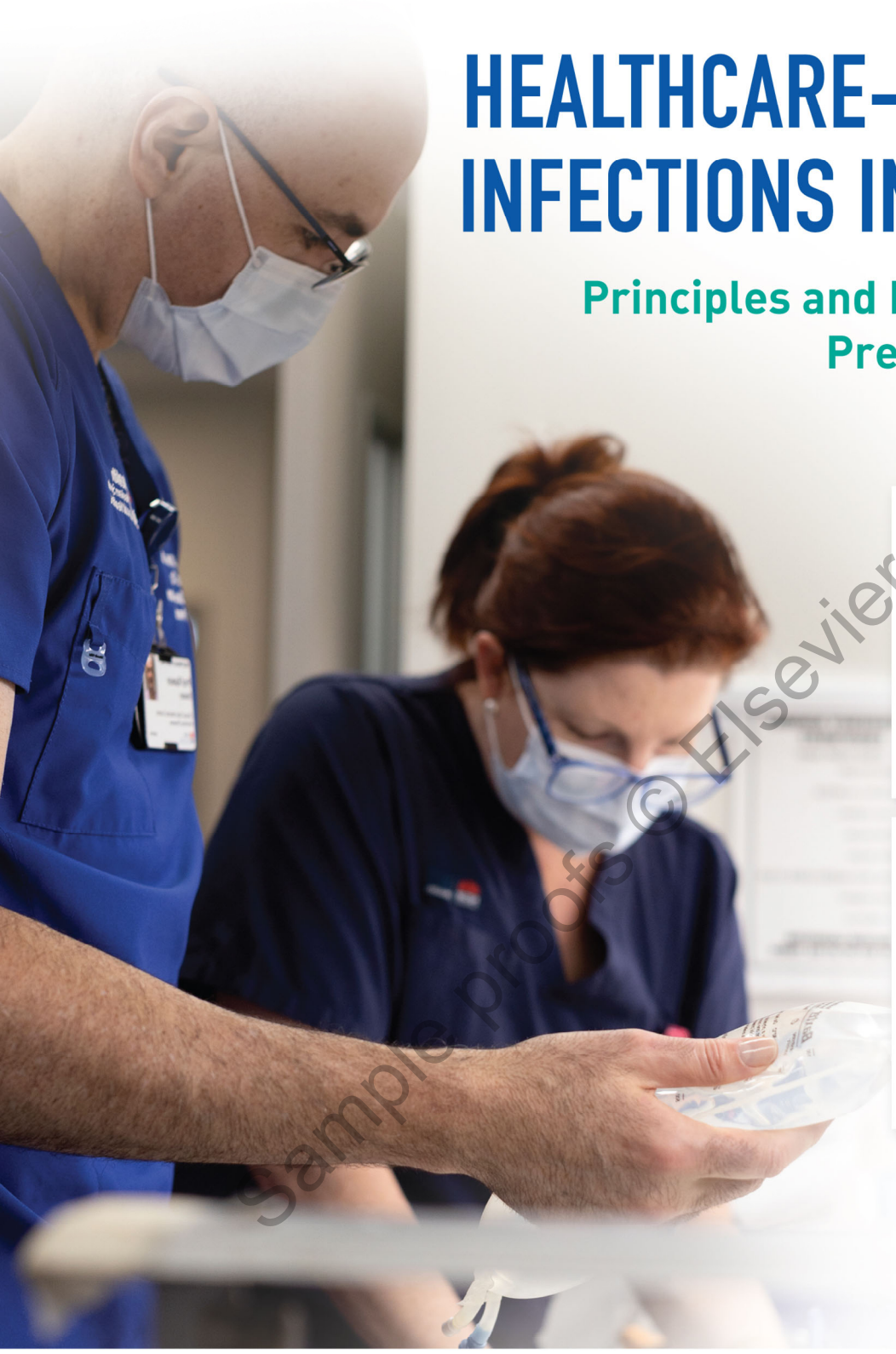
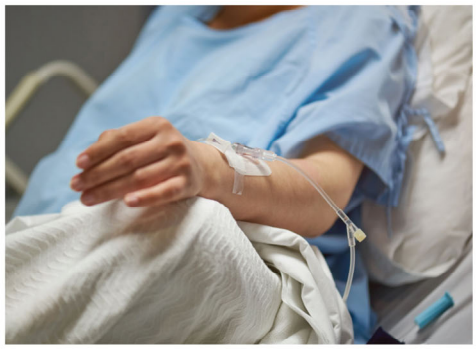
HEALTHCARE-ASSOCIATED INFECTIONS IN AUSTRALIA

Principles and Practice of Infection Prevention and Control





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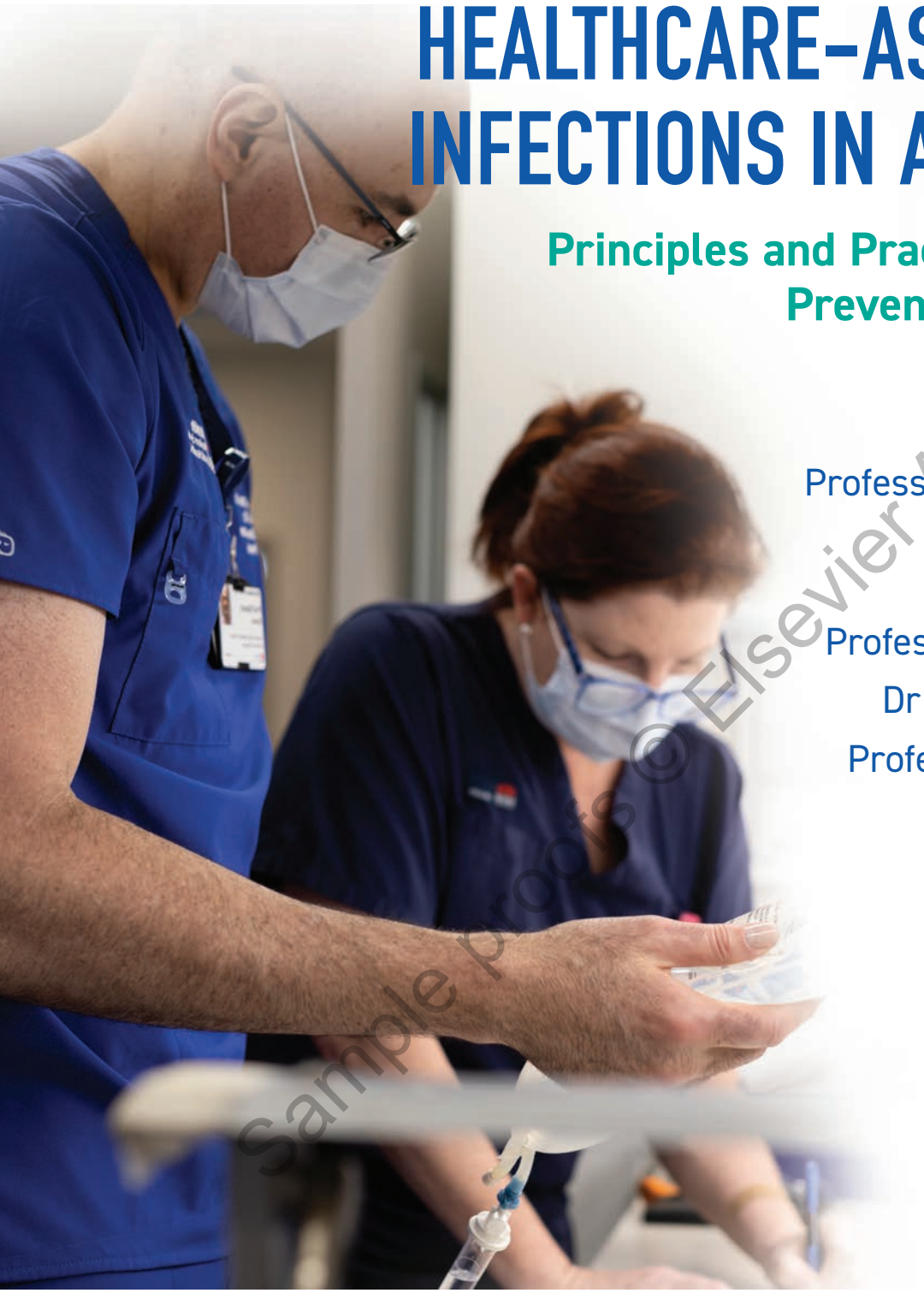

HEALTHCARE-ASSOCIATED INFECTIONS IN AUSTRALIA

Principles and Practice of Infection Prevention and Control

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Contents

Foreword Australasian College for Infection Prevention and Control (ACIPC)

This comprehensive work offers invaluable insights and practical guidance for infection prevention and control within the Australian healthcare system. By promoting knowledge, expertise and best practices, *Healthcare-Associated Infections in Australia: Principles and Practice of Infection Prevention and Control* will contribute to reducing the burden of healthcare-associated infections and enhance patient safety in Australia.

We congratulate and commend the authors of the chapters within this textbook, the first to be edited and authored by infection prevention and control experts and address the unique challenges faced within the Australian context.

We are confident the text will be an invaluable tool for infection control professionals, administrators, educators, academics and students.

We extend our congratulations to the editors of the text, Ramon Shaban, Brett Mitchell, Deborough Macbeth and Philip Russo, and all the contributors to this pivotal text.

Kristie Popkiss
President
Australasian College for Infection Prevention and Control Ltd

Foreword Australasian Society for Infectious Diseases (ASID)

The Australasian Society for Infectious Diseases is delighted to endorse this comprehensive and informative book *Healthcare-associated Infections in Australia: Principles and Practice of Infection Prevention and Control*. It provides a contemporary update on all matters relating to infection prevention and control in the Australian context, a topic of extreme importance as highlighted during the recent COVID-19 pandemic. Topics span from the guiding principles to contemporary practice across multiple healthcare settings including veterinary practice.

Optimal infection prevention and control practices are of utmost importance as we face the impact of global warming on global infectious disease spread and outbreak threats, increased outbreaks of vaccine-preventable diseases such as measles due to declining vaccine uptake and global travel and rising antimicrobial resistance, including pan-resistant organisms.

This book provides a cutting-edge practical toolkit for optimal Infection Prevention and Control in Australia and is highly recommended by society.

Katie Flanagan
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Preface

It is with great pleasure that we present the first edition of *Healthcare-Associated Infections in Australia: Principles and Practice of Infection Prevention and Control*. The release of this book heralds the introduction of the first comprehensive, contemporary, practical and evidence-based Australian text for infection control practitioners, healthcare workers and students to guide their everyday work, practice and study. It is an invaluable resource for the everyday clinician and healthcare professional, containing information that is specifically tailored to a wide range of local practice environments and informed by best-available global evidence.

This text addresses a critical gap in the context-specific academic literature with respect to healthcare-associated infection and the science and practice of IPC in Australia. Whereas our peer professions of infectious diseases, medical microbiology, nursing and others have comprehensive Australian textbooks on their subjects, up until now there has been no such resource on IPC. This notable void has been amplified by the COVID-19 pandemic, which resulted in unparalleled disruption to the health and wellbeing of individuals globally. As new issues related to communicable diseases and IPC continue to emerge and evolve—such as increasing antimicrobial resistance and the dawning of a global 'post antibiotic era'—the need for such a resource became paramount. Here, we thoroughly address this gap in the literature but also prepare professionals and clinicians to respond effectively to the ever-changing landscape of disease and infection control.

Each of the 45 chapters contains information derived from the most recent published research as well as benefitting from the author team's extensive wealth of experience in the practice of IPC in Australia. Our 72 contributing authors are recognised professionals of standing in Australia or New Zealand and all were chosen for their expertise.

The text is organised into two sections. Section 1 comprehensively documents the core principles that underpin contemporary IPC practice in Australia. These chapters include the origins and history of IPC; manifestations of its contemporary construct; IPC programs and plans; the role of the infection control professional; governance and standards; risk assessment and management; and One Health. In addition, their underpinning sciences are discussed, including microbiology and sciences of infection and disease, clinical infectious diseases, epidemiology and surveillance, outbreak management, public health and research for evidence-based practice.

Building on this, Section 2 covers the contemporary practice of IPC in Australia, beginning with comprehensive overviews of core practices, such as Standard and Transmission-based Precautions, followed by an examination of the most relevant contemporary healthcare-associated infections. The final 20 chapters examine contemporary practices and systems in 20 different healthcare contexts in Australia. The context-specific environments and unique risks associated with these are described, and guidance is provided on the specific practices that should feature in their associated infection control management plans.

