# 1 General Aspects of Medical Microbiology

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Infectious diseases are caused by subcellular infectious entities (prions, viruses), prokaryotic bacteria, eukaryotic fungi and protozoans, metazoan animals, such as parasitic worms (helminths), and some arthropods. Definitive proof that one of these factors is the cause of a given infection is demonstrated by fulfillment of the three Henle-Koch postulates. For technical reasons, a number of infections cannot fulfill the postulates in their strictest sense as formulated by R. Koch, in these cases a modified form of the postulates is applied.

## The History of Infectious Diseases

### The Past

Infectious diseases have been known for thousands of years, although accurate information on their etiology has only been available for about a century. In the medical teachings of Hippocrates, the cause of infections occurring frequently in a certain locality or during a certain period (epidemics) was sought in "changes" in the air according to the theory of miasmas. This concept, still reflected in terms such as "swamp fever" or "malaria," was the predominant academic opinion until the end of the 19$^{th}$ century, despite the fact that the Dutch cloth merchant A. van Leeuwenhoek had seen and described bacteria as early as the 17$^{th}$ century, using a microscope he built himself with a single convex lens and a very short focal length. At the time, general acceptance of the notion of "spontaneous generation"—creation of life from dead organic material—stood in the way of implicating the bacteria found in the corpses of infection victims as the cause of the deadly diseases. It was not until Pasteur disproved the doctrine of spontaneous generation in the second half of the 19$^{th}$ century that a new way of thinking became possible. By the end of that century, microorganisms had been identified as the causal agents in many familiar diseases by applying the Henle-Koch postulates formulated by R. Koch in 1890.

## The Henle–Koch Postulates

The postulates can be freely formulated as follows:

- The microorganism must be found under conditions corresponding to the pathological changes and clinical course of the disease in question.
- It must be possible to cause an identical (human) or similar (animal) disease with pure cultures of the pathogen.
- The pathogen must not occur within the framework of other diseases as an "accidental parasite."

These postulates are still used today to confirm the cause of an infectious disease. However, the fact that these conditions are not met does not necessarily exclude a contribution to disease etiology by a pathogen found in context. In particular, many infections caused by subcellular entities do not fulfill the postulates in their classic form.

## The Present

The frequency and deadliness of infectious diseases throughout thousands of years of human history have kept them at the focus of medical science. The development of effective preventive and therapeutic measures in recent decades has diminished, and sometimes eliminated entirely, the grim epidemics of smallpox, plague, spotted fever, diphtheria, and other such contagions. Today we have specific drug treatments for many infectious diseases. As a result of these developments, the attention of medical researchers was diverted to other fields: it seemed we had tamed the infectious diseases. Recent years have proved this assumption false. Previously unknown pathogens causing new diseases are being found and familiar organisms have demonstrated an ability to evolve new forms and reassert themselves. The origins of this reversal are many and complex: human behavior has changed, particularly in terms of mobility and nutrition. Further contributory factors were the introduction of invasive and aggressive medical therapies, neglect of established methods of infection control and, of course, the ability of pathogens to make full use of their specific genetic variability to adapt to changing conditions. The upshot is that physicians in particular, as well as other medical professionals and staff, urgently require a basic knowledge of the pathogens involved and the genesis of infectious diseases if they are to respond effectively to this dynamism in the field of infectiology. The aim of this textbook is to impart these essentials to them.

Table 1.**1** provides an overview of the causes of human infectious diseases.

Table 1.1 Human Pathogens

| Subcellular biological entities | Prokaryotic microorganisms | Eukaryotic microorganisms | Animals |
|---|---|---|---|
| Prions (infection proteins) | Chlamydiae (0.3–1 µm) | Fungi (yeasts 5–10 µm, size of mold fungi indeterminable) | Helminths (parasitic worms) |
| Viruses (20–200 nm) | Rickettsiae (0.3–1 µm) | Protozoa (1–150 µm) | Arthropods |
| | Mycoplasmas | | |
| | Classic bacteria (1–5 µm) | | |

## Pathogens

### Subcellular Infectious Entities

**Prions (proteinaceous infectious particles).** The evidence indicates that prions are protein molecules that cause degenerative central nervous system (CNS) diseases such as Creutzfeldt-Jakob disease, kuru, scrapie in sheep, and bovine spongiform encephalopathy (BSE) (general term: transmissible spongiform encephalopathies [TSE]).

**Viruses.** Ultramicroscopic, obligate intracellular parasites that:

- contain only one type of nucleic acid, either DNA or RNA,
- possess no enzymatic energy-producing system and no protein-synthesizing apparatus, and
- force infected host cells to synthesize virus particles.

### Prokaryotic and Eukaryotic Microorganisms

According to a proposal by Woese that has been gaining general acceptance in recent years, the world of living things is classified in the three domains *bacteria*, *archaea*, and *eucarya*. In this system, each domain is subdivided into

kingdoms. Pathogenic microorganisms are found in the domains bacteria and eucarya.

**Bacteria, Archaea, Eucarya**

**Bacteria.** This domain includes the kingdom of the heterotrophic eubacteria and includes all human pathogen bacteria. The other kingdoms, for instance that of the photosynthetic cyanobacteria, are not pathogenic. It is estimated that bacterial species on Earth number in the hundreds of thousands, of which only about 5500 have been discovered and described in detail.

**Archaea.** This domain includes forms that live under extreme environmental conditions, including thermophilic, hyperthermophilic, halophilic, and methanogenic microorganisms. The earlier term for the archaea was archaebacteria (ancient bacteria), and they are indeed a kind of living fossil. Thermophilic archaea thrive mainly in warm, moist biotopes such as the hot springs at the top of geothermal vents. The hyperthermophilic archaea, a more recent discovery, live near deep-sea volcanic plumes at temperatures exceeding 100 °C.

**Eucarya.** This domain includes all life forms with cells possessing a genuine nucleus. The plant and animal kingdoms (animales and plantales) are all eukaryotic life forms. Pathogenic eukaryotic microorganisms include fungal and protozoan species.

Table 1.**2** lists the main differences between prokaryotic (bacteria and archaea) and eukaryotic pathogens.

## Bacteria

- **Classic bacteria.** These organisms reproduce asexually by binary transverse fission. They do not possess the nucleus typical of eucarya. The cell walls of these organisms are rigid (with some exceptions, e.g., the mycoplasma).
- **Chlamydiae.** These organisms are obligate intracellular parasites that are able to reproduce in certain human cells only and are found in two stages: the infectious, nonreproductive particles called elementary bodies (0.3 μm) and the noninfectious, intracytoplasmic, reproductive forms known as initial (or reticulate) bodies (1 μm).
- **Rickettsiae.** These organisms are obligate intracellular parasites, rod-shaped to coccoid, that reproduce by binary transverse fission. The diameter of the individual cell is from 0.3–1 μm.

Table 1.2 Characteristics of Prokaryotic (Eubacteria) and Eukaryotic (Fungi, Protozoans) Microorganisms

| Characteristic | Prokaryotes (bacteria) | Eukaryotes (fungi, protozoans) |
|---|---|---|
| Nuclear structure | Circular DNA molecule not covered with proteins | Complex of DNA and basic proteins |
| Localization of nuclear structure | Dense tangle of DNA in cytoplasm; no nuclear membrane; nucleoid or nuclear equivalent | In nucleus surrounded by nuclear membrane |
| DNA | Nucleoid and plasmids | In nucleus and in mitochondria |
| Cytoplasm | No mitochondria and no endoplasmic reticulum, 70S ribosomes | Mitochondria and endoplasmic reticulum, 80S ribosomes |
| Cell wall | Usually rigid wall with murein layer; exception: mycoplasmas | Present only in fungi: glucans, mannans, chitin, chitosan, cellulose |
| Reproduction | Asexual, by binary transverse fission | In most cases sexual, possibly asexual |

**Mycoplasmas.** Mycoplasmas are bacteria without rigid cell walls. They are found in a wide variety of forms, the most common being the coccoid cell (0.3–0.8 μm). Threadlike forms also occur in various lengths.

## Fungi and Protozoa

**Fungi.** Fungi (*Mycophyta*) are nonmotile eukaryotes with rigid cell walls and a classic cell nucleus. They contain no photosynthetic pigments and are carbon heterotrophic, that is, they utilize various organic nutrient substrates (in contrast to carbon autotrophic plants). Of more than 50 000 fungal species, only about 300 are known to be human pathogens. Most fungal infections occur as a result of weakened host immune defenses.

**Protozoa.** Protozoa are microorganisms in various sizes and forms that may be free-living or parasitic. They possess a nucleus containing chromosomes and organelles such as mitochondria (lacking in some cases), an en-

doplasmic reticulum, pseudopods, flagella, cilia, kinetoplasts, etc. Many parasitic protozoa are transmitted by arthropods, whereby multiplication and transformation into the infectious stage take place in the vector.

### Animals

**Helminths.** Parasitic worms belong to the animal kingdom. These are metazoan organisms with highly differentiated structures. Medically significant groups include the trematodes (flukes or flatworms), cestodes (tapeworms), and nematodes (roundworms).

**Arthropods.** These animals are characterized by an external chitin skeleton, segmented bodies, jointed legs, special mouthparts, and other specific features. Their role as direct causative agents of diseases is a minor one (mites, for instance, cause scabies) as compared to their role as vectors transmitting viruses, bacteria, protozoa, and helminths.

