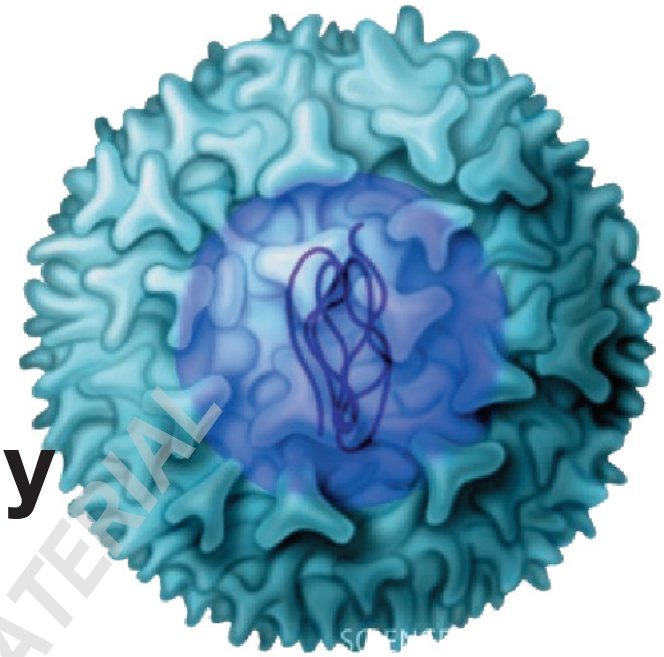

Chapter 1

Microbial etiology of disease

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Microbes and their habitats have held a peculiar fascination for mankind ever since **Antony van Leeuwenhoek** (1632–1723) recorded some of the most important discoveries in the history of biology. Once Leeuwenhoek succeeded in creating the simple microscope, he described bacteria, free-living and parasitic creatures, sperm cells, blood cells, microscopic nematodes and much more. His publications opened up an entire world of microscopic life for scientific study. Microbes continue to excite intense research because of their virulence; their ability to cause tissue damage and death. They have been responsible for the great plagues and epidemics and have often changed the course of human history. The HIV pandemic has emerged as the single most defining occurrence in the history of infectious diseases of the late 20th and early 21st centuries. Microbes continue to baffle human ingenuity; they defy attempts at control by chemotherapeutic agents, vaccines, and the human immune system. The threat of a future pestilence is never far away.

In order to study the microbial etiology of infectious disease, an understanding of the basic principles of microbiology and their interaction with the human host are essential. There are four basic groups of microbes:

- Bacteria
- Viruses
- Fungi: yeasts and molds
- Protozoa.

Multicellular organisms such as helminths also need to be included in the broad description of infectious disease agents.

Table 1.1 Differences between prokaryotic and eukaryotic cells

Cell structure	Prokaryotic	Eukaryotic
Cell wall	Complex cell wall containing peptidoglycan/lipopolysaccaride Some cells have a capsule	Animal cells lack cell walls; plants, algae and fungi do have cell walls, but they differ in composition from those of bacteria
Cytoplasmic membrane	Cytoplasmic membrane without carbohydrates and usually lacking sterols. Incapable of endocytosis and exocytosis	Cytoplasmic membrane contains sterols and carbohydrates and is capable of endocytosis (phagocytosis and pinocytosis) and exocytosis
Nuclear body	Not bounded by a nuclear membrane Usually contains one circular chromosome composed of deoxyribonucleic acid (DNA). No nucleolus. May contain extrachromosomal DNA – the plasmid	Nucleus is bounded by a nuclear membrane, connecting it with the endoplasmic reticulum. Contains one or more paired, linear chromosomes composed of DNA. Nucleolus present
Cytoplasmic structures	70S ribosomes composed of a 50S and a 30S subunit. Mitochondria, endoplasmic reticulum, Golgi apparatus, vacuoles, and lysosomes are absent. No microtubules. Contains only actin-like protein that contribute to cell shape. Spore formation may occur	Ribosomes composed of a 60S and a 40S subunit; mitochondria, endoplasmic reticulum, Golgi apparatus, vacuoles, and lysosomes present. Mitotic spindle involved in mitosis. Cytoskeleton with microtubules, actin, and intermediate filaments
Respiratory enzymes and electron transport chains	Located in the cell membrane	Located in the mitochondria
Cell division	Usually by binary fission. No mitosis or meiosis	By mitosis; sex cells in diploid organisms are produced through meiosis
Organelles of locomotion	Some have hair-like flagellae, fimbriae or pili may also be present. No cilia	May have flagella or cilia: organelles involved in locomotion; consist of a distinct arrangement of sliding microtubules surrounded by a membrane

Prokaryotic and eukaryotic cells

The cell is the basic unit of life, whether it is of human or bacterial origin. Differences in bacterial (prokaryotic cells) and human (eukaryotic cells) have been exploited for diagnostic and treatment purposes. It is important to understand what these differences are and how they contribute to disease pathogenesis (Table 1.1).

