1

Introduction to Microbiology

1.1 Historical

Only a few years after the first descriptions and isolations of microorganisms by Louis Pasteur, Robert Koch, Gerhard Hansen, and others, the public was already aware of an essential property of these mostly unicellular microorganisms: They are ubiquitously distributed, i.e. everywhere! Also, that they can cause fever in the bodies of humans and animals or cause diseases, partly with death consequences, was already known, likewise already disinfection measures like the use of the agents carbolic acid and iodine. Still in 1906, in the 8th edition of the "Lehrbuch der Botanik für Hochschulen" by Eduard Strasburger, among other names of fission fungi (Schizomycetes), the cyanobacteria are called fission algae [1].

Humans have been taking advantage of the services of microorganisms for thousands of years, but without knowing of their existence for very long. The Sumerians brewed a beer-like beverage as early as 5000 years ago, and the Assyrians fermented grape juice into wine about 3500 years ago.

The first person to see microorganisms with his own eyes was probably the Dutch draper Antony van Leeuwenhoek (1632–1723). He experimented with homemade single-lens microscopes, with which he achieved magnifications up to 270× and resolutions down to 1.5 µm. In 1675, he examined an infusion of peppercorns and discovered tiny creatures. He discovered more of these creatures, then called "*animalcula*," in dental plaque. Van Leeuwenhoek made drawings of these creatures, which he sent by letter to the Royal Society in London in 1683 [2].

The French chemist Louis Pasteur (1822–1895) made several groundbreaking discoveries in the field of microbiology. He experimentally disproved the primordial hypothesis, explained the nature of fermentation using the examples of alcoholic fermentation and lactic acid fermentation, developed methods for disinfection and sterilization, and introduced procedures for combating infectious diseases by vaccination (e.g. rabies vaccination in 1885).

In 1873, the Norwegian physician Gerhard Hansen (1841–1912) microscopically discovered the causative agent of leprosy, *Mycobacterium leprae,* as one of the first bacteria to be recognized as a pathogen [3]. To this day, this bacterium cannot be cultured in culture media. Diagnosis is made with the microscope on biopsy material or scrapings of the nasal mucosa. The propagation of these mycobacteria is only

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successful in the paws of mice and the *Armadillo*. Pathogen-specific DNA can be detected using the polymerase chain reaction (PCR).

In 1876, the German physician Robert Koch (1843–1910) proved that microorganisms are the causative agents of infectious diseases, using the anthrax pathogen *Bacillus anthracis* as an example. He established the following four postulates:

1) Bacteria must be detectable in the infected organism.
2) These bacteria must be isolated and brought into pure culture.
3) Infection with these isolated bacteria will cause the disease again in the healthy organism.
4) The same infectious agent can be isolated from the host again.

Koch developed culture media, e.g. meat extract broth, which he initially solidified with gelatin and later with agar–agar. Koch's plate-casting method, still used today in all bacteriological laboratories, goes back to him.

Microorganisms are grouped into two taxonomic domains of their own (Bacteria and Archaea) and thus distinguished from the domain Eukarya (fungi, animals, and plants). Based on the cell structure of microorganisms, they are divided into prokaryotes (Bacteria and Archaea; Greek: *bakteria* = rod; Greek: *archaios* = ancient, original) and eukaryotes (fungi, yeasts, algae, and protozoa).

1.2 Importance

Medical microbiology is concerned with the study of pathogens of significance to humans and animals, their habits, and their effects on the human or animal organism; it is thus primarily concerned with obligate pathogens (pathogenic in any case) and facultative pathogens (pathogenic under certain circumstances), i.e. with germs that are to be regarded as dangerous or as "pests" due to cell destruction or the release of toxic metabolic products. However, microorganisms are generally much more likely to be considered "beneficial organisms." Biological equilibrium without microorganisms is not possible at all. By mineralizing organic matter (e.g. plant material), they ensure the recovery of carbon, nitrogen, sulfur, phosphorus, etc., which are then available again to the plants (material cycles). In the gastrointestinal tract of humans and animals, microorganisms play an important role in the digestion of food. The skin and mucous membranes of humans are also colonized. To illustrate the orders of magnitude: A human being consists of about 10^{13} cells. The gastrointestinal tract is home to about 10^{14} and the skin to about 10^{12} microorganisms, which together weigh about 1.25 kg [4]. The human body thus harbors more microorganisms than it has cells of its own.

Microorganisms find application in the food industry. Examples are:

- Yeasts in the manufacture of bread, beer, sake, and wine;
- Lactic acid bacteria in the production of yogurt, kefir, sauerkraut, and salami;
- Acetic acid bacteria for the preparation of vinegar;
- Molds in cheese production (Gorgonzola, Roquefort, etc.) and for the preparation of soybeans (in East Asia).

Microorganisms are used for the recovery of:

- Vitamins;
- Amino acids;
- Hormones;
- Steroids;
- Enzymes, e.g. amylases (starch cleavage), proteases (digestion, leather tanning), lipases (fat cleavage), and pectinases (fruit juice clarification);
- Antibiotics;
- Alcohols (ethanol, butanol, butanediol, glycerol, etc.);
- Active ingredients, some of which are also produced by genetically modified microorganisms (e.g. insulin).

Microorganisms are essential in the treatment of wastewater and waste composting.

1.3 World of Microorganisms

An overview of the various groups of microorganisms and other causative agents of infectious diseases is given in Table 1.1. Microorganisms are not visible to the naked eye; for their observation, a light microscope is required, and in the case of viruses – with very few exceptions – an even higher magnification electron microscope is required.

The average size of bacteria is between 0.3 and 10 µm. The diameter of cocci, which belong to the human skin flora, is approx. 1 µm. If you think of 500 cocci of this size strung together, the diameter of the dot at the end of the sentence would be reached. Another size comparison: a hair on the head is approx. 40–120 µm, on average 80 µm, thick (see Table 1.2). The human eye can recognize objects up to approx. 25 µm (resolving power).

Table 1.1 Groups of microorganisms and biological agents.

Subcellular biological objects	Mostly unicellular organisms (microorganisms)
Prions	**Prokaryotes**
Viroid	Eubacteria
Bacteriophages	Chlamydia
Viruses	Rickettsia
	Mycoplasma
	Archaea
	Eukaryotes
	Fungi, yeasts, algae, and protozoa

Table 1.2 Sizes of particles and cells.

Cell or particle	Size
Egg (bird)	In the centimeter range (ostrich egg: $d = 15$ cm)
Ovum (human)	200 µm
Human hair	$d = 40$–120 µm, average 80 µm
Human and animal cells	20–30 µm
Human erythrocyte	7.5 µm
Human sperm cell	6.5 µm long
Pollen	7–100 µm
Dust	0.1–100 µm
Aerosols when sneezing	10–300 µm
Protozoa	5–150 µm
Mushrooms	5–10 µm
Bacteria	0.3–10 µm
Nanobacterium equitum (Archaeon)	0.4 µm
Mycoplasma	0.3–0.8 µm
Chlamydia	0.3–1.0 µm
Rickettsia	0.5–1.0 µm
Viruses	0.016–2.0 µm
Viroid	2 × 40 nm
Macromolecules	1–10 nm
Prions	<5 nm
Atoms	0.1 nm

d, diameter.

The world of microorganisms consists of the following groupings (although the following first three groupings are not living organisms in the strict sense, but biological agents).

