1 Microbiology and medicine

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KEY POINTS

- Microbes are too small to be seen directly and special methods are needed to investigate them. In daily life and in clinical practice we are forced to use our imagination to understand how our behaviour influences and is influenced by them.
- Infections and microbes were considered as separate phenomena until the late nineteenth century when Pasteur reconciled previous observations on the physical requirements for the transmission of infection with the nature of microbes and established the necessity of a chain of transmission in infection.
- Some infections can be prevented by interrupting transmission and/or by immunization.
- The role of specific microbes in specific infective conditions may be established by propagating the microbe in pure laboratory culture and subsequently reproducing the disease in a suitable model.
- Molecular biology has opened up new ways of identifying microbes and establishing causality in infection.
- Transmission of infection is related to the *reservoir*, *immediate source* and *mode of transmission* of the causal agent.
- Approximately 10^{14} bacterial, fungal and protozoan cells live on and in healthy human bodies. Most are harmless or even beneficial. Those that cause disease in otherwise healthy individuals are termed *pathogens.* The *normal microbiota* constitutes the reservoir and immediate source for *endogenous* infection. Infections in which the source of the causal organism is external are termed *exogenous* infections.
- Many infections can now be treated with antimicrobial agents that possess *selective toxicity.* None the less, infection remains the most common cause of morbidity and premature death in the world.

Read this paragraph, then close your eyes and think through the following: inside your intestines, in your mouth and on your skin, there reside more than 100 000 000 000 000 microbial cells – 100-fold more than the number of cells that make up the human body. We are not conscious of these companions any more than we are conscious of passing them round every time we shake hands, speak or touch a surface. Inoculation with just one microbe of the wrong type in the wrong way may kill you, yet we tolerate and indeed thrive on constant appropriate exposure to this unseen world.

Because microbes are generally hidden from our senses, an appreciation of microbiology and infection demands imagination. Our forebears who established the discipline lavished imagination on the problems they studied. Sadly it is that lack of imagination that now underpins serious problems such as hospital-acquired infection and antibiotic resistance.

Hard-won advances in microbiology have transformed the diagnosis, prevention and cure of infection and have made key contributions to improved human health and a doubling in life expectancy. The conquest of epidemic and fatal infections has sometimes seemed so conclusive that infections may be dismissed as of minor concern to modern doctors in wealthy countries. However, infection is far from defeated. In resource-poor countries, an estimated 10 million young children die each year from the effects of infectious diarrhoea, measles, malaria, tetanus, diphtheria and whooping cough alone. Many other classical scourges, such as tuberculosis, cholera, typhoid and leprosy, continue to take their toll. Although we have the potential to prevent nearly all of these deaths, political and social issues constantly hinder progress, and more effective and economic means of delivery provide a constant challenge.

Even in wealthy nations, infection is still extremely common: at least a quarter of all illnesses for which patients consult their doctors in the UK are infective and around one in ten patients acquire infection while

in hospital, sometimes with multiresistant organisms. Global communications and changes in production systems, particularly those affecting food, can have a profound effect on the spread of infectious disease. The emergence of human immunodeficiency virus (HIV), new-variant Creutzfeldt–Jakob disease (CJD), severe acute respiratory syndrome (SARS), avian and most recently so-called swine influenza illustrate the need for continued vigilance.

The relative freedom of wealthy societies from fatal infections has been won through great struggles, which are all too easily forgotten. As generations grow up without the experience of losing friends and relatives through infection, so the balance of perceived risk and benefit looks different. So now, in addition to the old threats which are ever present, we constantly face pressure to drop or modify measures such as public immunization. A historical understanding of infection is as important in maintaining and improving the present status as is knowledge of contemporary progress.

AN OUTLINE HISTORY OF MICROBIOLOGY AND INFECTION

Micro-organisms and infection

Infection and microbiology followed different strands of development for centuries (Fig. 1.1). We tend to map this story against the recorded efforts of prominent individuals, though many others doubtless contributed.

Ideas of infection and epidemics were recorded by Hippocrates, but it was nearly 2000 years before Girolamo Fracastoro (1483–1553) proposed in his classic tome 'De Contagione' that 'seeds of contagion' (as opposed to spirits in the ether) might be responsible. Quite separately, the early microscopists began to make observations on objects too small to be seen by the naked eye. Foremost among these was the Dutchman Antonie van Leeuwenhoek (1632–1723). With his remarkable home-made and hand-held microscope, he found many micro-organisms in materials such as water, mud, saliva and the intestinal contents of healthy subjects, and recognized them as living creatures ('animalcules') because they swam about actively. That he saw bacteria as well as the larger microbes is known from his measurements of their size ('one-sixth the diameter of a red blood corpuscle').

