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

actinomycosis, caused by oral cavity colonizer *A. israelii*, is the most frequent form of actinomycosis. Treatment includes surgical procedures and antibiosis with aminopenicillins.

The group of Gram-positive, irregular (pleomorphic), nonsporing rod bacteria includes many different genera that are normal components of the skin and mucosal flora (Table 4.**3**, p. 261). Pathogens in this group cause two characteristic diseases: diphtheria, caused by *Corynebacterium diphtheriae* and actinomycosis, caused mainly by *Actinomyces israelii*.

Corynebacterium diphtheriae (Diphtheria)

Morphology and culturing. Diphtheria bacteria are Gram-positive, pleomorphic, often club-shaped rods. The individual cells tend to group in V, Y, or palisade arrangements (Fig. 4.**9**). Neisser staining reveals the polar bodies (polyphosphates stored at one end of the rod).

Löffler nutrient medium, which consists of coagulated serum and nutrient broth, is still used for the primary cultures. Selective indicator mediums containing tellurite are used in selective culturing. K tellurite is used to inhibit the accompanying flora. The K tellurite is also reduced to tellurium, coloring the colonies a brownish black.

Extracellular toxin. Diphtheria toxin consists of two functionally distinct fragments, A and B, whereby **B** stands for **binding** to receptors of target cells and **A** stands for toxic **activity**. Fragment A irreversibly blocks protein synthesis translation in the target cells, which then die. The toxin gene is always a prophage genome component (see lysogenic conversion, p. 186).

Corynebacterium diphtheriae

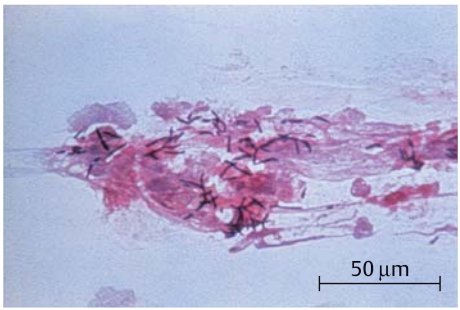


Fig. 4.**9** Gram staining of a wound secretion preparation in wound diphtheria: typical configuration of Gram-positive rods of irregular thickness, often with a clublike enlargement at one end.

Diphtheria toxin

Fragment A is an ADP ribosyl transferase. The enzyme transfers adenosine diphosphate ribose from NAD to the elongation factor eEF2, thereby inactivating it:

$$NAD + eEF2 \rightarrow ADP\ ribosyl\ eEF2 + nicotinamide + H^+$$

eEF2 "translocates" the peptidyl tRNA from the amino acid position A to the peptide position P on the eukaryotic ribosome. Although the toxin gene is integrated in a phage genome, its activity is regulated by the gene product DtxR of the *dtxR* gene of the bacterial cell's genome. DtxR combines with Fe^{2+} to become an active repressor that switches off the transcription of the toxin gene.

4

Pathogenesis and Clinical Picture

Local infection. Infection of the mucosa of tonsils, pharynx, nose, and conjunctiva (Fig. 4.**10**). Wounds and skin lesions can also be infected. The pathogens invade the host through these portals, reproduce, and produce toxin, resulting in local cell damage. The inflammatory reaction leads to collection of a grayish-white exudate, the matrix of the "diphtherial pseudomembrane" consisting of fibrin, dead granulocytes, and necrotic epithelial cells. This coating adheres quite strongly to the mucosa. It may extend into the larynx, thus eventually hindering respiration. Regional lymph nodes are highly swollen.

Systemic intoxication. Parenchymal degeneration in the cardiac muscle, liver, kidneys, and adrenal glands. Motor cranial nerve paralysis. Late sequel damage due to the intoxication is frequently seen after the acute infection has subsided.

Toxin-negative strains of *C. diphtheriae* are occasionally observed as pathogens in endocarditis or dermal infections. The pathogenicity of such strains corresponds to that of commensal corynebacteria (see Table 4.**3**, p. 261).

Diagnosis. The method of choice is detection and identification of the pathogen in cultures from local infection foci. The culture smear, which arrives at the laboratory in transport medium, is plated out on Löffler medium and a selective indicator medium. Identification is based on both morphological and physiological characteristics. The toxin is detected by the Elek-Ouchterlony immunodiffusion test. A molecular method is now also being used to identify the toxin gene. Toxin detection is necessary for a laboratory diagnosis of diphtheria because of the occurrence of toxin-negative strains.

Therapy. Antitoxic serum therapy is the primary treatment and it must commence as soon as possible if diphtheria is suspected. This treatment is supplemented by administration of penicillin or erythromycin.

Nose and Throat (Nasopharyngeal) Diphtheria

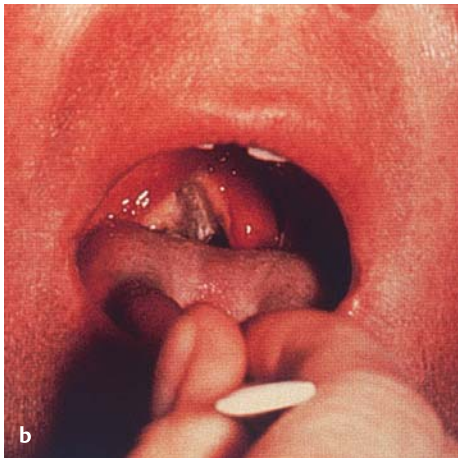


Fig. 4.**10** **a** Hemorrhaging of the nasal mucosa (endothelial damage). Pronounced cervical adenopathy and swelling, creating a bull neck appearance. **b** Thick coating (membrane) on highly swollen tonsils (so-called diphtherial pseudomembrane), causing respiratory stridor.

Epidemiology and prevention. Humans are the sole *pathogen reservoir* for diphtheria. *Infection sources* include infected persons and carriers (rare). The disease is usually *transmitted* by droplet infection, or less frequently indirectly via contaminated objects. The *incubation period* is two to five days. Incidence levels in central Europe are low. From 1975 to 1984, only 113 cases were reported in Germany. Incidence levels are higher in other countries (Russia). *Protective immunization* with diphtheria toxoid is the most important preventive measure (see Table 1.**13**, p. 33). *Exposure prophylaxis* involves isolation of infected persons until two cultures from specimens taken at least 24 hours apart are negative.

Actinomyces

Actinomycetes are Gram-positive bacteria that tend to grow in the form of branched filaments. The resulting mycelial masses are, however, not observed in older cultures, which strongly resemble those of corynebacteria in their morphology.

Occurrence. Actinomycetes are part of the normal mucosal flora in humans and animals. They colonize mainly the oral cavity, and an actinomycosis infection is therefore always endogenous. Ninety percent of actinomycetes infections in humans are caused by *A. israelii*, with far fewer cases caused by *A. naeslundii* and other species.

Morphology and culture. Actinomycetes are Gram-positive, pleomorphic rod bacteria that sometimes also show genuine branching (Fig. 4.**11**). The yellowish **sulfur granules**, measuring 1–2 mm, can be observed macroscopically in actinomycetes pus. These particles are conglomerates of small *Actinomyces* colonies surrounded by a wall of leukocytes. Mycelial filaments extend radially from the colonies (actinium = Greek for raylike). Culturing the organism requires enriched mediums and an anaerobic milieu containing 5–10% CO_2. Mycelial microcolonies form only during the first days. Whitish macrocolonies, often with a rough surface, begin to appear after two weeks.

Pathogenesis and clinical picture. The pathogens breach mucosa (perhaps normal dermis as well) and are able to establish themselves in tissue in the presence of a low redox potential. The factors responsible for these conditions include poor blood perfusion and, above all, contributing bacterial

Actinomyces israelii

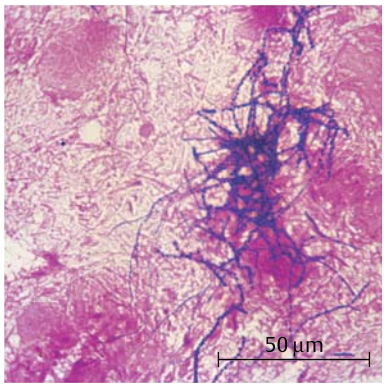


Fig. 4.**11** Gram staining of a pus preparation in cervicofacial actinomycosis: mass of Gram-positive, branched rods; next to them mixed Gram-negative flora. Tentative clinical diagnosis: actinomycosis.

pathogens. Genuine actinomycoses are actually always polymicrobial. The mixed flora found includes mainly the anaerobes of the oral cavity. *Actinobacillus actinomycetemcomitans* is frequently isolated along with various species of *Bacteroidaceae*. Facultative anaerobes such as staphylococci, streptococci, and *Enterobacteriaceae* are, however, also found among the contributing flora.

- **Cervicofacial actinomycosis.** This is the most frequent form of actinomycetes infection (>90%). The abscesses are hard and tumorlike at first, then they necrotize. They may also break through to the dermal surface to create fistulae.

- **Thoracic actinomycosis.** This rare form results from aspiration of saliva; sometimes this type also develops from an actinomycosis in the throat or hematogenous spread.

- **Abdominal actinomycosis.** This type results from injuries to the intestine or female genitals.

- **Genital actinomycosis.** May result from use of intrauterine contraceptive devices.

- **Canaliculitis.** An inflammation of the lacrimal canaliculi caused by any of several *Actinomyces* species.

- **Caries.** The *Actinomyces* species involved in caries development are *A. viscosus*, *A. naeslundii*, and *A. odontolyticus* (p. 243f.). A possible contribution to periodontitis is also under discussion.

Diagnosis involves identification of the pathogen by microscopy and culturing in pus, fistula secretion, granulation tissue, or bronchial secretion. The samples must not be contaminated with other patient flora, in particular from the oral cavity and must be transported to the laboratory in special anaerobe containers. Microscopic detection of branched rods suffices for a tentative diagnosis. Detection of mycelial microcolonies on enriched nutrient mediums after one to two weeks further consolidates this diagnosis. Final identification by means of direct immunofluorescence, cell wall analysis, and metabolic analysis requires several weeks.

Therapy. Treatment includes both surgical and antibiotic measures. The antibiotic of choice is an aminopenicillin. Antibiosis that also covers the contributing bacterial pathogens is important.

Epidemiology and prevention. Actinomycoses occur sporadically worldwide. Average morbidity (incidence) levels are between 2.5 and five cases per 100 000 inhabitants per year. Men are infected twice as often as women. Prophylactic considerations are irrelevant due to the endogenous nature of actinomycetes infections.

Other Gram-Positive Rod Bacteria

Table 4.**3** lists bacteria that are rarely involved in infections and normally infect only persons with defective immune defenses. Recent years have seen considerable changes in their classification and nomenclature—still an ongoing process. Many of these bacteria are part of the normal dermal and mucosal flora. They are frequently found in sampled materials as contaminants, but also occasionally cause infections. Some of these bacteria are designated by collective terms such as "diphtheroid rods" or "coryneform bacteria."

Table 4.3 Gram-Positive Rods with (Generally) Low-Level Pathogenicity

Actinomyces pyogenes	Cutaneous and subcutaneous purulent infections.
Arcanobacterium hemolyticum	Purulent dermal infections; pharyngitis?
Corynebacterium ulcerans	Can produce diphtheria toxin and therefore cause diphtherialike clinical symptoms
C. jeikeium	Dermal pathogen. Occasionally isolated from blood, wounds, or intravasal catheters. Often shows multiple antibiotic resistance.
C. xerosis *C. pseudodiphtheriticum*	Rare endocarditis pathogens.
Gordona bronchialis	Colonizes and infects the respiratory tract.
Rhodococcus equi	Infections of the respiratory tract in immunosuppressed persons.
Tsukamurella sp.	Infections of the respiratory tract in immunosuppressed persons; meningitis.
Turicella otitidis	Infections of the ear in predisposed persons.
Propionibacterium acnes *P. granulosum* *P. avidum*	Anaerobic or microaerophilic. Rarely involved in endocarditis. *P. acnes* is thought to be involved in the development of acne.
Eubacterium sp.	Obligate anaerobe. Normal flora of the intestinal tract. Sometimes component of an anaerobic mixed flora.
Tropheryma whipplei (nov. gen.; nov. spec.; formerly *T. whippelii)*	Causal pathogen in Whipple's disease. Culture growth of this organism has not been possible to date. Probable taxonomic classification in proximity to actinomycetes. Little is known about this organism. Rare, chronic systemic disease. Dystrophy of small intestine mucosa (100%). Also involvement of cardiovascular system (55%), respiratory tract (50%), central nervous system (25%), and eyes (10%). Primary clinical symptoms are weight loss, arthralgias, diarrhea, abdominal pain. Microscopic detection and identification in small intestine biopsies, other biopsies or cerebrospinal fluid (PAS staining) or by molecular methods (see p. 216). Cotrimoxazole is the antibiotic agent of choice.
Mobiluncus mulieri *M. curtisii*	Obligate anaerobic. Colonize the vagina; frequently isolated in cases of bacterial vaginosis together with *Gardnerella vaginalis* and other bacteria.

Mycobacterium

■ Mycobacteria are slender rod bacteria that are stained with special differential stains (Ziehl-Neelsen). Once the staining has taken, they cannot be destained with dilute acids, hence the designation acid-fast. In terms of human disease, the most important mycobacteria are the tuberculosis bacteria (TB) *M. tuberculosis* and *M. bovis* and the leprosy pathogen (LB) *M. leprae*.