## Host–Pathogen Interactions

The factors determining the genesis, clinical picture and outcome of an infection include complex relationships between the host and invading organisms that differ widely depending on the pathogen involved. Despite this variability, a number of general principles apply to the interactions between the invading pathogen with its aggression factors and the host with its defenses. Since the pathogenesis of bacterial infectious diseases has been researched very thoroughly, the following summary is based on the host–invader interactions seen in this type of infection.

The determinants of bacterial pathogenicity and virulence can be outlined as follows:

- Adhesion to host cells (adhesins).
- Breaching of host anatomical barriers (invasins) and colonization of tissues (aggressins).
- Strategies to overcome nonspecific defenses, especially antiphagocytic mechanisms (impedins).
- Strategies to overcome specific immunity, the most important of which is production of IgA proteases (impedins), molecular mimicry, and immunogen variability.

- Damage to host tissues due to direct bacterial cytotoxicity, exotoxins, and exoenzymes (aggressins).
- Damage due to inflammatory reactions in the macroorganism: activation of complement and phagocytosis; induction of cytokine production (modulins).

The above bacterial pathogenicity factors are confronted by the following host defense mechanisms:

- Nonspecific defenses including mechanical, humoral, and cellular systems. Phagocytosis is the most important process in this context.
- Specific immune responses based on antibodies and specific reactions of T lymphocytes (see chapter on immunology).

The response of these defenses to infection thus involves the correlation of a number of different mechanisms. Defective defenses make it easier for an infection to take hold. Primary, innate defects are rare, whereas acquired, secondary immune defects occur frequently, paving the way for infections by microorganisms known as "facultative pathogens" (opportunists).

## Basic Terminology of Infectiology

Tables 1.**3** and 1.**4** list the most important infectiological terms together with brief explanations.

The terms **pathogenicity** and **virulence** are not clearly defined in their relevance to microorganisms. They are sometimes even used synonymously. It has been proposed that pathogenicity be used to characterize a particular species and that virulence be used to describe the sum of the disease-causing properties of a population (strain) of a pathogenic species (Fig. 1.**1**)

Pathogenicity and virulence in the microorganism correspond to **susceptibility** in a host species and **disposition** in a specific host organism, whereby an individual may be anywhere from highly disposed to resistant.

## Determinants of Bacterial Pathogenicity and Virulence

Relatively little is known about the factors determining the pathogenicity and virulence of microorganisms, and most of what we do know concerns the disease-causing mechanisms of bacteria.

Table 1.**3** Basic Infectiological Terminology I (Pathogen)

| Term | Explanation |
|---|---|
| Saprophytes | These microorganisms are nonpathogenic; their natural habitat is dead organic matter |
| Parasites | Unicellular or metazoan organism living in or on an organism of another species (host) on the expense of the host |
| – Commensals | Normal inhabitants of skin and mucosa; the normal flora is thus the total commensal population (see Table 1.**7**, p. 25) |
| – Pathogenic microorganisms | Classic disease-causing pathogens |
| – Opportunists or facultatively pathogenic microorganisms | Can cause disease in immunocompromised individuals given an "opportune" situation; these are frequently germs of the normal flora or occasionally from the surrounding environment, animals, or other germ carriers |
| Pathogenicity | Capacity of a pathogen species to cause disease |
| Virulence | Sum of the disease-causing properties of a strain of a pathogenic species |
| Incubation period | Time between infection and manifestation of disease symptoms; this specific disease characteristic can be measured in hours, days, weeks, or even years |
| Prepatency | A parasitological term: time between infection and first appearance of products of sexual reproduction of the pathogen (e.g., worm eggs in stool of a host with helminthosis) |
| Infection spectrum | The totality of host species "susceptible" to infection by a given pathogen |
| Minimum infective dose | Smallest number of pathogens sufficient to cause an infection |
| Mode of infection | Method or pathway used by pathogen to invade host |

Tab 1.4 Basic Infectiological Terminology II (Host)

| Term | Explanation |
|---|---|
| Contamination | Microbiological presence of microorganisms on objects, in the environment, or in samples for analysis |
| Colonization | Presence of microorganisms on skin or mucosa; no penetration into tissues; typical of normal flora; pathogenic microorganisms occasionally also show colonization behavior |
| Infection | Invasion of a host organism by microorganisms, proliferation of the invading organisms, and host reaction |
| Inapparent (or subclinical) infection | Infection without outbreak of clinical symptoms |
| Infectious disease (or clinical infection) | Infection with outbreak of clinical symptoms |
| Probability of manifestation | Frequency of clinical manifestation of an infection in disposed individuals (%) |
| Endogenous infection | Infection arising from the colonizing flora |
| Exogenous infection | Infection arising from invasion of host by microorganisms from sources external to it |
| Nosocomial infection | Infection acquired during hospitalization (urinary tract infections, infections of the respiratory organs, wound infection, sepsis) |
| Local infection | Infection that remains restricted to the portal of entry and surrounding area |
| Generalized infection | Lymphogenous and/or hematogenous spread of invading pathogen starting from the portal of entry; infection of organs to which pathogen shows a specific affinity (organotropism); three stages: incubation, generalization, organ manifestation |
| Sepsis | Systemic disease caused by microorganisms and/or their toxic products; there is often a localized focus of infection from which pathogens or toxic products enter the bloodstream continuously or in intermittent phases |
| Transitory bacteremia/viremia/parasitemia | Brief presence of microorganisms in the bloodstream |
| Superinfection | Occurrence of a second infection in the course of a first infection |
| Relapses | Series of infections by the same pathogen |
| Reinfection | Series of infections by different pathogens |

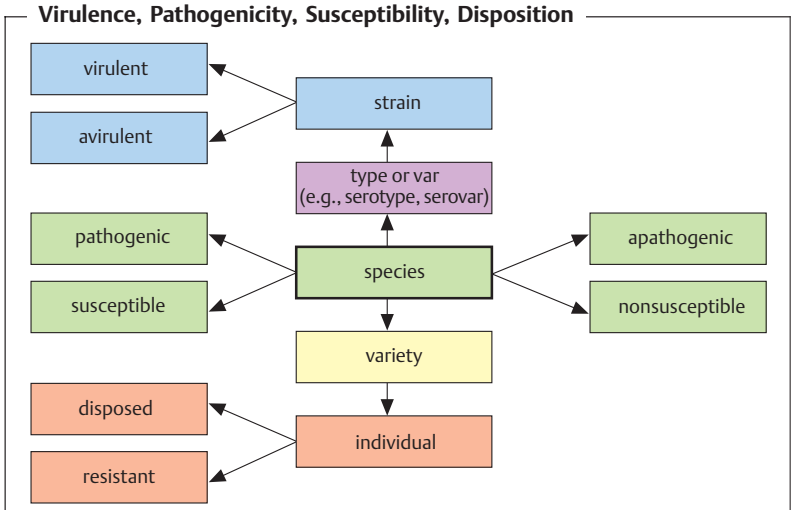



Fig. 1.1 Pathogenicity refers to pathogens, susceptibility to host species. The term virulence refers to individual strains of a pathogen species. The terms disposition and resistance are used to characterize the status of individuals of a susceptible host species.

There are five groups of potential bacterial contributors to the pathogenesis of infectious diseases:

1. **Adhesins.** They facilitate adhesion to specific target cells.
2. **Invasins.** They are responsible for active invasion of the cells of the macroorganism.
3. **Impedins.** These components disable host immune defenses in some cases.
4. **Aggressins.** These substances include toxins and tissue-damaging enzymes.
5. **Modulins.** Substances that induce excess cytokine production (i.e., lipopolysaccharides of Gram-negative bacteria, superantigens, murein fragments).

## Adhesion

When pathogenic bacteria come into contact with intact human surface tissues (e.g., mucosa), they contrive to adhere to receptors on the surface of the target cells by means of various surface structures of their own (attachment

pili, attachment fimbriae, adhesion proteins in the outer membrane of Gram-negative bacteria, cell wall-associated proteins in Gram-positive bacteria). This is a specific process, meaning that the adhesion structure (or ligand) and the receptor must fit together like a key in a keyhole.

## Invasion and Spread

**Invasion.** Bacteria may invade a host passively through microtraumata or macrotraumata in the skin or mucosa. On the other hand, bacteria that invade through intact mucosa first adhere to this anatomical barrier, then actively breach it. Different bacterial species deploy a variety of mechanisms to reach this end:

- Production of tissue-damaging exoenzymes that destroy anatomical barriers.
- Parasite-directed endocytosis, initiated by invasins on the surface of the bacterial cells, causes the cytoskeleton of the epithelial cell to form pseudopods that bring about endocytosis.
- Phagocytosis of enteropathogenic bacteria by M cells in the intestinal mucosa (cells that can ingest substances from the intestinal lumen by way of phagocytosis).

**Spread.**

- Local tissue spread beginning at the portal of entry, helped along by tissue-damaging exoenzymes (hyaluronidase, collagenase, elastase, and other proteases).
- Cell-to-cell spread. Bacteria translocated into the intracellular space by endocytosis cause actin to condense into filaments, which then array at one end of the bacterium and push up against the inner side of the cell membrane. This is followed by fusion with the membrane of the neighboring tissue cell, whereupon the bacterium enters the new cell (typical of *Listeria* and *Shigella*).
- Translocation of macrophage-resistant bacteria with macrophages into intestinal lymphoid tissue following their ingestion by M cells.
- Lymphogenous or hematogenous generalization. The bacteria then invade organs for which they possess a specific tropism.