Before the discipline of microbiology was formally established in the second half of the nineteenth century, three key aspects of infection were

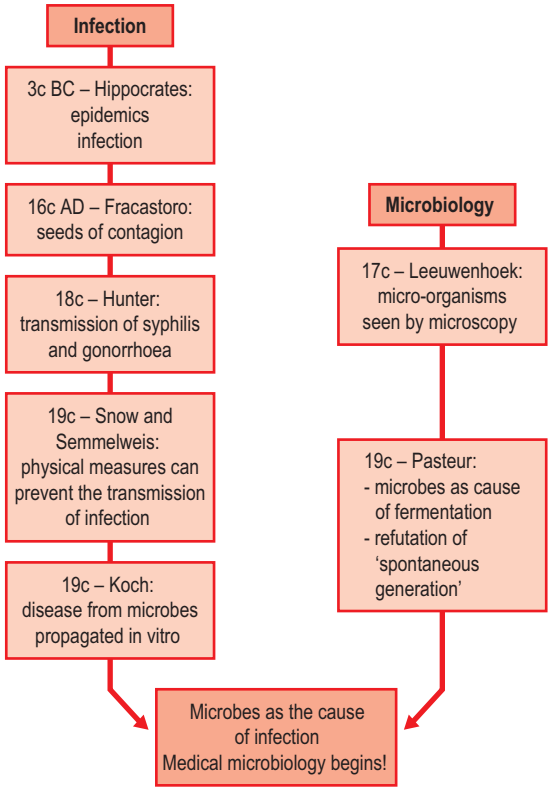

Fig. 1.1 Timelines for the history of infection and microbiology.

brought into stark relief by publicly acknowledged demonstrations:

1. John Hunter (1728–1793) inoculated secretions from sores around a prostitute's genitals into a penis (his own according to some sources) and demonstrated a *physical reality to the transmission of infection*, in this case syphilis and gonorrhoea (the prostitute had both, leading to a mistaken belief that the distinctive symptoms were manifestations of the same disease).
2. Edward Jenner (1749–1823) adapted the long-established oriental practice of variolation (inoculation with material from a mild case of smallpox) for the prevention of smallpox, by showing that cowpox was as effective and safer. The procedure, termed *vaccination* (Latin vacca = cow) established the concept of *immunization* in Europe.
3. John Snow (1813–1858) showed that, by preventing access to a water source epidemiologically linked to a cholera outbreak, further infections could be terminated. This established that *physical measures could prevent the transmission of infection*, a point further

illustrated by Ignaz Semmelweis (1818–1865) in Vienna and others who showed that fatal streptococcal infections (puerperal fever) affecting mothers following childbirth could be substantially reduced if those attending the birth applied simple hygienic measures.

Later two towering figures, Louis Pasteur (1822–1895) and Robert Koch (1843–1910), played central roles in establishing the microbial causation of infectious disease. The brilliant French chemist Louis Pasteur crushed two prevailing dogmas: that the fermentation responsible for alcohol formation was a purely chemical process (by demonstrating that the presence of living micro-organisms was essential), and that life could be spontaneously generated (by showing that nutrient solutions remained sterile if microbes were excluded). Refutation of spontaneous generation established unequivocally that all life must come from progenitors of the same species and, therefore, the need for a *chain of transmission* where infection is concerned. Pasteur made many other seminal contributions, including the identification of several causal agents of disease and the recognition that microbes could be rendered less capable of causing disease (less *virulent*) or *attenuated* by artificial subculture. He used the principle of attenuation to develop a successful vaccine against anthrax for use in animals. It was the influence of Pasteur's work that inspired the British surgeon Joseph Lister (1827–1912) to establish *antisepsis*, aimed at destroying the micro-organisms responsible for infection during surgery.

The other great founding father of medical microbiology, Robert Koch, came to microbiology through medicine. Working originally as a country doctor in East Prussia, he established the techniques required to isolate and propagate pure cultures of specific bacteria. His numerous contributions include establishment of the bacterial causes of anthrax, tuberculosis and cholera. He also formulated more precisely proposals first put forward by one of his mentors, Jacob Henle (1809–1885), describing how specific microbes might be recognized as the cause of specific diseases. These principles, often referred to as *Koch's postulates*, are used to substantiate claims that a particular organism causes a specific ailment. They require that:

- The organism is demonstrable in every case of the disease.
- It can be isolated and propagated in pure culture in vitro.
- Inoculation of the pure culture by a suitable route into a suitable host should reproduce the disease.
- The organism can be re-isolated from the new host.