TB can be grown on lipid-rich culture mediums. Their generation time is 12–18 hours. Initial droplet infection results in **primary tuberculosis**, localized mainly in the apices of the lungs. The primary disease develops with the Ghon focus (Ghon's complex), whereby the hilar lymph nodes are involved as well. Ninety percent of primary infection foci remain clinically silent. In 10% of persons infected, primary tuberculosis progresses to the **secondary** stage (reactivation or organ tuberculosis) after a few months or even years, which is characterized by extensive tissue necrosis, for example pulmonary caverns. The specific immunity and allergy that develop in the course of an infection reflect T lymphocyte functions. The allergy is measured in terms of the tuberculin reaction to check for clinically inapparent infections with TB. Diagnosis of tuberculosis requires identification of the pathogen by means of microscopy and culturing. Modern molecular methods are now coming to the fore in TB detection. Manifest tuberculosis is treated with two to four antituberculé chemotherapeutics in either a short regimen lasting six months or a standard regimen lasting nine months.

In contrast to TB, the LB pathogens do not lend themselves to culturing on artificial nutrient mediums. **Leprosy** is manifested mainly in skin, mucosa, and nerves. In clinical terms, there is a (malignant) lepromatous type leprosy and a (benign) tuberculoid type. Nondifferential forms are also frequent. Humans are the sole infection reservoir. Transmission of the disease is by close contact with skin or mucosa. ■

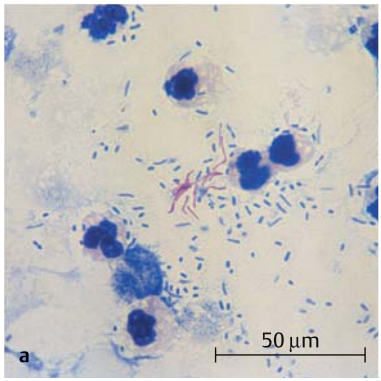
The genus *Mycobacterium* belongs to the *Mycobacteriaceae* family. This genus includes saprophytic species that are widespread in nature as well as the causative pathogens of the major human disease complexes tuberculosis and leprosy. Mycobacteria are Gram-positive, although they do not take gram staining well. The explanation for this is a cell wall structure rich in lipids that does not allow the alkaline stains to penetrate well. At any rate, once mycobacteria have been stained (using radical methods), they resist destaining, even with HCl-alcohol. This property is known as **acid fastness**.

Tuberculosis Bacteria (TB)

History. The tuberculosis bacteria complex includes the species *Mycobacterium tuberculosis*, *M. bovis*, and the rare species *M. africanum*. The clinical etiology of tuberculosis, a disease long known to man, was worked out in 1982 by R. Koch based on regular isolation of pathogens from lesions. Tuberculosis is unquestionably among the most intensively studied of all human diseases. In view of the fact that tuberculosis can infect practically any organ in the body, it is understandable why a number of other clinical disciplines profit from these studies in addition to microbiology and pathology.

Morphology and culturing. TB are slender, acid-fast rods, 0.4 µm wide, and 3–4 µm long, nonsporing and nonmotile. They can be stained with special agents (Ziehl-Neelsen, Kinyoun, fluorescence, p. 212f.) (Fig. 4.**12a**).

Mycobacterium Tuberculosis



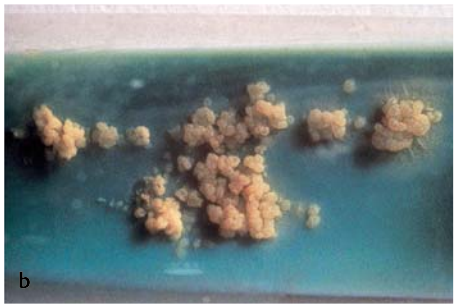


Fig. 4.**12** **a** Ziehl-Neelsen staining of a urine preparation: Fine, red, acid-fast rods, which tend to stick together. Clinical diagnosis: renal tuberculosis.
b Culture of *M. tuberculosis* on egg nutrient substrate according to Löwenstein-Jensen: after four weeks of incubation rough, yellowish, cauliflowerlike colonies.

TB are obligate anaerobes. Their reproduction is enhanced by the presence of 5–10% CO_2 in the atmosphere. They are grown on culture mediums with a high lipid content, e.g., egg-enriched glycerol mediums according to Löwenstein-Jensen (Fig. 4.**12b**). The generation time of TB is approximately 12–18 hours, so that cultures must be incubated for three to six or eight weeks at 37 °C until proliferation becomes macroscopically visible.

Cell wall. Many of the special characteristics of TB are ascribed to the chemistry of their cell wall, which features a murein layer as well as numerous lipids, the most important being the glycolipids (e.g., lipoarabinogalactan), the mycolic acids, mycosides, and wax D.

Glycolipids and wax D.
- Responsible for **resistance** to chemical and physical noxae.
- **Adjuvant effect** (wax D), i.e., enhancement of antigen immunogenicity.
- **Intracellular persistence** in nonactivated macrophages by means of inhibition of phagosome-lysosome fusion.
- **Complement resistance**.
- **Virulence.** Cord factor (trehalose 6,6-dimycolate).

Tuberculoproteins.
- **Immunogens.** The most important of these is the 65 kDa protein.
- **Tuberculin.** Partially purified tuberculin contains a mixture of small proteins (10 kDa). Tuberculin is used to test for TB exposure. Delayed allergic reaction.

Polysaccharides. Of unknown biological significance.

Pathogenesis and clinical picture. It is necessary to differentiate between primary and secondary tuberculosis (reactivation or postprimary tuberculosis) (Fig. 4.**13**). The clinical symptoms are based on reactions of the cellular immune system with TB antigens.

Primary tuberculosis. In the majority of cases, the pathogens enter the lung in droplets, where they are phagocytosed by alveolar macrophages. TB bacteria are able to reproduce in these macrophages due to their ability to inhibit formation of the phagolysosome. Within 10–14 days a reactive inflammatory focus develops, the so-called primary focus from which the TB bacteria move into the regional hilar lymph nodes, where they reproduce and stimulate a cellular immune response, which in turn results in clonal expansion of specific T lymphocytes and attendant lymph node swelling. The Ghon's complex (primary complex, PC) develops between six and 14 weeks after infection. At the same time, granulomas form at the primary infection site and in the affected lymph nodes, and macrophages are activated by the cytokine MAF (macrophage activating factor). A tuberculin allergy also develops in the macroorganism.

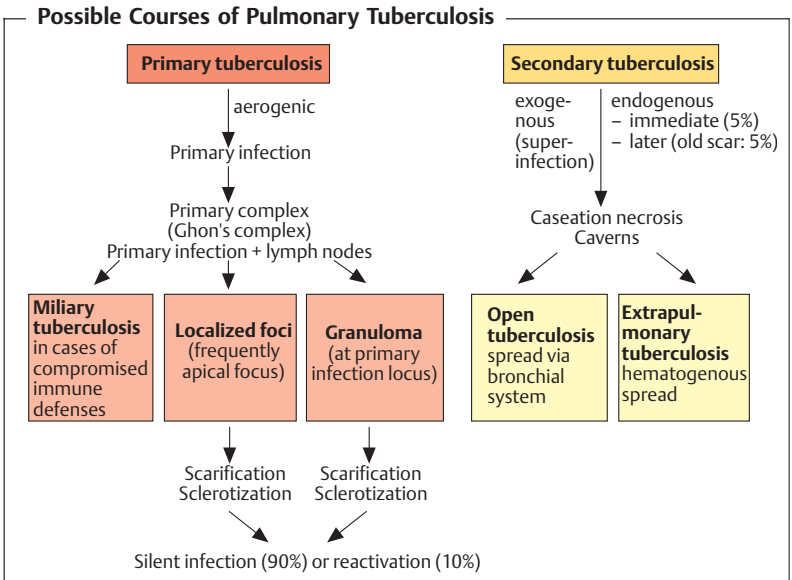


Fig. 4.**13** The primary tuberculosis that develops immediately post infection is clinically silent in most cases thanks to an effective immune response. However, in 10% of cases a so-called secondary tuberculosis develops, either immediately or years later, and may spread to the entire bronchial system or other organ systems.

The further course of the disease depends on the outcome of the battle between the TB and the specific cellular immune defenses. Postprimary dissemination foci are sometimes observed as well, i.e., development of local tissue defect foci at other localizations, typically the apices of the lungs. Mycobacteria may also be transported to other organs via the lymph vessels or bloodstream and produce dissemination foci there. The host eventually prevails in over 90% of cases: the granulomas and foci fibrose, scar, and calcify, and the infection remains clinically silent.

■ **Secondary tuberculosis.** In about 10% of infected persons the primary tuberculosis reactivates to become an organ tuberculosis, either within months (5%) or after a number of years (5%). Exogenous reinfection is rare in the populations of developed countries. Reactivation begins with a caseation necrosis in the center of the granulomas (also called tubercles) that may progress to cavitation (formation of caverns). Tissue destruction is caused by cytokines, among which tumor necrosis factor α (TNFα) appears

to play an important role. This cytokine is also responsible for the cachexia associated with tuberculosis. Reactivation frequently stems from old foci in the lung apices.

The body's immune defenses have a hard time containing necrotic tissue lesions in which large numbers of TB cells occur (e.g., up to 10^9 bacteria and more per cavern); the resulting lymphogenous or hematogenous dissemination may result in infection foci in other organs. Virtually all types of organs and tissues are at risk for this kind of secondary TB infection. Such infection courses are subsumed under the term extrapulmonary tuberculosis.

Immunity. Humans show a considerable degree of genetically determined resistance to TB. Besides this inherited faculty, an organism acquires an (incomplete) specific immunity during initial exposure (first infection). This acquired immunity is characterized by localization of the TB at an old or new infection focus with limited dissemination (Koch's phenomenon). This immunity is solely a function of the T lymphocytes. The level of immunity is high while the body is fending off the disease, but falls off rapidly afterwards. It is therefore speculated that resistance lasts only as long as TB or the immunogens remain in the organism (= infection immunity).

Tuberculin reaction. Parallel to this specific immunity, an organism infected with TB shows an altered reaction mechanism, the tuberculin allergy, which also develops in the cellular immune system only. The tuberculin reaction, positive six to 14 weeks after infection, confirms the allergy. The tuberculin proteins are isolated as purified tuberculin (PPD = purified protein derivative). Five tuberculin units (TU) are applied intracutaneously in the tuberculin test (Mantoux tuberculin skin test, the "gold standard"). If the reaction is negative, the dose is sequentially increased to 250 TU. A positive reaction appears within 48 to 72 hours as an inflammatory reaction (induration) at least 10 mm in diameter at the site of antigen application. A positive reaction means that the person has either been infected with TB or vaccinated with BCG. It is important to understand that a positive test is not an indicator for an active infection or immune status. While a positive test person can be assumed to have a certain level of specific immunity, it will by no means be complete. One-half of the clinically manifest cases of tuberculosis in the population are secondary reactivation tuberculoses that develop in tuberculin-positive persons.

Diagnosis requires microscopic and cultural identification of the pathogen or pathogen-specific DNA.

Traditional method

■ **Workup of test material**, for example with *N*-acetyl-L-cysteine-NaOH (NALC-NaOH method) to liquefy viscous mucus and eliminate rapidly prolif-

erating accompanying flora, followed by centrifugation to enrich the concentration.

- **Microscopy.** Ziehl-Neelsen and/or auramine fluorescent staining (p. 212). This method produces rapid results but has a low level of sensitivity ($>10^4$–10^5/ml) and specificity (acid-fast rods only).

- **Culture** on special solid and in special liquid mediums. Time requirement: four to eight weeks.

- **Identification.** Biochemical tests with pure culture if necessary. Time requirement: one to three weeks.

- **Resistance test** with pure culture. Time requirement: three weeks.

Rapid methods. A number of different rapid TB diagnostic methods have been introduced in recent years that require less time than the traditional methods.

- **Culture.** Early-stage growth detection in liquid mediums involving identification of TB metabolic products with highly sensitive, semi-automated equipment. Time requirement: one to three weeks. Tentative diagnosis.

- **Identification.** Analysis of cellular fatty acids by means of gas chromatography and of mycolic acids by means of HPLC. Time requirement: 12 days with a pure culture.

- **DNA probes.** Used to identify *M. tuberculosis* complex and other mycobacteria. Time requirement: several hours with a pure culture.

- **Resistance test.** Use of semi-automated equipment (see above). Proliferation/nonproliferation determination in liquid mediums containing standard antituberculotic agents (Table 4.**4**). Time requirement: 7–10 days.

Table 4.**4** Scheme for Chemotherapy of Tuberculosis

	Standard scheme	Months	Short scheme *	Months
Initial phase	isoniazid (INH) rifampicin (RMP) ethambutol (EMB)	2	isoniazid rifampicin ethambutol pyrazinamide (PZA)	2
Continuation phase	isoniazid rifampicin	7	isoniazid rifampicin	4

* Alternative in cases of confirmed INH sensitivity or mild clinical picture: initial treatment with a combination of fixed INH + RMP + PZA for two months

Direct identification in patient material. Molecular methods used for direct detection of the *M. tuberculosis* complex in (uncultured) test material. These methods involve amplification of the search sequence.