## Strategies against Nonspecific Immunity

Establishment of a bacterial infection in a host presupposes the capacity of the invaders to overcome the host's nonspecific immune defenses. The most important mechanisms used by pathogenic bacteria are:

■ **Antiphagocytosis** (see also Fig. 1.**6**, p. 23)**.**

– *Capsule*. Renders phagocytosis more difficult. Capsule components may block alternative activation of complement so that C3b is lacking (ligand for C3b receptor of phagocytes) on the surface of encapsulated bacteria. Microorganisms that use this strategy include *Streptococcus pneumoniae* and *Haemophilus influenzae*.

– *Phagocyte toxins*. Examples: leukocidin from staphylococci, streptolysin from streptococci.

– Macrophages may be disabled by the type III secretion system (see p. 17) of certain Gram-negative bacteria (for example salmonellae, shigellae, yersiniae, and coli bacteria). This system is used to inject toxic proteins into the macrophages.

– *Inhibition of phagosome-lysosome fusion*. Examples: tuberculosis bacteria, gonococci, *Chlamydia psittaci*.

– *Inhibition of the phagocytic "oxidative burst."* No formation of reactive $O_2$ radicals in phagocytes. Examples: *Legionella pneumophilia*, *Salmonella typhi*.

■ **Serum resistance.** Resistance of Gram-negative bacteria to complement. A lipopolysaccharide in the outer membrane is modified in such a way that it cannot initiate alternative activation of the complement system. As a result, the membrane attack complex (C5b6789), which would otherwise lyse holes in the outer membrane, is no longer produced (see p. 86ff.).

■ **Siderophores.** Siderophores (e.g., enterochelin, aerobactin) are low-molecular-weight iron-binding molecules that transport $Fe^{3+}$ actively into the intracellular space. They complex with iron, thereby stealing this element from proteins containing iron (transferrin, lactoferrin). The intricate iron transport system is localized in the cytoplasmic membrane, and in Gram-negative bacteria in the outer membrane as well. To thrive, bacteria require $10^{-5}$ mol/l free iron ions. The free availability of only about $10^{-20}$ mol/l iron in human body fluids thus presents a challenge to them.

## Strategies against Specific Immunity

■ **Immunotolerance.**

– *Prenatal infection*. At this stage of development, the immune system is unable to recognize bacterial immunogens as foreign.

– *Molecular mimicry*. Molecular mimicry refers to the presence of molecules on the surface of bacteria that are not recognized as foreign by the immune system. Examples of this strategy are the hyaluronic acid capsule of *Streptococcus pyogenes* or the neuraminic acid capsule of *Escherichia coli* K1 and serotype B *Neisseria meningitidis*.

**Mechanism of Molecular Variation of Pilin in Gonococci**

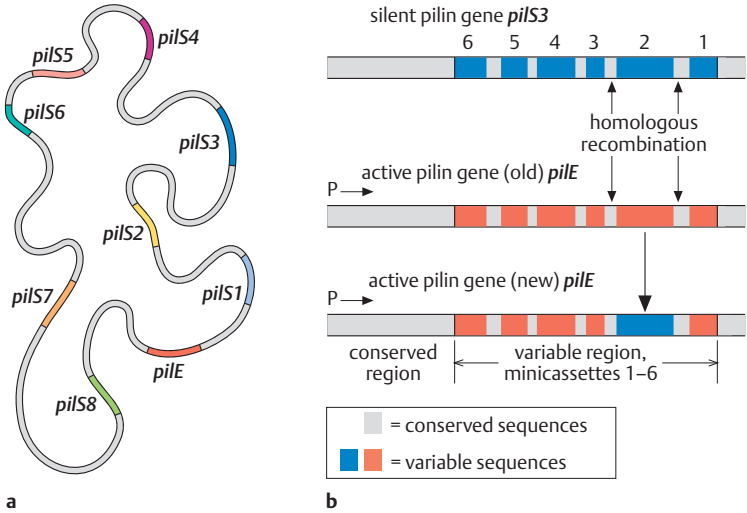



Fig. 1.2 The structural element of the attachment pili of gonococci is the polypeptide monomer pilin. Mucosal immunity to gonococci depends on antibodies in the secretions of the urogenital mucosa that attach to the immunodominant segment of the pilin, thus blocking adhesion of gonococci to the target cells.
**a** Model of the gonococcal genome. The primary structure of the pilin is determined by the expressed gene *pilE*. The gonococcal genome has many other *pil* genes besides the *pilE* without promoters, i.e., "silent" genes that are not transcribed (*pilS1*, *pilS2*, *pilS3*, etc.).
**b** *pil* genes have both a conserved and a variable region. The variable region of all *pil* genes has a mosaic structure, i.e., it consists of minicassettes. Minicassette 2 codes for the most important immunodominant segment of the pilin. Intracellular homologous recombination of conserved regions of silent *pil* genes and corresponding sequences of the expressed gene results in *pilE* genes with changed cassettes. These code for a pilin with a changed immunodominant segment. Therefore, antibodies to the "old" pilin can no longer bind to the "new" pilin.

**Antigen variation.** Some bacteria are characterized by a pronounced variability of their immunogens (= immune antigens) due to the genetic variability of the structural genes coding the antigen proteins. This results in production of a series of antigen variants in the course of an infection that no longer "match" with the antibodies to the "old" antigen. Examples: gonococci can modify the primary structure of the pilin of their attachment

pili at a high rate (Fig. 1.**2**). The borreliae that cause relapsing fevers have the capacity to change the structure of one of the adhesion proteins in their outer membrane (vmp = variable major protein), resulting in the typical "recurrences" of fever. Similarly, meningococci can change the chemistry of their capsule polysaccharides ("capsule switching").

**IgA proteases.** Mucosal secretions contain the secretory antibodies of the $sIgA_1$ class responsible for the specific local immunity of the mucosa. Classic mucosal parasites such as gonococci, meningococci and *Haemophilus influenzae* produce proteases that destroy this immunoglobulin.

## Clinical Disease

The clinical symptoms of a bacterial infection arise from the effects of damaging noxae produced by the bacteria as well as from excessive host immune responses, both nonspecific and specific. Immune reactions can thus potentially damage the host's health as well as protect it (see Immunology, p. 103ff.).

**Cytopathic effect.** Obligate intracellular parasites (rickettsiae, chlamydiae) may kill the invaded host cells when they reproduce.

**Exotoxins.** Pathogenic bacteria can produce a variety of toxins that are either the only pathogenic factor (e.g., in diphtheria, cholera, and tetanus) or at least a major factor in the unfolding of the disease. One aspect the classification and nomenclature of these toxins must reflect is the type of cell affected: **cytotoxins** produce toxic effects in many different host cells; **neurotoxins** affect the neurons; **enterotoxins** affect enterocytes. The structures and mechanisms of action of the toxins are also considered in their classification (Table 1.**5**):

- *AB toxins*. They consist of a binding subunit "B" responsible for binding to specific surface receptors on target host cells, and a catalytic subunit "A" representing the active agent. Only cells presenting the "B" receptors are damaged by these toxins.
- *Membrane toxins*. These toxins disrupt biological membranes, either by attaching to them and assembling to form pores, or in the form of phospholipases that destroy membrane structure enzymatically.
- *Superantigens* (see p. 72). These antigens stimulate T lymphocytes and macrophages to produce excessive amounts of harmful cytokines.

**Hydrolytic exoenzymes.** Proteases (e.g., collagenase, elastase, nonspecific proteases), hyaluronidase, neuraminidase (synonymous with sialidase), lecithinase and DNases contribute at varying levels to the pathogenesis of an infection.

Table 1.**5** Examples of Bacterial Toxins; Mechanisms of Action and Contribution to Clinical Picture

| Toxin | Cell specificity | Molecular effect | Contribution to clinical picture |
|---|---|---|---|
| ***AB toxins*** | | | |
| **Diphtheria toxin** (*Corynebacterium diphtheriae*) | Many different cell types | ADP-ribosyl transferase. Inactivation of ribosomal elongation factor eEF2 resulting from ADP-ribosylation during protein synthesis; leads to cell death. | Death of mucosal cells. Damage to heart musculature, kidneys, adrenal glands, liver, motor nerves of the head. |
| **Cholera toxin** (*Vibrio cholerae*) | Enterocytes | ADP-ribosyl transferase. ADP-ribosylation of regulatory protein $G_s$ of adenylate cyclase, resulting in permanent activation of this enzyme and increased levels of cAMP (second messenger) (see Fig. 4.**20**, p. 298). Result: increased secretion of electrolytes. | Massive watery diarrhea; severe loss of electrolytes and water. |
| **Tetanus toxin** (*Clostridium tetani*) | Neurons (synapses) | Metalloprotease. Proteolytic cleavage of protein components from the neuroexocytosis apparatus in the synapses of the anterior horn that normally transmit inhibiting impulses to the motor nerve terminal. | Increased muscle tone; cramps in striated musculature. |
| ***Membrane toxins*** | | | |
| **Alpha toxin** (*Clostridium perfringens*) | Many different cell types | Phospholipase. | Cytolysis, resulting tissue damage. |
| **Lysteriolysin** (*Listeria monocytogenes*) | Many different cell types | Pore formation in membranes. | Destruction of phagosome membrane; intracellular release of phagocytosed listeriae. |

Table 1.**5** *Continued: Examples of Bacterial Toxins*

| Toxin | Cell specificity | Molecular effect | Contribution to clinical picture |
|---|---|---|---|
| ***Superantigen toxins*** | | | |
| **Toxic shock syndrome toxin-1 (TSST-1)** (*Staphylococcus aureus*) | T lymphocytes; macrophages | Stimulation of secretion of cytokines in T cells and macrophages. | Fever; exanthem; hypotension. |

■ **Secretion of virulence proteins.** Proteins are synthesized at the ribosomes in the bacterial cytoplasm. They must then be secreted through the cytoplasmic membrane, and in Gram-negative bacteria through the outer membrane as well. The secretion process is implemented by complex protein secretion systems (I-IV) with differing compositions and functional pathways. The type III (virulence-related) secretion system in certain Gram-negative bacteria (*Salmonella*, *Shigella*, *Yersinia*, *Bordetella*, *Escherichia coli*, *Chlamydia*) is particularly important in this connection (see Fig. 1.**3**).