Bacteria

Sizes, shapes and arrangement of bacteria

Bacteria are unicellular organisms, ranging from 0.4 μm to 2.0 μm in size. They exist broadly in one of three morphological forms, spheres (cocci), rods (bacilli), or spirals. All of these forms are subject to variation depending on existing growth conditions. The morphology of a bacterium is maintained by a unique cell wall structure and it is the chemical nature of this cell wall that is exploited by the Gram staining

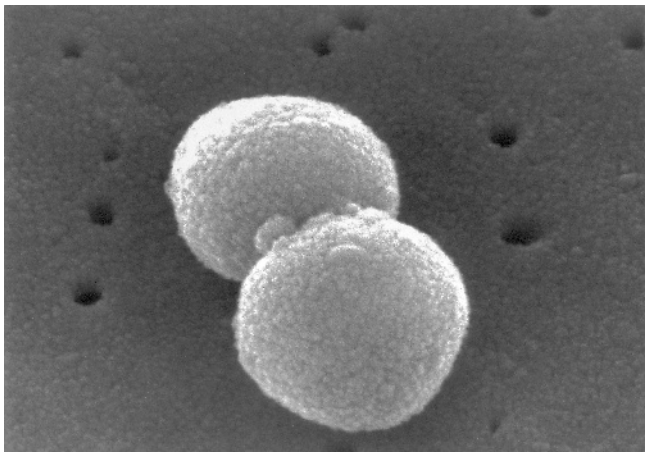


Figure 1.1 Scanning electron micrograph of a diplococcus. Image provided by Dr Richard Facklam. Courtesy of the Centers for Disease Control and Prevention; from CDC website: http://phil.cdc.gov/phil/details.asp

technique (see Chapter 4). The Gram stain remains the single most important diagnostic test in the study of infection – dividing bacteria into two basic groups: **Gram positive** bacteria and **Gram negative** bacteria – thereby influencing the all too important decision: which antibiotic does the clinician use immediately and empirically before full microbiological results are available.

With the help of the Gram stain and a microscope it is possible to visualize the size (relative to a human red or white cell), the shape, the arrangement (if distinctive), and the Gram reaction of the bacterial cell. All the above features are important clues that help identify the infectious agent from a patient's clinical specimen.

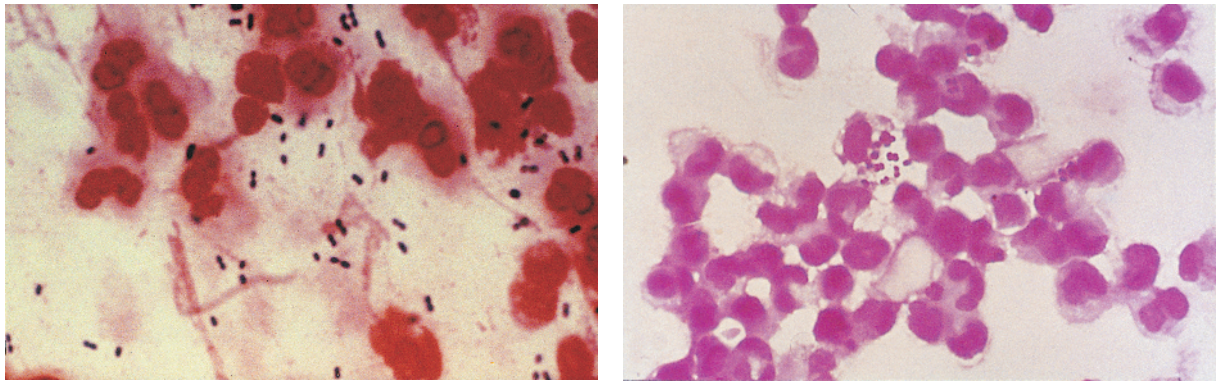
Cocci are spherical or oval bacteria having one of several distinct arrangements based on their planes of division:

1. Division in **one plane** produces either a **diplococcus** (paired; Figure 1.1) or **streptococcus** (chain) arrangement.
   If you were to Gram stain a smear of the specimen containing a putative diplococcus you would be able to see a Gram positive (purple) or -negative (pink) coccus in pairs; note the size relative to a polymorphonuclear leukocyte (Figure 1.2a and b). Streptococci, including medically important ones such as *Streptococcus pyogenes*, are Gram positive (Figure 1.3). The streptococci can be arranged in pairs (e.g. *Streptococcus pneumoniae*, Figure 1.2a) or in chains (e.g. *S. pyogenes*, Figure 1.3).
2. Division in **random planes** produces a **staphylococcus** arrangement. Note the Gram stained smear of a pus sample showing numerous polymorphs 'pus cells' and staphylococci: cocci in irregular, grape-like clusters (Figure 1.4). Ordered division in two or three planes can result in sarcinial arrangements (tetrads) respectively.

