constipation, and dry mucosa. Lethality rates range from 25–70%, depending on the amount of toxin ingested. Death usually results from respiratory paralysis. **Wound botulism** results from wound infection by *C. botulinum* and is very rare. **Infant botulism**, first described in 1976, results from ingestion of spores with food (e.g., honey). Probably due to the conditions prevailing in the intestines of infants up to the age of six months, the spores are able to proliferate there and produce the toxin. The lethality of infant botulism is low (<1%).

**Diagnosis.** Based on toxin detection by means of the mouse neutralization test.

**Therapy.** Urgent administration of a polyvalent antitoxin.

**Epidemiology and prevention.** Botulism is a rare disease. Exposure to the toxin is a food hygiene problem that can be avoided by taking appropriate precautions during food production. Aerosolized botulinum toxin has been used experimentally as a bioweapon.

### Clostridium difficile (Pseudomembranous Colitis)

*C. difficile* occurs in the fecal flora of 1–4% of healthy adults and in 30–50% of children during the first year of life. The factors that lead to development of the disease are not known with certainty. Cases of pseudomembranous colitis are observed frequently under treatment with clindamycin, aminopenicillins, and cephalosporins (hence the designation **antibiotic-associated colitis**), but also occur in persons not taking antibiotics. Occasional outbreaks are seen in hospitals. The **pathological mechanism** is based on formation of two toxins. Toxin A is an enterotoxin that causes a dysfunction characterized by increased secretion of electrolytes and fluids. Toxin B is a cytotoxin that damages the mucosa of the colon.

The **clinical course** includes fever, diarrhea, and spasmodic abdominal pains. Coloscopy reveals edematous changes in the colon mucosa, which is also covered with yellowish-whitish matter. **Laboratory diagnosis** involves culturing the pathogen from patient stool and detection of the cytotoxin in bacteria-free stool filtrates on the basis of a cytopathic effect (CPE) observed in cell cultures, which CPE is then no longer observed after neutralization with an antiserum. Toxins A and B can also be detected with immunological test kits (ELISA tests, see p. 127f.). A specific **therapy** is not required in many cases. Antibiotic treatment is indicated in severe cases. The agent of choice is currently metronidazole.



## Listeria, Erysipelothrix, and Gardnerella

■ ***Listeria monocytogenes*** are diminutive Gram-positive rods with peritrichous flagellation that are quite motile at 20 °C and can be cultured aerobically on blood agar. They occur ubiquitously in nature. Human infections may result if $10^6$–$10^9$ pathogens enter the gastrointestinal tract with food. Listeriae are classic opportunists. In immunocompetent persons, an infection will either be clinically silent or present the picture of a mild flu. In immunocompromised patients, the disease manifests as a primary sepsis and/or meningoencephalitis. More rarely, listeriae cause endocarditis. Listeriosis during pregnancy may result in spontaneous abortion or connatal listeriosis (granulomatosis infantiseptica). Penicillins (amoxicillin) and cotrimoxazole, sometimes in combination with aminoglycosides, are used in therapy. Listeriosis is a rare infection characterized by sporadic occurrence. Occasional gastrointestinal epidemics due to contaminated food may result from the coincidence of unfortunate circumstances.

***Erysipelothrix rhusiopathiae***, the pathogen that causes the zoonosis swine erysipelas, is the causative organism in the human infection erysipeloid, now a rare occupational disease.

***Gardnerella vaginalis*** is usually responsible, in combination with other bacteria, for nonspecific vaginitis (vaginosis).

## Listeria monocytogenes



The only listeriae that cause human disease are *L. monocytogenes* and the rare species *L. ivanovii*. The designation *L. monocytogenes* results from the observation that infections of rodents, which are much more susceptible than humans, are accompanied by a monocytosis.

**Morphology and culturing.** The small Gram-positive rods feature peritrichous flagellation. They show greater motility at 20 °C than at 37 °C. Culturing is most successful under aerobic conditions on blood agar. Following incubation for 18 hours, small gray colonies surrounded by inconspicuous hemolytic zones appear. The zones are caused by listeriolysin O. Listeriae can also reproduce at 5–10 °C, which fact can be used in their selective enrichment ("cold enrichment").

**Pathogenesis.** Studies of the molecular processes involved have used mainly systemically infected mice.

- **Adherence.** To phagocytic cells (e.g., macrophages) and nonphagocytic cells (e.g., enterocytes).
- **Invasion.** Endocytosis, induced by the protein internalin on the surface of the listeriae. Formation of the endosome.
- **Destruction of the endosome.** The virulence factor listeriolysin forms pores in the endosomal membrane, releasing the listeriae into the cytoplasm.
- **Replication** of the listeriae in the cytoplasm of infected cells.
- **Local intercellular dissemination.** Polymerization of the actin of infected cells at one pole of the listeriae to form so-called actin tails that move the listeriae toward the membrane. Formation of long membrane protuberances (known as listeriopods) containing listeriae. Neighboring cells engulf the listeriopods, whereupon the process of listeria release by means of endosome destruction is repeated.
- **Dissemination is generally** by means of hematogenous spread.