**Needle Complex of Type III Secretion System**

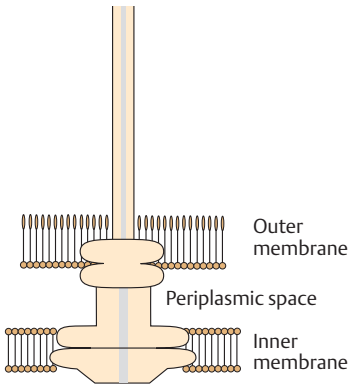



Fig. 1.**3** When certain Gram-negative rod bacteria make contact with eukaryotic target cells, a sensor molecule interacts with a receptor on the target cells. This interaction results in the opening of a secretion channel of the so-called "needle complex" (extending through both the cytoplasmic membrane and outer membrane) and in formation of a pore in the membrane of the target cell. Through the pore and channel, cytotoxic molecules are then translocated into the cytosol of the target cell where they, for example, inhibit phagocytosis and cytokine production (in macrophages), destroy the cytoskeleton of the target cell, and generally work to induce apoptosis.

**Cell wall.** The endotoxin of Gram-negative bacteria (lipopolysaccharide) plays an important role in the manifestation of clinical symptoms. On the one hand, it can activate complement by the alternative pathway and, by releasing the chemotactic components C3a and C5a, initiate an inflammatory reaction at the infection site. On the other hand, it also stimulates macrophages to produce endogenous pyrogens (interleukin 1, tumor necrosis factor), thus inducing fever centrally. Production of these and other cytokines is increased, resulting in hypotension, intravasal coagulation, thrombocyte aggregation and stimulation of granulopoiesis. Increased production of cytokines by macrophages is also induced by soluble murein fragments and, in the case of Gram-positive bacteria, by teichoic acids.

**Inflammation.** Inflammation results from the combined effects of the nonspecific and specific immune responses of the host organism. Activation of complement by way of both the classic and alternative pathways induces phagocyte migration to the infection site. Purulent tissue necrosis follows. The development of typical granulomas and caseous necrosis in the course of tuberculosis are the results of excessive reaction by the cellular immune system to the immunogens of tuberculosis bacteria. Textbooks of general pathology should be consulted for detailed descriptions of these inflammatory processes.

## Regulation of Bacterial Virulence

Many pathogenic bacteria are capable of living either outside or inside a host and of attacking a variety of host species. Proliferation in these differing environments demands an efficient regulation of virulence, the aim being to have virulence factors available as required. Four different regulatory mechanisms have been described:

**DNA changes.** The nucleotide sequences of virulence determinants are changed. Examples of this include pilin gene variability involving intracellular recombination as described above in gonococci and inverting a leader sequence to switch genes on and off in the phase variations of H antigens in salmonellae (see p. 284).

**Transcriptional regulation.** The principle of transcriptional control of virulence determinants is essentially the same as that applying to the regulation of metabolic genes, namely repression and activation (see p. 169f.):

– *Simple regulation*. Regulation of the diphtheria toxin gene has been thoroughly researched. A specific concentration of iron in the cytoplasm activates the diphtheria toxin regulator (DtxR). The resulting active repressor prevents transcription of the toxin gene by binding to the promoter

region. Other virulence genes can also be activated by regulators using this mechanism.

– *Complex regulation, virulence regulon*. In many cases, several virulence genes are switched on and off by the same regulator protein. The virulence determinants involved are either components of the same operon or are located at different genome sites. Several vir (virulence) genes with promoter regions that respond to the same regulator protein form a so-called vir regulon. Regulation of the virulence regulon of *Bordetella pertussis* by means of gene activation is a case in point that has been studied in great detail. This particular regulon comprises over 20 virulence determinants, all controlled by the same vir regulator protein (or BvgA coding region) (Fig. 1.**4**).

■ **Posttranscriptional regulation.** This term refers to regulation by mRNA or a posttranslational protein modification.

■ **Quorum sensing.** This term refers to determination of gene expression by bacterial cell density (Fig. 1.**5**). Quorum sensing is observed in both

**Regulation of Bacterial Virulence: Two-Component Regulator System**

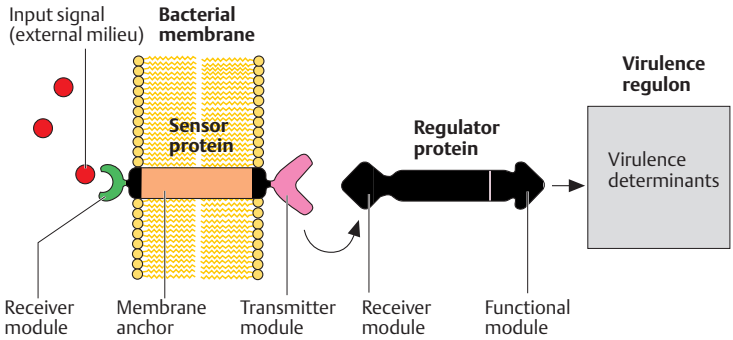



Fig. 1.**4** A sensor protein integrated in the cytoplasmic membrane receives signals from a receiver module extending into the external milieu, activating the transmitter module. These signals from the external milieu can carry a wide variety of information: pH, temperature, osmolarity, $Ca^{2+}$, $CO_2$, stationary-phase growth, hunger stress, etc. The transmitter module effects a change in the receiver module of the regulator protein, switching the functional module of the regulator to active status, in which it can then repress or activate the various virulence determinants of a virulence regulon by binding to the different promoter regions. Phosphorylation is commonly used to activate the corresponding sensor and regulator modules.

**Quorum-Sensing Communication in Bacteria (Cell-to-Cell Signals)**

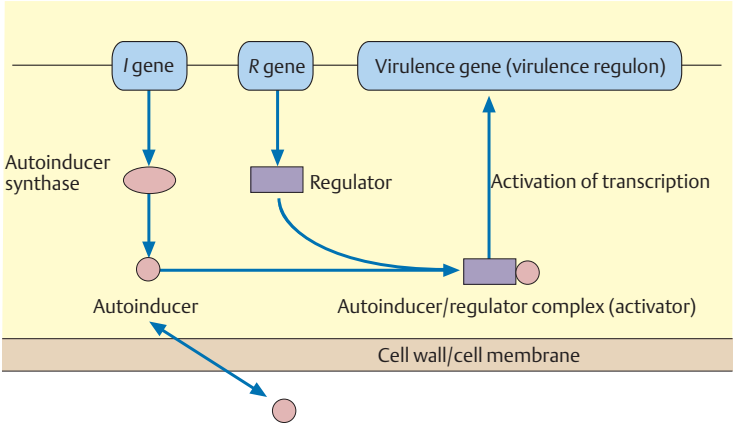



Fig. 1.5 Cell-to-cell signaling is made possible by activation of two genes. The *I* gene codes for the synthase responsible for synthesis of the autoinducer. The autoinducer (often an *N*-acyl homoserine lactone) can diffuse freely through the cell membrane. The *R* gene codes for a transcriptional regulator protein that combines with the autoinducer to become an activator for transcription of various virulence genes.

Gram-positive and Gram-negative bacteria. It denotes a mode of communication between bacterial cells that enables a bacterial population to react analogously to a multicellular organism.

Accumulation of a given density of a low-molecular-weight pheromone (autoinducer) enables a bacterial population to sense when the critical cell density (quorum) has been reached that will enable it to invade the host successfully, at which point transcription of virulence determinants is initiated.

## The Genetics of Bacterial Pathogenicity

The virulence genes of pathogenic bacteria are frequently components of mobile genetic elements such as plasmids, bacteriophage genomes, or conjugative transposons (see p. 170ff.). This makes lateral transfer of these genes between bacterial cells possible. Regions showing a high frequency of virulence genes in a bacterial chromosome are called pathogenicity islands (PI).

PIs are found in both Gram-positive and Gram-negative bacteria. These are DNA regions up to 200 kb that often bear several different vir genes and have specific sequences located at their ends (e.g., IS elements) that facilitate lateral translocation of the islands between bacterial cells. The role played by lateral transfer of these islands in the evolutionary process is further underlined by the fact that the GC contents in PIs often differ from those in chromosomal DNA.

## Defenses against Infection

A macroorganism manifests defensive reactions against invasion by microorganisms in two forms: as **specific, acquired immunity** and as **nonspecific, innate resistance** (see also Chapter 2, Basic Principles of Immunology, p. 43).

### Nonspecific Defense Mechanisms

Table 1.**6** lists the most important mechanisms.

- **Primary defenses.** The main factors in the first line of defense against infection are mechanical, accompanied by some humoral and cellular factors. These defenses represent an attempt on the part of the host organism to prevent microorganisms from colonizing its skin and mucosa and thus stave off a generalized invasion.

- **Secondary defenses.** The second line of defense consists of humoral and cellular factors in the blood and tissues, the most important of which are the professional phagocytes.