Bacilli are rod-shaped bacteria (Figure 1.5). Bacilli divide in one plane and are arranged singly or in chains as in *Bacillus anthracis*. For many clinically important bacilli the arrangement is not distinctive; some bacilli may be rounded off looking more coccoid; they are often called cocco-bacillary forms. Bacilli, like cocci, can be Gram positive or -negative (Figure 1.6)

Other common shapes of bacteria are: curved bacteria as in *Campylobacter* (Figure 1.7) and *Vibrio* species; *Spirillum* species have thick rigid spirals; and spirochaete forms such as *Leptospira* species have flexible spirals (Figure 1.8). Spirals range in size from 1 μm to over 100 μm in length.

It is worth remembering that not all bacteria stain with the Gram stain. In Chapter 2 we will discuss organisms that do not take up the Gram stain readily, those that do not have typical bacterial cell wall structures or arrangements, and those that are obligate intracellular microorganisms.



(a) (b)

Figure 1.2 (a) Gram stain of sputum (1000×) showing numerous polymorphs and Gram positive cocci in pairs (e.g. *Streptococcus pneumoniae* or pneumococci). (b) Gram stain of CSF (1000×) showing numerous polymorphs and Gram negative cocci in pairs (e.g. *Neisseria meningitidis* or meningococci). © Bayer

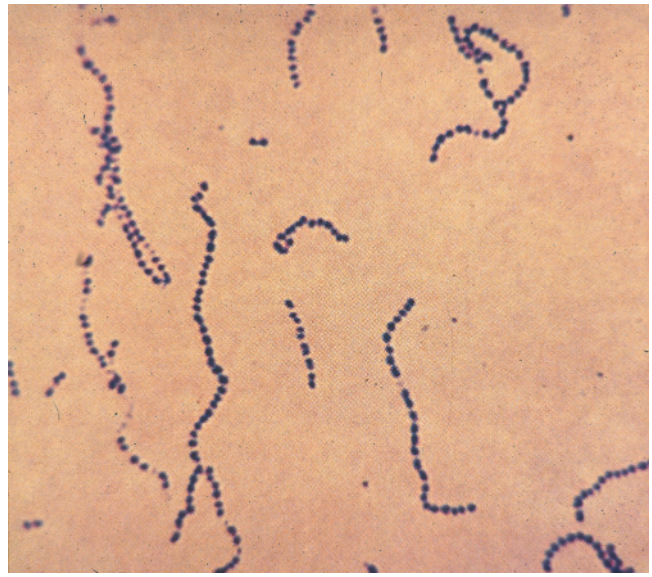


Figure 1.3 Gram positive cocci in chains: the streptococci

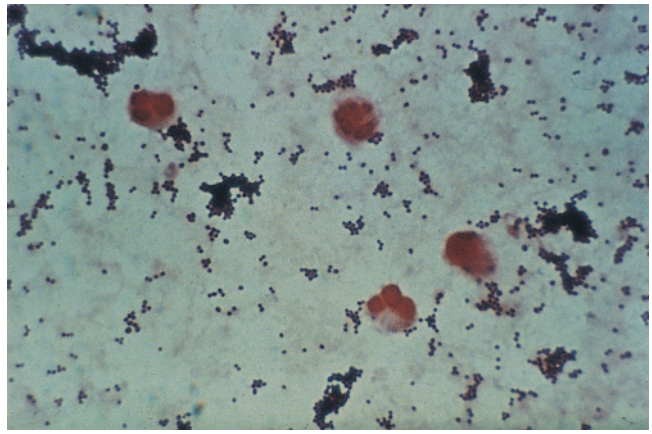


Figure 1.4 Gram stain of a smear (1000×) from pus showing numerous polymorphs and Gram positive cocci in clusters (e.g. *Staphylococcus aureus*)