The chapters are indexed and cross-referenced so that readers can readily integrate relevant sections in ways that mirror what happens in practice, and case studies enable consolidation of knowledge for practice. As with all textbooks and all forms of infection, it is critical that readers continue to search for the most recent sources of appropriate information to guide their practice. To assist with this, useful websites are provided throughout the text.

We commend *Healthcare-Associated Infections in Australia: Principles and Practice of Infection Prevention and Control* to you in support of our shared efforts to prevent and control healthcare-associated infections—the leading complication and challenge to the provision of high-quality, safe and effective care for patients, their families and the community.

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Acknowledgements

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CHAPTER 3

Sciences of infection and disease

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Chapter highlights

- An overview of pathogens important in Australian healthcare, including their structure, taxonomy and methods of transmission
- General strategies of specific groups of pathogens, showing how they cause disease in human and animal hosts
- Details of how the host immune system works against pathogens and therapies that assist in the treatment and prevention of infectious disease
- A summary of common assays used in the diagnosis of pathogens, and the therapies associated with their identification and management

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Introduction

COVID-19 has reminded us that pathogens are devastating and have significant individual, societal and population implications for human health and wellbeing. Pathogens that affect humans are diverse and can be cellular microorganisms, biological particles or multicellular parasites; however, the vast majority are harmless and are fundamental to environmental, animal and human health. In humans, normal microbial flora play integral roles in maintaining health although they may cause disease in circumstances where the usual defence mechanisms are disrupted or where there is a disruption in the normal microbial flora. For example, immunosuppressive drugs, such as steroids or antimicrobial therapeutics, can result in opportunistic infections from natural microbiota such as the yeast *Candida albicans*, causing thrush.

In Australia, pathogens that are significant to human health are formally identified and managed in accordance with a range of public health legislation and regulation. A notifiable disease is one that must be reported to state and territory health departments. Table 3.1 lists notifiable infections in Australia from 2009–19, not including infections from COVID-19 which emerged in late 2019. Over this time, the most prevalent infections were influenza, *Chlamydia* and campylobacteriosis.

The epidemiology of infections varies according to local and regional, environmental and population, and societal factors. For example, the warmer conditions of Australia's tropical north predispose it to mosquito-borne infections such as malaria and dengue. Aboriginal and Torres Strait Island peoples experience disproportionately higher rates of infection and disease than the non-Indigenous population due to inequities such as poverty derived from centuries of systemic privilege and racism.[1]

As noted in Table 3.1, the majority of notifiable infections in Australia are bacterial and viral, and reflect their importance in human health. These pathogens infect via a number of routes that include the gastrointestinal tract, respiratory system, sexual transmission, contact with animals and humans, and bites from insects and blood (e.g. sharing needles) (see 3.4 Transmission of pathogens). The epidemiology of these and other infections is influenced by a range of complex, interconnected factors that span prevention, such as vaccine reluctance, to control, such as modifying risk-based behaviours, to treatment, including empiric and targeted therapeutics.

3.1 Pathogenic microorganisms

Generally speaking, microorganisms are unicellular cells that are too small to be seen by the naked eye and whose visualisation requires the use of a microscope. Although organisms such as fleas and mites are small, these are multicellular organisms that contain cells as part of tissues and detailed body plans and are not defined as microorganisms. In this chapter we will briefly cover some of these multicellular parasites, due to their clinical importance.

Pathogenic microorganisms can be prokaryotic or eukaryotic and are defined as 'living' as they are capable of self-replication if provided with appropriate nutrients. Prokaryotic cells are simpler than eukaryotic cells and differ in a variety of ways including their size and sub-cellular structures (Fig 3.1). Typically, eukaryotic cells are about five to ten times larger than prokaryotic cells and contain a variety of membrane-bound structures called organelles, with the nucleus containing the DNA being most notable. As we will learn later, differences in sub-cellular structures between these cell types are important in the effectiveness of chemotherapeutics used to treat bacterial infections. Prokaryotic cells include bacteria and archaea; however, as there are no known human or animal archaeal pathogens, these will not be discussed here. In the context of eukaryotic cells, fungi and protozoa cause infections in humans and animals.

3.1.1 Prokaryotic cellular pathogens

Many of the important pathogens affecting humans, particularly in clinical environments, are bacterial. These microorganisms are diverse in structure with bacillus (rod-shaped) and coccus (sphere-shaped) shapes being the most common. Most bacteria can be subdivided into Gram negative or Gram positive on the basis of a staining procedure called the Gram stain that differentiates bacterial cells according to their envelope structure and which has important historical roots in diagnostics (Fig 3.2). Gram-positive cells stain purple due to their thick cell wall (20–80 nm) made of a polymer called peptidoglycan positioned outside of the cytoplasmic membrane, whereas Gram-negative cells stain pink due to a thin peptidoglycan cell wall (2–7 nm) in between the cytoplasmic and outer membranes. Some bacterial pathogens lack a cell wall, including *Mycoplasma* and *Chlamydia* species. *Mycobacteria* species, which includes the bacterium responsible for tuberculosis, contains a modified peptidoglycan cell

TABLE 3.1 Main notifiable infectious diseases affecting Australians from 2009–19

Disease	Main route of infection	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Average
All		239,475	214,260	242,391	249,127	230,067	278,891	338,372	345,076	519,103	328,762	593,055	325,325
Bacterial													
Campylobacteriosis	Gastrointestinal	16,106	16,994	17,723	15,704	14,689	19,938	22,551	24,241	28,698	32,133	35,869	22,241
Listeriosis	Gastrointestinal	92	71	70	93	76	80	70	85	71	73	52	76
Paratyphoid	Gastrointestinal	61	86	69	77	74	70	76	79	68	80	117	78
Salmonellosis	Gastrointestinal	9,400	11,777	12,166	11,120	12,667	16,191	16,886	18,014	16,376	14,149	14,682	13,948
Shigellosis	Gastrointestinal	617	552	493	548	537	1,035	1,037	1,410	1,748	2,508	3,157	1,240
STEC	Gastrointestinal	128	80	95	112	180	115	136	341	497	562	655	264
Typhoid fever	Gastrointestinal	115	96	135	122	150	117	114	104	144	176	202	134
Legionellosis	Respiratory	297	307	356	382	507	425	364	368	387	449	431	388
Meningococcal disease (invasive)	Respiratory	256	226	241	223	147	167	181	252	380	281	206	233
Tuberculosis	Respiratory	1,307	1,362	1,386	1,315	1,262	1,339	1,249	1,362	1,436	1,436	1,520	1,361
Chlamydial infection	STI	63,193	74,367	81,081	83,238	83,837	86,818	86,420	94,627	101,244	104,796	102,514	87,467
Gonococcal infection	STI	8,269	10,321	12,087	13,963	15,062	15,692	18,478	23,874	28,371	30,889	34,317	19,211
Syphilis	STI	1,285	1,097	1,243	1,532	1,763	2,063	2,791	3,376	4,415	5,080	5,795	2,767
Haemophilus influenzae type b*	Respiratory	19	23	13	16	20	21	16	17	16	18	22	18
Pertussis*	Respiratory	30,185	34,832	38,752	24,093	12,376	11,892	22,572	20,120	12,236	12,583	12,024	21,060
Pneumococcal disease (invasive)*	Respiratory	1,554	1,640	1,883	1,824	1,553	1,563	1,498	1,664	2,050	2,031	2,131	1,763
Brucellosis	Zoonosis	32	21	37	31	14	17	19	18	19	28	9	22
Leptospirosis	Zoonosis	141	133	214	105	85	84	72	130	146	142	85	122
Ornithosis	Zoonosis	63	58	89	76	47	41	16	22	21	9	21	42
Q fever	Zoonosis	317	338	359	371	491	474	605	560	478	513	563	461

Data sourced from: Infectious and communicable diseases – Australian Institute of Health and Welfare (aihw.gov.au).

Viral													
Hepatitis B (newly acquired)	Blood-borne	248	231	192	194	176	180	150	176	146	137	163	181
Hepatitis C (newly acquired)	Blood-borne	400	384	620	705	668	710	820	738	620	615	787	642
Hepatitis D	Blood-borne	65	52	53	47	73	66	49	71	65	79	70	63
Hepatitis A	Gastrointestinal	564	267	145	166	190	231	179	145	217	434	246	253
Hepatitis E	Gastrointestinal	33	37	41	32	34	58	41	43	48	39	52	42
Barmah Forest virus infection	Vectorborne	1,472	1,465	1,865	1,728	4,236	741	628	329	448	343	255	1,228
Dengue virus infection	Vectorborne	1,404	1,228	821	1,539	1,838	1,721	1,714	2,238	1,136	932	1,462	1,458
Flavivirus infection (unspecified)	Vectorborne	4	14	13	6	16	20	11	116	17	8	11	21
Ross River virus infection	Vectorborne	4,739	5,120	5,129	4,678	4,312	5,310	9,544	3,734	6,930	3,139	2,961	5,054
Influenza (laboratory confirmed)*	Respiratory	59,040	13,457	27,213	44,539	28,303	67,686	100,583	90,884	251,290	58,869	313,428	95,936
Measles*	Respiratory	104	70	194	199	158	339	74	99	81	103	285	155
Mumps*	Respiratory	166	97	153	201	217	186	645	804	812	634	171	371
Rotavirus*	Gastrointestinal	1,820	3,918	3,331	3,797	3,208	3,022	4,136	2,733	7,266	3,163	6,173	3,870
Rubella*	Respiratory	27	44	58	35	25	16	17	17	10	9	22	25
Varicella zoster (chicken pox)*	Direct touch	1,798	1,797	2,103	1,991	2,134	2,112	2,489	3,030	3,172	4,600	4,386	2,692
Protozoal													
Cryptosporidiosis	Gastrointestinal	4,624	1,482	1,811	3,142	3,852	2,408	4,064	5,421	4,695	3,011	2,678	3,381
Malaria	Vectorborne	504	404	419	344	423	325	234	305	364	408	379	374

STI: sexually transmitted infection;: vaccine-preventable disease.*
Data sourced from: Infectious and communicable diseases. Australian Institute of Health and Welfare. Available: https://aihw.gov.au. 1 Sep 2022.

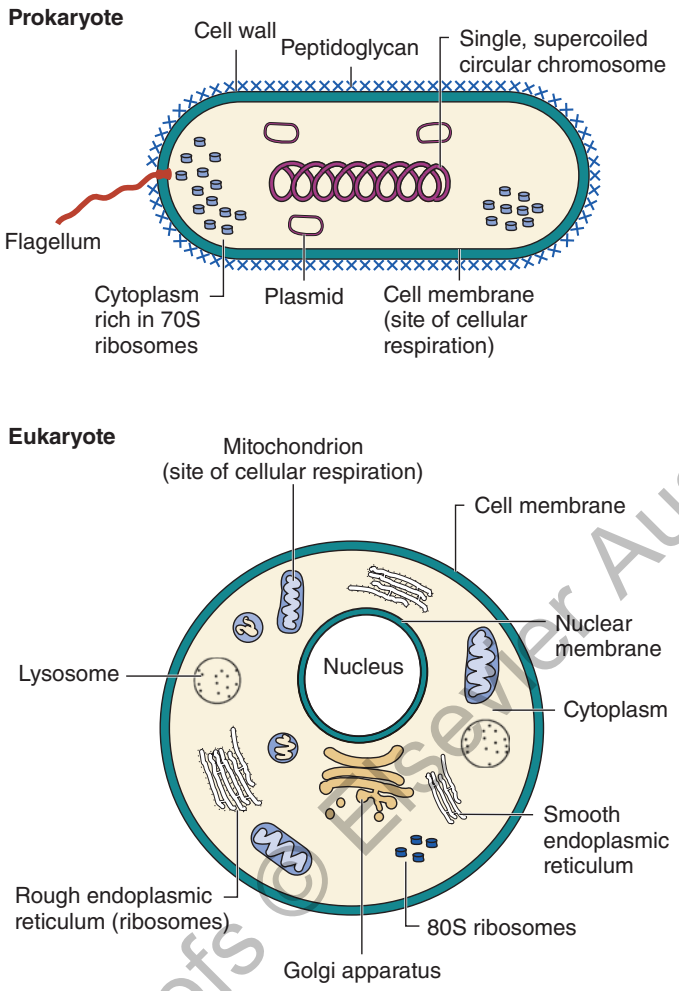


FIGURE 3.1 Prokaryotic and eukaryotic cellular structure. Generally, prokaryotes are 0.5–2 μm in size consisting of a rigid peptidoglycan cell wall and membrane-enclosed cytoplasm containing cellular DNA and other sub-cellular structures including chromosomal DNA, 70S ribosomes and sometimes a plasmid(s) of which the functions are summarised in Table 3.2. Eukaryotic cells are approximately 5–10x larger than prokaryotic cells and more complex containing a membrane-bound nucleus where the DNA resides, 80S ribosomes and diverse organelles that fulfil important cellular roles (see text for details)

Source: George, A., & Charleman, J. E. (2017). Elsevier's Surgical Technology Exam Review. Elsevier.

wall with mycolic acids covalently bound, giving the cell surface a waxy finish.