For various reasons, universal application of the postulates is impossible and greater subtleties in establishing causal relationships in infection are now recognized. Austin Bradford Hill (1897–1991) developed a sophisticated algorithm to recognize a biological gradient of association; most recently an approach to determining the role of specific molecules in pathogenesis has been enshrined in 'molecular Koch's postulates'.

Organisms for which Koch's postulates and later modifications have been fulfilled are clearly capable of inducing disease and are designated as *pathogens* to distinguish them from the vast majority of *non-pathogenic* micro-organisms. It should be emphasized that fulfilment of the postulates and the diagnostic process in which a given patient's illness is attributed to a known pathogen are profoundly different processes. In the former, many experiments are done to provide robust scientific evidence, whereas in the latter circumstantial evidence is obtained, which, in the light of experience, identifies a particular micro-organism as the most likely cause of the illness.

In the century following Pasteur and Koch's work, the list of specific human pathogens has extended to include several hundred organisms. Early on, fungal and protozoan pathogens were recognized, as were macroscopic agents including parasitic worms and insects. Technological breakthroughs, including tissue culture and electron microscopy, were required to enable recognition of viruses. In the early days, viral pathogens were termed filterable agents, because they passed through filters designed to retain bacteria. In many cases pathogens of insects, animals or even plants were described before their medical equivalents were recognized.

Many further advances in technology through the twentieth century provided more precise understanding of the nature and function of microbes. The revolution in molecular biology that followed the elucidation of the structure of DNA by James Watson, Francis Crick, Maurice Wilkins and Rosalind Franklin in 1953 ultimately enabled a leap forward in analytical capability. For three decades this did not radically change the understanding of microbes and infection. However, almost exactly a century after Pasteur and Koch initiated what has been called the 'golden era of bacteriology', three interconnected breakthroughs once again altered the perspective:

1. The recognition, principally by the American molecular biologist Carl Woese, that ribosomal ribonucleic acid (rRNA), which has essentially the same core structure in all cells, carries unique signatures indicating its evolutionary

relationships. It transpires that all cellular forms of life can be classified according to the DNA sequence encoding their rRNA (rDNA). Determination of this sequence provides a means of identifying all microbes and has led to the discovery of a previously unsuspected third 'domain' of life, the *Archaea* (see Ch. 2).

2. Technological advances made possible by molecular genetics. The molecular basis for the pathogenesis of infection now enables recognition of the specific roles of individual genes and their products in both the pathogen and the host. This offers the promise of new approaches to treatment and prevention of infection. The discovery of mobile genetic elements that convey genes from one organism to another (see Ch. 6) confronted our biological sense of what makes up an individual. Mobile bacterial genes encoding antibiotic resistance present a major problem to the practice of medicine.
3. The development of ultra-sensitive means of detecting specific DNA or RNA sequences and the development, by Kary Mullis, of the polymerase chain reaction (PCR) in 1986. The analytical capacity of nucleic acid amplification techniques offers the prospect that it may be possible to diagnose microbial disease routinely by these methods. However, there are many challenges to be met before this shift away from detection of micro-organisms by isolation in laboratory cultures can be accepted.

Our capacity to exploit these breakthroughs has been enhanced enormously by the development of DNA sequencing by the double Nobel Laureate Fred Sanger in Cambridge. Most recently, advances in DNA analytical technologies offer real prospect that rapid sequence analyses will be performed in clinical diagnostic labs. This type of analysis has the potential to transform our insights into the epidemiology of infection and the ongoing evolution of the microbes that cause disease.

Hygiene, treatment and prevention of infection

The work of Snow, Semmelweis, Lister and others led to an appreciation of the benefits of hygiene in the prevention of infection. Nursing practices rooted in almost obsessive cleanliness became the norm and *aseptic* practice (avoidance of contact between sterile body tissues and materials contaminated with live micro-organisms) was introduced to supplement the use of antisepsis. Before the advent of antibiotics, hygiene was a matter of life and death; institutes of hygiene were established around the world. When treatment of infection later became reliable and routine, hygiene standards were often allowed to drop, leading to present problems, notably with hospital-acquired infection.