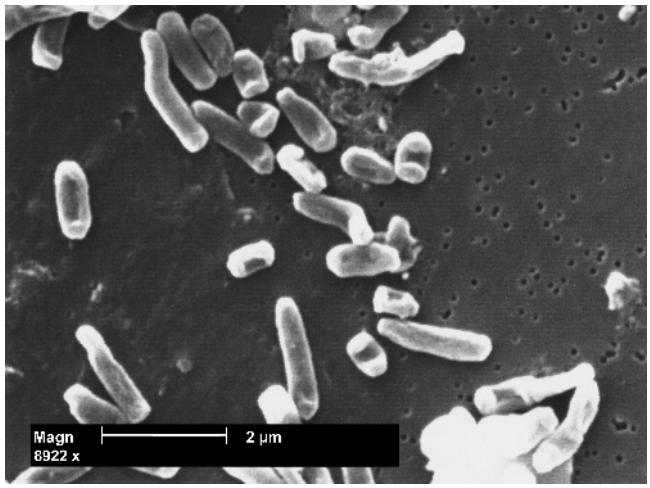


Figure 1.5 Scanning electron image of a bacillus (rod). Image provided by Ray Butler and Janice Carr. Courtesy of the Centers for Disease Control and Prevention; from CDC website: http://phil.cdc.gov/phil/details.asp

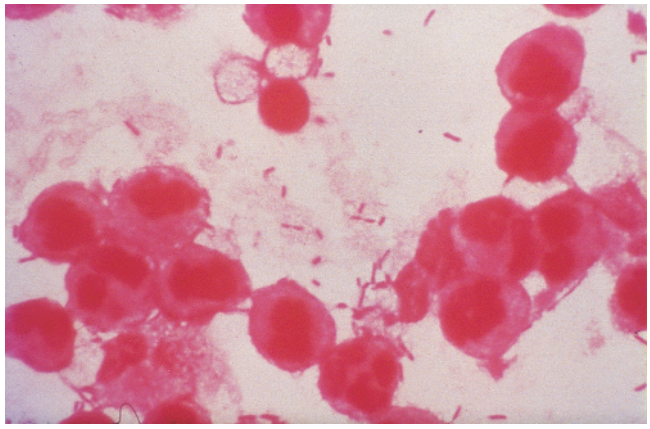


Figure 1.6 Gram stained smear of urine (1000×) showing polymorphs and Gram negative rods (e.g. *Escherichia coli*)

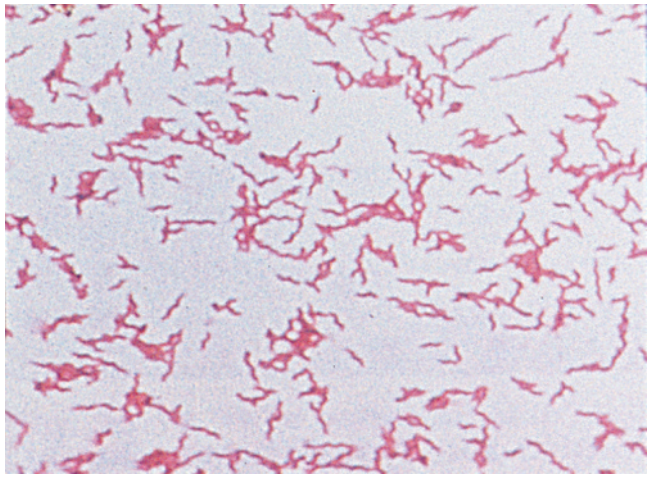


Figure 1.7 Gram stain image (1000×) of *Campylobacter* species, showing curved Gram negative rods

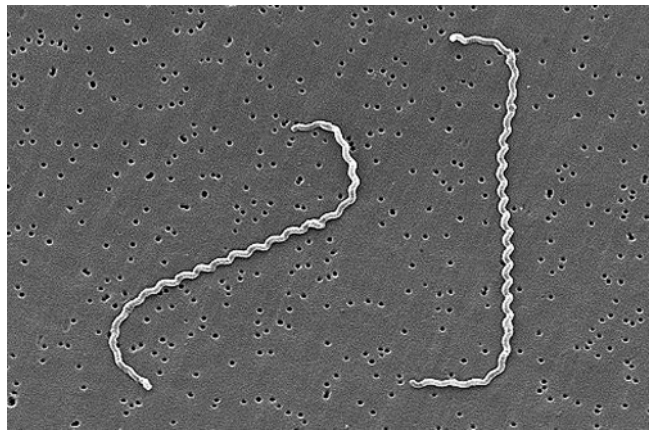


Figure 1.8 Scanning electron micrograph of *Leptospira* species. Image provided by Rob Weyant. Courtesy of the Center for Disease Control and Prevention; from CDC website: http://phil.cdc.gov/phil/details.asp

Phases of bacterial growth

When an organism is inoculated into suitable media such as a liquid culture medium in the laboratory or if it were to encounter a susceptible human/animal host it will exhibit a growth curve (Figure 1.9).