In addition to the cell wall, bacteria are differentiated from eukaryotic cells with their ribosomes being smaller (i.e. 70S versus 80S) and having a different composition of proteins and RNA. They contain a variety of cellular structures that are important in virulence, some of which are summarised in Table 3.2 and further discussed in Section 3.5 Host–pathogen interactions. Of note is the ability of some bacterial pathogens to form spores, dormant structures that are highly resistant to general methods of pathogen control, such as chemical disinfection. They can survive for long periods of time without nutrition and have the ability to convert back into a growing state when conditions improve. Some pathogens, such as *Bacillus anthracis* (anthrax), *Clostridium tetani* (tetanus) and *Clostridium botulinum* (botulism), are spore formers. For example, *C. botulinum* spores are widely found in soil and water and, if ingested or enter a wound, produce a toxin that causes muscle paralysis and possibly death. On the positive side, botulinum toxin has been repurposed in multiple medical procedures, including

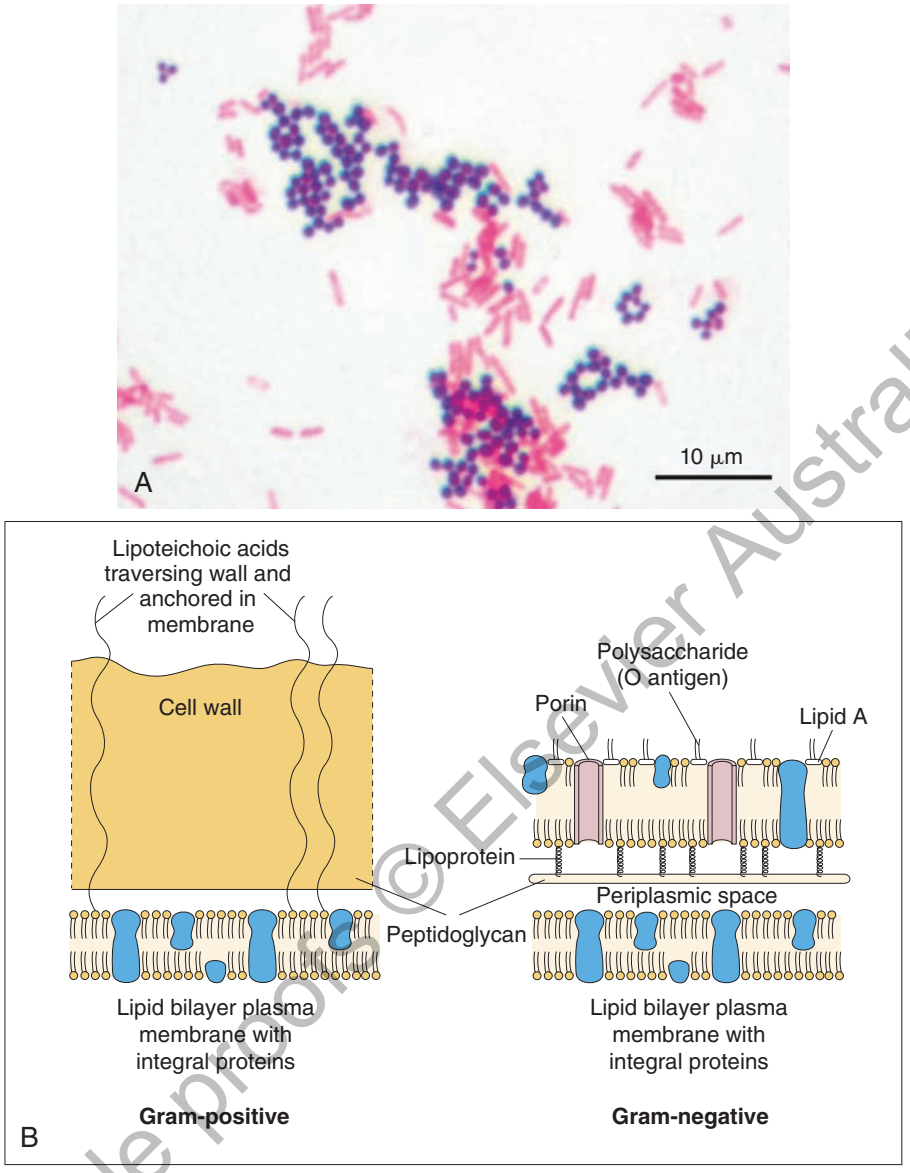


FIGURE 3.2a and b Gram stain of a mixture of the Gram-negative bacillus *Escherichia coli* and the Gram-positive coccus *Staphylococcus aureus* staining pink and purple respectively: (a) Envelope structure of Gram-positive cells (left) and Gram-negative cells (right). Gram-positive cells contain a cytoplasmic membrane (plasma membrane) and a thick cell wall with teichoic acids and lipoteichoic acids sometimes anchored in the cell wall and cytoplasmic membrane respectively (b). Gram-negative cells contain a cytoplasmic membrane, an outer membrane and a periplasmic space in between where a thin cell wall resides. The outer membrane in Gram-negative bacteria is the site for LPS

Source: (a) Godbey, W. T. (2022). Biotechnology and its applications: using cells to change the world. Elsevier. (b) Bassert, J. M. (2022). McCurnin's Clinical Textbook for Veterinary Technicians and Nurses. Elsevier.

controlling migraines, excessive sweating, muscle spasticity and in cosmetic procedures (i.e Botox). To avoid foodborne botulism, the canned vegetable food industry must take precautions, including high temperature treatment, to ensure the destruction of *C. botulinum* spores. Additionally, there are concerns regarding the weaponisation of bacterial spores, such as in the 2001 Anthrax attacks in the United States of America where a series of letters were sent containing anthrax spores in the form of a powder resulting in five deaths.

TABLE 3.2 Sub-cellular structures in bacteria important for survival and virulence

Structure	Details	Function(s)
Membrane and surface-associated structures		
Fimbriae	Short hair-like appendage extending from the cell surface	• Adhesion to surfaces
Pilus	Short appendage extending from the cell surface	• Adhesion to surfaces • Surface twitching motility • DNA transfer (conjugation)
Flagellum	Thread-like appendage extending from the cell surface	• Swimming motility
Capsule/ slime layer	Made up of polymeric substances such as polysaccharide and found on the cell surface. A capsule is tightly adhered to the cell surface, whereas slime layers are easily washed off	• Adhesion and biofilm formation to surfaces • Resists desiccation and phagocytosis by immune cells
Lipopolysaccharide (LPS)	Found only in Gram-negative bacteria, LPS extends from the cell surface. Consists of a lipid portion (lipid A) embedded in the outer membrane connected to a variable polysaccharide component called the O antigen	• Adhesion to surfaces • Creates a permeability barrier • Lipid A portion is highly inflammatory in bloodstream. Also called endotoxin
Teichoic acids	Found only in some Gram-positive bacteria, teichoic acids are a phosphorylated polyalcohol present in the cell wall	• Adhesion to surfaces
Cell wall	Rigid peptidoglycan structure positioned outside the bacterial plasma membrane	• Provides the cell with integrity and protects from osmotic stress
Internal structures		
Plasmid	Small, circular DNA that replicates independently of the bacterial chromosome	• Can move between bacteria and transfer genetic traits such as antibiotic resistance and virulence genes
Spores	Highly resistant dormant structure produced by some Gram-positive bacteria	• Highly resistant to environmental stress. Hard to destroy

3.1.2 Eukaryotic cellular pathogens

Eukaryotic microorganisms are more complex than prokaryotic microorganisms and are therefore substantially more structurally diverse. Figure 3.1 illustrates the general characteristics of a eukaryotic cell shown with a series of labelled organelles holding important cellular roles. Mitochondria are involved in energy (i.e. adenosine triphosphate [ATP]) production. The endoplasmic reticulum (ER) are flattened, enclosed membrane sacs that are involved in the synthesis and modification of multiple cellular products such as lipids and proteins. Rough ER is so named due to its appearance in having ribosomes attached to it and is therefore important in protein and glycoprotein synthesis, whereas smooth ER is important for the synthesis of lipids, phospholipids and steroids. The golgi apparatus transports ER-produced products and the lysosome contains enzymes that digest proteins, fats and polysaccharide for reuse. In addition, eukaryotic microorganisms can harbour flagella and cilia that are involved in motility. Compared to flagella, cilia are much shorter and tend to coat the cell and move synchronously.

Of the eukaryotic microorganisms, the members of fungi and protozoa are human pathogens. Microscopically, fungi can appear as unicellular cells called yeast, or as moulds that are long, branched filaments of cells called hyphae forming a mass called mycelium (Fig 3.3). Fungi may also produce spores that aid in dispersion and are organised into elaborate structures that are useful in diagnostic microscopy. Fungi possess cell walls that are usually made of chitin although some may produce cell walls of other polysaccharides, such as cellulose. Importantly, some fungi are dimorphic and can morph between a mould and yeast form, including pathogens such

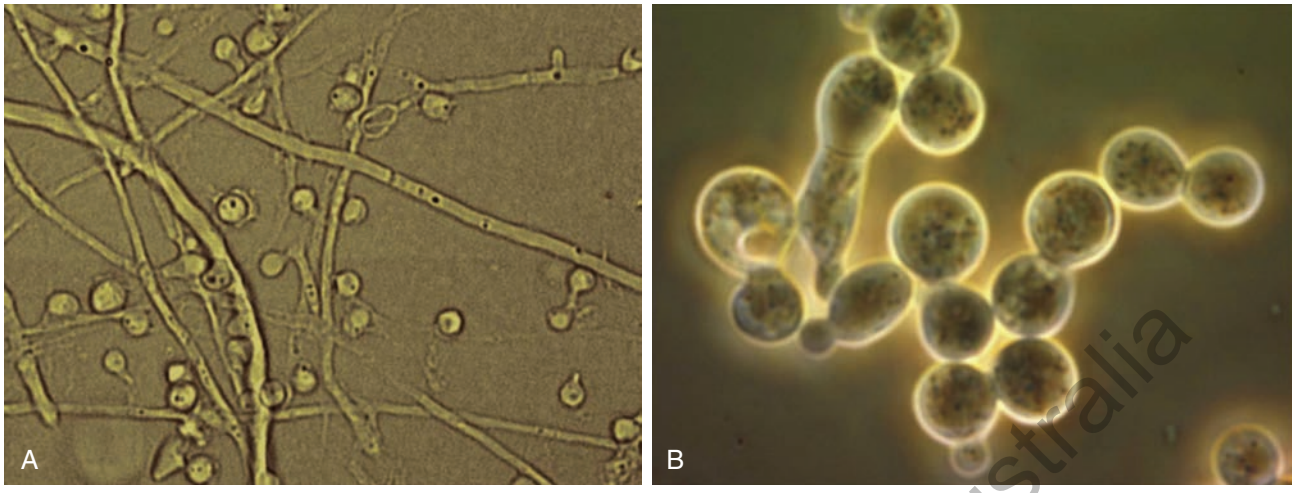


FIGURE 3.3 Microscopic images of *Blastomyces dermatitidis* in mould (a) and yeast (b) forms. As a mould, long cells called hyphae are observed. Sacs called conidia containing spores branching from the hyphae. In yeast form, cells are thick-walled and oval-shaped

Source: Images courtesy of Bruce Klein, University of Wisconsin.

as *Blastomyces dermatitidis* that convert to a yeast form at a human body temperature of 37°C (Fig 3.3).