1.3.1 Prions

Infectious prions PrP^{sc} are misfolded forms of a small (molar mass about 30 000 Da) cellular glycoprotein. The misfolding occurs in cattle between amino acids 121 and 230 and is inaccessible to protease digestion [5]. Stanley Prusiner derived the name from "proteinaceous infectious particle" [6]. PrP^{sc} causes diseases in sheep and goats (scrapie), cattle and cats (bovine spongiform encephalopathy (BSE) and feline spongiform encephalopathy (FSE)), mink, deer, and ungulates. Humans can also be infected (Kuru, Creutzfeldt–Jakob disease, and Gerstmann–Sträussler–Scheinker syndrome). Incubation periods can last for many years. In the course of these diseases, the brain tissue decays in a spongiform manner. BSE first appeared on a larger

scale in the United Kindom toward the end of the 1980s, while scrapie has been known for more than 260 years [7]. Presumably, the prions were transmitted via insufficiently heated meat-and-bone meal containing PrP^{sc} from scrapie-infected sheep, which was fed to cattle.

1.3.2 Viroid

Viroids are circular, single-stranded RNA molecules of low molar mass (approx. 12×10^4 Da, approx. 360 nucleotides). The RNA is "naked," i.e. not coated by protein. Viroids cause plant diseases, e.g. potato spindle tuber viroid.

1.3.3 Viruses

Viruses (lat. virus = poison, mucus) are predominantly ultramicroscopic, obligate cell parasites that contain only either DNA (e.g. poxvirus and herpes simplex) or RNA (e.g. influenza, rhinitis, and rabies viruses), have no enzyme systems for energy production and no systems for protein synthesis, and cause infected host cells to synthesize the virus building blocks. Viruses consist of at least a nucleic acid-containing inner body and a protein coat called a capsid. They may be enveloped, i.e. surrounded by a lipid bilayer (such as pathogens of smallpox, herpes, measles, influenza, rabies, acquired immunodeficiency syndrome [AIDS], and severe acute respiratory syndrome [SARS]) or be unenveloped (such as pathogens of polio, hepatitis A, rhinitis, and foot-and-mouth disease). Poliovirus can be characterized by the chemical molecular formula $C_{332652}H_{492388}N_{98245}\ O_{131196}P_{7501}\ S_{2340}$ [8]. On December 9, 1979, the WHO declared the world free of smallpox.

The size of viruses varies from 20 nm (picornaviruses and arboviruses) to 2000 nm (plant viruses such as the *Citrus tristeza* virus). Viruses that infect bacteria are called bacteriophages. T phages (coli phages) are well studied in molecular biology; their size is 70 nm × 200 nm. The bacteriophage T4 contains linear double-stranded DNA (dsDNA), and its genome size is 168 903 (see Figure 1.1).

In 2003, large viruses were found in amoebae. They were called mimiviruses. With sizes up to 800 nm, they are visible under the light microscope [8]. Viruses are detected using tissue culture, animal experiments, egg culture methods, PCR, and immunological methods. Approximately 1500 viruses are currently known, of which slightly more than 200 are human pathogens [9].

1.3.4 Archaea

Archaea (Greek: *archaios* for old, original) live in extreme locations, for example, in salt lakes (e.g. Dead Sea with approx. 30% of different salts, corresponding to an a_w value of 0.75), hot sulfur springs, and the deep sea. Archaea include methanogenic (produce methane, CH_4), thermophilic (live at high ambient temperatures), and halophilic representatives. Their cell wall structure is different from that of bacteria. So far, more than 250 species of archaea have been described, although pathogenic representatives are not yet known [9].

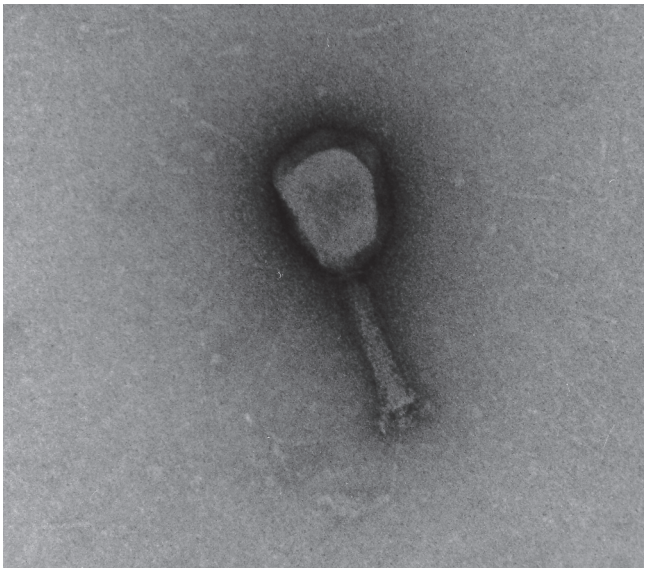

Figure 1.1 Bacteriophage T4, acceleration voltage 80 kV, uranyl acetate as contrast medium, magnification 184 800×. Electron Microscope EM 301, Philips, Eindhoven, The Netherlands.

The smallest representative of the archaea is *Nanoarchaeum equitans*. Although this organism has its own ribosomes, it uses part of the metabolic functions of the host cell. Archaea were defined as a separate bacterial kingdom (domain) by the American microbiologist C.R. Woese in 1977 [10].

1.3.5 Bacteria

Bacteria reproduce asexually by transverse division. They have a rigid cell wall of varying thickness that ensures shape and stability. The nuclear structure (which is not a true nucleus) is called a nucleoid. Meanwhile, more than 1000 bacterial genomes have been sequenced (the first sequence analysis was achieved in 1995 on the genome of *Haemophilus influenzae*). To date, more than 10 000 bacterial species have been described [11], and several hundred are added each year. Approximately 340 of the species known to date are human pathogens, and among the causes of death, infectious diseases occupy second place, with the consequences of tobacco consumption in the first place [12].

1.3.6 Chlamydia

They are obligate cell parasites that possess all the typical structural elements of bacteria. Chlamydiae undergo a developmental cycle (from the 0.3 µm elementary bodies to the 1 µm initial bodies). An example is the causative agent of the parrot disease psittacosis, *Chlamydia psittaci,* which can also infect humans, developing flu-like symptoms. Infection happens through inhalation of dust containing chlamydia from bird excrement. Many pigeons in cities are infected with *Chlamydia* species.

1.3.7 Rickettsia

They are also obligate cell parasites of 0.5–1 μm size. They reproduce by transverse division with the aid of host cell cofactors. An example is the causative agent of spotted fever, *Rickettsia prowazekii*. The bacteria are transmitted by ticks, mites, lice, and fleas. Another pathogen is *Coxiella burnetii*. Domestic and wild animals are infected by tick bites. Humans become infected by dust containing *Coxiella* from animal feces. The disease is called Q fever, and its diagnosis is done serologically.

1.3.8 Mycoplasma

This group includes bacteria without a rigid cell wall; as a result, they appear polymorphic and show high plasticity. Their size is 0.3–0.8 μm. Examples are the pathogens of pneumonia (*Mycoplasma pneumoniae*) and urinary tract infections (*Ureaplasma urealyticum*). Normal flora include *Mycoplasma buccale* (on the oral mucosa) and *Mycoplasma hominis* (on the mucosa of the intestine). From *Mycoplasma genitalium*, the genome was sequenced. It is 580 kb in size and contains only about 500 genes. In the Gram stain, the mycoplasmas react variably. They are resistant to penicillins and sulfonamides but not to tetracyclines and streptomycin.