Therapy. The previous method of long-term therapy in sanatoriums has been replaced by a standardized chemotherapy (see Table 4.**4** for examples), often on an outpatient basis.

Epidemiology and prevention. Tuberculosis is endemic worldwide. The disease has become much less frequent in developed countries in recent decades, where its **incidence** is now about five to 15 new infections per 100 000 inhabitants per year and **mortality** rates are usually below one per 100 000 inhabitants per year. Seen from a worldwide perspective, however, tuberculosis is still a major medical problem. It is estimated that every year approximately 15 million persons contract tuberculosis and that three million die of the disease. The main **source of infection** is the human carrier. There are no healthy carriers. Diseased cattle are not a significant source of infection in the developed world. **Transmission** of the disease is generally direct, in most cases by droplet infection. Indirect transmission via dust or milk (udder tuberculosis in cattle) is the exception rather than the rule. The **incubation period** is four to 12 weeks.

Exposure prophylaxis. Patients with open tuberculosis must be isolated during the secretory phase. Secretions containing TB must be disinfected. Tuberculous cattle must be eliminated.

Disposition prophylaxis. An active vaccine is available that reduces the risk of contracting the disease by about one-half. It contains the live vaccine BCG (lyophilized bovine TB of the Calmette-Guérin type). Vaccination of tuberculin-negative persons induces allergy and (incomplete) immunity that persist for about five to 10 years. In countries with low levels of tuberculosis prevalence, the advisory committees on immunization practices no longer recommend vaccination with BCG, either in tuberculin-negative children at high risk or in adults who have been exposed to TB. Preventive chemotherapy of clinically inapparent infections (latent tuberculosis bacteria infection, LTBI) with INH (300 mg/d) over a period of six months has proved effective in high-risk persons, e.g., contact persons who therefore became tuberculin-positive, in tuberculin-positive persons with increased susceptibility (immunosuppressive therapy, therapy with corticosteroids, diabetes, alcoholism) and in persons with radiologically confirmed residual tuberculosis. Compliance with the therapeutic regimen is a problem in preventive chemotherapy.

Leprosy Bacteria (LB)

Morphology and culture. *Mycobacterium leprae* (Hansen, 1873) is the causative pathogen of leprosy. In morphological terms, these acid-fast rods are identical to tuberculosis bacteria. They differ, however, in that they cannot be grown on nutrient mediums or in cell cultures.

Pathogenesis. The pathomechanisms of LB are identical to those of TB. The host organism attempts to localize and isolate infection foci by forming granulomas. Leprous granulomas are histopathologically identical to tuberculous granulomas. High counts of leprosy bacteria are often found in the macrophages of the granulomas.

Immunity. The immune defenses mobilized against a leprosy infection are strictly of the cellular type. The lepromin skin test can detect a postinfection allergy. This test is not, however, very specific (i.e., positive reactions in cases in

Tuberculoid Leprosy

Fig. 4.**14** Tuberculoid leprosy is the benign, nonprogressive form of the disease, characterized by spotty dermal depigmentations.

Lepromatous Leprosy

Fig. 4.**15** In lepromatous leprosy, nodular dermal and mucosal lesions develop. Nerve inflammation and neuroparalysis follow, eventually resulting in mutilations.

which no leprosy infection is present). The clinically differentiated infection course forms observed are probably due to individual immune response variants.

Clinical picture. Leprosy is manifested mainly on the skin, mucosa, and peripheral nerves.

A clinical differentiation is made between tuberculoid leprosy (TL, Fig. 4.**14**) and lepromatous leprosy (LL, Fig. 4.**15**). There are many intermediate forms. TL is the benign, nonprogressive form characterized by spotty dermal lesions. The LL form, on the other hand, is characterized by a malignant, progressive course with nodular skin lesions and cordlike nerve thickenings that finally lead to neuroparalysis. The inflammatory foci contain large numbers of leprosy bacteria.

Diagnosis. Detection of the pathogens in skin or nasal mucosa scrapings under the microscope using Ziehl-Neelsen staining (p. 212). Molecular confirmation of DNA sequences specific to leprosy bacteria in a polymerase chain reaction is possible.

Therapy. Paucibacillary forms are treated with dapson plus rifampicin for six months. Multibacillary forms require treatment with dapson, rifampicin, and clofazimine over a period of at least two years.

Epidemiology and prevention. Leprosy is now rare in socially developed countries, although still frequent in developing countries. There are an estimated 11 million victims worldwide. Infected humans are the only source of infection. The details of the transmission pathways are unknown. Discussion of the topic is considering transmission by direct contact with skin or mucosa injuries and aerogenic transmission. The incubation period is 2–5–20 years. Isolation of patients under treatment is no longer required. An effective epidemiological reaction requires early recognition of the disease in contact persons by means of periodical examinations every six to 12 months up to five years following contact.

Nontuberculous Mycobacteria (NTM)

Mycobacteria that are neither tuberculosis nor leprosy bacteria are categorized as atypical mycobacteria (old designation), nontuberculous mycobacteria (NTM) or MOTT (mycobacteria other than tubercle bacilli).

Morphology and culture. In their morphology and staining behavior, NTM are generally indistinguishable from tuberculosis bacteria. With the exception of the rapidly growing NTM, their culturing characteristics are also similar to TB. Some species proliferate only at 30 °C. NTM are frequent inhabitants of the natural environment (water, soil) and also contribute to human and animal mucosal flora. Most of these species show resistance to the antituberculoid agents in common use.

Clinical pictures and diagnosis. Some NTM species are apathogenic, others can cause mycobacterioses in humans that usually follow a chronic course (Table 4.**5**). NTM infections are generally rare. Their occurrence is encouraged by compromised cellular immunity. Frequent occurrence is observed together with certain malignancies, in immunosuppressed patients and in AIDS patients, whereby the NTM isolated in 80% of cases are *M. avium* or *M. intercellulare*. As a rule, NTM infections are indistinguishable from tuberculous lesions in clinical, radiological, and histological terms. Diagnosis therefore requires culturing and positive identification. The clinical significance of a positive result is difficult to determine due to the ubiquitous occurrence of these pathogens. They are frequent culture contaminants. Only about 10% of all persons in whom NTM are detected actually turn out to have a mycobacteriosis.

Therapy. Surgical removal of the infection focus is often indicated. Chemotherapy depends on the pathogen species, for instance a triple combination (e.g., INH, ethambutol, rifampicin) or, for resistant strains, a combination of four or five antituberculoid agents.

Table 4.5 Infections Caused by Nontuberculous Mycobacteria

Disease	Frequent species	Rare species
Chronic pulmonary disease (adults)	*M. kansasii* *M. avium/M. intracellulare (M. avium complex)* *M. abscessus*	*M. malmoense* *M. xenopi* *M. scrofulaceum* *M. fortuitum* *M. chelonae* and others
Local lymphadenitis (children, adolescents)	*M. avium complex*	*M. kansasii,* *M. malmoense* *M. fortuitum*
Skin and soft tissue infections	*M. marinum* *M. fortuitum* *M. chelonae* *M. ulcerans*	*M. haemophilum* *M. smegmatis* *M. hansasii*
Bone, joint, tendon infections	*M. kansasii* *M. avium complex* *M. fortuitum* *M. abscessus*	*M. smegmatis* *M. chelonae* *M. marinum* *M. malmoense*
Disseminated diseases in immunocompromised patients	*M. kansasii* *M. avium complex*	*M. fortuitum* *M. chelonae* *M. genavense* *M. xenopi* and others

Nocardia

Occurrence. The genus *Nocardia* includes species with morphology similar to that of the actinomycetes, differing from them in that the natural habitat of these obligate aerobes is the soil and damp biotopes. The pathogens known for involvement in nocardioses, a generally very rare type of infection, include *N. asteroides*, *N. brasiliensis*, *N. farcinia*, *N. nova*, and *N. otitidiscaviarum*.

Morphology and culture. Nocardia are Gram-positive, fine, pleomorphic rods that sometimes show branching. They can be cultured on standard nutrient mediums and proliferate particularly well at 30 °C. Nocardia are obligate aerobes.

Pathogenesis and clinical picture. Nocardia penetrate from the environment into the macroorganism via the respiratory tract or dermal wounds. An infection develops only in patients with predisposing primary diseases directly

affecting the immune defenses. Monoinfections are the rule. There are no typical clinical symptoms. Most cases of infection involve pyogenic inflammations with central necroses. The following types have been described: **pulmonary nocardioses** (bronchial pneumonia, pulmonary abscess), **systemic nocardioses** (sepsis, cerebral abscess, abscesses in the kidneys and musculature), and **surface nocardioses** (cutaneous and subcutaneous abscesses, lymphocutaneous syndrome).

Actinomycetomas are tumorlike processes affecting the extremities, including bone. An example of such an infection is Madura foot, caused by *Nocardia* species, the related species *Actinomadura madurae*, and *Streptomyces somaliensis*. Fungi (p. 355) can also be a causal factor in this clinical picture.

Diagnosis. Detection of the pathogen by means of microscopy and culturing techniques is required in materials varying with the specific disease. Due to the long generation time of these species, cultures have to be incubated for at least one week. Precise identification to differentiate pathogenic and apathogenic species is desirable, but difficult.

Therapy. The anti-infective agents of choice are sulfonamides and cotrimoxazole. Surgery may be required.

Epidemiology and prevention. Nocardioses are rare infections. Annual incidence levels range from about 0.5 to 1 case per 1 000 000 inhabitants. The pathogens, which are present in the natural environment, are carried by dust to susceptible patients. There are no practicable prophylactic measures.

Neisseria, Moraxella, and Acinetobacter

■ Neisseria are Gram-negative, aerobic cocci that are often arranged in pairs. They are typical mucosal parasites that die rapidly outside the human organism. Culturing on enriched nutrient mediums is readily feasible.

Neisseria gonorrheae is the pathogen responsible for gonorrhea ("clap"). Infection results from sexual intercourse. The organisms adhere to cells of the urogenital tract by means of attachment pili and the protein Opa, penetrate into the organism using parasite-directed endocytosis and cause a pyogenic infection, mainly of the urogenital epithelium. An infection is diagnosed mainly by means of microscopy and culturing of purulent secretions. The therapeutic of choice is penicillin G. Alternatives for use against penicillinase-positive gonococci include third-generation cephalosporins and 4-quinolones.

N. meningitidis is a parasite of the nasopharyngeal mucosa. These meningococci cause meningitis and sepsis. Diagnosis involves detection of the

pathogens in cerebrospinal fluid and blood. The disease occurs sporadically or in the form of minor epidemics in children, youths, and young adults. The antibiotics of choice are penicillin G and third-generation cephalosporins.

The family *Neisseriaceae* includes aerobic, Gram-negative cocci and rods (see Table 3.**9**, p. 222), the most important of which are the human pathogens *N. gonorrheae* and *N. meningitidis*. Other species in the genus *Neisseria* are elements of the normal mucosal flora.

Neisseria gonorrheae (Gonorrhea)

Morphology and culture. Gonococci are Gram-negative, coffee-bean-shaped cocci that are usually paired and have a diameter of approximately 1 µm (Fig. 4.**16**). Attachment pili on the bacterial cell surface are responsible for their adhesion to mucosal cells.

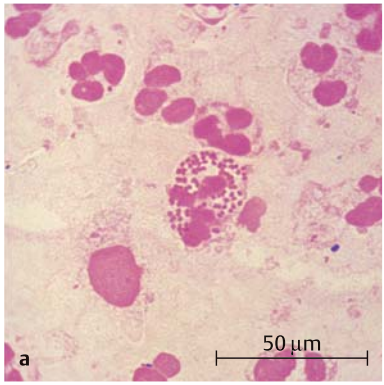
Gonococci can be grown on moist culture mediums enriched with protein (blood). The atmosphere for primary culturing must contain 5–10% CO_2.

Pathogenesis and clinical picture. Gonorrhea is a sexually transmitted disease. The pathogens penetrate into the urogenital mucosa, causing a local purulent infection. In men, the prostate and epididymis can also become infected. In women, the gonococci can also cause salpingitis, oophoritis, or even peritonitis. Gonococci reaching the conjunctival membrane may cause a purulent conjunctivitis, seen mainly in newborn children. Gonococci can also infect the rectal or pharyngeal mucosa. Hematogenously disseminated gonococci may also cause arthritis or even endocarditis.

Determinants of the Pathogenicity of Gonococci

Attachment pili on the surface and the outer membrane protein Opa are responsible for adhesion to cells of the urogenital tract. Opa also directs the invasion process by means of endocytosis. Immune defenses against granulocytes are based on the outer membrane porin Por that prevents the phagosome from fusing with lysosomes, resulting in the survival—and proliferation—of phagocytosed gonococci in granulocytes. The lipo-oligosaccharide (LOS) in the outer membrane is responsible for resistance to complement (serum resistance) as well as for the inflammatory tissue reaction in a manner analogous to the more complexly structured LPS of enterobacteria. Gonococci can capture iron from the siderophilic proteins lactoferrin and transferrin, accumulating it inside the bacterial cells to facilitate their rapid proliferation. An IgA_1 protease produced by the gonococci hydrolyzes secretory antibodies in the mucosal secretions. The pronounced antigen variability of the attachment pili (p. 14) and the Opa protein make it possible for gonococci to thwart specific immune defense mechanisms repeatedly.