Pathogenic protozoa are responsible for some important human diseases, and these include malaria and trypanosomiasis caused by species of the *Plasmodium* and *Trypanosoma* genera respectively. Protozoa are unicellular eukaryotes that feed on organic material and in this state they are called trophozoites (Fig 3.4a). Many protozoa are capable of encystment, a process where the trophozoite becomes simpler and converts into a cyst that is marked by a low metabolic rate and the formation of a cell wall (Fig 3.4b). Cysts are more resistant to environmental stress and in pathogenic protozoa are important in transmission between human and animal hosts. Protozoa covert back into a trophozoite state in favourable conditions in a process called excystment.

3.1.3 Horizontal gene transfer in bacteria

An important behavioural feature of bacteria is their ability to share DNA in a process called horizontal gene transfer (HGT) which has been pivotal in the emergence of many problematic pathogens and antibiotic-resistant pathogens.[2] There are three mechanisms of HGT: i) conjugation; ii) transformation; and iii) transduction (Fig 3.5). Conjugation requires cell-to-cell contact and through a pilus, DNA (usually a plasmid) is transferred. Transformation is the uptake of naked DNA and requires the bacterium to change physiology and become competent, a state where DNA uptake is possible. Transduction is DNA transfer from one bacterial cell to another with a bacterial virus (bacteriophage) being the intermediate.

Except for plasmids which can self-replicate, transferred DNA must integrate into the chromosome or a residing plasmid in order to be replicated and be passed on to progeny during cell division. There are multiple ways by which transferred DNA may integrate that go beyond the scope of this chapter; however, a common method is homologous recombination where a natural cellular process recombines DNA with high homology.

All mechanisms of HGT are common and there are numerous examples of each driving the evolution of pathogens and antibiotic resistance. For example, *Escherichia coli* are natural inhabitants of the gastrointestinal tract of humans and animals; however, some are capable of causing diarrhoeal disease and urinary tract infections of which horizontally acquired genes are often identified as being involved in disease.[3] Important to infection prevention and control is antibiotic resistance and many bacterial pathogens show evidence of horizontally receiving plasmids and other genetic elements that provide them with resistance to multiple antibiotics.[4]

3.1.4 Koch's postulates

Traditionally, infectious diseases were investigated as a single pathogen causing disease and formed the basis for Koch's postulates, a series of logical truths designed by the microbiologist Robert Koch that are applied to

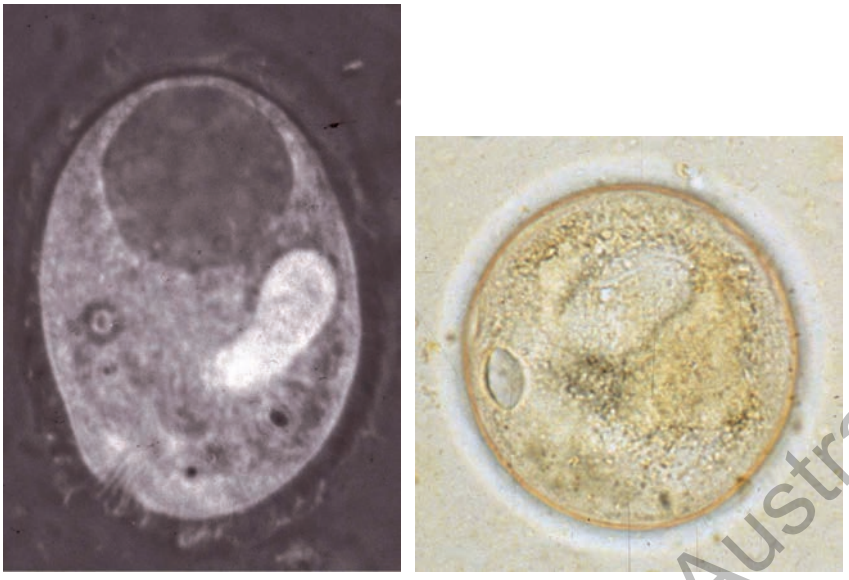


FIGURE 3.4 Parasitic intestinal pathogen *Balantidium coli* in trophozoite (left) and cyst (right) form. Infection occurs through oral consumption of cysts that excyst into trophozoites. Trophozoites multiply in the intestine and are passed out in faeces where they encyst as the faeces dry out

Source: Sirois, M. (2020). Laboratory Procedures for Veterinary Technicians. Elsevier.

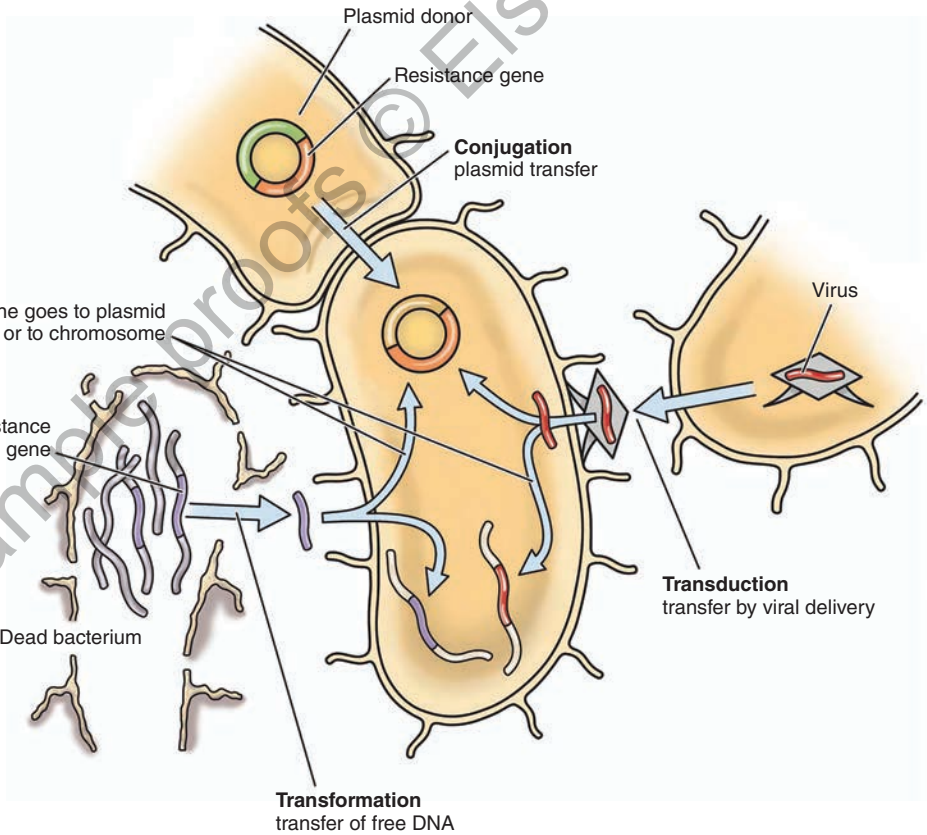


FIGURE 3.5 Horizontal gene transfer in bacteria can occur through transformation, conjugation or transduction. Transformation is the uptake of free DNA sources from a lysed bacterium. Conjugation requires cell-to-cell contact and the transfer of DNA (usually a plasmid) through a pilus. Transduction is transfer of DNA from one bacterial cell to another through a bacterial virus (bacteriophage) intermediate

Source: Zachary, J. F., & McGavin, M. D. (Eds.). (2012). Pathologic Basis of Veterinary Disease. Elsevier.

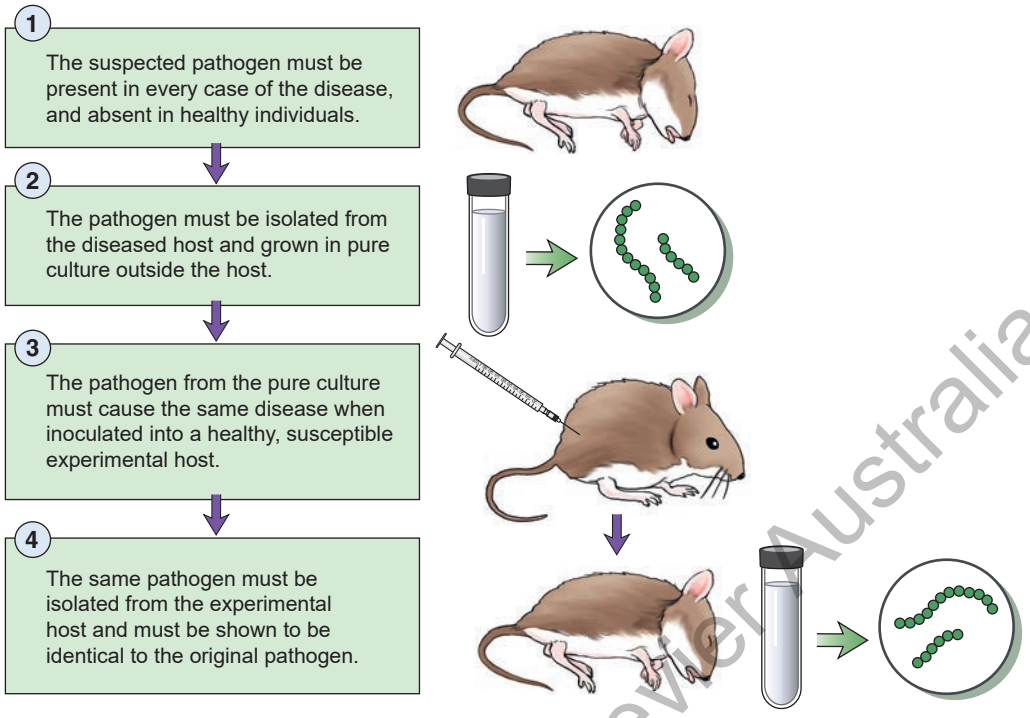


FIGURE 3.6 Koch's postulates used for proving a single pathogen is responsible for an infectious disease
Source: VanMeter, K. C., Hubert, R. J. & VanMeter, W. (2010). Microbiology for the healthcare professional. Elsevier.

prove that a single pathogen is responsible for a single disease (Fig 3.6). Koch's postulates require that a pathogen be culturable and that a host model system be available for infection experiments, and where this is not possible, the postulates cannot be applied. Nevertheless, causality can be proved with a variety of techniques that allow tracking the pathogen within the infected human host without the need for cultivating the pathogen or infecting a host model. Additionally, some diseases can be complex and go beyond a single pathogen being responsible for a disease, making it difficult to apply Koch's postulates. The human immunodeficiency virus (HIV) is a pathogen that diminishes host immunity, but disease is often caused by secondary infection with other pathogens. Another example is dental diseases that are caused by complex microbial communities adhered to the teeth and gums. Other diseases are opportunistic and only infect when the host conditions change, such as a disruption of the host microbiome (see 3.1.5 Microbiomes) or host immune system. Due to these complexities, research increasingly examines how host microbiome and immunity changes affect susceptibility to, or the severity of, an infectious disease.

3.1.5 Microbiomes

Microorganisms are found in almost every niche on Earth and play essential roles in global nutrient cycling. They are involved in numerous interactions that support the growth and health of higher organisms. As a collective, these microorganisms are known as the microbiome. Microorganisms are naturally found in most parts of the human body including skin, gastrointestinal tract, nose, mouth and vagina, and combined these are referred to as the 'human microbiome'. If referring to the microorganisms associated with a part of the body, then we refer to them as the 'human gut microbiome' or 'human skin microbiome'. The human microbiome is estimated to hold about 100 times more genes than those in the human genome, with research supporting important roles in digestion of nutrients, synthesis of vitamins, protection from pathogens, and immune stimulation and regulation.[5] If we consider the colon, the number of bacteria can number 10^{11}–10^{12} per gram of contents, and imbalances in microbiome composition are linked to a variety of gastrointestinal conditions that include inflammatory bowel syndrome and inflammatory bowel disease. Identifying the exact mechanism(s) by which the gut microbiome might influence disease is complex and remains elusive. Studies into microbiome composition largely utilise DNA-based techniques that require extraction of DNA from an environment (e.g. faeces) and this is called metagenomic DNA. Metagenomic DNA is then subjected to other DNA sequencing techniques that identify the

composition of microorganisms or functional microbial genes in the sample. Comparisons between healthy and diseased samples help identify those microorganisms or genes that correlate with disease; however, there is substantial inter-individual variation that can make identifying trends tricky.