**Clinical characteristics.** Listeriae are classic opportunists. The course of most infections is clinically silent. Symptoms resembling a mild flu do not occur in immunocompetent persons until large numbers of pathogens ($10^6$–$10^9$) enter the gastrointestinal tract with food. Massive infections frequently cause symptoms of gastroenteritis.

Listeriosis can take on the form of a **sepsis** and/or **meningoencephalitis** in persons with T cell defects or malignancies, in alcoholics, during cortisone therapy, during pregnancy, in elderly persons and in infants.

**Connatal listeriosis** is characterized by sepsis with multiple abscesses and granulomas in many different organs of the infant (**granulomatosis infantiseptica**).

The lethality rate in severe cases of listeriosis varies between 10% and 40%. The incubation period can vary from one to three days to weeks.



**Diagnosis** requires pathogen identification by means of microscopy and culturing.

**Therapy.** Amoxicillin, penicillin G, or cotrimoxazole.

**Epidemiology and prevention.** Listeriae occur ubiquitously in soil, surface water, plants, and animals and are also found with some frequency (10%) in the intestines of healthy humans. Despite the fact that contact with listeriae is, therefore, quite normal and even frequent, listeriosis is not at all common. The incidence of severe infections is estimated at six cases per $10^6$ inhabitants per year. Occurrence is generally sporadic. Small-scale epidemics caused by food products—such as milk, milk products (cheese), meat products, and other foods (e.g., coleslaw)—contaminated with very high numbers of listeriae have been described. Preventive measures include proper processing and storage of food products in keeping with relevant hygienic principles.

## Erysipelothrix rhusiopathiae

This bacterium is a slender, nonmotile, Gram-positive rod. *E. rhusiopathiae* causes a septic disease in pigs, swine erysipelas. The correlate in humans is now quite rare and is a recognized occupational disease. Following contact with infectious animal material, the pathogens enter body tissues through dermal injuries. After an incubation period of one to three days, the so-called **erysipeloid**—a hivelike, bluish-red swelling—develops at the site of entry. The lymph nodes are also affected. These benign infections often heal spontaneously and disappear rapidly under treatment with penicillin G. Laboratory diagnostic procedures involve identification of the pathogen in wound secretion using the methods of microscopy and culturing.

### Gardnerella vaginalis

*G. vaginalis* is a Gram-variable, nonmotile, nonencapsulated rod bacterium. Its taxonomy has changed repeatedly in recent decades. It has thus also been designated as *Corynebacterium vaginalis* and *Haemophilus vaginalis*. Based on DNA hybridization, the pathogen is now classified with the regularly shaped, Gram-positive, nonsporing rod bacteria. The natural habitat of this organism is the vagina of sexually mature women. It can also cause vulvovaginitis (vaginosis). *G. vaginalis* is found in over 90 % of women showing the symptoms of this infection, usually together with other bacteria including in particular obligate anaerobes (*Mobiluncus*, *Bacteroides*, *Peptostreptococcus*). The organism can be detected in vaginal discharge by means of microscopy and culturing. In the microscopic analysis, so-called clue cells (vaginal epithelia densely covered with Gram-labile rods) provide evidence of the role played by *G. vaginalis*. This bacterium can be cultured on blood-enriched agar incubated in an atmosphere containing 5 % $CO_2$. The therapeutic agent of choice is metronidazole.



## Corynebacterium, Actinomyces, Other Gram-Positive Rod Bacteria

**Diphtheria bacteria** are pleomorphic, club-shaped rod bacteria that often have polar bodies and group in V, Y, or palisade forms. They can be grown on enriched nutrient media. Their pathogenicity derives from diphtheria toxin, which binds to receptors of sensitive cells with the B fragment. Once the binding process is completed, the active A fragment invades the cell. This substance irreversibly blocks translation in the protein biosynthesis chain. The toxin gene is a component of the β prophage. Local and systemic intoxications are differentiated when evaluating the clinical picture. Local infection usually affects the tonsils, on which the diphtherial pseudomembrane develops. Systemic intoxications affect mainly the liver, kidneys, adrenal glands, cardiac muscle, and cranial nerves. Laboratory diagnosis is based on pathogen identification. The most important treatment is antitoxin therapy. Diphtheria occurs only in humans. Thanks to extensive diphtheria toxoid vaccination programs, it is now rare.

**Actinomycetes** are part of the normal mucosal flora. These are Gram-positive rods that often occur in the form of branched filaments in young cultures. Conglomerates of microcolonies in pus form so-called sulfur granules. Actinomycetes are obligate anaerobes. The pathogens enter body tissues through mucosa defects. Monoinfections are rare, the most frequent case being actinomycetes-dominated endogenous polyinfections. Cervicofacial