In the **lag phase** the microorganism adapts to a new and often more favourable environment. During this phase, there is a marked increase in enzymes and intermediates, in preparation for active growth. The lag phase is a period of adjustment necessary for the accumulation of metabolites until they are present in concentrations that permit cell division to resume.

In the exponential or **logarithmic phase**, cells are in a state of balanced growth. The cells increase in number and there is a logarithmic expansion of mass and volume. In other words imagine a single cell dividing into two, each further divides in a binary manner and two becomes four, four to eight, eight to sixteen, and so on. A steady state is reached where one of many factors come into play; either essential nutrients become exhausted, there is accumulation of waste products, change in pH, induction of host immune mechanisms and other obscure factors exert a deleterious effect on the culture, and growth is progressively slowed.

During the **stationary phase**, accumulation of toxic products or exhaustion of nutrients causes net growth to cease. The viable cell count remains constant. The formation of new organisms equals the death of organisms in the system. The stationary phase is important to the clinical microbiologist as microbial toxins, antimicrobial substances and other proteins such as bacteriocins and lysins accumulate to significant levels at the end of the stationary phase. Thus, they affect not only growth in the laboratory but also pathogenesis of disease in the host.

As factors detrimental to the bacteria accumulate, more bacteria are killed than are formed. During the **phase of decline** there is a negative exponential phase, which results in a decrease in the numbers of viable bacteria within the system.

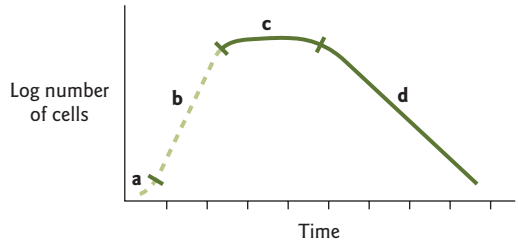


Figure 1.9 The bacterial growth curve showing the four phases of growth. (a) The lag phase; (b) the exponential phase; (c) the stationary phase; (d) the phase of decline

Viruses

General properties

Of all the agents infectious to man, and indeed to other living things, viruses are the smallest. (See Box 1.1 for a description of prions.) An individual infectious unit, comprising a nucleic acid genome, packaged inside a protein coat with or without a surrounding lipid-containing envelope membrane, is known as a viral particle or **virion** (Figure 1.10).

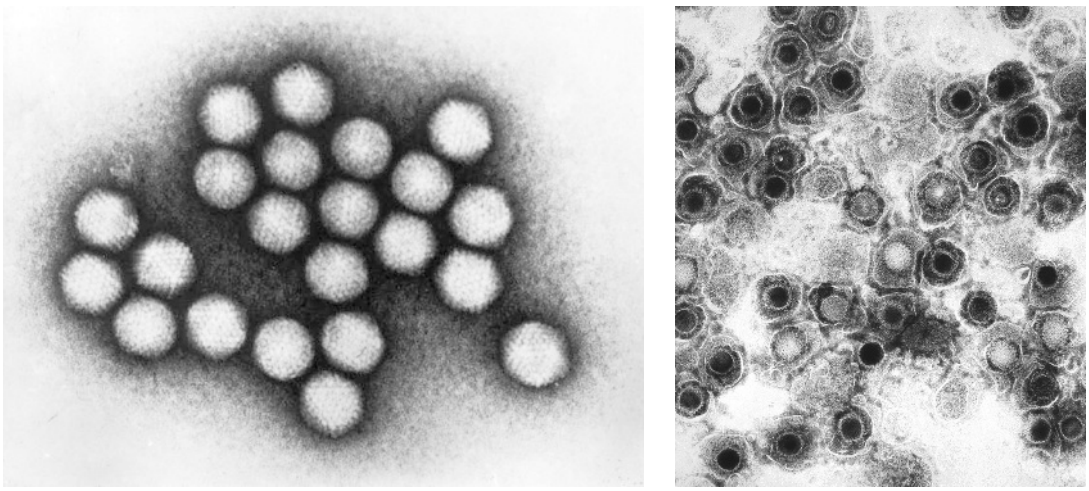
Only the very largest of these, the poxviruses measuring up to 400 nm in their longest dimension and the even larger mimivirus, can be visualized with a light microscope. Cell-free, intact virions are entirely

Box 1.1 Prions

Even smaller infectious agents have been identified that, amazingly, lack a nucleic acid genome. It would appear that the protein alone is the infectious agent. This infectious agent has been called a **prion**, short for **pro**teinaceous **in**fectious particle. They are unique structures as they lack DNA or RNA (the very code for life in a living microorganism) and they resist all conventional attempts at inactivation. The discovery that proteins alone can transmit an infectious disease has led to much debate and controversy in the scientific community.