3.1.6 Taxonomy of microorganisms

Taxonomy is the science of classifying and naming organisms using defined criteria. Microorganisms in a classification group will share more characteristics than those outside of the group, and so classifying a microorganism provides the advantage of ascribing what we know about a microorganism in a group to others within that group. In this way, decisions can be made about a microorganism whether it be prescribing appropriate treatment for an infection or protecting Australia's agriculture industry through quarantine procedures.

Historically, phenotypic characteristics such as cellular morphology and metabolism were used in classification and are still useful today in pathogen diagnostics; however, genetic-based methods comparing the sequence of a taxonomic marker gene(s) or the entire microbial genome are becoming commonplace and have the benefit of reflecting the phylogenetic relationships that microorganisms have with one another. A useful gene in taxonomy and phylogeny is the single subunit ribosomal RNA (SSU rRNA) gene that is found in all cells and encodes an RNA molecule required for the protein synthesis function of ribosomes. Based on SSU RNA sequences, all cells can be classified into three domains that include the prokaryotes Archaea and Bacteria, and a third domain called Eukarya which is made up of the eukaryotes (Fig 3.7).

Below 'domain' are further subclassifications, and the microorganisms grouped into these will progressively share more characteristics. The names given to microorganisms (and all organisms) follow the binomial naming system developed by Carolus Linnaeus where the genus and species classifications are used to derive the name. For example, *Escherichia coli* is in the *Escherichia* genus and *coli* species groups (Fig 3.8). What defines a species is sometimes controversial and there is much discussion and debate about how best to classify a microorganism into a species. In general, a 2–3% divergence in the SSU rRNA sequence separates species. An additional subclassification below 'species' is 'strain' and this describes microorganisms that are in the same species group but have a different phenotypic characteristic(s). These differences are often derived from HGT events (see 3.1.3 Horizontal gene transfer in bacteria) and so the genomes of microorganisms within a species group are said to contain a core genome and a pan genome. A core genome are all those genes that are common among all strains within a species, whereas a pan genome are those genes that are uncommon and are often derived from HGT.

3.2 Pathogenic biological particles

In addition to cellular pathogens there are pathogenic biological particles which are incapable of self-replication.

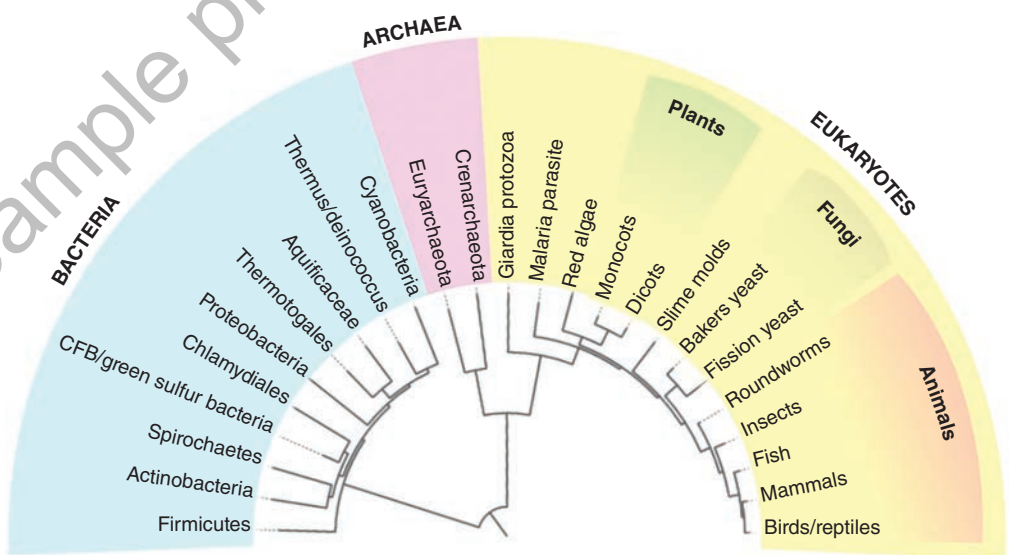


FIGURE 3.7 Tree of life derived from the SSU rRNA sequence of all cells showing three clades that classify all into the three domains of Bacteria, Archaea and Eukarya

Soltis, D., & Soltis, P. (2019). The great tree of life. Academic Press.

Domain	Bacteria	Archaea	Eukarya
Kingdom	Monera		
Phylum	Proteobacteria		
Class	Gammaproteobacteria		
Order	Enterobacteriales		
Family	Enterobacteriaceae		
Genus	*Escherichia*		
Species	*Escherichia coli*		

FIGURE 3.8 The binomial system developed by Carolus Linnaeus is used to name organisms including microorganisms. On the left are the various classifications and the names of the genus and species groups are used to derive the name of the organisms which in this instance is *Escherichia coli*

Source: VanMeter, K. C., & Hubert, R. J. (2022). Microbiology for the Healthcare Professional. Elsevier.

They are intracellular obligate parasites that must infect a living cell and use the host's cellular machinery for their reproduction. Human pathogenic biological particles include viruses and prions. Viruses are defined as nucleic acid (DNA or RNA) enclosed within a protein coat, although some may also contain a membrane envelope. Prions are infective protein particles.

3.2.1 Viruses

Viruses are important pathogens responsible for devastating pandemics in humans including influenza virus causing flu, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing COVID-19, and HIV causing acquired immune deficiency syndrome (AIDS). Viruses are very small and range from approximately 20–400 nm in size with a single viral particle known as a virion. A virion, at minimum, consists of a nucleocapsid which is nucleic acid surrounded by a protein coat (Fig 3.9). The capsid acts to protect the nucleic acid although it may also aid in adhesion to a host cell. Capsid structures are mostly icosahedral or helical, with other shapes known as 'complex'.

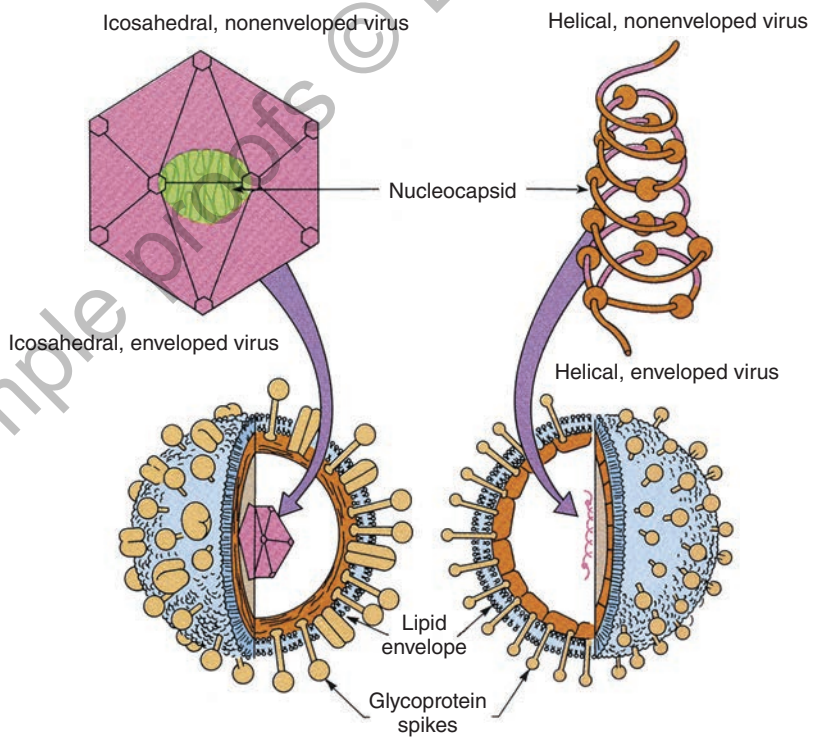


FIGURE 3.9 Viral particles. The capsids of both enveloped and non-enveloped virions mostly have an icosahedral or helical shape and act to protect the nucleic acid. Many eukaryotic viruses are enveloped with the membrane derived from the host cell nuclear or plasma membrane during infection. The envelope contains glycoprotein spikes (sometimes called peplomers) that are important for adhesion to the host cell which initiates the infectious process

Source: Tille, P. (2022). Bailey & Scott's diagnostic microbiology. Elsevier.

Many eukaryotic viruses contain a membrane envelope surrounding the nucleocapsid which is acquired from the host nuclear or plasma membrane during infection. The viral protein contains proteins called spikes or peplomers that are required for host cell adhesion, a critical step in infection (see 3.5.4 Viral pathogens). The viral nucleic acid may be double- or single-stranded DNA (ssDNA or dsDNA) or RNA (ssRNA or dsRNA) and will encode genes that are required for host cell infection and for virion replication and synthesis.

Taxonomy of viruses is mostly based on virion structure, type of nucleic acid, mode of replication, host cell range and the kind of disease it causes. Figure 3.10 shows the range of viral families that causes disease in humans and animals, demonstrating structural and nucleic acid diversity.

3.2.2 Prions

Prions are infective proteins that are linked to a series of neurodegenerative disorders including kuru and variant Creutzfeldt-Jakob (vCJD) in humans, and bovine spongiform encephalopathy (BSE) and scrapie in sheep.[6] These diseases are collectively known as transmissible spongiform encephalopathies (TSEs) due to their effect on the brain resulting in a spongy appearance when brain slices are observed histologically. Prions are spread through consumption of contaminated

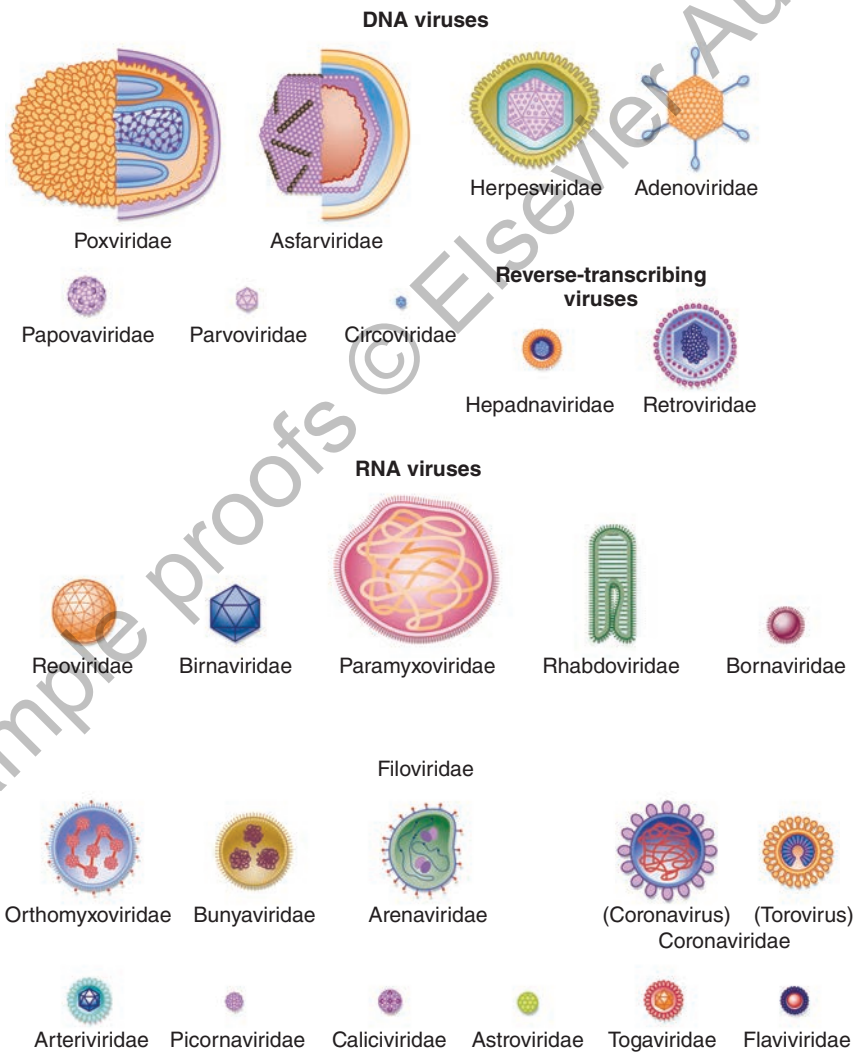


FIGURE 3.10 Shapes and sizes of viral families that infect humans and animals. In this figure, virions are grouped according to their nucleic acid type and in some, a cross-section of capsid and envelope is shown. Artistic licence has been used in representing viral structures

Source: Cann, A. J. (2016). Principles of molecular virology. Academic press.

meat containing the infectious protein that is thought to force a conformational change of a normal protein in brain cells, presumably affecting its normal function. vCJD emerged in the 1990s and was linked to the consumption of beef sourced from cattle suffering from BSE or 'mad cow disease' as it was colloquially known. Kuru affected specific tribes in Papua New Guinea due to a cannibalistic ritual where tribe members would consume body parts of a deceased family member. TSE pathology usually takes years to develop post exposure. There are no effective therapies and prions are highly resistant to standard methods of infection control.