Neisseria gonorrhoeae* and *Neisseria meningitidis



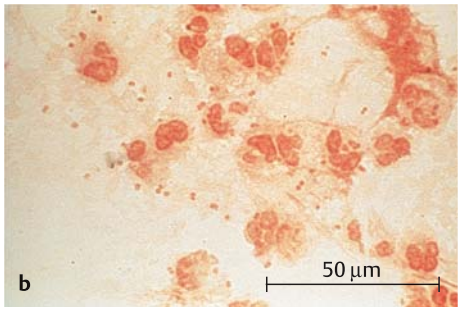


Fig. 4.**16** **a** *N. gonorrheae*: gram staining of a preparation of urethral secretion: coffee-bean-shaped diplococci, grouped within a granulocyte. Clinical diagnosis: gonorrhea. **b** *N. meningitidis*: gram staining of a preparation of cerebrospinal fluid sediment. Clinical diagnosis: acute purulent meningitis.

Diagnosis. The method of choice is detection of the pathogens by means of methylene blue and gram staining and culturing. Gonococci are sensitive in cultures and the material must be used immediately after they are obtained to inoculate Thayer-Martin blood agar with antibiotics added to eliminate accompanying flora, on which medium the cultures are then transported to the laboratory. The identification procedure involves both morphology and biochemical characteristics. Techniques developed recently utilize immunofluorescence or coagglutination methods (p. 217) utilizing monoclonal antibodies to the main protein of the outer membrane, Por.

Direct detection in pus and secretion samples is possible using an enzymatic immunosorbence test or detection of gonococcus-specific DNA sequences coding for rRNA using a gene probe.

Therapy. The agent of choice used to be penicillin G. In recent years, however, the percentage of penicillinase-producing strains has increased considerably all over the world. For this reason, third-generation cephalosporins are now used to treat uncomplicated cases of gonorrhea. They are applied in a single dose (e.g., ceftriaxone, 250–500 mg i.m.). Good results have also been reported with single-dose oral application of fluorinated 4-quinolones (e.g., 0.5 g ciprofloxacin or 0.4 g ofloxacin).

Penicillin Resistance in Gonococci

The determinants of high-level penicillin resistance in gonococci are small, nonconjugative plasmids, which are mobilized by a conjugative helper plasmid for transmission from one gonococcal cell to another. The penicillin resistance plasmids code for the TEM betalactamase that occurs frequently in *Enterobacteriaceae*. It is therefore assumed that the *penicillinase* gene in gonococci derived from the *Enterobacteriaceae* gene pool. Low-level, inherent resistance to penicillin is based on chromosomal genes (*penA*, *penB*) that code for penicillin-binding proteins with reduced affinity to penicillin. These genes are products of mutations.

Epidemiology and prevention. Gonorrhea is a worldwide sexually transmitted disease that occurs only in humans. Its level of annual incidence in developed countries is estimated at 12 cases per 1000 inhabitants. The actual figures are likely to be much higher due to large numbers of unreported cases. A reduction in incidence seen in recent years may be due to AIDS prophylaxis. Protective immunization for high-risk persons is not feasible due to the antigen variability of the organism as described above. Stopping the spread of gonorrhea involves mainly rapid recognition of infections and treatment accordingly.

One hundred percent prevention of ophthalmia neonatorum is possible with a single parenteral dose of 125 mg ceftriaxone. Local prophylaxis is also practiced using a 1% solution of silver nitrate or eye ointments containing 1% tetracycline or 0.5% erythromycin.

Neisseria meningitidis (Meningitis, Sepsis)

Morphology and culture. Meningococci are Gram-negative, coffee-bean-shaped cocci that are frequently pleomorphic and have a diameter of 1 µm (Fig. 4.**16b**). They are nonmotile and feature a polysaccharide capsule.

Growing meningococci in cultures requires mediums containing blood. A concentration of 5–10% CO_2 encourages proliferation.

Antigen structure. Serogroups A, B, C, D, etc. (a total of 12) are differentiated based on the capsule chemistry. Epidemics are caused mainly by strains of

serogroup A, sometimes by B strains as well and, more rarely, by group C strains. Serogroups are divided into serovars based on differences in the outer membrane protein antigens.

Pathogenesis and clinical picture. Meningococci are parasites of the nasopharynx. These microorganisms are carried by 5–10% of the population. If virulent meningococci colonize the nasopharyngeal mucosa of a host lacking the antibodies, pathogen invasion of the mucosa by means of "parasite-directed endocytosis" becomes possible (see p. 12). The CNS is doubtless the preferred compartment for secondary infections, although hematogenously disseminated pathogens can also infect the lungs, the endocardium, or major joints.

Onset of the meningitis is usually sudden, after an incubation period of two to three days, with severe headache, fever, neck stiffness, and severe malaise. Severe hemorrhagic sepsis sometimes develops (Waterhouse-Friedrichsen syndrome).

Diagnosis requires detection of the pathogen in cerebrospinal fluid or blood by means of microscopy and culturing techniques. For success in culturing, the material must be used to inoculate blood agar without delay. Identification of the pathogen is based on identification of metabolic properties. The slide agglutination test is used to determine the serogroup.

Latex agglutination or coagglutination (p. 217) can be used for direct antigen detection in cerebrospinal fluid.

Therapy. The antibiotic of choice is penicillin G. Very good results have also been obtained with third-generation cephalosporins, e.g., cefotaxime or ceftriaxone. It is important to start treatment as quickly as possible to prevent delayed damage.

The advantage of cephalosporins is that they are also effective against other meningitis pathogens due to their broad spectrum of action (with the exception of *Listeria monocytogenes*).

Epidemiology and prevention. Meningococcal infections are more frequent in the winter and spring months. **Transmission** of meningococci is by droplet infection. Humans are the only pathogen reservoir. **Sources of infection** include both carriers and infected persons with manifest disease. In developed countries, meningitis occurs sporadically or in the form of minor epidemics in more or less isolated collectives (work camps, recruiting camps, school camping facilities). The **incidence** level is approximately 12 cases per 100 000 inhabitants per year. In parts of the developing world (African meningitis belt) the level is higher. Lethality runs to 85% if the disease is left untreated, but is reduced to less than 1% if treatment is begun early enough. **Prophylactic antibiosis** is indicated for those in close contact with diseased persons (e.g., in the same family). Prophylactic measures also include treatment of

carriers to eliminate this reservoir, whereby minocylin or rifampicin must be used instead of penicillin G. **Prophylactic immunization** can be achieved with a vaccine made from the purified capsule polysaccharides A, C, Y, and W-135. There is no serogroup B vaccine, since the capsule in serogroup B consists of polyneuraminic acid, which the immune system does not recognize as a foreign substance.

Moraxella and Acinetobacter

The taxonomic definitions of these genera are still inconclusive. Bergey's *Manual of Systematic Bacteriology* groups both under the family *Moraxellaceae*. These bacteria are short, rounded rods, often coccoid, sometimes also diplococcoid. Their natural habitat is either human mucosa (*Moraxella*) or the natural environment (*Acinetobacter*).

- ***Moraxella.*** The genus comprises two medically important species:
  - ***Moraxella catarrhalis.*** Component of the normal flora of the upper respiratory tract. May be responsible for: pneumonia, acute exacerbation of chronic bronchitis, otitis media (up to 20% in children), and sinusitis. About 90% of all strains produce one of the so-called BRO penicillinases, so that therapy with a penicillinase-stable betalactam antibiotic is indicated.
  - ***Moraxella lacunata.*** Formerly *Diplobacterium Morax-Axenfeld*. Can cause conjunctivitis and keratitis. The reason why this organism is now rarely found as a pathogen in these eye infections is unknown.

- ***Acinetobacter.*** In immunodeficient persons, *A. baumannii*, *A. calcoaceticus*, and other species can cause nosocomial infections (urinary tract infections, pneumonias, wound infections, sepsis). Clinical strains of these species often show multiresistance to antibiotics, so that treatment of these infections may prove difficult.

Enterobacteriaceae, Overview

The most important bacterial family in human medicine is the *Enterobacteriaceae*. This family includes genera and species that cause well-defined diseases with typical clinical symptoms (typhoid fever, dysentery, plague) as well as many opportunists that cause mainly nosocomial infections (urinary tract infections, pneumonias, wound infections, sepsis). *Enterobacteriaceae* are Gram-negative, usually motile, facultatively anaerobic rod bacteria. The high levels of metabolic activity observed in them are made use of in identification procedures. The species are subdivided into epidemiologically

significant serovars based on O, H, and K antigens. The most important pathogenicity factors of *Enterobacteriaceae* are colonizing factors, invasins, endotoxin, and various exotoxins. *Enterobacteriaceae* are the most significant contributors to intestinal infections, which are among the most frequent diseases of all among the developing world populace.

Definition and significance. Together with the families *Vibrionaceae* and others (p. 224), the *Enterobacteriaceae* form the group of Gram-negative, facultatively anaerobic rod bacteria. Their natural habitat is the intestinal tract of humans and animals. Some species cause characteristic diseases. While others are facultatively pathogenic, they are still among the bacteria most frequently isolated as pathogens (e.g., *E. coli*). They are often responsible for nosocomial diseases (see p. 343ff.).

Taxonomy. The taxonomy of the *Enterobacteriaceae* has seen repeated changes in recent decades and has doubtless not yet assumed its final form. The family includes 41 genera with hundreds of species. Table 4.**6** provides an overview of the most important *Enterobacteriaceae* in the field of human medicine.

The taxonomic system applied to *Enterobacteriaceae* is based on varying patterns of metabolic processes (Fig. 3.**36**, p. 214). One of the important characteristics of this bacterial family is lactose breakdown (presence of the lac operon). The lac operon includes the genes *lacZ* (codes for β-galactosidase), *lacY* (codes for β-galactoside permease), and *lacA* (codes for transacetylase). Lactose-positive *Enterobacteriaceae* are grouped together as coliform *Enterobacteriaceae*. Salmonellae and most of the shigellae are lactose-negative.

Morphology and culture. *Enterobacteriaceae* are short Gram-negative rods with rounded ends, 0.5–1.5 μm thick, and 24 μm long (Fig. 4.**17a**). Many have peritrichous flagellation. Species with many flagella (e.g., *Proteus* species) show motility on the agar surface, which phenomenon is known as "swarming." Some *Enterobacteriaceae* possess a capsule.

All bacteria in this family can readily be cultured on simple nutrient mediums. They are rapidly growing facultative anaerobes. Their mean generation time in vitro is 20–30 minutes. They show resistance to various chemicals (bile salts, crystal violet), which fact is made use of in selective culturing. Endo agar is an important selective indicator medium; it allows only Gram-negative rod bacteria to grow and indicates lactose breakdown (Fig. 4.**17b**).

Table 4.6 The Most Important Genera/Species/Vars of *Enterobacteriaceae* and the Corresponding Clinical Pictures

Genera/species/var	Disease	Remarks
Salmonella enterica		
S. Typhi	Typhus abdominalis (syn. typhoid fever)	Generalized septic infection
S. Typhimurium *S. Enteritidis* and others	Gastroenteritis (diarrhea)	Profuse watery diarrhea
Shigella	Bacterial dysentery	Diarrhea, abdominal cramping, tenesmus, stool frequently contains blood and mucus
Klebsiella pneumoniae	Pneumonia (Friedländer's)	Severe pneumonia in predisposed persons
Escherichia coli *Citrobacter* *Klebsiella* *Enterobacter* *Serratia* *Proteus* *Providencia* *Morganella* and others	Sepsis, wound infections, infections of the urinary tract and respiratory tract	Facultatively pathogenic bacteria; disease only manifests if host organism immune defenses are weakened; often cause nosocomial infections; frequently resistant to antibiotics
Yersinia		
Y. pestis	Plague	Generalized systemic infection; rare
Y. enterocolitica *Y. pseudotuberculosis*	Enterocolitis, lymphadenitis of the mesenteric lymph nodes	Pseudoappendicitis, reactive arthritis, erythema nodosum
Escherichia coli	Intestinal infections	
Enteropathogenic *E. coli* (EPEC)	Classic infant diarrhea	Epidemics in hospitals, children's homes
Enterotoxic *E. coli* (ETEC)	Diarrhea, choleralike	Cause of travelers' diarrhea (50%)
Enteroinvasive *E. coli* (EIEC)	Dysenterylike	Invasion and verocytotoxins
Enterohemorrhagic *E. coli* (EHEC)	Hemorrhagic colitis	Hemolytic–uremic syndrome (HUS) in 5% of EHEC cases
Enteroaggregative *E. coli* (EAggEC)	Watery diarrhea, mainly in infants	Adhesion to small intestine mucosa; production of a toxin

Escherichia coli

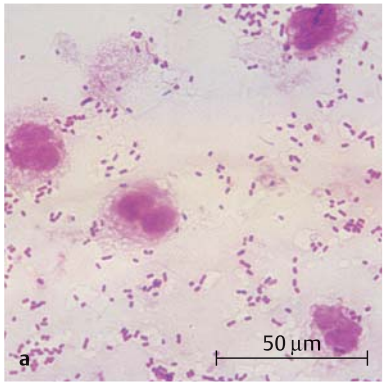


Fig. 4.**17** **a** Gram staining of a urine sediment preparation: rounded gram-negative rods, some coccoid. Clinical diagnosis: acute cystitis. **b** Culture on endo agar, a combined selective/indicator medium. The red color of the colony and agar indicates the lactose breakdown process.