Prion diseases target the brain and are often called **spongiform encephalopathies**. The post mortem appearance of the brain is characteristic, with large vacuoles in the cortex and cerebellum. Many mammalian species develop these diseases. Specific examples include:

- **Scrapie** in sheep
- **TME** (transmissible mink encephalopathy) in mink
- **CWD** (chronic wasting disease) in muledeer and elk
- **BSE** (bovine spongiform encephalopathy) in cows; this led to banning of British beef worldwide (commonly known as mad cow disease).



(a) (b)

Figure 1.10 Electron photomicrograph of: (a) a nonenveloped virus, the adenovirus; (b) an enveloped virus, the herpes virus. Courtesy of: (a) Dr G. William Gray Jr; (b) Dr Fred Murphy; from CDC website: http://phil.cdc.gov/phil/details.asp

metabolically inert: they cannot be said to be "alive" at all. Yet, on entering an appropriate cell (which is anything but a matter of chance proximity), the cellular machinery is hijacked and diverted towards production of new viral particles. Normal cellular function may be disrupted to a greater or lesser extent and the consequences of this may be manifest as disease.

As viruses can replicate themselves only inside living prokaryotic or eukaryotic cells, they have evolved alongside cellular organisms and specific viral infections are known in mycoplasma and other bacteria, algae, fungi, plants, and animals. In that certain viruses depend on co-infection of their cellular targets with other viruses, viruses might be said to even parasitize each other (e.g. hepatitis D virus cannot replicate in the absence of hepatitis B virus (HBV) because it requires the HBV protein coat for the packaging of its own nucleic acid).

The **host range** for a given virus may be relatively broad (e.g. influenza A, which can infect ducks, chickens, pigs and horses as well as humans), or extremely narrow (e.g. measles, which infects only humans). This is known as **host-specificity**. Within a multicellular host, a particular virus may be able to infect many types of cell (e.g. Ebola virus) or be restricted to the cells of only certain tissues. This phenomenon is known as **tissue tropism**. Both host-specificity and tissue tropism are determined largely by the molecular properties of the viral surface (the viral envelope proteins or in the absence of an envelope, the viral coat proteins) precisely interacting with specific cell surface molecules. In the absence of the cell surface molecule(s) required, the virus cannot enter the cell. This will be discussed further later in this chapter.

The "purpose" of a virus, then, is to find a susceptible cell, enter it and replicate in such a way as to facilitate its progeny finding susceptible cells in new hosts, i.e. transmission. The strategies used to achieve this end are hugely diverse, and accomplished with the most extraordinary economy of material. A virion may carry an enzyme or two, necessary for the initiation of the replication cycle, but is essentially just a sophisticatedly addressed package bearing an auto-start program: a blueprint for making more of itself. Compared with the genomes of cellular organisms, viral genomes are minute. For comparison, the human genome comprises 3×10^6 kilobases (kb), that of *Haemophilus influenzae* 1.8×10^3 kb, that of a pox virus around 200 kb, and that of hepatitis B virus (HBV) only 3.2 kb. The numbers of genes encoded by viral genomes are commensurately small. HBV encodes just four. Moreover, there is almost no redundancy in viral genomes and, not infrequently, genes overlap, with different proteins being transcribed from different open reading frames (see Chapter 19). The possession of relatively so few genes does not however imply that viruses are easily understood and hence eliminated by human intervention. It should be noted that while the 9.7-kb genome of the human immunodeficiency virus (HIV) was first fully sequenced in 1985 by Ratner and his co-workers, over 20 years later, we do not entirely understand its pathogenesis, have no cure, and no preventative vaccine.

Viral transmission

Many viral diseases that occur in humans are **zoonoses**, i.e. they are communicable from animals to humans under natural conditions. Unlike most other human pathogens, viruses have no free-living form outside their host(s). In the environment, a virus particle can do no more than passively survive intact and any damage is liable to render it noninfectious. In this respect it is important to note that the lipid bilayer of enveloped viruses is very vulnerable to disruption by desiccation, detergents, and solvents. Because infectivity depends on the integrity of the envelope bearing its viral attachment (glyco)proteins, these viruses do not survive long in the environment and are readily susceptible to decontamination methods (e.g. even simple soap and water). Conversely, nonenveloped viruses tend to be considerably more durable. Caliciviruses are particularly resilient and the difficulty of adequately decontaminating **fomites** (contaminated inanimate objects) in the context of norovirus outbreaks has no doubt contributed to outbreak persistence on many occasions.