- **Phagocytosis.** "Professional" phagocytosis is realized by polymorphonuclear, neutrophilic, eosinophilic granulocytes—also known as microphages—and by mononuclear phagocytes (macrophages). The latter also play an important role in antigen presentation (see p. 62). The total microphage cell count in an adult is approximately $2.5 \times 10^{12}$. Only 5% of these cells are located in the blood. They are characterized by a half-life of only a few hours. Microphages contain both primary granules, which are lysosomes containing lysosomal enzymes and cationic peptides, and secondary granules. Both microphages and macrophages are capable of ameboid motility and chemotactic migration, i.e., directed movement along a concentration gradient toward a source of chemotactic substances, in most cases the complement components C3a and C5a. Other potentially chemotactic substances include secretory products of lymphocytes, products of infected and damaged cells or the *N*-formyl peptides (fMet-Phe and fMet-Leu-Phe).

Table 1.**6** The Most Important Mechanisms in Nonspecific Defenses Against Infection

| | |
|---|---|
| **a** | **Mechanical factors** |
| | Anatomical structure of skin and mucosa |
| | Mucus secretion and mucus flow from mucosa |
| | Mucociliary movement of the ciliated epithelium in the lower respiratory tract |
| | Digestive tract peristalsis |
| | Urine flow in the urogenital tract |
| **b** | **Humoral factors** |
| | Microbicidal effect of the dermal acidic mantle, lactic acid from sweat glands, hydrochloric acid in the stomach, and the unsaturated fatty acids secreted by the sebaceous glands |
| | Lysozyme in saliva and tear fluid: splitting of bacterial murein |
| | Complement (alternative activation pathway) |
| | Serum proteins known as acute phase reactants, for example C-reactive protein, haptoglobin, serum amyloid A, fibrinogen, and transferrin (iron-binding protein) |
| | Fibronectin (a nonspecific opsonin); antiviral interferon |
| | Mannose-binding protein: binds to mannose on the outer bacterial surface, thus altering the configuration and triggering alternative activation of complement |
| **c** | **Cellular factors** |
| | Normal flora of skin and mucosa |
| | Natural killer cells (large, granulated lymphocytes; null cells) |
| | Professional phagocytes: microphages (neutrophilic and eosinophilic granulocytes); mononuclear phagocytes (macrophages, monocytes, etc.) |

Phagocytes are capable of ingestion of both particulate matter (phagocytosis) and solute matter (pinocytosis). Receptors on the phagocyte membrane initiate contact (Fig. 1.**6**). Particles adhering to the membrane are engulfed, ingested and deposited in a membrane-bound vacuole, the so-called phagosome, which then fuses with lysosomes to form the phagolysosome. The bacteria are killed by a combination of lysosomal factors:

– *Mechanisms that require no oxygen.* Low pH; acid hydrolases, lysozyme; proteases; defensins (small cationic peptides).

**Phagocytosis of Bacteria**

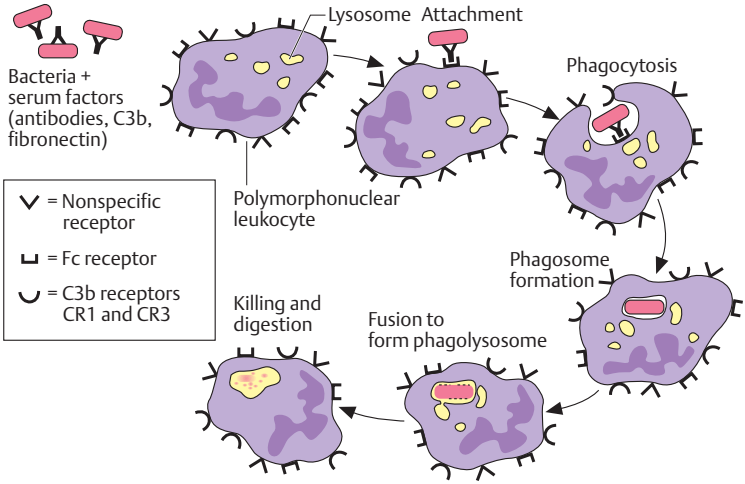



Fig. 1.6 Encapsulated bacteria can only be effectively phagocytosed if IgG-class antibodies (Fc ligand) or the complement component C3b, or both, are located on their surfaces. The Fc and C3b ligands bind to their specific receptors on the phagocyte surface.

– *Mechanisms that require oxygen.* Halogenation of essential bacterial components by the myeloperoxidase-$H_2O_2$–halide system; production of highly reactive $O_2$ radicals (oxidative burst) such as superoxide anion ($O_2^-$), hydroxyl radical (•OH), and singlet oxygen ($^1O_2$).

## Specific Defense Mechanisms

Specific immunity, based on antibodies and specifically reactive T lymphocytes, is acquired in a process of immune system stimulation by the corresponding microbial antigens. Humoral immunity is based on antitoxins, opsonins, microbicidal antibodies, neutralizing antibodies, etc. Cellular immunity is based on cytotoxic T lymphocytes (T killer cells) and T helper cells. See Chapter 2 on the principles of specific immunity.

## Defects in Immune Defenses

Hosts with defects in their specific and/or nonspecific immune defenses are prone to infection.

- **Primary defects.** Congenital defects in the complement-dependent phagocytosis system are rare, as are B and T lymphocyte defects.

- **Secondary defects.** Such effects are acquired, and they are much more frequent. Examples include malnutrition, very old and very young hosts, metabolic disturbances (diabetes, alcoholism), autoimmune diseases, malignancies (above all lymphomas and leukemias), immune system infections (HIV), severe primary diseases of parenchymatous organs, injury of skin or mucosa, immunosuppressive therapy with corticosteroids, cytostatics and immunosuppressants, and radiotherapy.

One result of progress in modern medicine is that increasing numbers of patients with secondary immune defects are now receiving hospital treatment. Such "problem patients" are frequently infected by opportunistic bacteria that would not present a serious threat to normal immune defenses. Often, the pathogens involved ("problem bacteria") have developed a resistance to numerous antibiotics, resulting in difficult courses of antibiotic treatment in this patient category.

## Normal Flora

Commensals (see Table 1.**3**, p. 9) are regularly found in certain human microbiotopes. The normal human microflora is thus the totality of these commensals. Table 1.**7** lists the most important microorganisms of the normal flora with their localizations.

Bacteria are the predominant component of the normal flora. They proliferate in varied profusion on the mucosa and most particularly in the gastrointestinal tract, where over 400 different species have been counted to date. The count of bacteria per gram of intestinal content is $10^1$–$10^5$ in the duodenum, $10^3$–$10^7$ in the small intestine, and $10^{10}$–$10^{12}$ in the colon. Over 99% of the normal mucosal flora are obligate anaerobes, dominated by the Gram-neg. anaerobes. Although life is possible without normal flora (e.g., pathogen-free experimental animals), commensals certainly benefit their hosts. One way they do so is when organisms of the normal flora manage to penetrate into the host through microtraumas, resulting in a continuous **stimulation of the immune system**. Commensals also compete for living space with overtly pathogenic species, a function known as **colo-**

Table 1.7 Normal Microbial Flora in Humans

| Microorganisms | Microbiotopes | | | | |
|---|---|---|---|---|---|
| | Skin | Oral cavity | Intestine | Upper respiratory tract | Genital tract |
| Staphylococci | +++ | + | + | ++ | ++ |
| Enterococci | | | ++ | | + |
| α-hemolytic streptococci | + | +++ | + | + | + |
| Anaerobic cocci | | + | + | | + |
| Pneumococci | | + | | + | |
| Apathogenic neisseriae | | + | | + | + |
| Apathogenic corynebacteria | ++ | + | + | + | + |
| Aerobic spore-forming bacteria | (+) | | | | |
| Clostridia | | | +++ | | (+) |
| Actinomycetes | | +++ | | | + |
| *Enterobacteriaceae* | (+) | (+) | +++ | (+) | + |
| *Pseudomonas* | | | + | | |
| *Haemophilus* | | + | | ++ | (+) |
| Gram-neg. anaerobes | | +++ | +++ | +++ | +++ |
| Spirochetes | | ++ | | + | (+) |
| Mycoplasmas | | ++ | + | + | ++ |
| Fungi (yeast) | ++ | + | + | + | + |
| *Entamoeba*, *Giardia*, *Trichomonas* | | + | | + | |

+++ = numerous, ++ = frequent, + = moderately frequent, (+) = occasional occurrence

**nization resistance**. On the other hand, a potentially harmful effect of the normal flora is that they can also cause infections in immunocompromised individuals.

## General Epidemiology

■ Within the context of medical microbiology, epidemiology is the study of the occurrence, causality, and prevention of infectious diseases in the populace. Infectious diseases occur either sporadically, in epidemics or pandemics,

or in endemic forms, depending on the time and place of their occurrence. The frequency of their occurrence (morbidity) is described as their *incidence* and *prevalence*. The term *mortality* is used to describe how many deaths are caused by a given disease in a given population. *Lethality* is a measure of how life-threatening an infection is. The most important sources of infection are infected persons and carriers. Pathogens are transmitted from these sources to susceptible persons either directly (person-to-person) or indirectly via inert objects or biological vectors. Control of infectious diseases within a populace must be supported by effective legislation that regulates mandatory reporting where required. Further measures must be implemented to prevent exposure, for example isolation, quarantine, disinfection, sterilization, use of insecticides, and dispositional prophylaxis (active and passive immunization, chemoprophylaxis).

## Epidemiological Terminology

Epidemiology investigates the distribution of diseases, their physiological variables and social consequences in human populations, and the factors that influence disease distribution (World Health Organization [WHO] definition). The field covered by this discipline can thus be defined as medical problems involving large collectives. The rule of thumb on infectious diseases is that their characteristic spread depends on the virulence of the pathogen involved, the susceptibility of the threatened host species population, and environmental factors. Table 1.**8** provides brief definitions of the most important epidemiological terms.