The discovery of phagocytic cells and humoral immunity (antibodies) as natural defence mechanisms at the end of the nineteenth century led to a re-assessment of the response to infection. One outcome was the use of antibodies produced in one host for the protection of another *(serum therapy)*. This produced some spectacular successes, notably in the life-saving use of antitoxin in diphtheria and tetanus. Unfortunately these foreign proteins often caused hypersensitivity reactions *(serum sickness)* and few diseases responded reliably to serum therapy. Nevertheless, the capacity of the immune system to achieve *selective toxicity*, and observations by the brilliant German doctor Paul Ehrlich (1854–1915) that dyes used to stain infected tissues selectively labelled parasites in preference to host tissues, contributed to the notion that systemic chemotherapy might be achievable.

In 1909 Ehrlich and his colleagues introduced the arsenical drug Salvarsan for the treatment of syphilis, but it fell short of his ideal of a *magic bullet* that would destroy the parasite without harming the host. A more important breakthrough than Ehrlich's came in 1935 with the publication of a paper by Gerhard Domagk (1895–1964) of the German dyestuffs consortium, IG Farbenindustrie. Domagk described the remarkable activity against streptococci of a dye derivative, prontosil, which turned out to owe its activity to a sulphonamide substituent previously unsuspected of antibacterial activity. Earlier, in 1928, Alexander Fleming had accidentally discovered the antibacterial properties of a fungal mould *Penicillium notatum*, but he was unable to purify the active component or exploit the therapeutic potential of his discovery. This was left to a team of scientists at Oxford led by the Australian experimental pathologist Howard Florey (1898–1968), heralding the start of the antibiotic era – the most important therapeutic development of the twentieth century.

Meanwhile, in America, the Ukrainian-born soil microbiologist Selman Waksman (1888–1973) undertook a systematic search for antibiotic substances produced by soil micro-organisms that achieved its greatest success in 1943 with the discovery of streptomycin by one of his PhD students, Albert Schatz (1920–2005). The hunt for antibiotics intensified after the Second World War, yielding chloramphenicol, tetracyclines and many other natural, synthetic and

semi-synthetic antibacterial compounds. Progress in the development of antiviral, antifungal and antiparasitic compounds has been much slower, despite the fact that two effective antiprotozoal drugs, quinine (cinchona bark) and emetine (ipecacuanha root), and some natural anthelminthic agents have been recognized for centuries. Therapeutic choice in non-bacterial infection consequently remains severely limited, although the human immunodeficiency virus (HIV) pandemic has stimulated much work in the antiviral field that has been rewarded with significant success. Meanwhile, the explosion of knowledge in immunology has renewed hopes that it may be possible to manipulate immunological processes triggered by infection to the benefit of the host.

SOURCES AND SPREAD OF INFECTION

To adequately grasp the ways in which the microbial world intersects with human lives it is necessary to understand different microbial lifestyles and the degree to which they depend on human beings. Thus there are some pathogens for which an association with man is essential in order for them to propagate, whereas for others human association is of little significance compared with their propagation in other species or environments. Microbes that depend on human beings are *obligate parasites.* A few actually need to cause disease to propagate themselves; these are termed *obligate pathogens.* In most cases, disease is accidental, or even detrimental, to the microbe's long-term survival. Viruses that cause disease in man are obligate parasites, although they often cause inapparent, subclinical or *asymptomatic* infection. Many viruses rely on infecting a particular host species. Smallpox was eradicated, not only because of the availability of an effective vaccine, but also because man was the only host. Some bacteria, fungi, protozoa and helminths are also species-specific. Among bacteria, the agent of tuberculosis, which is harboured by one-third of humanity, has an absolute requirement to cause disease for its natural transmission to continue.

Since Pasteur established the need for a chain of transmission in infection, it has been possible to fit the sources and spread of infection into a relatively simple framework. All infection recently transmitted has an *immediate source* and reaches the newly infected individual via one or more specific *mode(s) of transmission.* Behind these events, the organism, which of course does not care how we choose to classify it or its activities, lives and propagates in its natural habitat(s). These may or may not be the same as the immediate source but, in considering the control of infection, the natural habitat of the causal organism constitutes the *reservoir of infection.* These points are illustrated in Figure 1.2. Elimination of the organism from the reservoir will lead to eradication of the infection, whereas elimination from the immediate source, if this is distinct from the reservoir, provides one means by which control of infection can be achieved.