Despite their passivity in the environment, viruses have evolved so as to maximize their chances of transmitting from host to host. Some viruses, **ar**thropod **bo**rne or **arboviruses**, multiply to high viral loads in the bloodstream and are transferred to new hosts by arthropods that feed on human blood. Transmission is also assured if viruses shed in vast numbers into human body fluids – respiratory secretions, saliva, genital

secretions, urine, and stool. Human behavior takes care of the rest: face to face conversation, sexual activity, use of the hands for eating as well as for toileting facilitates transmission and continued survival. Certain viruses, influenza and rotavirus for example, go even further by causing the volume of the infectious fluid to be considerably increased and literally sprayed into the environment by sneezing or explosive, watery diarrhea respectively. Mother-to-child transmission (also known as **vertical transmission**) is usually an incidental means of transmission rather than the predominant one. The transmission of **blood-borne viruses** through parenteral exposure (blood transfusion, contaminated surgical implements, tattooing, sharing of equipment for IV drug use, etc.) is of course an artifact of very recent (in evolutionary terms) human behavior, where the major means of transmission is mucosal or skin lesion contact with blood or genital secretions. In the absence of universal immunization, knowledge of the route by which a viral disease is transmitted is essential to the control of that infection in human populations.

Fungi

Fungi are an extremely diverse group of organisms, ubiquitous in the environment. They are found as two main forms, **yeasts** and **molds**. They are nonphotosynthetic organisms with the ability to absorb soluble nutrients by diffusion from living or dead organic matter. Molds consist of branching filaments (**hyphae**), which interlace to form a mycelium. The hyphae of the more primitive molds remain aseptate (without walls) whereas those of the more developed groups are septate with a central pore in each cross wall. Yeasts are unicellular organisms consisting of separate round or oval cells. They do not form a mycelium, although the intermediate yeast-like fungi form a pseudomycelium consisting of chains of elongated cells.

Many fungi, including some of clinical importance, can exist in both forms dependent on temperature and other environmental conditions. These are known as **dimorphic** fungi.

Like mammalian cells, fungi are **eukaryotes** (Table 1.1) with DNA organized into chromosomes within the cell nucleus. Fungi also have distinct cytoplasmic organelles including Golgi apparatus, mitochrondria, and storage vacuoles. Homology with mammalian cells also extends to biosynthesis, where fungi share similar pathways for both protein synthesis and DNA replication.

A formal classification scheme of fungi has little medical relevance so a simplified clinical classification for **pathogenic** fungi, based on initial site of infection, is more commonly used (Table 1.2).

Cutaneous superficial fungal infections are very common. The majority are caused by three groups of fungi: mold dermatophytes such as *Microsporium* spp. and *Trichophyton* spp., *Candida albicans*, and *Malassezia* spp. Keratin-containing structures such as hair shafts, nails, and skin are affected. Dermatophyte skin infection (sometimes called ringworm) is commonly named after the area affected, for example tinea capitis (head) or tinea corporis (body).

The **systemic** fungi include *Coccidioides immitis*, *Paracoccidioides braziliensis*, and *Histoplasma capsulatum*. These are thermally dimorphic fungi, meaning they have both yeast-like and filamentous forms. They are environmental organisms, which enter the body usually via inhalation. Infection is geographically

Table 1.2 Classification of fungi associated with human infection (mycoses)

Classification	Examples (and form)
Cutaneous and mucocutaneous infection	*Microsporium* spp. (mold) *Trichophyton* spp. (mold) Candida vaginal infection (vaginal thrush) Candida mouth infection in babies (oral thrush)
Systemic infection	*Coccidioides immitis* (dimorphic) *Histoplasma capsulatum* (dimorphic)
Infections of the immunocompromised host	*Candida* spp. (yeast) *Aspergillus* spp. (mold)

circumscribed and often clinically mild. Severe disseminated disease can occur, however, particularly in immunocompromised patients.

The main fungi that cause disease in immunocompromised patients are the yeasts ***C. albicans*** and related species such as *Candida krusei*. ***Aspergillus*** species are important environmental filamentous fungi, which may cause pulmonary or disseminated infection. The yeast-like fungi ***Cryptococcus neoformans*** can cause chronic meningitis in patients with HIV infection.

Fungi of medical importance

Over 200 000 species of fungi have been described although only about 200 have been associated with human disease. With a few exceptions, fungal infections of humans originate from an exogenous source in the environment and are acquired through inhalation, ingestion, or traumatic implantation.