3.3 Multicellular parasites

Beyond unicellular pathogens and biological particles that detrimentally affect humans are multicellular parasites that include helminths (worms) and ectoparasites, which are arthropods living on the surface of the human body.

3.3.1 Helminths

Worm infections are common in parts of the world where access to clean drinking water is limited and where there is poor sanitation. They tend to cause intestinal infestations leading to gut symptoms such as diarrhoea; however, some can migrate to other parts of the body causing more serious complications. Although worm infections are rarely fatal, a high worm load in the intestine can deprive a person of substantial nutrition and in a child can lead to growth and mental retardation. On the other hand, *Schistosoma* is a flatworm that kills approximately 200,000 people per year, with 230 million requiring treatment. Long periods of infection by this worm lead to severe inflammatory immune reactions in organs where eggs have been deposited. In Australia, worm infections are rare; however, remote Indigenous communities are particularly affected by a worm infection called *Strongyloidiasis* where it migrates to the lungs and then the gut, causing a cough or wheeze and a variety of gut-related symptoms such as abdominal pain and diarrhoea. Additionally, hookworm is prevalent in Australian Indigenous communities.

There are three types of helminths that cause disease in humans, namely cestodes (tapeworms), trematodes (flukes) and nematodes (roundworms) (Fig 3.11). Table 3.3 provides a list of worm-related infections in humans. Tapeworms are segmented containing a head (celled scolex) that is used to latch onto the host's intestinal wall and then consists of segments called proglottids that grow from the neck of the head. The proglottids contain male and female gonads that produce thousands of eggs that are shed into faeces as mature proglottids. Tapeworms have no digestive tracts and rely on absorption of nutrients from the host intestine. Flukes are non-segmented flattened worms that have an incomplete digestive system consisting of a mouth and a digestive tract but no anus. They are muscular suckers which allows attachment to tissue. Nematodes are non-segmented cylindrical worms with tapered ends. They are coated in a chitinous cuticle and have a

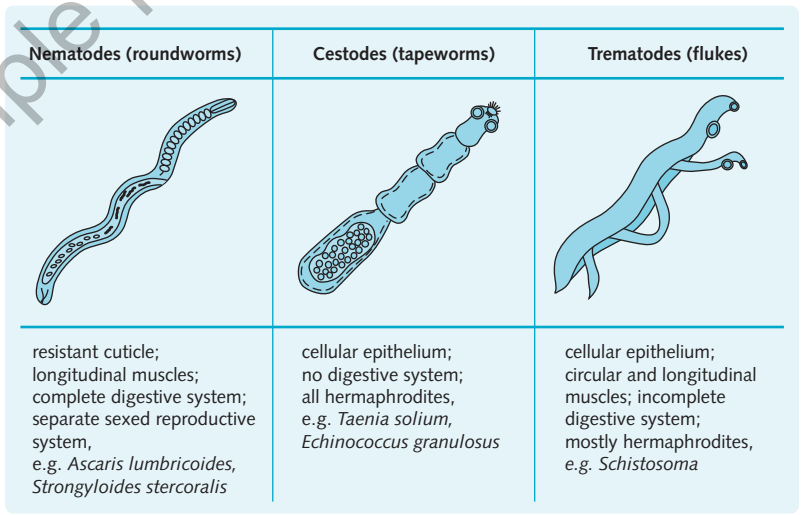


FIGURE 3.11 Main groups of helminths affecting humans
Source: Xiu, P. (2016). Crash Course Pathology. Mosby Ltd.

TABLE 3.3 Examples of helminth infections

Helminth	Disease and symptoms	Transmission
Tapeworms		
Taenia saginata	Larvae attach to intestinal gut and mature into adult worms. May be asymptomatic but high worm loads in the gut may result in weight loss and other gut-related symptoms such as abdominal pain and diarrhoea	Consumption of undercooked beef containing larvae
Hymenolepsis nana	Eggs hatch in intestine maturing into adult worms. May be asymptomatic but high worm loads in the gut may result in weight loss and other gut-related symptoms such as abdominal pain and diarrhoea	Consumption of eggs
Flukes		
Schistosoma species	Schistosomiasis occurs when larvae invade skin, migrating through blood to liver and urinary bladder, maturing into worms in blood vessels. Complications occur from eggs becoming trapped in tissues causing severe inflammatory reactions	Exposure to water environments containing larvae
Fasciola hepatica	Excystation in duodenum then burrow through the lining of the intestine and migrate to the bile ducts causing inflammation and blockage	Consumption of cysts
Nematodes		
Wuchereria bancrofti	Filariasis occurs when larvae in the bloodstream enter tissues and mature into adults. The worms reside in the lymph system causing blockages and fluid accumulation, possibly leading to lymphedema and elephantiasis	Transmitted from the bite of a mosquito
Strongyloides stercoralis	Larvae migrate through blood to lungs where they are coughed up and swallowed into the digestive tract where they develop into adults. Symptoms are gut-related with infection in children affecting growth and mental retardation	Larvae in soil penetrate through skin
Ancylostoma duodenale and *Necator americanus* (hookworm)	Similar disease and symptoms to *S. stercoralis*	Larvae in soil penetrate through skin

complete digestive tract including a mouth and anus. While nematodes only produce worms of male or female sex, tapeworms and flukes are capable of hermaphrodism where one worm contains both sets of gonads and is capable of self-fertilisation.

3.3.2 Ectoparasites

Ectoparasites are arthropods that colonise the surface of the skin where they feed and reproduce causing skin irritation and allergic reactions. The main ectoparasites affecting humans are scabies (a mite), fleas, lice, bedbugs and ticks (Fig 3.12). Table 3.4 provides a summary of these ectoparasites and their effect on humans. Ectoparasites are not lethal; however, they can be vectors for other pathogens or produce an environment for secondary infections. For example, fleas are the vector for the bacterium *Yersinia pestis* responsible for bubonic plague and the human body louse (*Pediculus humanus*) is a vector for the bacterium *Rickettsia prowazekii* that causes typhus. Ticks are also carriers of bacterial pathogens including *Rickettsia* species and in North America, *Borrelia burgdorferi* causing Lyme disease. Of particular importance are scabies which is endemic in Australia's northern Indigenous communities but is also common in urban areas where there is human crowding. Extensive scratching from scabies may cause serious secondary infections such as staphylococcal and group A streptococcal bacterial infections.

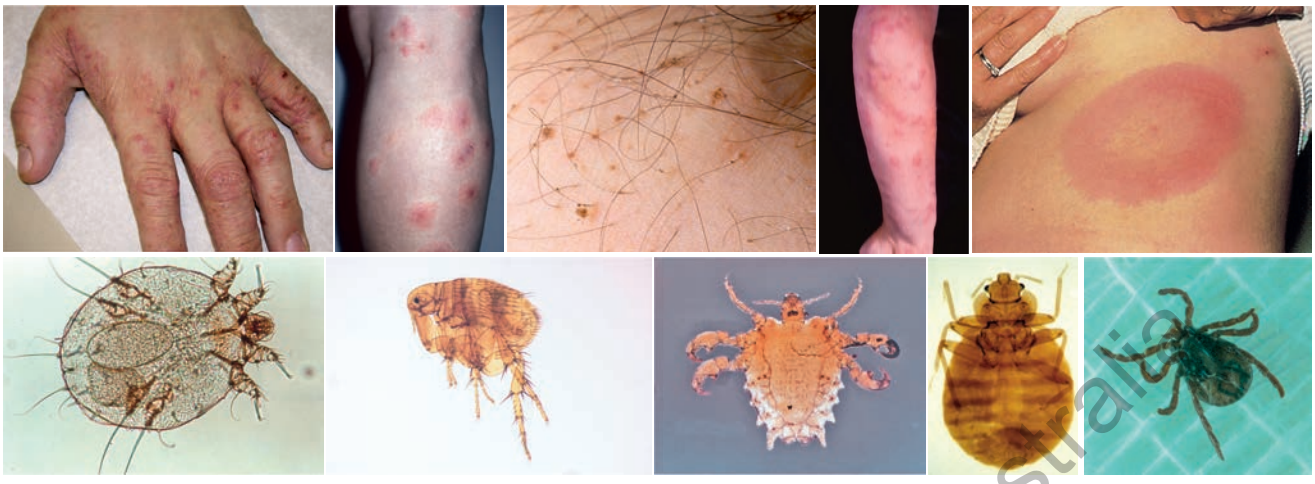


FIGURE 3.12 The effect of bites or colonisation by ectoparasites (in order from right to left scabies, flea, crab louse, bedbug, tick) on human skin surfaces (above) and images of the ectoparasites (below). Most ectoparasites produce minor irritation at site(s) of bite or colonisation. The upper tick bite image shows radiating redness from the bite site as a result of a bacterial infection by *B. burgdorferi* causing Lyme disease

Source: Kaji, A., & Pedigo, R. A. (2021). Emergency Medicine Board Review. Elsevier; Micheletti, R. G., James, W. D., Elston, D., & McMahon, P. J. (2021). Andrews' Diseases of the Skin Clinical Atlas. Elsevier; Ko, C. J. (2021). Dermatology: Visual Recognition and Case Reviews. Elsevier; Micheletti, R. G., James, W. D., Elston, D., & McMahon, P. J. (2021). Andrews' Diseases of the Skin Clinical Atlas. Elsevier; Chintamani, M., & Mani, M. (Eds.). (2021). Lewis's Medical-Surgical Nursing, Fourth South Asia Edition. Elsevier; Paller, A. S., & Mancini, A. J. (2020). Paller and Mancini-Hurwitz Clinical Pediatric Dermatology. Elsevier; Chambers, J. A. (2021). Field Guide to Global Health & Disaster Medicine. Elsevier; Micheletti, R. G., James, W. D., Elston, D., & McMahon, P. J. (2021). Andrews' Diseases of the Skin Clinical Atlas. Elsevier; Hendrix, C. M., & Robinson, E. D. (2022). Diagnostic parasitology for veterinary technicians. Elsevier; Paller, A. S., & Mancini, A. J. (2020). Paller and Mancini-Hurwitz Clinical Pediatric Dermatology. Elsevier.

TABLE 3.4 Summary of ectoparasites affecting humans

Ectoparasite	Disease and symptoms	Transmission
Scabies (*Sarcoptes scabei*)	Female mite burrows under skin to lay eggs causing irritation. May occur anywhere on the body but tends to affect hands and forearms	Direct contact with infected individuals or by coming into contact with their bedding or clothing
Fleas (Siphonaptera)	Minor irritation from bite	Contact with animals, usually pets, that carry fleas
Lice Head louse (*Pediculus humanus capitis*) Body louse (*Pediculus humanus corporis*) Crab louse (*Phthirus pubis*)	Bite causes irritation. Different lice affect different parts of the body including head (head louse), body (body louse) and pubic (crab louse) regions	Direct contact with affected individuals or coming into contact with their bedding or clothing
Bedbugs (*Cimex lectularius*)	Intense itching and irritation from bite which may develop into a painful welt	Bedbugs do not live on human skin. Instead, they infest unhygienic environments, mainly coming out at night to feed on the blood of sleeping individuals
Ticks (Ixodidae)	Minor irritation from bite	Live in humid bush areas and colonise passing humans

3.4 Transmission of pathogens

When discussing transmission of pathogens, it is important to note that some are capable of human-to-human transmission and others are not. Arguably, pathogens that spread between humans are more problematic but depending on the source, pathogens that do not spread between humans can still have a major impact. The mass production of food means that contaminated food sources can be the major cause of infectious outbreaks without human-to-human transmission. Infection proceeds through six interconnected steps called the 'chain of infection' that describes the transmission of a pathogen from its natural reservoir, through a portal of exit, and transmission to a susceptible host through an appropriate portal of entry (Fig 3.13). Breaking one or more of the links connecting the steps helps prevent further infection.