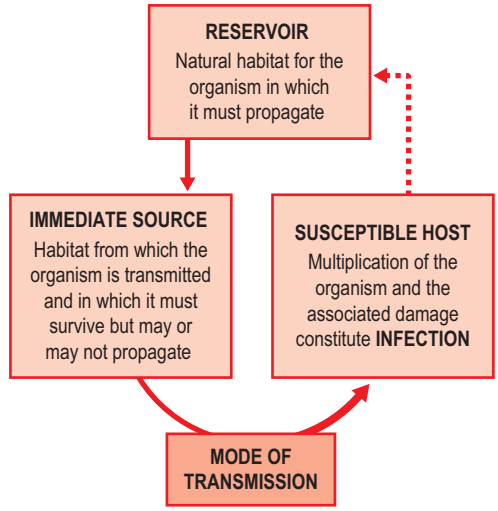

Fig. 1.2 Reservoir, immediate source and mode of transmission in infection.

The mode of transmission can involve: other infected individuals in the case of *contagious* infections; food in the case of foodborne infections; water in waterborne infection; aerosol generation by an infected individual; or contamination of an inanimate object *(fomites)* such as medical equipment or bed linen. The possible sources and modes of transmission of infection are enormous, and new variants are continually being recognized. Engagement of all healthcare workers in recognizing and controlling these hazards is a vital part of medical practice. Fortunately, most infections are transmitted by well recognized pathways (Table 1.1), and these must be clearly understood and learnt.

In the public mind, most infection is seen as contagious, but a large proportion of infections result from *endogenous infection* with a bacterium or fungus that is normally resident in the patient concerned. These resident organisms constitute the *normal microbiota* of the host (the term 'normal flora' cannot be considered appropriate any longer as it denotes plant life and does not reflect the relationships between microbes

Table 1.1 Examples of reservoirs, sources and modes of transmission

Infective disease	Agent of infection	Reservoir	Immediate source	Mode of transmission
Sore throat	*Streptococcus pyogenes*[a] (bacterium)	Human upper respiratory tract	Human upper respiratory tract	Exogenous: airborne droplets
Oral thrush	*Candida albicans* (fungus)	Most human mucosal surfaces	Normal microbiota of oral mucosa	Endogenous: overgrowth in antibiotic-treated or immunocompromised patient
Tetanus	*Clostridium tetani* (bacterium)	Soil or animal intestine	Any environment contaminated with soil or animal faeces	Exogenous: penetrating injury
Syphilis	*Treponema pallidum* (bacterium)	Infected human beings	Patients with genital ulcers or secondary syphilis	Exogenous: sexual contact
Yellow fever[b]	Yellow fever virus (virus)	Monkeys	Usually infected human beings, occasionally monkeys	Exogenous: mosquito-borne
AIDS	Human immunodeficiency virus (virus)	Infected human beings	Usually human blood	Exogenous: mainly blood-borne and by sexual contact
Toxoplasmosis[b]	*Toxoplasma gondii* (protozoon)	Cats	Undercooked meat or contact with areas contaminated by cat faeces	Exogenous: ingestion

[a]One of many causes of sore throat.
[b]Example of a zoonosis.
AIDS, acquired immune deficiency syndrome.

and other organisms). These abundant fellow travellers generally cause infection when they get into the wrong place, often as a result of traumatic wounds (including surgery) or other types of impairment of the host's ability to prevent the spread of organisms to sites where they may cause mischief. Disturbance of the normal microbiota by antibiotics may also allow unaffected *opportunist* pathogens from the endogenous microbiota or the environment to cause infection.

In the case of endogenous infection, the reservoir and source of infection are the same and transmission is unnecessary. When infection comes from an external source it is termed *exogenous infection* and the reservoir reflects the natural habitat of the organism. Where other animals constitute that habitat, the infection is termed a *zoonosis*. In many countries bacteria, protozoa, helminths and viruses are commonly transmitted by insects or other arthropods and the conditions they cause are classified as *vector-borne* diseases.

Study of the ecology and transmission of disease, including infectious disease, is the province of the important public health discipline of *epidemiology*. Important tools include surveillance of the *prevalence* (total cases in a defined population at a particular time) and *incidence* (number of new cases occurring during a defined period) of disease. Knowledge of the ways in which micro-organisms spread and cause disease in communities has produced vital insights that can be used to inform effective control programmes in hospitals and the wider community. Monitoring of the prevalence and incidence of infection on an institutional, local, national or global basis can similarly help in the formulation of policies that reduce the impact of specific infections (monitoring of influenza virus variants to forestall global pandemics is a good example) or of drug-resistant micro-organisms such as those causing malaria, tuberculosis or staphylococcal infections. The World Health Organization and other national or international surveillance agencies carry out much of this important work and deserve full support. For, make no mistake, despite antibiotics, immunization and – for the fortunate – improved living conditions and effective health services, infection will remain the most common cause of sickness and premature death for the foreseeable future.

RECOMMENDED READING

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