Antigen structure. The most important antigens of the *Enterobacteriaceae* are:

- **O antigens.** Specific polysaccharide chains in the lipopolysaccharide complex of the outer membrane (p. 156).
- **H antigens.** Flagellar antigens consisting of protein.
- **K antigens.** Linear polymers of the outer membrane built up of a repeated series of carbohydrate units (sometimes proteins as well). They can cover the cell densely and render them O inagglutinable (p. 155).
- **F antigens.** Antigens of protein attachment fimbriae.

Pathogenicity determinants. A number of factors are known to play a role in the pathogenicity of various *Enterobacteriaceae* infections. The most important are:

- **Adhesion factors.** Attachment fimbriae, attachment pili, colonizing factor antigens (CFAs).
- **Invasive factors.** Proteins localized in the outer membrane (invasins) that facilitate the invasion of target cells.
- **Exotoxins.**
  - *Enterotoxins* disturb the normal functioning of enterocytes. Stimulation of adenylate or guanylate cyclase; increased production of cAMP (see p. 298). This results in the loss of large amounts of electrolytes and water.
  - *Cytotoxins* exert a direct toxic effect on cells (enterocytes, endothelial cells).
- **Endotoxin.** Toxic effect of lipoid A as a component of LPS (p. 156).
- **Serum resistance.** Resistance to the membrane attack complex C5b6789 of the complement system (p. 86ff.).
- **Phagocyte resistance.** Makes survival in phagocytes possible. Resistance against defensins and/or oxygen radicals (p. 23).
- **Cumulation of Fe^{2+}.** Active transport of Fe^{2+} by siderophores in the bacterial cell (p. 13).

Salmonella (Gastroenteritis, Typhoid Fever, Paratyphoid Fever)

All salmonellae are classified in the species *Salmonella enterica* with seven subspecies. Nearly all human pathogen salmonellae are grouped under *S. enterica*, subsp. *enterica*. Salmonellae are further subclassified in over 2000 serovars based on their O and H antigens, which used to be (incorrectly) designated as species.

Typhoid salmonelloses are caused by the serovars *typhi* and *paratyphi A*, *B*, and *C*. The salmonellae are taken up orally and the invasion pathway is through the intestinal tract, from where they enter lymphatic tissue, first spreading lymphogenously, then hematogenously. A generalized septic clinical picture results. Human carriers are the only source of infection. Transmission is either direct by smear infection or indirect via food and drinking water. Anti-infective agents are required for therapy (ampicillin, cotrimoxazole, 4-quinolones). An active vaccine is available to protect against typhoid fever.

Enteric salmonelloses develop when pathogens are taken up with food. The primary infection source is usually livestock. These relatively frequent infections remain restricted to the gastrointestinal tract. Treatment with antiinfective agents is necessary in exceptional cases only.

Taxonomy. The salmonellae that cause significant human disease are classified in most countries under the taxon *Salmonella enterica,* subsp. *enterica* (synonymous with *S. choleraesuis*, subsp. *choleraesuis*). However, this nomenclature has still not been officially adopted by the *Enterobacteriaceae* Subcommittee. *Salmonella enterica*, spp. *enterica* includes over 2000 serovars, which were formerly (incorrectly) designated with species names. The serovars are capitalized to differentiate them from species.

Taxonomy of the Salmonellae

The problems involved in the taxonomy and nomenclature of this group of bacteria can only be understood in the historical perspective. At first, the genus *Salmonella* appeared to comprise species that differed only in their antigen structures. Species names were therefore used for what turned out to be serovars. More recent molecular studies have demonstrated that the genus *Salmonella* contains only a single species that can be subdivided into seven subspecies. All of the important human pathogen salmonellae belong to the subspecies *enterica*. The (false) species names for the serovars had, however, already become normal usage. In view of the fact that the causative pathogens in typhoid salmonelloses, a clinical picture clearly differentiated from *Salmonella* gastroenteritis, are only serovars of the same species/subspecies, the official committee has, however, not adopted the new nomenclature as yet.

The serovars are determined by O and H antigens. The Kauffman–White scheme is used to arrange them (see Table 4.**7** for an excerpt).

This taxonomic arrangement classifies the serovars in groups characterized by certain O antigens (semibold). This results in a clinically and epidemiologically useful grouping, since certain serovars are responsible for typhoid salmonelloses and others for enteric salmonelloses. The serovars are determined by means of antisera in the slide agglutination test.

Phase Variations of the H Antigens

H antigens occur with two different antigen structures. The primary structure of flagellin is determined by two genes on the chromosome, only one of which is read off. Whether a gene is read off or not is determined by spontaneous inversion of a DNA sequence before the *H2* gene, which inversion occurs with a frequency of approximately 10^{-4} per cell division (Fig. 4.**18**).

Table 4.**7** Excerpt from the Kauffmann–White Scheme which Covers Over 2000 Serovars

Group	Serovar	O antigens	H antigens Phase 1	Phase 2
A	*Paratyphi A*	1, **2**, 12	a	–
B	*Schottmuelleri* (syn. *Paratyphi B)*	1, **4**, (5), 12	b	1, 2
	Typhimurium	1, **4**, (5), 12	i	1, 2
C1	*Hirschfeldii* (syn. *Paratyphi C)*	**6**, 7, (Vi)	c	1, 5
	Choleraesuis	**6**, 7	(c)	1, 5
C2	*Newport*	**6**, 8	e, h	1, 2
D1	*Typhi*	**9**, 12, (Vi)	d	–
	Enteritidis	1, **9**, 12, (Vi)	g, m	(1, 7)
	Dublin	1, **9**, 12, (Vi)	g, p	–
	Gallinarum	1, **9**, 12	–	–
	Panama	1, **9**, 12	l, v	1, 5
E1	*Oxford*	**3**, 10	a	1, 7

Parentheses indicate that the antigen is often not present. The Vi antigen is, strictly speaking, actually a K antigen. The numbers in bold type indicate the antigen that characterizes the O group.

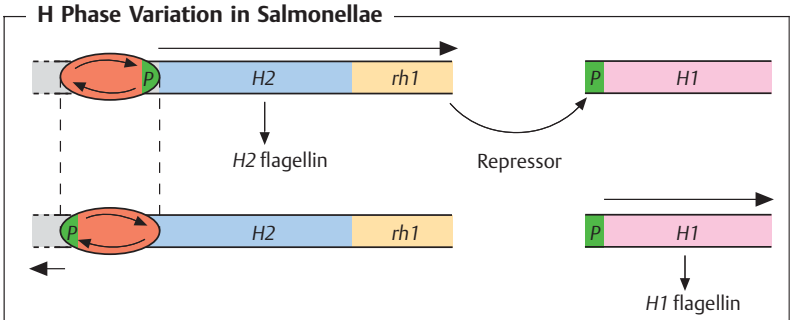


Fig. 4.**18** *P* = promoter, *H2* = H2 phase gene, *H1* = H1 phase gene, *rh1* = regulator gene for *H1, hin* = gene coding for the DNA invertase. The horizontal arrows show the direction in which the genetic information is read off.

Pathogenesis and clinical pictures. Salmonellae are classified as either typhoid or enteric regarding the relevant clinical pictures and epidemiologies. It is not known why typhoid salmonellae only cause systemic disease in humans, whereas enteric salmonella infections occur in animals as well and are usually restricted to the intestinal tract.

Typhoid salmonelloses. Attachment of typhoid salmonellae to cells of the jejunum (M cells). Invasion by means of endocytosis, transfer, and exocytosis. Phagocytosis in the subserosa by macrophages and translocation into the mesenteric lymph nodes. Proliferation occurs. Lymphogenous and hematogenous dissemination. Secondary foci in the spleen, liver, bone marrow, bile ducts, skin (roseola), Peyer's patches.

Manifest illness begins with fever, rising in stages throughout the first week to 39/40/41 °C. Further symptoms: stupor (typhos [greek] = fog), leukopenia, bradycardia, splenic swelling, abdominal roseola, beginning in the third week diarrhea, sometimes with intestinal bleeding due to ulceration of the Peyer's patches.

Enteric salmonelloses. Attachment to enterocytes of the ileum and colon. Invasion of mucosa induced by invasin proteins on the surface of the salmonella cells. Persistence in epithelial cells, possibly in macrophages as well. Production of *Salmonella* enterotoxin. Local inflammation. Manifest illness usually begins suddenly with diarrhea and vomiting, accompanied in some cases by high fever. The symptoms abate after several days without specific therapy. In cases of massive diarrhea, symptoms may be observed that result from the loss of water and electrolytes (Table 4.**8**).

Diagnosis. The method of choice is detection of the pathogens in cultures. Selective indicator mediums are used to isolate salmonellae in stool. Identification is done using metabolic patterns (see Fig. 3.**36**, p. 214). Serovar classification is determined with specific antisera in the slide agglutination test. Culturing requires at least two days. Typhoid salmonelloses can be diagnosed indirectly by measuring the titer of agglutinating antibodies to O and H antigens (according to Gruber-Widal). To provide conclusive proof the titer must rise by at least fourfold from blood sampled at disease onset to a sample taken at least one week later.

Therapy. Typhoid salmonelloses must be treated with anti-infective agents, whereas symptomatic treatment will suffice for enteric infections. Symptomatic treatment encompasses slowing down intestinal activity (e.g., with loperamide) and replacing fluid and electrolyte losses orally as required (WHO formula: 3.5 g NaCl, 2.5 g $NaHCO_3$, 1.5 g KCl, 20 g glucose per liter of water).

Table 4.**8** Overview of the Most Important Differences between Typhoid and Enteric Salmonellae and Salmonelloses

Parameter	Typhoid salmonellae/salmonelloses	Enteric salmonellae/salmonelloses
Serovars	*Typhi*; *Paratyphi A*, *B*, *C* (see Table 4.**7**)	Often *Enteritidis* and *Typhimurium*; more rarely: numerous other serovars
Infection spectrum	Humans	Animals and humans
Source of infection	Humans: infected persons, chronic carriers	Mainly livestock; possibly humans as well
Mode of infection	Oral	Oral
Transmission	Indirect: water, contaminated food Direct: smear infection	Indirect: contaminated food
Infective dose	Small: 10^2–10^3 bacteria	Large: $>10^6$ bacteria; in most cases proliferation in food
Incubation time	1–3 weeks	1–2 days
Clinical picture	**Generalized infection.** Sepsis	**Acute diarrhea with vomiting.** Fever. Self-limiting infection in most cases
Diagnosis	Identification of pathogen in blood, stool, urine. Antibody detection using Gruber-Widal quantitative agglutination reaction	Identification of pathogen in stool
Therapy	Antibiotics: aminopenicillins, 4-quinolones	Symptomatic therapy: loperamide, replacement of water and electrolyte losses as required (WHO formula)
Occurrence	Sporadic; usually imported from countries with endemic typhoid fever	Endemic, epidemics in small groups (family, cafeteria, etc.) or as mass infection
Prevention	**Exposure prophylaxis:** Drinking water and food hygiene; elimination of pathogen in chronic carriers. **Immunization prophylaxis:** Active immunization possible (travelers) (see p. 287f.)	**Exposure prophylaxis:** Food hygiene

Eliminating the infection in chronic stool carriers of typhoid salmonellae, 2–5% of cases, presents a problem. Chronic carriers are defined as convalescents who are still eliminating pathogens three months after the end of the manifest illness. The organisms usually persist in the scarified wall of the gallbladder. Success is sometimes achieved with high-dose administration of anti-infective agents, e.g., 4-quinolones or aminopenicillins. A cholecystectomy is required in refractory cases.

Epidemiology. The cases of typhoid salmonellosis seen in northern and central Europe are imported by travelers. Cases arise only sporadically or in form of an epidemic because of a chain of unfortunate circumstances. Humans are the only primary source of infection.

By contrast, enteric salmonelloses occur in this population both endemically and epidemically. Case counts are steadily increasing. Exact morbidity data are hard to come by due to the large numbers of unreported cases. Livestock represents the most important source of infection. The pathogens are transmitted to humans in food.

Prevention. The main method of effective prevention is to avoid exposure: this means clean drinking water, prevention of food contamination, avoidance of uncooked foods in countries where salmonellae occur frequently, disinfection of excreta containing salmonellae or from chronic carriers, etc. It is also important to report all cases to health authorities so that appropriate measures can be taken.

Typhoid fever vaccinations for travelers to endemic areas can best be done with the oral attenuated vaccine Virotif Ty 21a.

Shigella (Bacterial Dysentery)

■ Shigella is the causative pathogen in bacterial dysentery. The genus comprises the species *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. Shigellae are nonmotile. The three primary species can be classified in serovars based on the fine structure of their O antigens. Shigellae are characterized by invasive properties. They can penetrate the colonic mucosa to cause local necrotic infections. Humans are the sole source of infection since shigellae are pathologically active in humans only. The pathogens are transmitted directly, more frequently indirectly, via food and drinking water. Antibiotics can be used therapeutically. ■

Classification. The genus *Shigella* includes four species: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. The first three are subdivided into 10, six, and

15 serovars, respectively, based on their antigen structures. Shigellae are nonmotile and therefore have no flagellar (H) antigens.