1.3.9 Fungi

Fungi (mycobionta, molds, and yeasts) are a very heterogeneous group of ubiquitous eukaryotic organisms in many forms and colors, with more than 110 000 species. They can be divided into the following four groups: Basidiomycota with about 30 000 species, Ascomycota with about 46 000 species (including about 1000 species of yeasts or Endomycetes), Zygomycota with about 650 species, and *Fungi imperfecti* (or Deuteromycota) with about 30 000 species. Almost all human and animal pathogenic fungi, as well as most molds, belong to this last group [13]. Fungi are estimated to make up 25% of the biomass of our planet. Fungi can even colonize optical lenses in objective lenses. About 300 species of fungi are pathogenic to humans [9], and most diseases of crops are due to fungi. Fungi can produce toxins (more than 500 mycotoxins are known to date), some of which are lethal to humans and animals (in Germany, about 50 people die annually as a result of fungal poisoning). In addition, toxic and carcinogenic metabolites can be produced, especially by molds (e.g. Aflatoxins, Ochratoxins, Patulin, and Fusarium toxins). The Food and Agricultural Organization estimates that up to a quarter of the world's food production is contaminated with mycotoxins. The allergenic potential of the fungi, on the other hand, has so far been classified as low.

Common to all fungi is a rigid cell wall containing chitin (a polysaccharide), cellulose, glucans, etc., and the true nucleus. Fungi cannot photosynthesize, so they feed on finished organic matter: they are C-heterotrophs. Fungi either feed on dead organic matter (see Figure 1.2) or live as parasites on or in other living organisms. While most of the fungi reproduce asexually, some fungi also reproduce sexually. *Fungi imperfecti* are only known to reproduce asexually, for example,

Figure 1.2 White mold on damp furniture wood in the basement, after rainwater penetration.

by sprouting or conidiospores. Fungi are unicellular (e.g. sprout fungi such as the brewer's yeast *Saccharomyces cerevisiae* and the various *Candida* species) or multicellular (e.g. pathogens of dermatomycoses). The fungal cells are significantly larger than bacterial cells. Sprout fungi can appear in severely ill patients, for example, on the tongue, in the throat, in the bronchi, and in the esophagus. They also cause dangerous diseases of the meninges, lungs, kidneys, intestines, and other organs. Aspergillosis, caused by *Aspergillus fumigatus*, is feared in hospitals: this infectious disease has the worst prognosis of all [14]. A photograph of an Aspergillus is shown on Figure 1.3. In nature, the fungus lives on dead plants, in compost heaps, organic waste garbage cans, cereals, hay, tea leaves, and nuts. The living range for molds is shown in Table 1.3. The fungal spores are inhaled through the lungs. In healthy people, the spores are destroyed by macrophages, but in immunocompromised patients, the defense against fungal spores does not work, and they are transported through the bloodstream to the various organs. The lethality is high; about 2/3 of the infected patients die, which is about 2500 people in Germany every year.

Skin fungi belong to various species and, like sprout fungi, are very difficult to control. Fungi can multiply in damp places, for example, in bathing establishments.

Other fungal diseases are liver tumors caused by fungal metabolites (aflatoxins and patulin). Aflatoxin can be present in moldy food, patulin-containing spoiled apples, and juices.

As with foodstuffs, mold growth is also possible with pharmaceuticals, especially if they are stored improperly. Walls with mold growth pose a particular hazard, as measurably elevated levels of fungal spores can be found in the air in such rooms. This is a danger both for the people who have to stay in such rooms and for the medicines that are manufactured or stored in such rooms.

Figure 1.3 SEM image of *Aspergillus niger* on agar plate. Source: Courtesy of Dr. M.Rohde/HZI Braunschweig.

1.3.10 Protozoa

This group includes free-living or parasitic unicellular eukaryotes with most of the characteristics of animal cells. Reproduction usually occurs by bipartition. Transmission of parasitic protozoa to humans often occurs through arthropods: The causative agent of malaria (*Plasmodium*) is transmitted by anopheles mosquitoes, and the causative agent of sleeping sickness (*Trypanosoma brucei)* is transmitted by tsetse flies (*Glossina* ssp.). Sleeping sickness is one of the few infectious diseases with a 100% lethality rate.

1.4 The Bacterial Cell

The average weight of a bacterial cell is about 10^{-12} g, which is less than one-thousandth of the cell weight of an animal cell [15], and it is also much smaller than the eukaryote cell. The bacterial cell is composed of the following components:

Table 1.3 Living range for molds. In the area marked with **X**, the growth of molds is optimal.

Temperature (°C) 60	70	75	80	85	90	95	100	pH value
Increase in relative humidity (% r. h.) →								
80								12
70	×	×	×	×	×	×	×	11
60	×	×	×	×	×	×	×	10
50	×	×	×	×	×	×	×	9
40	×	×	×	×	×	×	×	8
30	×	×	×	**X**	**X**	**X**	×	7
20	×	×	×	**X**	**X**	**X**	×	6
10	×	×	×	**X**	**X**	**X**	×	5
0	×	×	×	×	×	×	×	4
Increase in the supply of nutrients→.								

- Prokaryotic nuclear substance (nucleoid)
  - The nucleoid is a naked, unraveled, right-handed, mostly circular DNA molecule with a molar mass of about 2.5×10^9 Da. In case of transverse division, doubling of the nucleoid always occurs first.
- Plasmids
  - Plasmids consist of extrachromosomal DNA. Between 1% and 5% of the genetic information of the bacterial cell may be plasmid-encoded. Of medical importance are the resistance plasmids (R plasmids), which contain genes that provide resistance to antibiotics. The F plasmids carry fertility factors.
- Cytoplasm
  - The cytoplasm contains many substances dissolved in water (proteins and minerals) and the 70S ribosomes. The ribosomes are responsible for protein synthesis. Their number in fast-growing bacteria is about 20 000, their size is 20–24 nm, and their sedimentation speed in ultracentrifuge is 70 Svedberg units.
- Reserves
  - Reserve substances include polyphosphates (volutin), poly-β-hydroxy-butyric acid (PHB), glycogen (in *Bacillus species* and enterobacteria), and lipid droplets.
  - Reserve substances are formed under certain environmental conditions and are used again in situations of deficiency.
- Cytoplasmic membrane
  - This semipermeable elemental membrane consists of a phospholipid bilayer in which folded protein molecules are embedded.
- Cell wall
  - It is 10–80 nm thick, gives the bacteria a solid shape, and forms an elastic protective shell against external injuries. The internal pressure can be between 500

and 2000 kPa [9]. The cell wall is permeable, i.e. largely permeable to food substances. The chemical structure of the cell wall is different in Gram-negative and Gram-positive bacteria. In Gram-positive bacteria, the cell wall consists of a lot of murein (mucopolysaccharide cross-linked by peptides). The thickness of the cell wall is 15–80 nm. The cell wall makes up 30% of the dry mass. In Gram-negative bacteria, there is little murein but many proteins and phospholipids. The thickness here is around 10 nm.