3.4.1 Reservoirs

Settings where pathogens naturally grow are called reservoirs and this includes humans, animals or the environment. Although a reservoir may be where an infectious agent is naturally found, it may not be the source of an infection. For example, the bacterium causing Legionnaire's disease is naturally found in freshwater environments; however, it is when cooling tanks that are using contaminated water aerosolise the pathogen through air-conditioning systems that lung infections occur. Another example is spores of *C. botulinum*, which are naturally present in soil, but improperly canned vegetables is the source of infection. Where humans are the only reservoir, infectious agents may be eradicated through vaccination, for example smallpox, although this is rare. Human reservoirs may be controlled if a person who is aware they are infectious isolates themselves. Importantly, some people do not show symptoms and are unaware they are infectious and in these circumstances, transmission may occur more freely.

Many infections that affect humans come from animal reservoirs in a process called 'zoonosis'. Many diseases that are endemic now are thought to have originated from animals, including smallpox, measles, tuberculosis and malaria. Importantly, 70% of new infectious diseases arise from zoonosis. COVID-19 is a likely zoonosis, although the exact reservoir is unknown. It is largely accepted that the SARS-CoV-2 virus, like SARS-CoV-1, emerged from an animal reservoir, probably bats. Other examples include new strains of influenza (pigs and birds), HIV (non-human primates) and Ebola virus (bats). In the environment, plants, soils and water are reservoirs. Many pathogenic *Vibrio* species such as *V. cholerae* (cholera diarrhoea), *V. parahaemolyticus* (diarrhoea) and *V. vulnificus* (wound infections) are naturally present in marine and estuarine environments. Additionally, many fungal pathogens originate from soil environments. In the case of animal and environmental reservoirs, infectious agents can be consistently reintroduced into the human community despite being cleared.

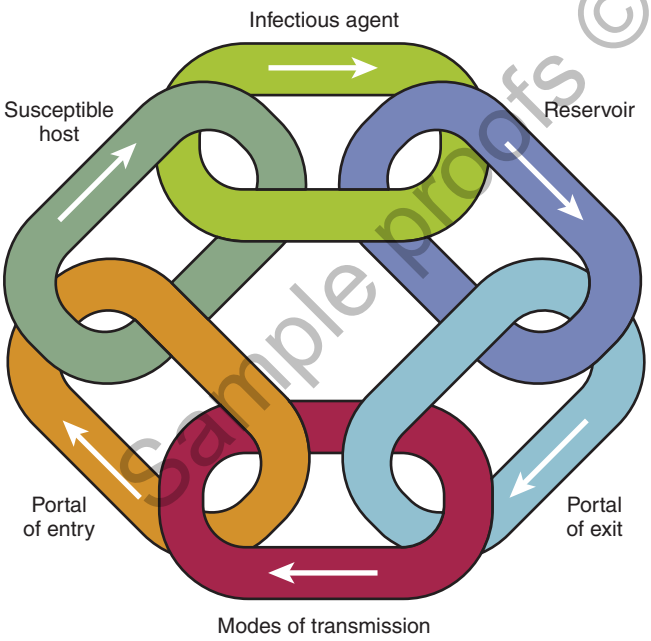


FIGURE 3.13 The chain of infection describes a series of events that leads to a susceptible host becoming infected with a pathogen. Any break in the link may prevent further spread

Source: Leifer, G., & Keenan-Lindsay, L. (2019). Leifer's Introduction to Maternity & Pediatric Nursing in Canada. Elsevier.

3.4.2 Portals of exit

Portals of exit define the method by which the infectious agent leaves its reservoir. In humans, the main portals of exit are: the upper respiratory tract, from saliva or respiratory droplets from sneezing, coughing and breathing; blood; the gastrointestinal tract, from vomit, diarrhoea/faeces; the urogenital tract, from urine or genital fluids; and the skin, from wounds containing infectious discharge and mucous membranes. For animal reservoirs, the portals of exit are largely the same. For environmental reservoirs, the portal of exit is dependent on the environment. If we consider pathogenic *Vibrio* bacteria found in marine/estuarine waters, the portal of exit is water and its movement or contact with humans or human food and drinking water sources.

3.4.3 Modes of transmission

Modes of transmission are subdivided into direct and indirect. Direct transmission refers to the infectious agents directly transferring from a reservoir to a host and this may occur through direct contact, droplet spread or vertical transmission. For human reservoirs, direct contact may occur through skin-to-skin contact, kissing and sex (Fig 3.14). Animal bites or contact with animal faeces and contact with environmental water, soil or plants may facilitate transfer from animal and environmental reservoirs, respectively. Droplet spread refers to short-range aerosols containing the infectious agent generated from coughing, singing, talking and breathing that is then spread to others nearby. The effectiveness of droplet spread is dependent on conditions such as distance between people and ventilation. Vertical transmission is the transfer of an infectious agent from mother to unborn child.

Indirect transmission refers to the transfer of infectious agents from a reservoir to a host via an intermediary and include suspended air particles, inanimate objects (fomites) and animate intermediaries (vectors). Infectious agents suspended on air particles facilitate airborne transmission and unlike droplet spread, can be carried over longer distances on air currents (e.g. measles virus). This may include infectious agents on dust particles or dried residue called droplet nuclei of less than 5 microns. Inanimate objects or fomites include food and drinking water or contaminated items such as bedding, surgical equipment, dental equipment or syringes. Technology can also be an efficient transmitter of diseases such as Legionnaire's disease, which is spread through evaporative condensers and air-conditioners. Vectors are arthropods such as mosquitoes and fleas that carry the infectious agent and deliver it to a host, usually through a bite. Many dangerous pathogens, such as those causing malaria and the plague, are vectorborne.

3.4.4 Portals of entry

The portal of entry is the method by which a pathogen enters a susceptible host. Pathogens generally infect specific tissues, and so for an infection to take hold, the portal of entry should provide appropriate access. For

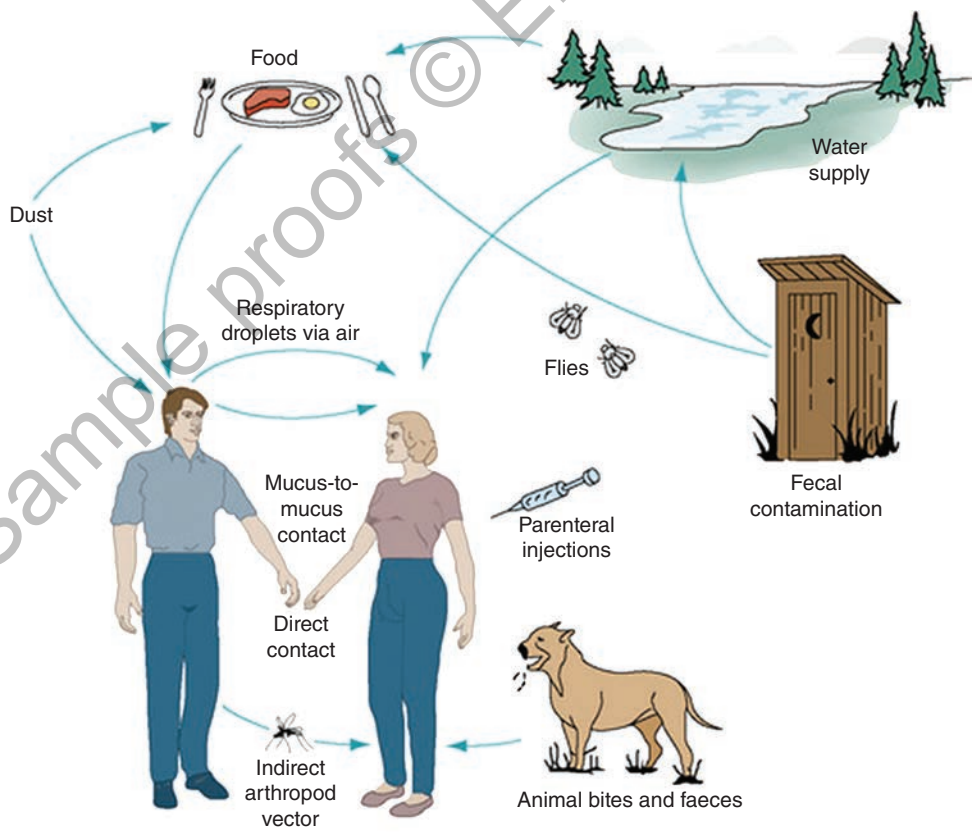


FIGURE 3.14 Modes of transmission for infectious disease

Source: Quah, S. R. & Cockerham, W. C. (2017). International encyclopedia of public health. Academic Press.

the most part, the portals of exit and portals of entry are the same and include the gastrointestinal, respiratory and urogenital tracts. Consumption of contaminated food and drinking water with faecal material from a portal of exit is often the portal of entry for many gastrointestinal pathogens and typifies the faecal-oral route. Respiratory infections are mostly spread through droplets, and sexual contact often facilitates transmission of disease affecting the urogenital tract. Human behaviours such as intravenous drug use and sharing of needles, or medical procedures such as blood transfusions, may spread blood-borne pathogens such as hepatitis viruses or HIV. Skin wounds permit entry of pathogens from direct contact with reservoirs (e.g. environment or other humans) or fomites. Some pathogens, such as hookworms, are capable of penetrating directly through skin and others enter through mucous membranes such as the eye (e.g. SARS-CoV-2 or Ebolavirus).

3.4.5 Susceptible host

Host susceptibility to an infection is an important link in the chain of transmission and is governed by a series of complex factors such as host genetics and host microbiome, age, pregnancy, use of medications, health status (e.g. malnutrition or dehydration) and underlying health conditions (e.g. diabetes). If we consider age, the very young have immature immune systems, increasing their vulnerability to infectious diseases whereas the very old harbour decreased immunity and generally accumulate more comorbidities such as high blood pressure, placing them at higher risk of disease complications. Factors such as nutrition, stress or environmental pollutants can reduce immune responses. Specific genetic conditions can adversely affect immunity whereas genetics generally dictate the variable response observed by populations to disease. Some modern medical treatments such as medications (e.g. immunosuppressants) or chemotherapy can dampen immunity and pregnancy may increase susceptibility to some diseases. Overall, host susceptibility is complex, and multiple colluding factors increase host susceptibility beyond a single factor.

3.4.6 Breaking links in the chain and public health

In battling infectious disease outbreaks, public health officials will attempt to break at least one link in the chain of infection, while targeting multiple breaks gives better outcomes. However, which link(s) to target is often dependent on the infectious disease and may be limited by cost or sociocultural barriers (e.g. human behavioural and cultural practices).

Controlling reservoirs or eliminating pathogens at source: In the context of medical health, infectious people are isolated or treated to eliminate the reservoir. In society, extensive testing can help identify human reservoirs for isolation and treatment. Where the source is a fomite, then effective waste disposal, disinfection or sterilisation methods such as washing bedding and cleaning dirty surfaces and equipment is key. Where the reservoir is animal or environmental, avoiding the reservoir is one strategy but in the case of food-producing animals, this may not be possible. For example, to control outbreaks of influenza in birds, extreme measures such as culling have been used to prevent spread. Outbreaks from contaminated food sources must first be identified and then controlled by closing the source and issuing alerts to those exposed.

Protecting portals of entry and disrupting transmission: Standard precautions and personal protection equipment is important in medical health. Standard precautions are designed to minimise risk of transmission to healthcare workers when engaging with patients. For example, strict precautions are in place when handling syringes to avoid accidental needlestick injuries. Face masks and gloves prevent transmission but also protect a portal of exit and have the additional benefit of minimising transmission to patients. Hand hygiene is another key tool in infection prevention that controls infectious agents on hand surfaces to minimise transmission to patients or common-touch surfaces. For some airborne infections, modifying ventilation or air pressure and air filtration can also be effective in preventing airborne transmission within a hospital.

In society, effective treatment and disposal of human faecal waste has played a major role in disrupting faecal-oral transmission. Additionally, good hygiene such as regular washing of hands disrupts transmission between people through direct touch or fomites or contamination during food preparation. Where the transmissive vehicle is a vector, using insecticides to control the vector population is common. Insect repellents, clothing and bed netting can protect from bites that provide a portal of entry for vectorborne pathogens.

Increasing host defences: The treatment or control of underlying diseases or prophylactic treatment with

antimicrobials are options. Patients may be given postoperative antimicrobials to decrease the likelihood of developing an infection; however, the rise of antimicrobial resistance requires prudent use of this approach. The most effective host defence-boosting intervention is vaccination. Vaccination of a population can dramatically decrease the transmission of an infectious agent and if rates are sufficiently high, can facilitate herd immunity, forcing the outbreak to burn out and thus protecting those who cannot be vaccinated or who are unable to mount an immune response in response to the vaccine.