## Transmission, Sources of Infection

### Transmission

Pathogens can be transmitted from a source of infection by direct contact or indirectly. Table 1.**9** lists the different direct and indirect transmission pathways of pathogenic microorganisms.

Person-to-person transmission constitutes a **homologous chain of infection**. The infections involved are called **anthroponoses**. In cases in which the pathogen is transmitted to humans from other vertebrates (and occasionally the other way around) we have a **heterologous chain of infection** and the infections are known as **zoonoses** (WHO definition) (Table 1.**10**).

Table 1.8 Epidemiological Terminology

| Term | Definition |
|---|---|
| **Sporadic occurrence** | Isolated occurrence of an infectious disease with no apparent connections between localities or times of occurrence |
| **Endemic occurrence** | Regular and continuing occurrence of infectious diseases in populations with no time limit |
| **Epidemic occurrence** | Significantly increased occurrence of an infectious disease within given localities and time periods |
| **Pandemic occurrence** | Significantly increased occurrence of an infectious disease within a given time period but without restriction to given localities |
| **Morbidity** | Number of cases of a disease within a given population (e.g., per 1000, 10 000 or 100 000 inhabitants) |
| Incidence | Number of new cases of a disease within a given time period |
| Prevalence | Number of cases of a disease at a given point in time (sampling date) |
| **Mortality** | Number of deaths due to a disease within a given population |
| **Lethality** | Number of deaths due to a disease in relation to total number of cases of the disease |
| **Manifestation index** | Number of manifest cases of a disease in relation to number of infections |
| **Incubation period** | Time from infection until occurrence of initial disease symptoms |
| **Prepatency** | Time between infection and first appearance of products of sexual reproduction of the pathogen (e.g., worm eggs in stool) |

Tab. 1.**9** Transmission Pathways of Pathogenic Microorganisms

| Direct transmission | Indirect transmission |
|---|---|
| Fecal-oral (smear infection) | Transmission via food |
| Aerogenic transmission (droplet infection) | Transmission via drinking water |
| Genital transmission (during sexual intercourse) | Transmission via contaminated inanimate objects or liquids |
| Transmission via skin (rare) | Transmission via vectors (arthropods) |
| Diaplacental transmission | Transmission via other persons (e.g., via the hands of hospital medical staff) |
| Perinatal transmission (in the course of birth) | |

Tab. 1.**10** Examples of Zoonoses Caused by Viruses, Bacteria, Protozoans, Helminths, and Arthropods

| Zoonoses | Pathogen | Reservoir hosts | Transmission |
|---|---|---|---|
| **Viral zoonoses** | | | |
| Rabies | *Rhabdoviridae* | Numerous animal species | Bite of diseased animals |
| Tickborne encephalitis (TBE) | *Flaviviridae* | Wild animals | Ticks |
| **Bacterial zoonoses** | | | |
| Brucellosis | *Brucella* spp. | Cattle, pig, goat, sheep, (dog) | Contact with tissues or secretions from diseased animals; milk and dairy products |
| Lyme disease | *Borrelia burgdorferi* | Wild rodents; red deer, roe deer | Ticks |
| Plague | *Yersinia pestis* | Rodents | Contact with diseased animals; bite of rat flea |
| Q fever | *Coxiella burnetii* | Sheep, goat, cattle | Dust; possibly milk or dairy products |
| Enteric salmonellosis | *Salmonella enterica* (enteric serovars) | Pig, cattle, poultry | Meat, milk, eggs |

Tab. 1.**10** *Continued: Examples of Zoonoses*

| Zoonoses | Pathogen | Reservoir hosts | Transmission |
|---|---|---|---|
| **Protozoan zoonoses** | | | |
| Toxoplasmosis | *Toxoplasma gondii* | Domestic cat, sheep, pigs, other slaughter animals | Postnatal toxoplasmosis: oral; prenatal toxoplasmosis: diaplacental |
| Cryptosporidiosis | *Cryptosporidium hominis*; *C. parvum* | Cattle (calves), domestic animals | Ingestion of oocysts |
| **Helminthic zoonoses** | | | |
| Echinococcosis | *Echinococcus granulosus, Echinococcus multilocularis* | Dog, wild canines, fox | Ingestion of eggs |
| Taeniosis | *Taenia saginata, Taenia solium, Taenia asiatica* | Cattle, buffalo, pigs<br>Pigs, cattle, goat | Ingestion of metacestodes with meat |
| **Zoonoses caused by arthropods** | | | |
| Pseudo scabies | *Sarcoptes* spp.; mite species from domestic animals | Dog, cat, guinea pig, domestic ruminants, pig | Contact with diseased animals |

**Other Zoonoses**

(For details see the corresponding chapters)

| | |
|---|---|
| Viral zoonoses | Hantavirus and other bunyavirus infections; infections by alphavirus, flavivirus, and arenavirus. |
| Bacterial zoonoses | Ehrlichiosis; erysipeloid; campylobacteriosis; cat scratch disease; leptospirosis; anthrax; ornithosis; rat-bite fever; rickettsioses (variety of types); tularemia; gastroenteritis caused by *Vibrio parahaemolyticus*; gastroenteritis caused by *Yersinia enterocolitica*. |
| Protozoan zoonoses | African trypanosomosis (sleeping sickness); American trypanosomosis (Chagas disease); babesiosis; balantidosis; cryptosporidosis; giardiosis; leishmaniosis; microsporidosis; sarcocystosis; toxoplasmosis. ▶ |

*Continued:* **Other Zoonoses**

| | |
|---|---|
| Helminthic zoonoses | Cercarial dermatitis; clonorchiosis; cysticercosis; dicrocoeliosis; diphyllobothriosis; echinococcosis; fasciolosis; hymenolepiosis; larva migrans interna; opisthorchiosis; paragonimosis; schistosomosis (bilharziosis); taeniosis; toxocariosis; trichinellosis. |
| Zoonoses caused by arthropods | Flea infestation; larva migrans externa; mite infestation; sand flea infestation. |

## Sources of Infection

Every infection has a source (Table 1.**11**). The **primary source of infection** is defined as the location at which the pathogen is present and reproduces. **Secondary sources of infection** are inanimate objects, materials, or third persons contributing to transmission of pathogens from the primary source to disposed persons.

Table 1.**11** Primary Sources of Infection

| Source of infection | Explanation |
|---|---|
| Infected person | The most important source; as a rule, pathogens are excreted by the organ system through which the infection entered; there are some exceptions |
| Carriers during incubation | Excretion during incubation period; typical of many viral diseases |
| Carriers in convalescence | Excretion after the disease has been overcome; typical of enteric salmonelloses |
| Chronic carriers | Continued excretion for three or more months (even years) after disease has been overcome; typical of typhoid fever |
| Asymptomatic carriers | They carry pathogenic germs on skin or mucosa without developing "infection" |
| Animal carriers | Diseased or healthy animals that excrete pathogenic germs |
| Environment | Soil, plants, water; primary source of microorganisms with natural habitat in these biotopes |

## The Fight against Infectious Diseases

### Legislation

Confronting and preventing infectious diseases can sometimes involve substantial incursions into the private sphere of those involved as well as economic consequences. For these reasons, such measures must be based on effective disease control legislation. In principle, these laws are similar in most countries, although the details vary.

The centerpiece of every disease prevention system is provision for reporting outbreaks. Basically, reporting is initiated at the periphery (individual patients) and moves toward the center of the system. Urgency level classifications of infections and laboratory findings are decided on by regional health centers, which are in turn required to report some diseases to the WHO to obtain a global picture within the shortest possible time.

Concrete countermeasures in the face of an epidemic take the form of prophylactic measures aimed at interrupting the chain of infection.

### Exposure Prophylaxis

Exposure prophylaxis begins with *isolation* of the source of infection, in particular of infected persons, as required for the disease at hand. *Quarantine* refers to a special form of isolation of healthy first-degree contact persons. These are persons who have been in contact with a source of infection. The quarantine period is equivalent to the incubation period of the infectious disease in question (see International Health Regulations, www.who.int/en/).

Further measures of exposure prophylaxis include *disinfection* and *sterilization*, use of insecticides and pesticides, and eradication of animal carriers.