Pathogenesis. Shigellae are only pathogenic in humans. The pathogens are ingested orally. Only a few hundred bacteria suffice for an infective dose. Shigellae enter the terminal ileum and colon, where they are taken up by the M cells in the intestinal mucosa, which in turn are in close vicinity to the macrophages. Following phagocytosis by the macrophages, the shigellae lyse the phagosome and actively induce macrophage apoptosis. The shigellae released from the dead macrophages are then taken up by enterocytes via the basolateral side of the mucosa (i.e., retrograde transport). The invasion is facilitated by outer membrane polypeptides, the invasins, which are coded by *inv* genes localized on 180–240 kb plasmids. Adjacent enterocytes are invaded by means of lateral transfer from infected cells. In the enterocytes, the shigellae reproduce, finally destroying the cells. *Shigella dysenteriae* produces **shigatoxin**, the prototype for the family of **shigalike toxins** (or **verocytotoxins**), which also occur in several other *Enterobacteriaceae*. The toxin inhibits protein synthesis in eukaryotic cells by splitting the 23S rRNA at a certain locus. Shigatoxin contributes to the colonic epithelial damage, the small intestine diarrhea with watery stools at the onset of shigellosis and (less frequent) the hemolytic-uremic syndrome (HUS).

Clinical picture. Following an incubation period of two to five days, the disease manifests with profuse watery diarrhea (= small intestine diarrhea). Later, stools may contain mucus, pus, and blood. Intestinal cramps, painful stool elimination (tenesmus), and fever are observed in the further course of the infection. Complications include massive intestinal bleeding and perforation peritonitis. These severe effects are caused mainly by *S. dysenteriae*, whereas *S. sonnei* infections usually involve only diarrhea.

Diagnosis requires identification of the pathogen in a culture. Combined selective/indicator mediums must be used for the primary culture. Suspected colonies are identified by using indicator media to detect certain metabolic characteristics (p. 214). The serovar is determined with specific antisera in the slide agglutination test.

Therapy. Anti-infective agents are the first line of treatment (aminopenicillins, 4-quinolones, cephalosporins). Losses of water and electrolytes may have to be replaced.

Epidemiology and prevention. Bacterial dysentery occurs worldwide, although it is usually seen only sporadically in developed countries. In developing countries, its occurrence is more likely to be endemic and even epidemic. The source of infection is always humans, in most cases infected persons whose stools contain pathogens for up to six weeks after the disease has

abated. Transmission is by direct contact (smear infection) or indirect uptake via food, surface water, or flies. Control of dysentery includes exposure prophylaxis measures geared to prevent susceptible persons from coming into contact with the pathogen.

Yersinia (Plague, Enteritis)

■ *Y. pestis* is the causative pathogen of plague (black death, bubonic plague). Plague is a classic rodent zoonosis. It occurred in epidemic proportions in the Middle Ages, but is seen today only sporadically in persons who have had direct contact with diseased wild rodents. The pathogens penetrate into the skin through microtraumata, from where they reach regional lymph nodes in which they proliferate, resulting in the characteristic buboes. In the next stage, the pathogens may enter the bloodstream or the infection may generalize to affect other organs. Laboratory diagnosis involves isolation and identification of the organism in pus, blood, or other material. Therapy requires use of antibiotics.

Y. enterocolitica and *Y. pseudotuberculosis* cause generalized zoonoses in wild animals and livestock. Diseased animals contaminate their surroundings. Humans then take up the pathogens orally in water or food. The organisms penetrate the mucosa of the lower intestinal tract, causing enteritis accompanied by mesenteric lymphadenitis.

Extramesenteric forms are observed in 20% of infected persons (sepsis, lymphadenopathies, various focal infections). Secondary immunopathological complications include arthritis and erythema nodosum. Diagnosis involves identification of the pathogen by means of selective culturing. ■

To date, 10 different species have been classified in the genus *Yersinia*. The species most frequently isolated is *Y. enterocolitica*. *Y. pestis*, the "black death" pathogen responsible for epidemics in the Middle Ages, today no longer presents a significant threat.

Yersinia pestis

Morphology and culture. *Y. pestis* is a nonflagellated, short, encapsulated, Gram-negative rod bacteria that often shows bipolar staining. This bacterium is readily cultured on standard nutrient mediums at 30 °C.

Pathogenesis and clinical picture. The plague is primarily a disease of rodents (rats). It spreads among them by direct contact or via the rat flea. Earlier

plague epidemics in humans resulted from these same transmission pathways. The rare human infections seen today result from contact with rodents that are infected with or have died of plague. The pathogen breaches the skin through dermal injuries. From such a location, the bacteria reach regional lymph nodes in which they proliferate. Two to five days after infection, hemorrhagically altered, blue, and swollen lymph nodes (buboes) are observed. Over 90% of *pestis* infections show the "bubonic plague" course. In 50–90% of untreated cases, the organisms break out into the bloodstream to cause a clinical sepsis, in the course of which they may invade many different organs. Dissemination into the pulmonary circulation results in secondary pulmonary plague with bloody, bacteria-rich, highly infectious sputum. Contact with such patients can result in primary pulmonary plague infections due to direct, aerogenic transmission. Left untreated, this form of plague is lethal in nearly 100% of cases.

Diagnosis. The pathogen must be identified in bubo punctate, sputum, or blood by means of microscopy and culturing.

Therapy. In addition to symptomatic treatment, antibiotics are the primary method (streptomycin, tetracyclines, in the case of meningitis, chloramphenicol). Incision of the buboes is contraindicated.

Epidemiology and prevention. Plague still occurs **endemically** in wild rodents over large areas of Asia, Africa, South America, and North America. Human plague infections have been reduced to sporadic instances. The **sources of infection** are mainly diseased rodents. **Transmission** of the disease is mainly via **direct contact** with such animals.

Prevention involves **exposure prophylactic** measures. Persons with manifest disease, in particular the pulmonary form, must be isolated. Contact persons must be quarantined for six days (= incubation period). Cases of plague infection must be reported to health authorities.

Yersinia enterocolitica and Yersinia pseudotuberculosis

Occurrence and significance. *Y. enterocolitica* and *Y. pseudotuberculosis* cause generalized infections in domestic and wild animals, especially rodents. The pathogens can be transmitted from animals to humans. *Y. enterocolitica* is responsible for about 1% of acute enteritis cases in Europe. *Y. pseudotuberculosis* is insignificant in terms of human pathology.

Morphology, culture, and antigen structure. These are pleomorphic, short rods with peritrichous flagellation. They can be cultured on all standard mediums. These *Yersinia* bacteria grow better at 20–30 °C than at 37 °C.

Pathogenesis and clinical pictures. All of the strains isolated as human pathogens bear a 70 kb virulence plasmid with several vir determinants. They code for polypeptides that direct the functions cell adhesion, phagocytosis resistance, serum resistance, and cytotoxicity. Yersiniae also have chromosomal virulence genes, for example markers for invasins, enterotoxins, and an iron capturing system. Exactly how these virulence factors interact to produce the disease is too complex to be described in detail here.

Yersiniae are usually ingested indirectly with food. Although much less frequent, infections can also occur by way of direct contact with diseased animals or animal carriers. The bacteria enter the lower intestinal tract, penetrate the mucosa and are transported with the macrophages into the mesenteric lymph nodes. A simplified overview of the resulting clinical pictures follows:

- **Intestinal yersinioses.** The clinically dominant symptom is enteritis together with mesenteric lymphadenitis. This form is frequently observed in youths and children. Other enteric forms include pseudoappendicitis in youths and children, ileitis (pseudo Crohn disease), and colitis in adults.

- **Extraintestinal yersinioses.** These infections account for about 20% of cases, usually adults. Notable features of the clinical picture include sepsis, lymphadenopathy, rarely hepatitis, and various local infections (pleuritis, endocarditis, osteomyelitis, cholecystitis, localized abscesses).

- **Other sequelae.** The immunopathological complications observed in about 20% of acutely infected patients one to six weeks after onset of the intestinal symptoms include reactive arthritis and erythema nodosum.

Diagnosis. A confirmed diagnosis is only possible with identification of the pathogen in a culture based on physiological characteristics. Special mediums are used to isolate the pathogen from stool. The agglutination reaction, an ELISA or immunoblot assay can be used to detect the antibodies.

Therapy. Generally, favorable courses require no chemotherapy. Clinically difficult cases can be treated with cotrimoxazole, second- or third-generation cephalosporins, or fluorinated 4-quinolones.

Epidemiology and prevention. Prevalence of *Y. enterocolitica* and *Y. pseudotuberculosis* in animals is widespread. The most important reservoirs in epidemiological terms are mammals that are diseased or carry latent infections. From these sources, vegetation, soil, and surface water are contaminated. Transmission is by the oral pathway in food. Contact zoonosis is possible, but rare. There are no specific prophylactic measures.

Escherichia coli

The natural habitat of *E. coli* is the intestinal tract of humans and animals. It is therefore considered an indicator organism for fecal contamination of water and foods. *E. coli* is the most frequent causative pathogen in human bacterial infections. **Extraintestinal infections** include urinary tract infections, which occur when the tract is obstructed or spontaneously caused by the pathovar UPEC. The most important other coli infections are cholecystitis, appendicitis, peritonitis, postoperative wound infections, and sepsis. **Intestinal infections** are caused by the pathovars EPEC, ETEC, EIEC, EHEC, and EAggEC. EPEC and EAggEC frequently cause diarrhea in infants. ETEC produce enterotoxins that cause a choleralike clinical picture. EIEC cause a dysenterylike infection of the large intestine. EHEC produce verocytotoxins and cause a hemorrhagic colitis as well as the rare hemolytic-uremic syndrome. *E. coli* bacteria infections are diagnosed by means of pathogen identification.

General characteristics. The natural habitat of *E. coli* is the intestines of animals and humans. This bacterium is therefore used as an indicator for fecal contamination of drinking water, bathing water, and foods. Guideline regulations: 100 ml of drinking water must not contain any *E. coli*. Surface water approved for bathing should not contain more than 100 (guideline value) to 2000 (absolute cutoff value) *E. coli* bacteria per 100 ml.

E. coli is also an important human pathogen. It is the bacterial species most frequently isolated from pathological materials.

Morphology, culture, and antigen structure. The Gram-negative, straight rods are peritrichously flagellated. Lactose is broken down rapidly. The complex antigen structure of these bacteria is based on O, K, and H antigens. Fimbrial antigens have also been described. Specific numbers have been assigned to the antigens, e.g., serovar O18:K1:H7.

Pathogenesis and clinical picture of extraintestinal infections. Extraintestinal infections result from relocation of *E. coli* bacteria from one's own flora to places on or in the macroorganism where they are not supposed to be but where conditions for their proliferation are favorable.

Urinary tract infection. Such an infection manifests either solely in the lower urinary tract (**urethritis, cystitis, urethrocystitis**) or affects the renal pelvis and kidneys (**cystopyelitis, pyelonephritis**). In acute urinary tract infections, *E. coli* is the causative organism in 70–80% of cases and in chronic, persistent infections in 40–50% of cases.

Urinary tract infections result from ascension of the pathogen from the ostium urethrae. Development of such an infection is also furthered by obstructive anomalies, a neurogenic bladder or a vesicoureteral reflux. Urinary tract infections that occur in the absence of any physical anomalies are often caused by the pathovar UPEC (uropathogenic *E. coli*). UPEC strains can attach specifically to receptors of the renal pelvis mucosa with pyelonephritis-associated pili (PAP, P fimbriae, p. 158) or nonfimbrial adhesins (NFA). They produce the hemolysin HlyA.

- **Sepsis.** *E. coli* causes about 15 % of all cases of nosocomial sepsis (*S. aureus* 20 %). An *E. coli* sepsis is frequently caused by the pathovar SEPEC, which shows serum resistance (p. 13).

- **Other *E. coli* infections.** Wound infections, infections of the gallbladder and bile ducts, appendicitis, peritonitis, meningitis in premature infants, neonates, and very elderly patients.

Pathogenesis and clinical pictures of intestinal infections. *E. coli* that cause intestinal infections are now classified in five pathovars with differing pathogenicity and clinical pictures:

- **Enteropathogenic *E. coli* (EPEC).** These bacteria cause epidemic or sporadic infant diarrheas, now rare in industrialized countries but still a main contributor to infant mortality in developing countries. EPEC attach themselves to the epithelial cells of the small intestine by means of the EPEC adhesion factor (EAF), then inject toxic molecules into the enterocytes by means of a type III secretion system (see p. 17).

- **Enterotoxic *E. coli* (ETEC).** The pathogenicity of these bacteria is due to the heat-labile enterotoxin LT (inactivation at 60 °C for 30 minutes) and the heat-stable toxins STa and STb (can tolerate temperatures up to 100 °C). Some strains produce all of these toxins, some only one. LT is very similar to cholera toxin. It stimulates the activity of adenylate cyclase (see p. 298). STa stimulates the activity of guanylate cyclase. (cGMP mediates the inhibition of Na^+ absorption and stimulates Cl^- secretion by enterocytes.) ETEC pathogenicity also derives from specific fimbriae, so-called colonizing factors (CFA) that allow these bacteria to attach themselves to small intestine epithelial cells, thus preventing their rapid removal by intestinal peristalsis. The enterotoxins and CFA are determined by plasmid genes. The clinical picture of an ETEC infection is characterized by massive watery diarrhea. The disease can occur at any age. Once the illness has abated, a local immunity is conferred lasting several months.