A few species of fungi are capable of causing significant disease in otherwise normal individuals. Many more are only able to produce disease when the host has some aspect of impaired immunity e.g. HIV seropositivity, or during treatment for malignancy. With the increasing number of **immunocompromised** patients, fungi previously considered to be nonpathogenic are being recognized as the cause of sporadic infections. Any fungus capable of growing at the temperature of the human host (37°C) must now be regarded as a potential human pathogen.

Recent DNA sequencing work has shown that ***Pneumocystis jiroveci***, long believed to be a protozoon, is in fact also a fungus. This organism is an important pathogen in immunocompromised patients, especially those with AIDS.

Protozoa

Protozoa are unicellular microorganisms, which are found in almost every type of environment. Almost two-thirds of the world population live in conditions in which infection with protozoa or **helminths** (see later) are thought to be unavoidable. Protozoan lifecycles are extremely diverse and can be complex and thus they display a much wider range of morphology than bacteria or viruses. Many species are parasites of higher plants and animals but this chapter will deal only with those that cause disease in humans.

Protozoans such as *Plasmodium* spp., *Leishmania* spp., and *Entamoeba histolytica* are important causes of morbidity and mortality in the tropics but can be seen in the developed world in the returning traveler. Pathogens such as *Cryptosporidium parvum* and *Giardia lamblia* are important causes of morbidity in both the developed and developing world. Protozoan organisms including *Toxoplasma gondii*, *Leishmania* spp., and *C. parvum* have been shown to cause particularly severe disease in patients with AIDS. With increased levels of international travel and the immunosuppressive effects of infection with HIV, one needs to have a raised awareness of diseases caused by protozoa.

Classification

The classification of protozoa that are medically important can be simplified by subdivision into four main groups, which in part relate to the method of locomotion (Table 1.3). These distinctions are by no means absolute, for example some organisms may be flagellate or amoeboid at different stages of their lifecycle. Flagellates are often divided into organisms causing intestinal or urogenital infection such as *Giardia lamblia* or *Trichomonas vaginalis*, and flagellates found in the blood or tissues (hemoflagellates) such as *Leishmania* spp. or *Trypansoma* spp.

Helminths

For completeness a short section on helminths has been included here. Helminths are multicellular organisms that range from less than 1 cm to more than 10 m in length. The helminths that infect man include the

Table 1.3 A simplified classification scheme for the medically important Protozoa and example(s) in each group

Classification	Most important examples
Sporozoa	*Plasmodium* spp. *Toxoplasma gondii* *Cryptosporidium parvum* *Isospora belli* *Cyclospora cayetanensis*
Flagellates **Intestinal/urogenital** **Blood/tissue**	 *Giardia lamblia*, *Trichomonas vaginalis* *Leishmania* spp., *Trypanosoma brucei*, *Trypanosoma cruzi*
Ameboid	*Entamoeba histolytica*
Ciliates	*Balantidium coli*

Table 1.4 Some medically important helminths and the associated infections

Species name	Infection caused
Roundworms (nematodes)	
Ascaris lumbricoides	Intestinal roundworm
Trichuris trichiura	Whipworm
Ancylostoma duodenale/Necator americanus	Hookworm
Strongyloides stercoralis	Strongyloidiasis
Enterobius vermicularis	Thread worm or pin worm
Trichinella spiralis	Trichinellosis
Wuchereria bancrofti	Filariasis
Loa loa	Loiasis
Onchocerca volvulus	River blindness
Toxocara canis	Visceral larva migrans
Tapeworms (cestodes)	
Taenia solium	Cysticercosis
Taenia saginata	Beef tapeworm
Hymenolepis nana	Dwarf tapeworm
Diphyllobothrium latum	Fish tapeworm
Echinococcus granulosus	Hydatid disease
Flukes (trematodes)	
***Schistosoma* spp.**	Schistosomiasis
Fasciola hepatica	Liver fluke
Paragonimus westermani	Lung fluke
Clonorchis sinensis	Chinese liver fluke

nematodes (roundworms) and platyhelminths (flatworms), the latter group consisting of **cestodes** (tapeworms) and **trematodes** (flukes). Worms are covered by a cuticle that protects them from environmental stresses. The lifecycle of all worms includes an egg, one or more larval stages, and the adult. The prevalence of helminthic infection is greatest in developing countries where poverty leads to increased exposure because of lack of clean water, poor sanitation, and inadequate housing. Table 1.4 demonstrates some of the most important helminths known to infect humans.

Further reading

Chiodini PL, Moody A, Manser DW. *Atlas of Medical Helminthology and Protozoology*, 4th edition. London: Churchill Livingstone, 2001

Winn WC, Koneman EW, Allen SD, et al. *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*, 6th edition. Baltimore, MD: Lippincott Williams and Wilkins, 2006