### Immunization Prophylaxis

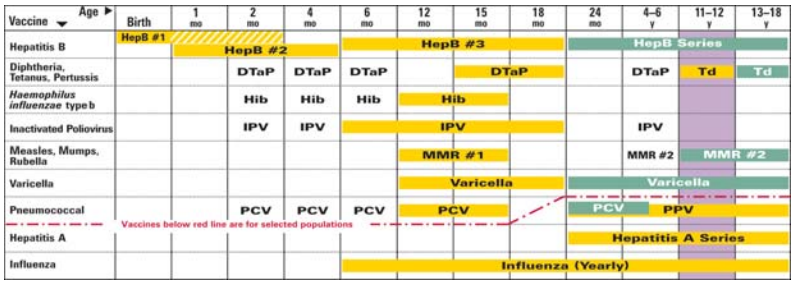
**Active immunization.** In active immunization, the immune system is stimulated by administration of vaccines to develop a disease-specific immunity. Table 1.**12** lists the vaccine groups used in active immunization. Table 1.**13** shows as an example the vaccination schedule recommended by the Advisory Committee on Immunization Practices of the USA (www.cdc.gov/nip). Recommended adult immunization schedules by age group and by medical conditions are also available in the National Immunization Program Website mentioned above. The vaccination calendars used in other countries deviate from these proposals in some details. For instance, routine varicella and

Table 1.12 Vaccine Groups Used in Active Immunization

| Vaccine group | Remarks |
|---|---|
| **Killed pathogens** | Vaccination protection often not optimum, vaccination has to be repeated several times |
| **Living pathogens with reduced virulence (attenuated)** | Optimum vaccination protection; a single application often suffices, since the microorganisms reproduce in the vaccinated person, providing very good stimulation of the immune system; do not use in immunocompromised persons and during pregnancy (some exceptions) |
| **Purified microbial immunogens** | |
| – Proteins | Often recombinant antigens, i.e., genetically engineered proteins; well-known example: hepatitis B surface (HBs) antigen |
| – Polysaccharides | Chemically purified capsular polysaccharides of pneumococci, meningococci, and *Haemophilus influenzae* serotype b; problem: these are T cell-independent antigens that do not stimulate antibody production in children younger than two years of age |
| – Conjugate vaccines | Coupling of bacterial capsular polysaccharide epitopes to proteins, e.g., to tetanus toxoid, diphtheria toxoid, or proteins of the outer membranes of meningococci; children between the ages of two months and two years can also be vaccinated against polysaccharide epitopes |
| **Toxoids** | Bacterial toxins detoxified by formaldehyde treatment that still retain their immunogen function |
| **Experimental vaccines** | DNA vaccines. Purified DNA that codes for the viral antigens (proteins) and is integrated in plasmid DNA or nonreplicating viral vector DNA. The vector must have genetic elements—for example a transcriptional promoter and RNA-processing elements—that enable expression of the insert in the cells of various tissues (epidermis, muscle cells) |
| | Anti-idiotype-specific monoclonal antibodies |
| | *Vaccinia* viruses as carriers of foreign genes that code for immunogens |

Table 1.**13** Recommended Childhood and Adolescent Immunization Schedule—United States, 2004

1. Hepatitis B vaccine (HepB).
   Infants born to HBs-Ag-positive mothers should receive HepB and 0.5 ml HepB Immune Globulin within 12 h of birth at separate sites.
2. Diphtheria (D) and tetanus (T) toxoids and acellular pertussis (aP) vaccine (DTaP).
   The term "d" refers to a reduced dose of diphtheria toxoid.
3. Haemophilus influenzae type b conjugate vaccine (see Table 1.**12**).
4. Measles, mumps, and rubella vaccine (MMR).
   Attenuated virus strains.
5. Varicella vaccine.
   Varicella vaccine is recommended for children who lack a reliable history of chickenpox.
6. Pneumococcal vaccine.
   The heptavalent conjugate vaccine (PCV) is recommended for all children age 2–23 months. Pneumococcal polysaccharide vaccine (PPV) can be used in elder children.
7. Hepatitis A vaccine.
   The "killed virus vaccine" is recommended in selected regions and for certain high-risk groups. Two doses should be administered at least six months apart.
8. Influenza vaccine.
   Influenza vaccine is recommended annually for children with certain risk factors (for instance asthma, cardiac disease, sickle cell disease, HIV, diabetes etc.). Children aged ≤ eight years who are receiving influenza vaccine for the first time should receive two doses separated at least four weeks.



pneumococcal vaccinations are not obligatory in Germany, Austria, and Switzerland (see www.rki.de). To simplify the application of vaccines, licensed combination vaccines may be used whenever any components of the combination are indicated and the vaccine's other components are not contraindicated. Providers should consult the manufacturers' inserts for detailed information.

**Passive immunization.** This vaccination method involves administration of antibodies produced in a different host. In most cases, homologous (human) hyperimmune sera (obtained from convalescent patients or patients with multiple vaccinations) are used. The passive immunity obtained by this method is limited to a few weeks (or months at most).

## Principles of Sterilization and Disinfection

■ Sterilization is defined as the killing or removal of all microorganisms and viruses from an object or product. Disinfection means rendering an object, the hands or skin free of pathogens. The term asepsis covers all measures aiming to prevent contamination of objects or wounds. Disinfection and sterilization makes use of both physical and chemical agents. The killing of microorganisms with these agents is exponential. A measure of the efficacy of this process is the D value (decimal reduction time), which expresses the time required to reduce the organism count by 90%. The sterilization agents of choice are hot air (180 °C, 30 minutes; 160 °C, 120 minutes) or saturated water vapor (121 °C, 15 minutes, $2.02 \times 10^5$ Pa; 134 °C, three minutes, $3.03 \times 10^5$ Pa). Gamma rays or high-energy electrons are used in radiosterilization at a recommended dose level of $2.5 \times 10^4$ Gy.

Disinfection is usually done with chemical agents, the most important of which are aldehydes (formaldehyde), alcohols, phenols, halogens (I, Cl), and surfactants (detergents). ■

### Terms and General Introduction

#### Terms

**Sterilization** is the killing of all microorganisms and viruses or their complete elimination from a material with the highest possible level of certainty.

An object that has been subjected to a sterilization process, then packaged so as to be contamination-proof, is considered **sterile**.

**Killing of Prions and Thermophilic Archaea**

The standard sterilization methods used in medical applications (see below) are capable of causing irreversible damage to medically relevant microorganisms such as bacteria, protozoans, fungi, and helminths including worm eggs. Much more extreme processes are required to inactivate prions, such as autoclaving at 121 °C for 4.5 hours or at 134 °C for 30 minutes. Hyperthermophilic archaea forms have also been discovered in recent years (see p. 5) that proliferate at temperatures of 100 °C and higher and can tolerate autoclaving at 121 °C for one hour. These extreme life forms, along with prions, are not covered by the standard definitions of sterilization and sterility.

**Disinfection** is a specifically targeted antimicrobial treatment with the objective of preventing transmission of certain microorganisms. The purpose of the disinfection procedure is to render an object incapable of spreading infection.

**Preservation** is a general term for measures taken to prevent microbe-caused spoilage of susceptible products (pharmaceuticals, foods).

**Decontamination** is the removal or count reduction of microorganisms contaminating an object.

The objective of **aseptic measures and techniques** is to prevent microbial contamination of materials or wounds.

**In antiseptic measures,** chemical agents are used to fight pathogens in or on living tissue, for example in a wound.

## The Kinetics of Pathogen Killing

Killing microorganisms with chemical agents or by physical means involves a first-order reaction. This implies that no pathogen-killing method kills off all the microorganisms in the target population all at once and instantaneously. Plotting the killing rate against exposure time in a semilog coordinate system results in a straight-line curve (Fig. 1.**7**).

Sigmoid and asymptotic killing curves are exceptions to the rule of exponential killing rates. The steepness of the killing curves depends on the sensitivity of the microorganisms to the agent as well as on the latter's effectiveness. The survivor/exposure curve drops at a steeper angle when heat is applied, and at a flatter angle with ionizing radiation or chemical disinfectants. Another contributing factor is the number of microorganisms contaminating a product (i.e., its *bioburden*): when applied to higher organism concentrations, an antimicrobial agent will require a longer exposure time to achieve the same killing effect.

**Bacterial Death Kinetics**

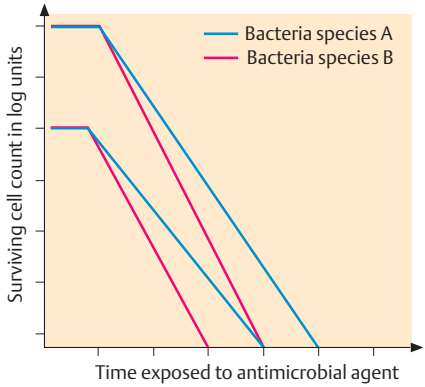



Fig. 1.**7** The death rate varies among bacterial species. The higher the initial concentration of a bacterial culture, the longer an applied antimicrobial agent will require to achieve the same effect.

Standard sterilization methods extend beyond killing all microorganisms on the target objects to project a theoretical reduction of risk, i.e., the number of organisms per sterilized unit should be equal to or less than $10^{-6}$.

The D value (decimal reduction time), which expresses the time required to reduce the organism count by 90%, is a handy index for killing effectiveness.

The concentration (c) of chemical agents plays a significant role in pathogen-killing kinetics. The relation between exposure time (t) and c is called the *dilution coefficient* (n): $t \cdot c^n$ = constant. Each agent has a characteristic coefficient n, for instance five for phenol, which means when c is halved the exposure time must be increased by a factor of 32 to achieve the same effect.

The *temperature coefficient* describes the influence of temperature on the effectiveness of chemical agents. The higher the temperature, the stronger the effect, i.e., the exposure time required to achieve the same effect is reduced. The coefficient of temperature must be determined experimentally for each combination of antimicrobial agent and pathogen species.

## Mechanisms of Action

When microorganisms are killed by heat, their proteins (enzymes) are irreversibly denatured. Ionizing radiation results in the formation of reactive groups that contribute to chemical reactions affecting DNA and proteins. Exposure to UV light results in structural changes in DNA (thymine dimers) that prevent it from replicating. This damage can be repaired to a certain extent

by light (photoreactivation). Most chemical agents (alcohols, phenols, aldehydes, heavy metals, oxidants) denature proteins irreversibly. Surfactant compounds (amphoteric and cationic) attack the cytoplasmic membrane. Acridine derivatives bind to DNA to prevent its replication and function (transcription).

## Physical Methods of Sterilization and Disinfection

### Heat

The application of heat is a simple, cheap and effective method of killing pathogens. Methods of heat application vary according to the specific application.