- **Enteroinvasive *E. coli* (EIEC).** These bacteria can penetrate into the colonic mucosa, where they cause ulcerous, inflammatory lesions. The pathogenesis and clinical picture of EIEC infections are the same as in bacterial dysentery (p. 288). EIEC strains are often lac-negative.

■ **Enterohemorrhagic *E. coli* (EHEC).** These bacteria are the causative pathogens in the hemorrhagic colitis and hemolytic-uremic syndrome (HUS) that occur in about 5 % of EHEC infections, accompanied by acute renal failure, thrombocytopenia, and anemia. EHEC possess specific, plasmid-coded fimbriae for adhesion to enterocytes. They can also produce prophage-determined cytotoxins (shigalike toxins or verocytotoxins). Some authors therefore designate them as VTEC (verotoxin-producing *E. coli*). EHEC strains have been found in the O serogroups O157, O26, O111, O145, and others. The serovar most frequently responsible for HUS is O157:H7.

■ **Enteroaggregative *E. coli* (EAggEC).** These bacteria cause watery, and sometimes hemorrhagic, diarrhea in infants and small children. Adhesion to enterocytes with specific attachment fimbriae. Production of a toxin identical to STa in ETEC.

Diagnosis. Extraintestinal infections are diagnosed by identifying the pathogen in relevant materials. Diagnosis of a urinary tract infection with midstream urine requires determination of the bacterial count to ensure that an infection can be distinguished from a contamination. Counts $\geq 10^5$/ml tend to indicate an infection, $\leq 10^3$/ml a contamination, 10^4/ml could go either way. Specific gene probes are now being used to make identification of intestinal pathogen *E. coli* bacteria less difficult.

Therapy. Antibiotic therapy must take into consideration the resistance pattern of the pathogen. Aminopenicillins, ureidopenicillins, cephalosporins, 4-quinolones, or cotrimoxazole are useful agents. Severe diarrhea necessitates oral replacement of fluid and electrolyte losses according to the WHO formula: 3.5 g NaCl, 2.5 g $NaHCO_3$, 1.5 g KCl, 20 g glucose per liter of water. When required, intestinal activity is slowed down with loperamide.

Epidemiology and prevention. Transmission of intestinal infections is usually indirect via food, drinking water, or surface water. Fifty percent of travelers' diarrhea cases are caused by *E. coli*, in most cases ETEC.

The most effective preventive measures against intestinal infections, e.g., when travelling in countries with warm climates, is to eat only thoroughly cooked foods and drink only disinfected water. Studies have demonstrated the efficacy of chemoprophylaxis with anti-infective agents in preventing traveler's diarrhea, whereby the agents used must not reduce the normal aerobic intestinal flora (4-quinolones and cotrimoxazole are suitable). This method is hardly practicable, however, in view of the large numbers of travelers.

Opportunistic Enterobacteriaceae

Many *Enterobacteriaceae* with minimum pathogenicity are classic opportunists. The most frequent opportunistic infections caused by them are: **urinary tract infections, respiratory tract infections, wound infections,**

Table 4.**9** Overview of the Most Important *Enterobacteriaceae* That Cause Opportunistic Infections

Bacterial species	Properties
Escherichia coli	See p. 280ff., p. 292ff.
Citrobacter freundii; C. divs.; C. amalonaticus	Can use citrate as its sole source of C; delayed breakdown of lactose; nonmotile
Klebsiella pneumoniae; K. oxytoca and others	Lactose-positive; nonmotile; many strains have a polysaccharide capsule. Cause approx. 10% of nosocomial infections. Causative organism in so-called Friedländer's pneumonia in predisposed persons, especially in the presence of chronic pulmonary diseases.
Klebsiella ozaenae	Causative pathogen in ozena; atrophy of nasal mucosa
Klebsiella rhinoscleromatis	Causative pathogen in rhinoscleroma; granuloma in the nose and pharynx
Enterobacter cloace; E. aerogenes; E. agglomerans; E. sakazakii, and others	Lactose-positive; motile; frequent multiple resistance to antibiotics
Serratia marcescens and others	Lactose-positive; motile; frequent multiple resistance to antibiotics, some strains produce red pigment at 20 °C
Proteus mirabilis *Proteus vulgaris*	Lactose-negative; highly motile; wanders on surface of nutrient agar (swarming). O antigens OX-2, OX-19, and OX-K from *P. vulgaris* are identical to rickettsiae antigens. For this reason, antibodies to rickettsiae were formerly identified using these strains (Weil-Felix agglutination test)
Morganella morganii	Lactose-negative; frequent multiple resistance to antibiotics
Providencia rettgeri; P. stuartii	Lactose-negative; frequent multiple resistance to antibiotics

dermal and subcutaneous infections, and sepsis. Such infections only occur in predisposed hosts, they are frequently seen in patients with severe primary diseases. Another reason why opportunistic *Enterobacteriaceae* have become so important in hospital medicine is the frequent development of resistance to anti-infective agents, which ability enables them to persist at locations where use of such agents is particularly intensive, i.e., in hospitals. Occurrence of multiple resistance in *Enterobacteriaceae* is due to the impressive genetic variability of these organisms (p. 170). Table 4.**9** provides an overview of the most important opportunistic *Enterobacteriaceae*.

4

Vibrio, Aeromonas, and Plesiomonas

■ *Vibrio cholerae* is the most important species in this group from a medical point of view. Cholera vibrios are Gram-negative, comma-shaped, monotrichously flagellated rods. They show alkali tolerance (pH 9), which is useful for selective culturing of *V. cholerae* in alkaline peptone water. The primary cholera pathogen is serovar O:1. NonO:1 strains (e.g., O:139) cause the typical clinical picture in rare cases. O:1 vibrios are further subdivided into the biovars *cholerae* and *eltor*. The disease develops when the pathogens enter the intestinal tract with food or drinking water in large numbers ($\geq 10^8$). The vibrios multiply in the proximal small intestine and produce an enterotoxin. This toxin stimulates a series of reactions in enterocytes, the end result of which is increased transport of electrolytes out of the enterocytes, whereby water is also lost passively. Massive watery diarrhea (up to 20 l/day) results in exsiccosis. The initial therapeutic focus is thus on replacement of lost electrolytes and water. Cholera occurs only in humans. Preventive measures concentrate on protection from exposure to the organism. A killed whole cell vaccine and an attenuated live vaccine are available. They provide only a moderate degree of protection over a period of only six months. International healthcare sources report an incubation period of five days. ■

The bacteria in these groups are Gram-negative rods with a comma or spiral shape. Their natural habitat is in most cases damp biotopes including the ocean. Some of them cause infections in fish (e.g., *Aeromonas salmonicida*). By far the most important species in terms of human medicine is *Vibrio cholerae*.

Vibrio cholerae (Cholera)

Morphology and culture. Cholera vibrios are Gram-negative rod bacteria, usually slightly bent (comma-shaped), 1.5–2 µm in length, and 0.3–0.5 µm wide, with monotrichous flagellation (Fig. 4.**19**).

Culturing of *V. cholerae* is possible on simple nutrient mediums at 37 °C in a normal atmosphere. Owing to its pronounced alkali stability, *V. cholerae* can be selectively cultured out of bacterial mixtures at pH 9.

Antigens and classification. *V. cholerae* bacteria are subdivided into serovars based on their O antigens (lipopolysaccharide antigens). The serovar pathogen is usually serovar O:1. Strains that do not react to an O:1 antiserum are grouped together as nonO:1 vibrios. NonO:1 strains were recently described in India (O:139) as also causing the classic clinical picture of cholera. O:1 vibrios are further subclassified in the biovars *cholerae* and *eltor* based on physiological characteristics. The var *eltor* has a very low level of virulence.

Cholera toxin. Cholera toxin is the sole cause of the clinical disease. This substance induces the enterocytes to increase secretion of electrolytes, above all Cl^- ions, whereby passive water loss also occurs. The toxin belongs to the group of AB toxins (see p. 16). Subunit **B** of the toxin **b**inds to enterocyte receptors, the **a**ctive toxin subunit **A** causes the adenylate cyclase in the enterocytes to produce cAMP continuously and in large amounts (Fig. 4.**20**). cAMP in turn acts as a second messenger to activate protein kinase A, which then activates the specific cell proteins that control secretion of electrolytes. The toxin genes *ctxA* and *ctxB* are components of the so-called CTX element, which is integrated in the nucleoid of toxic cholera vibrios (see lysogenic conversion, p. 186) as part of the genome of the filamentous prophage CTXϕ. The CTX element also includes several regulator genes that regulate both produc-

Vibrio cholerae

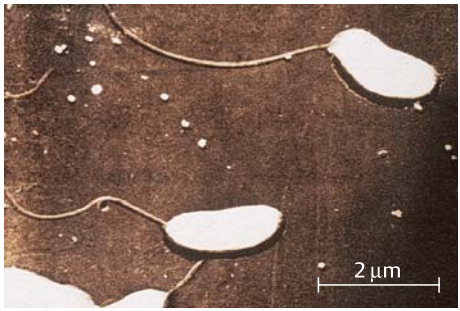


Fig. 4.**19** Comma-shaped rod bacteria with monotrichous flagellation (SEM image).

Mechanism of Action of Cholera Toxin

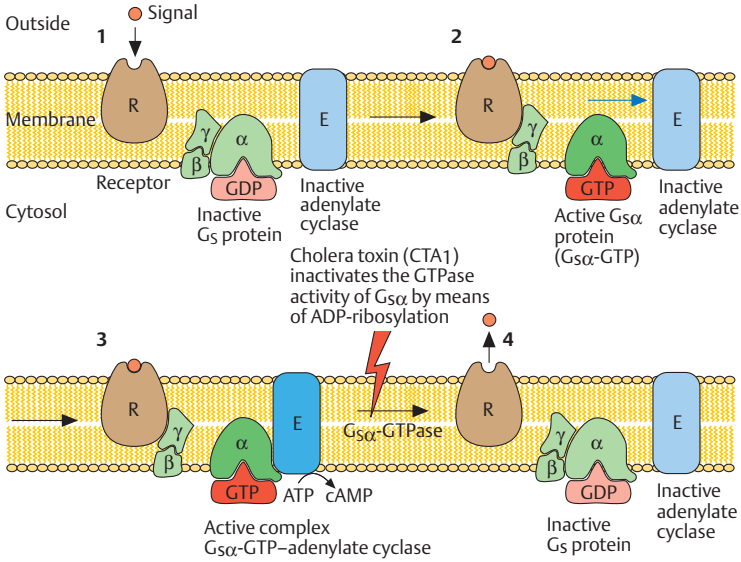


Fig. 4.**20** Cholera toxin disrupts the G_s protein-mediated signal cascade.
1 The G_s proteins in the membrane comprise the three subunits α, β, and γ. GDP is bound to subunit α. G_s is inactive in this configuration.
2 After a signal molecule is bound to the membrane receptor R, the subunits dissociate from G_s; also, the GDP on the $G_{s\alpha}$ is phosphorylated to GTP.
3 $G_{s\alpha}$–GTP then combines with adenylate cyclase to form the active enzyme that transforms ATP into the second messenger cAMP.
4 When the signal molecule once again dissociates from the receptor, the GTP bound to the $G_{s\alpha}$ is dephosphorylated to GDP, i.e., inactive status is restored. This is the step that cholera toxin prevents: the A_1 subunit of the cholera toxin (CTA_1) catalyzes ADP-ribosylation of $G_{s\alpha}$, which thus loses its GTPase activity so that the adenylate cyclase is not "switched off" and synthesis of cAMP continues unchecked.

tion of the toxin and formation of the so-called toxin-coregulated pili (TCP) on the surface of the *Vibrio* cells.

Pathogenesis and clinical picture. Infection results from oral ingestion of the pathogen. The infective dose must be large ($\geq 10^8$), since many vibrios are killed by the hydrochloric acid in gastric juice. Based on their pronounced stability in alkaline environments, vibrios are able to colonize the mucosa

of the proximal small intestine with the help of TCP (see above) and secrete cholera toxin (see Fig. 4.**20**). The pathogen does not invade the mucosa.

The incubation period of cholera is two to five days. The clinical picture is characterized by voluminous, watery diarrhea and vomiting. The amount of fluids lost per day can be as high as 20 l. Further symptoms derive from the resulting exsiccosis: hypotension, tachycardia, anuria, and hypothermia. Lethality can be as high as 50% in untreated cases.

Diagnosis requires identification of the pathogen in stool or vomit. Sometimes a rapid microscopical diagnosis succeeds in finding numerous Gram-negative, bent rods in swarm patterns. Culturing is done on liquid or solid selective mediums, e.g., alkaline peptone water or taurocholate gelatin agar. Suspected colonies are identified by biochemical means or by detection of the O:1 antigen in an agglutination reaction.

Therapy. The most important measure is restoration of the disturbed water and electrolyte balance in the body. Secondly, tetracyclines and cotrimoxazole can be used, above all to reduce fecal elimination levels and shorten the period of pathogen secretion.

Epidemiology and prevention. Nineteenth-century Europe experienced several cholera pandemics, all of which were caused by the classic *cholerae* biovar. An increasing number of cases caused by the biovar *eltor*, which is characterized by a lower level of virulence, have been observed since 1961. With the exception of minor epidemics in Italy and Spain, Europe, and the USA have been spared major outbreaks of cholera in more recent times. South America has for a number of years been the venue of epidemics of the disease.