1.4.1 Capsule

Many bacteria form a capsule of polysaccharide polymer outside the cell in vivo with the aid of extracellular enzymes (except *Bacillus anthracis*: D-GLUTAMIC ACID). The capsule largely protects against phagocytosis (uptake by white blood cells) and thus against nonspecific infection defense.

1.4.2 Flagella

Most motile bacteria have flagella. These are made up of the linear protein flagellin. Flagella are anchored in the cell envelope via a complex structure and are able to rotate around their axis (at frequencies of up to 300 Hz), resulting in forward movement. In water, bacteria can thus advance at up to 100 µm s^{-1}. *Escherichia coli* has four to six flagella (see Figure 13.3), whose lengths can be up to 45 µm [16]. Flagella of bacteria are thin (15–20 nm), and archaeal flagella are only 10–13 nm in width [20].

The flagella can be arranged as follows:

- Monotrich (e.g. *Vibrio*)
- Lophotrich (e.g. *Pseudomonas*)
- Peritrich (e.g. *Salmonella*)

1.4.3 Fimbriae and Pili

Many bacteria form surface structures that are shorter and finer than flagella. Fimbriae are responsible for attaching to specific host cell receptors. Sex pili are filamentous hollow protein tubes responsible for cell-to-cell contact during conjugation (transfer of DNA). The pili (see Figure 1.4) are 0.2–1.2 µm long and 10 nm thick [16].

1.4.4 Endotoxins

Endotoxins are lipopolysaccharides (LPS) localized in the outer membrane of the cell wall of Gram-negative bacteria. They enter the milieu through the release of membrane vesicles by living bacteria or when the bacterial cell dies. Endotoxins have a fever-producing (pyrogenic) effect in humans and in many mammals (rabbits, dogs, cats, horses, cows, etc.), but not, for example, in birds.

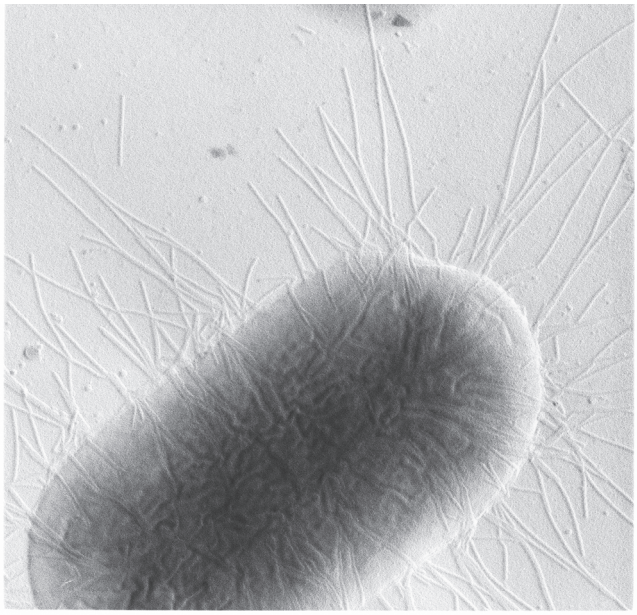

Figure 1.4 *Escherichia coli* BS5 with pili. Electron microscope EM 301, Philips, Eindhoven, The Netherlands.

1.4.5 Exotoxins

The Exotoxins are microbial virulence factors. As the prefix "exo" indicates, these toxins are secreted into the environment or are associated with the outside of the microbial cell. Exotoxins are mostly proteins/enzymes. *Vibrio cholera* is an example of a bacterium that contains endotoxins and exotoxins. Differential characteristics of exotoxins and endotoxins are shown in the following table.

Characteristic	Exotoxin	Endotoxin
Chemical nature	Protein	Lipopolysaccharide
Part of Gram(−) cell	No	Yes
Most from Gram(+) bacteria	Yes	No
Usually extracellular	Yes	No
Phage or plasmid coded	Many	No
Antigenic	Yes	Weakly
Can be inverted to toxoid	Many	No
Neutralized by antibody	Yes	Weakly
Differing pharmacologic specificities	Yes	No
Stable to boiling	No[a]	Yes

a) Exception is the enterotoxin of *Staphylococcus aureus*; the enterotoxins withstand boiling.
Source: Reference [34]/with permission of McGraw-Hill.

1.4.6 Bacterial Morphology

The size range of bacteria and other microorganisms is shown in Table 1.2. The size of all living organisms ranges between 0.3 µm (smallest bacteria such as *Corynebacterium diphtheriae,* the causative agent of diphtheria, or *Brevundimonas diminuta,* a rod-shaped aquatic bacterium) and the Hallimasch fungus (*Armillaria ostoyae*), whose mycelial extent under the ground is 600 ha, discovered in 1992 in the US state of Washington [6].

1.4.7 Bacteria Forms

Bacterial cells may appear in the following forms: Cocci alone or in clusters, racemes, chains, or lanceolate diplococci (the latter with capsule). They can also be found as straight rods, straight rod angular, club-shaped rods, rods with pointed ends, simple curved rods, or spiral rods.

1.4.8 Endospores

Bacterial endospores are not propagules like fungal spores but permanent forms in some aerobic and anaerobic bacterial genera. They protect the bacterial genome in unfavorable conditions. More than 200 genes are involved in sporulation. The widely distributed genera *Bacillus, Geobacillus, Paenibacillus, Sporolactobacillus, Sporosarcina, Sporobacter, Sporotomaculum, Halobacillus, Thermoactinomyces, Thermoanaerobacter, Desulfotomaculum,* and *Clostridium,* totaling over 30 genera, are capable of sporulation. The animal pathogenic genera *Actinobacillus* and *Streptobacillus* are not capable of forming endospores (the suffix "-bacillus" in their names leads to this assumption).

The formation of an endospore begins in the vegetative cell when environmental conditions become adverse. For sporulation, the dry matter of the cell condenses to 1/10 of its volume to form a spore protoplast. The double-enveloping cytoplasmic membrane forms the spore wall. In the final stage, the remnants of the vegetative cell dissolve. Endospores have considerable resistance to disinfectants and high temperatures and can remain viable for years or decades.

The causes of heat resistance are the thick spore wall and the lack of water, which makes denaturation of the proteins difficult.

If the endospore enters an environment where conditions are favorable for bacterial life, reversion to the vegetative cell form occurs.

Possible locations of endospores are as follows:

- Endospore formation centrally, without distension of the vegetative cell.
- Endospore formation terminal, without distension of the vegetative cell.
- Endospore formation terminal, with distension of the vegetative cell.
- Endospore formation central, with distension of the vegetative cell.
- Endospores released.

1.4.9 Bacterial Physiology

The metabolism and the growth and survival of bacteria are influenced by a variety of environmental factors, just as in higher organisms.

1.4.9.1 Nutrition and Metabolism

The basic needs of bacteria are very similar to those of higher organisms. They need:

- A source of energy for the metabolism.
- A carbon source for building proteins, polysaccharides, and nucleic acids.
- A nitrogen source for building proteins, polysaccharides, and nucleic acids.
- A phosphate source for building ATP, nucleotides, nucleic acids, and phospholipids.
- A source of sulfur for building amino acids cysteine and methionine, as well as thiamine pyrophosphate, coenzyme A, biotin, and α-lipoic acid.
- A series of inorganic salts and trace elements that are needed for enzymes.
- Vitamins and other growth factors.