- **Pasteurization.** This is the antimicrobial treatment used for foods in liquid form (milk):
  - Low-temperature pasteurization: 61.5 °C, 30 minutes; 71 °C, 15 seconds.
  - High-temperature pasteurization: brief (seconds) of exposure to 80–85 °C in continuous operation.
  - Uperization: heating to 150 °C for 2.5 seconds in a pressurized container using steam injection.

- **Disinfection.** Application of temperatures below what would be required for sterilization. Important: boiling medical instruments, needles, syringes, etc. does not constitute sterilization! Many bacterial spores are not killed by this method.

- **Dry heat sterilization.** The guideline values for hot-air sterilizers are as follows: 180 °C for 30 minutes, 160 °C for 120 minutes, whereby the objects to be sterilized must themselves reach these temperatures for the entire prescribed period.

- **Moist heat sterilization.** Autoclaves charged with saturated, pressurized steam are used for this purpose:
  - 121 °C, 15 minutes, one atmosphere of pressure (total: 202 kPa).
  - 134 °C, three minutes, two atmospheres of pressure (total: 303 kPa).

In practical operation, the heating and equalibriating heatup and equalizing times must be added to these, i.e., the time required for the temperature in the most inaccessible part of the item(s) to be sterilized to reach sterilization level. When sterilizing liquids, a cooling time is also required to avoid boiling point retardation.

The significant heat energy content of steam, which is transferred to the cooler sterilization items when the steam condenses on them, explains why it is such an effective pathogen killer. In addition, the proteins of microorganisms are much more readily denatured in a moist environment than under dry conditions.

## Radiation

- **Nonionizing radiation**. Ultra-violet (UV) rays (280–200 nm) are a type of nonionizing radiation that is rapidly absorbed by a variety of materials. UV rays are therefore used only to reduce airborne pathogen counts (surgical theaters, filling equipment) and for disinfection of smooth surfaces.

- **Ionizing radiation**. Two types are used:
  - Gamma radiation consists of electromagnetic waves produced by nuclear disintegration (e.g., of radioisotope $^{60}Co$).
  - Corpuscular radiation consists of electrons produced in generators and accelerated to raise their energy level.

Radiosterilization equipment is expensive. On a large scale, such systems are used only to sterilize bandages, suture material, plastic medical items, and heat-sensitive pharmaceuticals. The required dose depends on the level of product contamination (bioburden) and on how sensitive the contaminating microbes are to the radiation. As a rule, a dose of $2.5 \times 10^4$ Gy (Gray) is considered sufficient.

One Gy is defined as absorption of the energy quantum one joule (J) per kg.

## Filtration

Liquids and gases can also be sterilized by filtration. Most of the available filters catch only bacteria and fungi, but with ultrafine filters viruses and even large molecules can be filtered out as well. With membrane filters, retention takes place through small pores. The best-known type is the membrane filter made of organic colloids (e.g., cellulose ester). These materials can be processed to produce thin filter layers with gauged and calibrated pore sizes. In conventional depth filters, liquids are put through a layer of fibrous material (e.g., asbestos). The effectiveness of this type of filter is due largely to the principle of adsorption. Because of possible toxic side effects, they are now practically obsolete.

## Chemical Methods of Sterilization and Disinfection



**Ethylene oxide.** This highly reactive gas ($C_2H_4O$) is flammable, toxic, and a strong mucosal irritant. Ethylene oxide can be used for sterilization at low temperatures (20–60 °C). The gas has a high penetration capacity and can even get through some plastic foils. One drawback is that this gas cannot kill dried microorganisms and requires a relative humidity level of 40–90% in the sterilizing chamber. Ethylene oxide goes into solution in plastics, rubber, and similar materials, therefore sterilized items must be allowed to stand for a longer period to ensure complete desorption.

**Aldehydes.** *Formaldehyde* (HCHO) is the most important aldehyde. It can be used in a special apparatus for gas sterilization. Its main use, however, is in disinfection. Formaldehyde is a water-soluble gas. *Formalin* is a 35% solution of this gas in water. Formaldehyde irritates mucosa; skin contact may result in inflammations or allergic eczemas. Formaldehyde is a broad-spectrum germicide for bacteria, fungi, and viruses. At higher concentrations, spores are killed as well. This substance is used to disinfect surfaces and objects in 0.5–5% solutions. In the past, it was commonly used in gaseous form to disinfect the air inside rooms (5 g/m$^3$). The mechanism of action of formaldehyde is based on protein denaturation.

Another aldehyde used for disinfection purposes is *glutaraldehyde*.

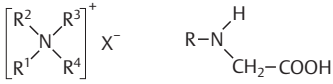
**Alcohols.** The types of alcohol used in disinfection are *ethanol* (80%), *propanol* (60%), and *isopropanol* (70%). Alcohols are quite effective against bacteria and fungi, less so against viruses. They do not kill bacterial spores. Due to their rapid action and good skin penetration, the main areas of application of alcohols are surgical and hygienic disinfection of the skin and hands. One disadvantage is that their effect is not long-lasting (no depot effect). Alcohols denature proteins.

**Phenols.** Lister was the first to use phenol (carbolic acid) in medical applications. Today, phenol derivatives substituted with organic groups and/or halogens (alkylated, arylated, and halogenated phenols), are widely used. One common feature of phenolic substances is their weak performance against spores and viruses. Phenols denature proteins. They bind to organic materials to a moderate degree only, making them suitable for disinfection of excreted materials.

**Halogens.** Chlorine, iodine, and derivatives of these halogens are suitable for use as disinfectants. Chlorine and iodine show a generalized microbicidal effect and also kill spores.

*Chlorine* denatures proteins by binding to free amino groups; hypochlorous acid (HOCl), on the other hand, is produced in aqueous solutions, then

**Surfactant Disinfectants**




a b

Fig. 1.**8** Quaternary ammonium compounds (**a**) and amphoteric substances (**b**) disrupt the integrity and function of microbial membranes.

disintegrates into HCl and $^1/_2$ $O_2$ and thus acts as a powerful oxidant. Chlorine is used to disinfect drinking water and swimming-pool water (up to 0.5 mg/l). Calcium hypochlorite (chlorinated lime) can be used in nonspecific disinfection of excretions. Chloramines are organic chlorine compounds that split off chlorine in aqueous solutions. They are used in cleaning and washing products and to disinfect excretions.

*Iodine* has qualities similar to those of chlorine. The most important iodine preparations are the solutions of iodine and potassium iodide in alcohol (tincture of iodine) used to disinfect skin and small wounds. Iodophores are complexes of iodine and surfactants (e.g., polyvinyl pyrrolidone). While iodophores are less irritant to the skin than pure iodine, they are also less effective as germicides.

**Oxidants.** This group includes ozone, hydrogen peroxide, potassium permanganate, and peracetic acid. Their relevant chemical activity is based on the splitting off of oxygen. Most are used as mild antiseptics to disinfect mucosa, skin, or wounds.

**Surfactants.** These substances (also known as surface-active agents, tensides, or detergents) include anionic, cationic, amphoteric, and nonionic detergent compounds, of which the cationic and amphoteric types are the most effective (Fig. 1.**8**).

The bactericidal effect of these substances is only moderate. They have no effect at all on tuberculosis bacteria (with the exception of amphotensides), spores, or nonencapsulated viruses. Their efficacy is good against Gram-positive bacteria, but less so against Gram-negative rods. Their advantages include low toxicity levels, lack of odor, good skin tolerance, and a cleaning effect.

## Practical Disinfection

The objective of **surgical hand disinfection** is to render a surgeon's hands as free of organisms as possible. The procedure is applied after washing the hands thoroughly. Alcoholic preparations are best suited for this purpose, although they are not sporicidal and have only a brief duration of action.

Alcohols are therefore often combined with other disinfectants (e.g., quaternary ammonium compounds). Iodophores are also used for this purpose.

The purpose of **hygienic hand disinfection** is to disinfect hands contaminated with pathogenic organisms. Here also, alcohols are the agent of choice.

Alcohols and/or iodine compounds are suitable for **disinfecting patient's skin** in preparation for surgery and injections.

Strong-smelling agents are the logical choice for **disinfection of excretions** (feces, sputum, urine, etc.). It is not necessary to kill spores in such applications. Phenolic preparations are therefore frequently used. Contaminated hospital sewage can also be thermally disinfected (80–100 °C) if necessary.

**Surface disinfection** is an important part of hospital hygiene. A combination of cleaning and disinfection is very effective. Suitable agents include aldehyde and phenol derivatives combined with surfactants.

**Instrument disinfection** is used only for instruments that do not cause injuries to skin or mucosa (e.g., dental instruments for work on hard tooth substance). The preparations used should also have a cleaning effect.

**Laundry disinfection** can be done by chemical means or in combination with heat treatment. The substances used include derivatives of phenols, aldehydes and chlorine as well as surfactant compounds. Disinfection should preferably take place during washing.

Chlorine is the agent of choice for **disinfection of drinking water and swimming-pool water**. It is easily dosed, acts quickly, and has a broad disinfectant range. The recommended concentration level for drinking water is 0.1–0.3 mg/l and for swimming-pool water 0.5 mg/l.

**Final room disinfection** is the procedure carried out after hospital care of an infection patient is completed and is applied to a room and all of its furnishings. Evaporation or atomization of formaldehyde (5 g/$m^3$), which used to be the preferred method, requires an exposure period of six hours. This procedure is now being superseded by methods involving surface and spray disinfection with products containing formaldehyde.

**Hospital disinfection** is an important tool in the prevention of cross-infections among hospital patients. The procedure must be set out in written form for each specific case.