Humans are the only **source of infection**. Infected persons in particular eliminate large numbers of pathogens. Convalescents may also shed *V. cholerae* for weeks or even months after the infection has abated. Chronic carriers as with typhoid fever are very rare. **Transmission** of the disease is usually via foods, and in particular drinking water. This explains why cholera can readily spread to epidemic proportions in countries with poor hygiene standards.

Protection from exposure to the pathogen is the main thrust of the relevant **preventive measures**. In general, control of cholera means ensuring adequate food and water hygiene and proper elimination of sewage. In case of an outbreak, infected persons must be isolated. Infectious excreta and contaminated objects must be disinfected. Even suspected cases of cholera must be reported to health authorities without delay. The incubation period of the cholera *vibrio* is reported in international health regulations to be five days. A vaccine containing killed cells as well an attenuated live vaccine are available. The level of immunization protection is, however, incomplete and lasts for only six months.

Other Vibrio Bacteria

Vibrio parahemolyticus is a halophilic (salt-friendly) species found in warm ocean shallows and brackish water. These bacteria can cause gastroenteritis epidemics. The pathogen is transmitted to humans with food (seafood, raw fish). The illness is transient in most cases and symptomatic therapy is sufficient.

Vibrio vulnificus is another aquatic organism that produces a very small number of septic infections, mainly in immunosuppressed patients.

Aeromonas and Plesiomonas

The bacteria of these two genera live in freshwater biotopes. Some are capable of causing infection in fish (*A. salmonicida*). They are occasionally observed as contaminants of moist parts of medical apparatus such as dialysis equipment, vaporizers, and respirators. They can cause nosocomial infections in hospitalized patients with weakened immune systems. Cases of gastroenteritis may result from eating foods contaminated with large numbers of these bacteria.

Haemophilus and Pasteurella

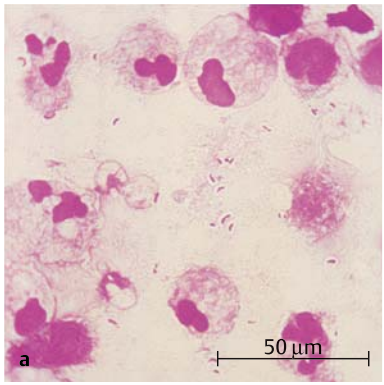
■ The most important species of *Pasteurellaceae* from the medical point of view is *Haemophilus influenzae*. This is a nonmotile, Gram-negative rod that is often encapsulated. Capsule serovar b is the main pathogenic form. *H. influenzae* is a facultative anaerobe that requires growth factors X (hemin) and V (NAD, NADP) in its culture medium. *H. influenzae* is a typical parasite of the respiratory tract mucosa. It occurs only in humans. It causes infections of the upper and lower respiratory tract in individuals with weakened immune defenses and in children under the age of four or five. Invasive infections—meningitis and sepsis—are also observed in small children. A betalactamase-stable betalactam antibiotic is required for treatment since the number of betalactamase-producing strains observed is increasing. Conjugate vaccines in which the capsule polysaccharide is coupled with proteins are available for prophylactic immunization. These vaccines can be administered beginning in the third month of life. ■

Haemophilus influenzae

Hemophilic bacteria are so designated because they require growth factors contained in blood. The most important human pathogen in this genus is *H. influenzae*. Other *Haemophilus* species either infect only animals or are found in the normal human mucosal flora. These latter include *H. parainfluenzae*, *H. hemolyticus*, *H. segnis*, *H. aphrophilus*, and *H. paraphrophilus*. These species can cause infections on occasion.

Morphology and culture. *Haemophilus* are small (length: 1.0–1.5 µm, width: 0.3 µm), often encapsulated, nonmotile, Gram-negative rods (Fig. 4.**21a**). The encapsulated strains are subclassified in serovars a-f based on the fine structure of their capsule polysaccharides. Serovar b (Hib) causes most *Haemophilus* infections in humans.

Haemophilus influenzae



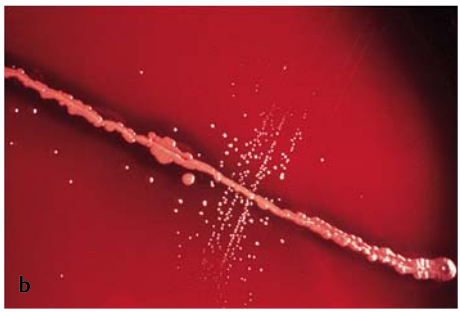


Fig. 4.**21** **a** Gram-stained cerebrospinal fluid sediment preparation. Fine, Gram-negative rods surrounded by a capsule (serovar b). Clinical diagnosis: purulent meningitis.
b Satellite colonies of *Haemophilus influenzae* surrounding the *Staphylococcus aureus* streak. *S. aureus* provides small amounts of V factor. The blood agar contains free X factor.

H. influenzae is a facultative anaerobe requiring growth factors X and V in its culture medium. The X factor is hemin, required by the bacteria to synthesize enzymes containing heme (cytochromes, catalase, oxidases). The X factor requirement is greatly reduced in anaerobic culturing. The V factor was identified as NAD or NADP. A standard blood agar plate does not contain sufficient free V factor. Some bacteria, in particular *Staphylococcus aureus*, produce excess NAD and even secrete this coenzyme into the medium. That is why *H. influenzae* can proliferate in the immediate vicinity of *S. aureus* colonies. This is known as the **satellite phenomenon** (Fig. 4.**21b**). The medium normally used to culture *H. influenzae* is chocolate agar containing sufficient amounts of the X and V factors.

Pathogenesis and clinical pictures. *H. influenzae* is a mucosal parasite of the upper respiratory tract present in 30–50% of healthy persons. The strains usually found are nonencapsulated and therefore hardly virulent. The capsule protects the cells from phagocytosis and is thus the primary determinant of pathogenicity. Others include the affinity of *H. influenzae* to respiratory tract mucosa and meninges and production of an IgA_1 protease (see p. 15).

H. influenzae infections are seen frequently in children aged from six months to four years of age due to the low levels of anticapsule antibodies in this age group. Maternal antibodies still protect children during the first months of life. The body has built up a sufficient store of antibodies by the age of four. Any list of potential clinical developments must begin with meningitis, followed by epiglottitis, pneumonia, empyema, septic arthritis, osteomyelitis, pericarditis, cellulitis, otitis media, and sinusitis. *Haemophilus* infections in adults are usually secondary complications of severe primary illnesses or the result of compromised immune defenses. The most frequent complication is an acute exacerbation of chronic bronchitis. Pneumonias caused by *H. influenzae* are also observed, often as superinfections following viral influenza. In immunocompromised adults, even the nonencapsulated strains can cause infections of the upper and lower respiratory tract.

Diagnosis. The method of choice is identification of the pathogen in cerebrospinal fluid, blood, pus, or purulent sputum using microscopy and culture assays. Satelliting on blood agar is an indication of a V factor requirement. An X factor requirement is confirmed most readily by the porphyrin test, with a negative result in the presence of *H. influenzae*.

Therapy. In view of the increasing number of betalactamase-producing *H. influenzae* strains observed in recent years, penicillinase-stable betalactam antibiotics should be used to treat these infections. The likelihood that a strain produces betalactamase is 5–30% in most countries. 4-quinolones are an alternative to betalactams that should not, however, be used in children. The agent of choice in meningitis is ceftriaxone.

Epidemiology and prevention. *H. influenzae* is found only in humans. The incidence of severe invasive infections (meningitis, sepsis, epiglottitis) in children has been reduced drastically—to about one in 10 of the numbers seen previously—since a vaccination program was started, and will continue to fall assuming the vaccinations are continued (see vaccination schedule, p. 33).

Immunization is achieved with the conjugate vaccine Hib in which the capsule polysaccharide epitope "b" conferring immunity is conjugated to protein. Such a conjugate vaccine can be administered as early as the first month of life. The immune system does not respond to pure polysaccharide vaccines until about the age of two, since polysaccharides are T-independent antigens against which hardly any antibodies are produced in the first two years of life. There is also no booster response. A four-day regimen of rifampicin has proved to be an effective chemoprophylactic treatment for nonvaccinated small children who have been exposed to the organism.

Haemophilus ducreyi and Haemophilus aegyptius

H. ducreyi are short, Gram-negative, nonmotile rods that are difficult to culture and require special mediums. This bacterium causes ulcus molle (soft chancre) a tropical venereal disease seen rarely in central Europe. The infection locus presents as a painful, readily bleeding ulcer occurring mainly in the genital area. Regional lymph nodes are quite swollen. Identification of the pathogen by means of microscopy and culturing are needed to confirm the diagnosis. Therapeutic alternatives include sulfonamides, streptomycin, and tetracyclines.

H. aegyptius (possibly identical with biovar III of ***Haemophilus influenzae***) causes a purulent conjunctivitis occurring mainly in northern Africa, in particular Egypt. A raised incidence of Brazilian purpuric fever, a systemic infection with this organism, has been observed in Brazil in recent years.

Pasteurella

Various different species belonging to the genus *Pasteurella* occur in the normal mucosal flora of animals and humans; some are pathogenic in animals. Their significance as human pathogens is minor. Infections by ***Pasteurella multocida*** are described here as examples of human pasteurelloses. The bacteria invade the organism through bite or scratch injuries or in droplets during contact with infected animals. Weakened immune defenses may then result in either local wound infections with lymphadenitis, subacute to chronic infections of the lower respiratory tract, or CNS infections (after cerebral trauma or brain surgery). Diagnosis is based on pathogen identification.

A penicillin or cephalosporin is recommended for therapy. Sources of infection include domestic animals (dogs, cats, birds, guinea pigs) and livestock (cattle, sheep, goats, pigs).

Gram-Negative Rod Bacteria with Low Pathogenic Potential

The bacterial species listed in Table 4.**10** are typical opportunists that occasionally cause infections in persons with defective specific or nonspecific immune defenses. When they are isolated from infective material, their pathological significance is in most cases difficult to interpret.

Table 4.**10** Overview of Gram-Negative Rod Bacteria with Low Pathogenic Potential

Bacterial species	Most important characteristics
HACEK group	
– *Hemophilus aphrophilus*	Endocarditis, cerebral abscesses
– *Actinobacillus actinomycetemcomitans*	Part of normal oral cavity flora. Nonmotile, slender rods; microaerophilic; colonies on blood agar with "starfish" appearance. Accompanying bacterium in approx. 25% of oral-cervicofacial **actinomycoses**. Penicillin G resistance. Also a pathogen in endocarditis.
– *Cardiobacterium hominis*	Nonmotile; pleomorphic. Normal flora of the respiratory tract. Culturing on blood agar in 5% CO_2 at 35 °C for 4 days. **Endocarditis**. Occasionally observed as component of mixed flora in facial purulent infections.
– *Eikenella corrodens*	Nonmotile, coccoid. Normal flora of respiratory and intestinal tracts. Cultures, on blood agar, show corrosion of the agar surface. **Abscesses, wound infections, peritonitis, empyemas, septic arthritis**, often as part of a mixed flora. Also reports of **endocarditis** and **meningitis**.
– *Kingella kingae*	Normal flora of the upper respiratory tract. Rare cases of endocarditis, arthritis, osteomyelitis.

Table 4.**10** *Continued: Overview of Other Gram-Negative Rod Bacteria*

Bacterial species	Most important characteristics
Calymmatobacterium granulomatis (syn. *Donovania granulomatis*)	Nonmotile, capsule, culturing on mediums containing egg yolk; facultative anaerobe. **Granuloma inguinale**. Venereal disease; indolent, ulcerogranulomatous lesions on skin and mucosa. Sporadic occurrence in Europe. Diagnosis involves identification of bacteria in vacuoles of large mononuclear cells using Giemsa staining (Donovan bodies). Antibiotics: aminoglycosides, tetracyclines
Streptobacillus moniliformis	Pronounced pleomorphism; frequent production of filaments because of defective cell walls. Culturing in enriched mediums at 35 °C, 5% CO_2, 3 days. Component of oral cavity flora in rats, mice, cats **Rat bite fever**. Incubation period 1–22 days. Fever, arthralgias, myalgias, exanthema. Possible inflammation at site of bite. Polyarthritis in 50% of patients. Therapy with penicillin G.
Chryseobacterium (formerly *Flavobacterium*) *meningosepticum* (and other flavobacteria)	Strictly aerobic; often with yellow pigment; nonfermenter. Natural habitat soil and natural bodies of water. **Meningitis.** In neonates. Poor prognosis. **Sepsis, pneumonia** in immunocompromised patients. All infections rare.
Alcaligenes faecalis (and other species of the genus *Alcaligenes*)	Strictly aerobic; nonfermenter. Natural habitat soil and surface water. Various **opportunistic infections** in patients with severe primary illnesses; usually isolated as a component in mixed flora; data difficult to interpret.
Capnocytophaga spp.	Component of normal oral cavity flora in humans and dogs. Long, thin, fusiform rods. Proliferation on blood agar in presence of 5–10% CO_2. Can contribute to pathogenesis of **periodontitis**. **Sepsis** in agranulocytosis, leukemias, malignancys. Wide variety of purulent processes. Often component of mixed flora.