In metabolism, a distinction is made between metabolism that builds up (anabolism or assimilation) and metabolism that breaks down (catabolism or dissimilation). However, the two processes cannot be strictly separated from each other, but run simultaneously side by side in the cell. Material degradation always takes place with the release of energy (exergonic process), and material buildup requires the supply of energy (endergonic process).

Both the anabolic and the catabolic processes are initiated and kept going by enzymes. Enzymes are high-molecular-weight proteins with catalyst functions, also called biocatalysts.

Examples include:

- Proteases = protein-cleaving enzymes (such as trypsin, pepsin, and papain),
- Carbohydrases = carbohydrate-splitting enzymes (such as amylase),
- Lipases = fat-cleaving enzymes (such as pancreatic lipase),
- Nucleases = nucleic acid-cleaving enzymes (such as DNase and RNase).

1.4.9.1.1 Assimilation Bacteria vary greatly in their ability to produce complicated organic compounds from simple building blocks. The unilaterally human-pathogenic gonococci are very dependent; in contrast, the tubercle bacilli (causative agent of tuberculosis) are capable of many syntheses. Certain soil bacteria can synthesize practically all compounds themselves.

1.4.9.1.2 Dissimilation The degradation processes for energy production only sporadically include fats and proteins as operating materials; the degradation of carbohydrates, especially glucose, occupies by far the most important position.

Fermentation is the decomposition of organic material with the release of energy in the absence of free oxygen, the hydrogen transfer function of which is taken over by other compounds.

Compared to respiration, fermentation yields only low energy. Often, the substrate (nutrient medium and degradable material) is not completely degraded to carbon dioxide and water, but typical fermentation products such as lactic acid, butyric acid, propionic acid, ethanol, and other compounds are formed in addition to CO_2.

During respiration and fermentation, a number of intermediate and end products are produced, so-called metabolites, which are secreted by the microorganisms,

sometimes in large quantities. Some of these metabolites (mostly proteins) – together with certain enzymes – constitute the bacterial poisons or toxins. Because they are released externally, they are also called exotoxins (e.g. hemolytic toxins of streptococci, diphtheria toxin, tetanus toxin, and botulinus toxin). These toxins are usually heat-labile, i.e. they are destroyed by heating. An exception is the staphylococcal toxins (e.g. in food), which are heat-stable and thus do not get destroyed by cooking.

The other group of bacterial toxins is the heat-stable endotoxins.

1.4.9.1.3 Respiration Metabolism is referred to as respiration when the hydrogen originating from various organic substances finally reacts with free oxygen to form water, releasing energy.

Most bacteria are tolerant of the presence or absence of free oxygen in their environment. They are facultatively anaerobic.

In addition, there are a number of bacteria that require the free oxygen in the air for their respiration: they are obligate aerobes, e.g. tubercle bacilli or many pseudomonads. A number of bacteria, in turn, obtain oxygen from organic compounds. For them, free oxygen is toxic. These bacteria are referred to as obligate anaerobes, e.g. *Clostridium tetani*, the causative agent of tetanus, and other clostridia. Finally, there are some species of bacteria that grow best in the presence of trace amounts of free oxygen. These bacteria are microaerophilic.

1.4.9.2 Ambient Condition

For optimal growth of microorganisms, they require suitable temperature, humidity, and pH conditions (Table 1.4).

1.4.9.2.1 Temperature The optimum growth temperature is not the same for all bacteria. Below are the optimum growth temperatures for some bacteria:

- 0–10 °C cold-loving, psychrophilic bacteria.
- 10–25 °C cold-bearing, psychrotolerant bacteria.
- 30–39 °C mesophilic bacteria (often pathogenic to humans and animals).
- >50 °C heat-loving, thermophilic bacteria.
- >80 °C (up to approx. 113 °C) hyperthermophilic microorganisms, mostly archaea.

Temperatures above the maximum temperature of the respective germ species have a harmful effect very soon. Even fever temperatures can have an inhibitory effect on certain pathogenic germs. Most bacteria tolerate cold well for long periods; however, some of the microorganisms perish during deep freezing, especially during thawing. Below –12 °C, bacteria cease to grow [17].

1.4.9.2.2 Humidity Bacteria consist of more than 80% by weight of water, so they are dependent on sufficient moisture for their growth – just like the higher organisms. The optimum moisture is offered to the microorganisms in liquid nutrient media. Solid culture media must also contain plenty of moisture; otherwise, most bacteria will dry out. Gram-negative bacteria (e.g. Pseudomonas and Gonococci) are

Table 1.4 Environmental conditions for microorganisms.

Parameter	Lower limit	Medium range	Upper limit
Temperature	<−12 °C no growth possible	e.g. *Escherichia coli*: 8–48 °C, optimal 39 °C	113 °C *Pyrolobus*, 84 °C *Bacillus* sp. from approx. 112 °C sporicidal effect in autoclave
pH value	pH 0.5 *Picrophilus oshimae* *Picrophilus torridus*	pH 5.5–8.0	pH 11.5 *Bacillus-like* isolates and pH 13.0 *Natronobacterium*
Print	—	Isolate MT41 grows optimally at 700 bar and 4 °C [24]	>1000 bar (deep sea), isolate MT41
Water activity	$a_w = 0.60$ *Saccharomyces rouxii*	$a_w = 0.80$–0.99	$a_w = 1.0$ (pure water) water microorganisms
Salt concentration		0.9 % (=0.16 M NaCl)	5 M NaCl (=29% w/v), $a_w = 0.75$ *Haloferax volcanii* (see Figure 1.5)
Radiation	—	—	18 000 Gy *Deinococcus radiodurans*

generally more sensitive to desiccation than Gram-positive ones. Bacteria with wax envelopes (*Mycobacterium*) are quite resistant, and endospores are even more resistant. Spores of the anthrax pathogen *Bacillus anthracis* have survived for more than 50 years in lab experiments.

1.4.9.2.3 Water Activity The viability and survivability of microorganisms depend on the actual available (active) water. The water activity a_w represents the physical quantity for the available water (Table 1.5).

The a_w value indicates the ratio of the water vapor pressure of a substrate to the vapor pressure of pure water; this value can be a maximum of 1.0.

$$a_w = \frac{p}{p_0}$$

p = Water vapor pressure of the substrate (e.g. tablet, raw material, and food).
p_0 = Vapor pressure of pure water at the same temperature.

ICH Q6A classifies microbial risk as follows:
High risk: $a_w > 0.95$
Medium risk: $a_w > 0.90$ to <0.95
Low risk: $a_w < 0.80$

Readily perishable substrates are those with a water activity of >0.95 (juices, liquid foods, fish, and fresh meat), while <0.90 microbial infestations are severely

Table 1.5 Minimum water activity values (a_w) for the growth of various microorganisms. Below 0.60, growth is no longer possible.

a_w value	Microorganisms	Substrate	Representative
0.98–1.00	Waterborne bacteria	Pure water 1.0; blood, parenterals, nasal spray, and hair shampoo 0.99	Caulobacter and Spirillum
0.96–0.97	Gram-negative rods	Juices, creams; 7.5% w/v NaCl solution 0.957	Pseudomonas, *Escherichia coli*, Shigella, Acinetobacter, Flavobacterium, and many other microorganisms
0.91–0.95	Most bacteria	Bread, ham 0.89–0.96	*Bacillus cereus*, Clostridium, Citrobacter, Corynebacterium, Salmonella, Lactobacillus, and Serratia
0.87–0.94	Most yeasts	Maple syrup, jam, and liquid oral formulations	Candida, Fusarium, Mucor, and Aspergillus
0.86–0.90	Gram-positive cocci	Salami	Micrococcus and *Staphylococcus aureus*
0.80	Most molds	Cake; jam	Penicillium, Rhizopus, and *Saccharomyces bailii*
0.70	Molds	Cereals	*Aspergillus glaucus*
0.75	Halophilic bacteria	Salt lakes; salted fish	Halobacterium and Halococcus
0.65	Xerophilic molds	Cereals; cookies; dried fruits	Aspergillus, Chrysosporium, Xeromyces, and Eurotium
0.61	Osmophilic yeasts	Ointments 0.55	*Zygosaccharomyces rouxii* and *Xeromyces bisporus*

Source: Compilation from Refs. [18–21]

restricted (e.g. hard cheese, hard sausage, dried foods, jams, and marzipan). Below $a_w = 0.55$ are film-coated tablets, capsules, ointments, suppositories, lipsticks, and raw materials such as flour, sugar, and salts. The excipient lactose monohydrate has $a_w = 0.38$, and calcium phosphate has $a_w = 0.3$. Below 0.60, growth and multiplication of microorganisms are not possible and endospores do not germinate.

For determination of water activity, see chapter 2.9.39 in Ph. Eur. and chapters <922> and <1112> in USP.

1.4.9.2.4 pH Value Most microorganisms multiply only within a narrow pH range, generally between pH 5.5 and 8.0. Pathogenic forms thrive best at pH 7.2–7.5 (human blood has a pH of 7.41). However, bacteria with an optimum pH <4.0 (lactobacilli and acetogenic bacteria) or pH 8.5 (Alcaligenes and Vibrio) also exist. *Bacillus cereus* tolerates pH values up to 9.3; molds can exist between pH 1.5 and 9. In nature, many fungi live on acidic forest soils and meadows. Among the archaea in particular, there are specialists that can live in highly acidic environments, e.g. the acidophilic and thermophilic *Picrophilus oshimae,* which grows at 60 °C and at pH 0.5 [22]. Lakes

Figure 1.5 Halobacteria color a saline on the Spanish island Lanzarote red. Source: With permission from Dr. Armin Quentmeier.

without any biological life are located in an East African desert named Danakil: The pH value is near 0, the concentration of salts is about 70%, and the temperature can reach 100 °C.

1.4.9.2.5 Radiation *Deinococcus radiodurans* was isolated in 1956 from canned meat sterilized with gamma rays. Cultures from the exponential growth phase can survive 18 000 Gy. For comparison, the LD_{50} for humans is 5 Gy. Deinococci have afforded their radiation resistance by their very effective repair of strand breaks in DNA. In addition, Deinococcus species are extremely resistant to UV radiation (up to 1000 J/m^2). The Gram-positive, pigmented, nonmotile cells grow between 4 and 45 °C with an optimum growth at 30 °C; the generation time is 80 minutes [23]. In addition to radiation, *D. radiodurans* has shown resistance to H_2O_2, desiccation, and DNA-damaging agents. Due to these facts, the microorganism found its way into the "Guinness Book of World Records" as one of the toughest microbes in the world.

1.5 Taxonomy of Microorganisms

Taxonomy of microorganisms means their classification and naming. Taxonomy is the science of classification and nomenclature.

1.5.1 Classification

Classification involves the ordering of bacteria into taxonomic groups (taxa) based on relationships. These are best revealed by knowledge about evolution. However, since very little is known about the phylogenetic relationships of bacteria, their classification is based on similarities in morphological, physiological, chemical, and,

more recently, increasingly on genetic characteristics. Especially due to the latter characteristics, renaming of numerous bacterial species became necessary.

Formally, prokaryotes are divided into domains, phyla, classes, orders, families, genera, and species with any subtaxa present. For this purpose, the following example is given:

Domain:	Bacteria
Phylum:	Proteobacteria
Class:	Gammaproteobacteria
Order:	Enterobacterales
Family (Familia):	Enterobacteriaceae (Greek: enteron = intestines)
Genus:	Escherichia
Species:	*Escherichia coli* (colon = intestine)
Var or type:	e.g. Serovar O157:H7
Strain:	xyz

This scheme is also applicable to humans. See the example below:

Order:	Primates
Family (Familia):	Hominidae
Genus:	Homo
Species:	*Homo sapiens*
Var or type:	e.g. Europide
Strain:	e.g. Hesse

Escherichia coli was named after the Bavarian pediatrician Theodor Escherich (1857–1911), who discovered this bacterium in 1885 and published his bacteriological studies in the book "The Intestinal Bacteria of the Child." *Escherichia coli* is the most common bacterial pathogen of urinary tract infections and travelers' diarrhea. Several thousand serovars are known.

Human-pathogenic *E. coli* include EHEC (see Figure 1.6 and Table 1.6), EAHEC, DAEC, STEC, VTEC, EIEC, EPEC, and ETEC. These pathovars have β-lactamase genes and virulence factors for toxins (e.g. Shiga toxins), adhesins (e.g. eae), and dispersin.

In May 2011, Germany experienced an outbreak of infections with EAHEC, a new hybrid of EAEC and EHEC; it was serotype O104:H4. O refers to the type of surface-specific side chain (=LPS) and H to the flagellar antigens. Over 4,300 people had become infected and over 50 died (as of July 2011). The source is thought to have been imported, contaminated fenugreek seed. The incubation period is two to ten days, and the infectious dose is very low at ten to one hundred cells. In Germany, there has been an obligation to report human-pathogenic *E. coli* since 2001. According to statistics from the Robert Koch Institute (RKI), Berlin, approximately 1000 people became ill annually in the years 2001–2010. Human-pathogenic *E. coli* live

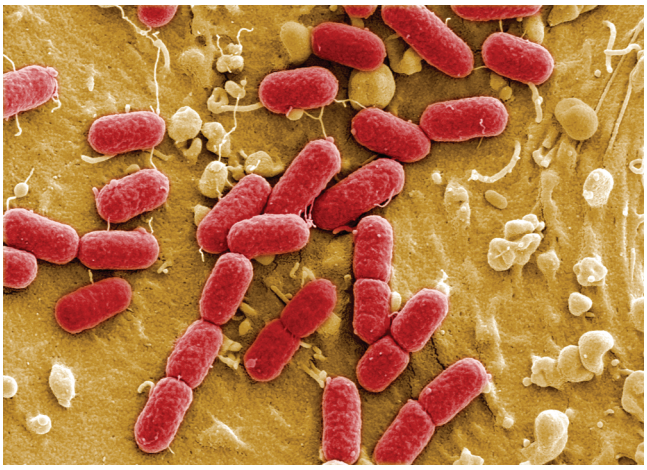

Figure 1.6 EHEC O157-H7 on fibroblast. SEM image, magnification 40,000×.
Source: Courtesy of Dr. M.Rohde/HZI Braunschweig.

Table 1.6 Human-pathogenic *Escherichia coli* strains.

Pathovar		Property	Disease
Adherent *Escherichia coli*	EAEC DAEC	Aggregation Adherence	Role of DAEC in intestinal Infections unclear
STEC/VTEC	EHEC	Adherence	Hemorrhagic colitis, HUS
EIEC		Invasion	Dysentery-like colon infection
EPEC		Adherence	Diarrhea in babies
ETEC		Colonization	Traveler's diarrhea

DAEC, diffusely adherent *Escherichia coli*; EAEC, enteroaggregative *Escherichia coli*; EHEC: enterohemorrhagic *Escherichia coli*; EIEC, enteroinvasive *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli;* ETEC, enterotoxigenic *Escherichia coli;* STEC, Shiga toxin-producing *Escherichia coli*; VTEC, verotoxin-producing *Escherichia coli*; HUS: hemolytic uremic syndrome.

naturally in the intestines of cattle, sheep, deer, and roe deer. Infections can happen through contaminated drinking water, raw milk, unrefrigerated meat, and contaminated vegetables. As a result of the EAHEC epidemic, the Infection Protection Act and the War Weapons Control Act ("dual use") are to be amended. Molecular biological detection of human-pathogenic *E. coli is* successful within two days. For the detection of STEC, PCR is considered the gold standard.

Globally, *E. coli* strains are responsible for approximately 160 million diarrheal cases annually, including approximately one million deaths (2013 figures, see Ref. [25]).

The Enterobacteriaceae are distributed over 47 genera with hundreds of species. They are Gram-negative, facultative anaerobic, peritrichous flagellated, or immobile rods. The historical term coliforms refers to the lactose-positive members of the

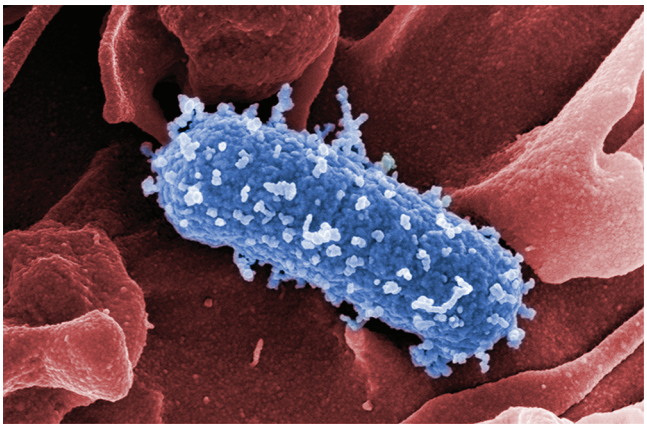

Figure 1.7 *Escherichia coli* cell on a macrophage. SEM image, magnification 40,000×. Source: Courtesy of Dr. M.Rohde/HZI Braunschweig.

Enterobacteriaceae. Coliform bacteria are Escherichia, Enterobacter, Klebsiella, Citrobacter, and Serratia, and non-coliform bacteria, being lactose-negative, are Salmonella, Shigella, Proteus, Providencia, and Morganella. Of Escherichia, six species have been described to date. Many Enterobacteriaceae cause opportunistic diseases in humans, such as infections of the skin, subcutis, urinary tract, respiratory tract, and wounds including sepsis.

Some data on the properties and composition of the *E. coli* cell (see Figure 1.7) are as follows [12, 16, 26]:

- Stick-shaped cell approximately 1 μm × 2 μm long, 0.5–0.6 μm wide, and volume approximately 2.5 μm^3.
- Approx. 70% water.
- Ring-shaped DNA molecule, approximately 1.7 mm long, 4 639 675 bp, and 4288 genes.
- Mutation rate 10^{-7}.
- One to several plasmids.
- Four to six flagella (up to 45 μm long).
- Numerous pili (0.2–1.2 μm long).
- Gram-negative, approximately 3.5×10^6 molecules of LPS.
- 20 000 ribosomes.
- Approx. 1250 different enzymes, which are present in the cell as copies ranging from tens to several thousand.
- Approx. 5000 RNA polymerase enzymes are active in the growth phase.
- 255 proteins involved in transport functions.
- 20 species t-RNA.
- Approx. 1220 organic compounds with $M_r < 1000$ Da (amino acids, sugars, nucleotides, etc.).
- Many inorganic ions.

The basis of classification is the species. Often it is necessary, especially in epidemiology, to subdivide a species into vare (synonym types). Cultures of a species that have certain characteristics in common are grouped together. Examples are biovar, phagovar, pathovar, morphovar, and serovar.

The term strain is used in different ways. In clinical bacteriology, it refers to the initial culture of a species isolated from a patient during an infection. In epidemiology, isolates of the same species from different patients are also referred to as belonging to the same epidemic strain.

It is important to know that there is no official, internationally valid classification of bacteria. Therefore, especially the higher taxa are often grouped according to practical aspects. For example, a classification based on the practical needs of medicine can be used, e.g. the classification used in the standard work "*Bergey's Manual of Systematic Bacteriology*" [27, 28].

1.5.2 Nomenclature

Nomenclature as the second subfield of taxonomy comprises the naming of the taxonomic groups. The "*International Code for the Nomenclature of Bacteria*" defines the rules for naming. Accordingly, a species is identified by two Latinized names: the first name characterizing the genus and the second the species. Families are designated with the suffix "-aceae." In contrast to the classification, names accepted by the "*International Committee of Systematic Bacteriology*" are considered official and binding.

1.6 Medical Microbiology

Some microorganisms can cause infectious diseases (see Table 1.7). Infection or contagion is the transmission, adherence, and invasion of microorganisms into a macroorganism such as humans, animals, or plants [29]. The site where the infectious agent resides is called the primary source of infection. Secondary sources of infection are objects or third persons involved in indirect transmission.

1.6.1 Infection Routes

Humans have several entry points for potential pathogens. The route of infection can be direct or indirect.

1.6.1.1 Direct Routes

- Fecal–oral (smear infection): Salmonella, Shigella, Vibriones, EHEC, and hepatitis A virus.
- Aerogenic (droplet infection): Mycobacterium *tuberculosis, Bacillus anthracis* (pulmonary anthrax), *Legionella pneumophila* (Pontiac fever), *Coxiella burnetii* (Q fever), *Francisella tularensis* (tularemia, rabbit plague), and *Chlamydia psittaci*.

Table 1.7 Infectious diseases and their modes of transmission, pathogens (causative agents), and infectious doses.

Disease	Incubation period	Initiating agent/infectious dose	Transmission
Tuberculosis	4–6 weeks	*Mycobacterium tuberculosis* 1 cell (guinea pig model)	Aerogenic (oral)
Legionellosis	2–10 days	1 cell of *Legionella pneumophila* in respirable aerosol droplets.	Aerogenic
Mite spotted fever	?	3 cells of *Orientia tsutsugamushi*	Bite
Q Fever	14–21 days	10 cells of *Coxiella burnetii*	Aerogenic
Tularemia	4 days	10 cells of *Francisella tularensis*	Aerogenic
Rubella	12–21 days	≥10 Rubella virus (portal of entry pharynx). 60 Rubella virus (entry nasal mucosa)	Aerogenic
Trichinosis	5–10 days	50–70 *Trichinella spiralis*	Oral
Syphilis	14–28 days	60 cells of *Treponema pallidum*	Mucosa
EHEC infection	2–10 days	10–100 enterohaemorrhagic *Escherichia coli*	Oral
Flu	1–3 days	340 influenza viruses	Aerogenic
Shigellose/Ruhr	2–7 days	10–200 cells of *Shigella flexneri* 10^9 cells of *Shigella dysenteriae*	Oral
Campylobacter	3–5 days	500 *Campylobacter jejuni*	Oral
Enteritis	2 weeks	10^3 *Giardia lamblia*	Oral
Pulmonary anthrax	1–7 days	≥1300 cells of *Bacillus anthracis*	aerogenic
Typhoid	12–14 days	10^5 cells of *Salmonella typhi*	Oral
Cholera	1–2 days	$>10^6$ *Vibrio cholerae*	Oral
Food poisoning	4–6 hours 1–3 days	(a) *Bacillus cereus*: oral 10^5–10^6 Bacilli/g food. b) *Clostridium botulinum*: lethal dose: 0.1–1 µg toxin A	Oral
Diarrhea	Hours	10^8 enterotoxigenic *Escherichia coli (ETEC)*	Oral
BSE, scrapie	Years	$>10^5$ infectious prions PrP^{sc}	Oral
Fever	After 20 minutes	Endotoxins of Gram−negative bacteria 1 ng = 0.1 EU from 5 EU/kg body weight fever reaction	Intravenously Intrathecal

- Genital (sexual intercourse): *Treponema pallidum,* Candida (thrush), HIV, and hepatitis B and D viruses.
- Cutaneous: staphylococci and dermatophytes.
- Pränatal: intrauterine infection of the fruit, infection routes via the placenta or from the fallopian tubes, infection after rupture of the membranes, with the abbreviation Toxoplasmosis, other, Rubella, Cytomegaly and Herpes (TORCH) the most important diseases are named: Toxoplasmosis (*Toxoplasma gondii*),

other (such as syphilis, listeriosis), Rubella (viruses), Cytomegaly (viruses), and Herpes simplex (viruses).
- Perinatal: hepatitis B, C, and D viruses; in premature infants, nosocomial infections due to Streptococcus, Klebsiella, and Listeria; umbilical wound infections; conjunctivitis due to chlamydia.
- Inoculation (through puncture wounds and cuts, animal bites, and stings): Rhabdoviruses (rabies), HIV, and hepatitis B viruses via infected needles; Rickettsia, Borrelia, TBE viruses, and Plasmodia via insect bites.

1.6.1.2 Indirect Route

Through

- Water: *Vibrio cholerae*.
- Food: *Bacillus cereus, Staphylococcus aureus,* Enterobacteriaceae, and *Clostridium perfringens*.
- Dust/soil: *Bacillus anthracis* and *Clostridium tetani*.
- Contaminated items: catheter infections (*Staphylococcus epidermidis*).
- Vectors: vectors are for example Ixodes ricinus, TBE viruses, Borrelia and others.
- The human (hand contacts): touching pus and blood.
- Medical/medical measures (iatrogenic): nonsterile instruments.

In sepsis (septicemia), microorganisms enter the bloodstream. The blood distributes them to the various organs so that foci of inflammation then form there. Typical sepsis pathogens are *Staphylococcus aureus, Streptococcus pyogenes, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Bacteroides fragilis* (an obligate anaerobic intestinal bacterium). Each year, more than 150 000 people in Germany develop sepsis, with nearly 50% dying as a result [30].

1.6.1.3 Nosocomial Diseases

The Section 2 of IfSG defines the term nosocomial infection as "an infection with local or systemic signs of infection as a reaction to the presence of pathogens or their toxins, which is temporally related to an inpatient or an outpatient medical procedure, as long as the infection did not already exist beforehand."

Nosocomial diseases can affect hospitalized patients (Greek: nosokomeion for hospital) by facultative pathogenic bacteria such as *Pseudomonas aeruginosa* and a number of other Gram-negative water bacteria (so-called "wet germs") as well as skin and mucous membrane germs (Staphylococcus and Streptococcus), yeasts such as *Candida albicans*, enteropathogenic fecal germs, and viruses (cytomegalovirus, coxsackievirus, ECHO virus, etc.) [31]. The source of infection is either the patient himself/herself or other patients, visitors, or medical staff. Nosocomial infections can be controlled by a strict hygiene regimen.

1.6.1.4 Zoonoses

Zoonoses are diseases of humans, with animals serving as sources of infection (see Table 1.8). In rare cases, the animal may infect the human, and then the human in turn may infect the animal, e.g. cat scratch disease; the causative agent is the

Table 1.8 Some human diseases and the probable animal sources of infection.

Animal source of infection	Disease	Pathogen
Fox, dog, and cat	Echinococcosis	*Echinococcus multilocularis*
Domestic cats and animals for slaughter	Toxoplasmosis	*Toxoplasma gondii*
Pork, beef, poultry, and eggs	Salmonellosis	*Salmonella enterica*
Rats (rat flea)	Pest	*Yersinia pestis*
Herbivorous animals (e.g. sheep and cows)	Anthrax	*Bacillus anthracis*
Chimpanzees	AIDS	HI virus
Great apes	Hepatitis B	HB virus
Cattle, sheep, and goats	EHEC infection, HUS	EHEC
Dogs, wolves, foxes, and bats	Rabies	Rhabdovirus
Bats	Ebola	Ebola virus
Bats and flying foxes	Hemorrhagic fever	Marburg virus
African monkeys	Yellow fever	Flavivirus
Wild and domesticated mammals	Chagas disease	*Trypanosoma cruzi*
Wild birds	Influenza A	Influenza viruses
Ticks	Lyme disease	*Borrelia burgdorferi*
Creeping cat (Viverridae)	SARS	Coronavirus

Source: References [9, 32, 33].

Gram-negative bacterium *Afipia felis*. Currently, approximately 800 zoonotic infectious diseases are known; among them, toxoplasmosis is the most common zoonosis [32]. Pet zoonoses include parrot disease, toxoplasmosis, echinococcosis, canine roundworm infection, and skin rashes caused by fungi transmitted from guinea pigs, rabbits, hamsters, mice, dogs, and cats. Hygiene measures such as daily cleaning of cages and toilets and thorough hand washing after animal contact, as well as regular worming and vaccination of pets, protect against infection. At least two new, unknown zoonoses are found in humans every year.

Substances of animal origin are used in the manufacture of drugs and medical devices, e.g. lactose, skim milk powder, and calcium lactate (from bovine milk); magnesium stearate (from animal fats); and gelatin (from bones, hides, and tendons). These examples are *excipients*. Active ingredients are derived from slaughterhouse materials such as porcine intestinal mucosa (heparin), pancreas (pancreatin or purified enzymes such as lipases, colipases, amylases, and proteases), gastric mucosa (pepsin), blood (hemin and albumin), liver (liver extract), thymus, bile (fel tauri, glycocholic acid, cholates, and deoxycholates), testes (hyaluronidase), and other organs (various organ extracts). These starting materials must be free of pathogenic microorganisms, viruses, and infectious prions, or the manufacturing process must include safe depletion or inactivation steps. This also applies to

pharmaceutical products manufactured from human material (e.g. clotting factors, immunoglobulins and albumin from blood, extracts, and enzymes from organs). Virus-depleting procedures include filtrations such as nanofiltration and chromatographic methods; inactivation procedures include irradiation with UV-C light (100-200 nm), precipitation, treatments with acid (e.g. 60 minutes at pH 3), and exposure to dry heat.

The three pillars of virus safety are as follows:

- Donor screening.
- Sufficient capacity for virus depletion/inactivation during the manufacturing process.
- Validated manufacturing process.

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