3.5 Host–pathogen interactions

This section gives an overview of the diverse methods that pathogens use to cause infection and disease, and an overview of host immunological responses to infection.

3.5.1 Bacterial pathogens

Bacterial strategies in infection are highly diverse and research into the methods by which bacterial pathogens cause disease is extensive. With that said, there are common themes that can be teased out. Exposure of the pathogen to its target tissue is critical and this is discussed in 3.4 Transmission of pathogens. However, it is important to note that some pathogens are opportunistic and only cause disease if the circumstances are right. Autoimmune conditions, disruptions in the microbiome or other bodily changes may allow commensal bacteria to opportunistically infect. Additionally, normal flora in one part of the body may cause infection if accidentally relocated to another part of the body. For example, faecal bacteria are commonly implicated in urinary tract infections.

Adhesion of the bacterium to host tissue is an important first step that helps the pathogen to establish a discrete focal point of infection. Without adhesion, a bacterium may be easily removed through natural bodily processes such as peristaltic motion of the intestine, passage of urine through the urethra, coughing or sneezing. A variety of cell surface structures are important in adhesion including fimbriae, pili, flagella and capsular polysaccharide and there is most often a specific interaction between the bacterium and a host tissue. There are multiple examples of adhesins such as fimbriae and pili used by certain strains of *E. coli* in adhesion to the urinary tract or the pilus used by diarrhoeal-causing strains of *Vibrio cholerae* in adherence to the intestine.

Following adhesion, bacteria use diverse strategies that assist in adjacent spread and invasion of tissue and/or spread into the bloodstream or other tissues. Secretion of toxins (exotoxins) is one strategy (see Table 3.5 for a representative list). Toxins are substances that disrupt the normal function of the host and include enzymes that break down polymeric substances holding

TABLE 3.5 Examples of toxins produced by bacteria and mechanism of action

Bacterium	Disease	Toxin	Mechanism of action
Secreted toxins			
Clostridium botulinum	Botulism	Neurotoxin	Blocks excitation of muscles causing flaccid paralysis
Clostridium tetani	Tetanus	Neurotoxin (tetanus toxin)	Prevents inhibition of motor neurons blocking relaxation of muscles
Corynebacterium diphtheriae	Diphtheria	Diphtheria toxin	Inhibition of protein synthesis
Listeria monocytogenes	Listeriosis	Listeriolysin	Lysis of multiple host cells
Shigella dysenteriae	Dysentery (Bloody diarrhoea)	Shiga toxin	Inhibition of protein synthesis. Mainly affects blood vessels
Vibrio cholerae	Cholera diarrhoea	Enterotoxin (cholera toxin)	Disrupts ion flow in the intestine causing massive water loss and diarrhoea
Injectosome delivered toxins			
Salmonella enterica Typhimurium	Inflammatory gastroenteritis	Multiple effector proteins delivered to intestinal cells by a Type III secretion injectosome system	Induces entry into host cell and manipulates multiple host cell pathways involved in actin arrangement, cytokine production and apoptosis

tissues together such as connective tissue, collagen and mucin, or substances that have specific impacts on host cells such as cell lysis, protein synthesis inhibition, interference with or inhibition of neurotransmitters or interference of host cell regulatory chemicals. Toxins are often named depending on the cell types they attack. Toxins that affect multiple host cell types are referred to as cytotoxic whereas toxins that attack specific host cell types are named accordingly, such as neurotoxin (nerve cells), hepatotoxin (liver cells), cardiotoxin (heart cells) or enterotoxin (enteric cells). Toxins may also be named according to the disease they cause (e.g. cholera toxin and tetanus toxin), activity or biochemical properties (e.g. heat-stable toxin). In some instances, people can be impacted by bacterially produced toxins without infection. Food intoxication occurs when food is consumed containing a toxin secreted by a bacterium such as staphylococcal intoxication, causing nausea and vomiting, or botulism, a severe paralysis disease caused by *Clostridium botulinum*-produced neurotoxin. Some toxins are directly injected by bacteria into host cells through injectosome needle-like secretion systems and these toxins are referred to as effector proteins. The lipid A portion of LPS in Gram-negative bacteria is often referred to as endotoxin and has inflammatory effects in the bloodstream. LPS may slough in small amounts but if released in large amounts from lysis due to phagocytosis or chemotherapeutic activity, may cause dangerous endotoxic shock.

Some bacteria grow on host tissue forming a slime layer called a biofilm which is defined as bacteria adhered to a surface in multi-layered communities encased in an extracellular polymeric matrix that is rich in polysaccharide. Biofilms can form on animate or inanimate surfaces and is a natural process that is widely considered the normal mode of bacterial growth rather than planktonic growth in a liquid medium (Fig 3.15). Biofilms have multiple advantages in infection including the coordinated and regulated efforts of a bacterial population to cause tissue damage and the polymeric matrix acting as a physical barrier to host immune cells and chemotherapeutics. As such, biofilms are often involved in chronic infection such as *Pseudomonas aeruginosa* infections in the lungs of cystic fibrosis sufferers or periodontal diseases. Implants such as hip joints or heart valves are prone to biofilm formation that often must be removed from the patient to clear the infection.[7] Temporarily implanted medical devices such as catheters are also prone to biofilm formation, increasing the risk of infection. Beyond infection, biofilms are important on hospital surfaces and within the water pipe infrastructure by acting as reservoirs of pathogens and antimicrobial resistance genes. Bacteria within biofilms are more resistant to disinfectants and the close cell-to-cell contact facilitates horizontal gene transfer processes that spread antimicrobial resistance genes, degrading options for treating bacterial pathogens (see Box 1 Resistance plasmids are everywhere!).

Some bacterial pathogens are adept at invading host cells, enabling evasion from the immune system and creating an intracellular replicative niche. Some intracellular pathogens are obligate, such as *Chlamydia trachomatis* spp. (*Chlamydia*) and *Mycobacterium tuberculosis* (tuberculosis), and can only replicate within a host cell whereas others are facultative, such as *Salmonella* typhi (typhoid), *Legionella pneumoniae* (Legionnaire's disease) and *Yersinia pestis*

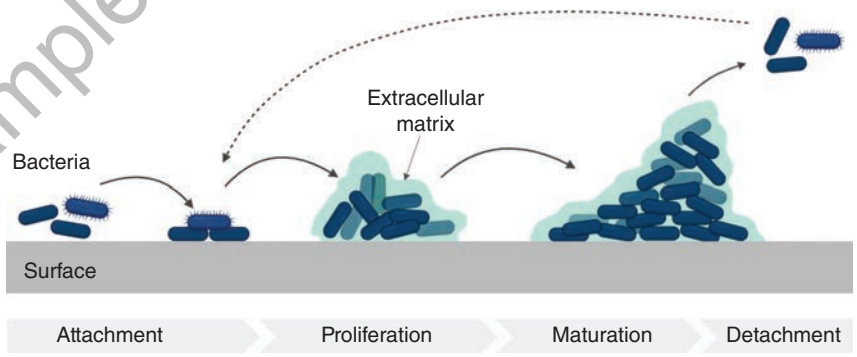


FIGURE 3.15 Biofilm formation in bacteria is initiated by adhesion of bacterial cells to a surface. Proliferation and maturation occur through cellular growth and the production of an extracellular matrix that encases the bacteria and helps protect them from immune cells, chemotherapeutic agents and disinfectants. Cells can detach from the biofilm and travel to other sites to reinitiate the process

Source: Costa, P., Costa, C. M., & Lanceros-Méndez, S. (Eds.). (2021). Advanced Lightweight Multifunctional Materials. Woodhead Publishing.

(plague) and can replicate inside and outside of a host cell. Intracellular lifestyle strategies are diverse, with some pathogens being internalised by phagocytosis whereas other pathogens induce host cells to uptake them. Intracellular bacterial pathogens use a number of virulence factors to subvert and control normal cellular functions including escaping destruction within immune cells and manipulating host actin to move directly from cell to cell.

BOX 3.1 Resistance plasmids are everywhere!

Bacteria can naturally evolve resistance to antimicrobials through mutation; however, many of the most problematic hospital pathogens acquire resistance from plasmids. Plasmids are circular pieces of DNA that replicate independently of the chromosome, with many containing conjugative genes (called conjugative plasmids) that facilitate transfer to other bacterial cells through a protein bridge called a pilus.

Due to the extensive use of antimicrobials in humans and animals, and their presence in waste streams, there is selective advantage for bacteria to acquire and keep a resistance plasmid. Additionally, waste streams are important in the dissemination of resistant bacteria and DNA including plasmids into the environment which are thought to re-enter into the community through water consumption or the food chain. As a result, resistance plasmids are not only common in pathogens but also in commensal and environmental bacteria, and these act as a source for pathogens. In fact, carriage of resistant plasmids in the human gut comes with a higher risk of developing a resistant infection due to possible transfer into an infecting pathogen.[8] Importantly, the passage of plasmids across different bacteria provides them with the opportunity to acquire novel antimicrobial resistance genes from the vast bacterial metagenome.

It is important to emphasise that resistance plasmids often contain multiple resistance genes thus providing resistance to multiple classes of antimicrobials. This means that a single conjugative transfer event into a pathogen can significantly limit treatment options for an infection. Therefore, effective infection prevention and control also includes the control of nefarious genetic material such as resistance plasmids.

3.5.2 Fungal pathogens

Despite being widely spread among humans, a small number of fungal species infect humans with very few causing life-threatening infections. Environmental fungi can infect humans if they are able to penetrate protective barriers such as skin, survive host immune defences, lyse tissue(s) and grow at human body temperature. With advancements in treatments that have allowed patients to survive where normally they would have succumbed, severe fungal infections have become more common in the immunocompromised or those with underlying health conditions.

Superficial, cutaneous and subcutaneous mycoses: Dermatophytoses are common mycoses also known as ringworm or tinea caused by fungi that usually localise at the level of skin, hair or nails (*Microsporum canis*: tinea capitis and tinea corporis). They are common in tropical regions including in Australia. Many predisposing factors can favour infection in the host including environmental factors (humidity, temperature), immunological and general health status of the host, and socio-economic factors. Recently, mask tinea was described in India and was linked to the use of masks for protection against COVID-19 infection.[9]

Dermatophytoses usually present as circular red scaly lesions on skin. Infected nails may be separated from the nail bed after discoloration, thickening and desquamation. Cutaneous infection may lead to invasive infections in immunocompromised patients, leading to ulcers and abscesses. Overall, these infections trigger a host inflammatory response at the level of the skin. Development of dermatophytosis includes three main steps: adherence, penetration and maintenance. Fungal cells attach to keratinised tissues via adhesins that connect it to the skin—this is often linked to a change in local skin pH which prevents the action of protective host enzymes that work better in acidic conditions. To penetrate the tissue, the fungal pathogen secretes several types of enzymes including keratolytic proteases. Once in the tissue, the fungus maintains itself by using nutrients from the host tissue and adapting to host metabolic changes. Dermatophytes are also able to produce biofilms which could explain why some infections are difficult to treat.

Systemic opportunistic fungal infections: Most severe to life-threatening fungal infections are systemic and occur in the immunocompromised. Fungi such as *Candida* spp., *Cryptococcus* spp. or *Aspergillus* spp. are major causes of systemic infections with high mortality rates when they cannot be cleared by the

host defence system. This evolution from asymptomatic to systemic is usually linked to a host's immune status. Individuals suffering from HIV/AIDS or treated with immunosuppressants for transplants or cancer are most at risk of developing these systemic diseases after being infected by an opportunistic fungus that would normally have caused a benign, subclinical infection in an otherwise healthy individual.

Both *Aspergillus* spp. and *Cryptococci* spp. can enter the lungs as spores. In invasive pulmonary aspergillosis, the pathogen enters the lung, then invades and damages the tissues before entering the blood, leading to a systemic infection allowing the dissemination of the infection to other organs such as the brain. Cryptococcosis is particularly severe in patients with low CD4 T-lymphocytes (see 3.6 Immunological responses) counts, making it a major cause of AIDS-related deaths, with the pathogen being able to spread to several organs with a high affinity for the central nervous system. *Candida albicans* is part of the microbiome that colonise humans; however, this colonisation can evolve into infection, notably among hospitalised patients in intensive care. Bloodstream infection occurring during disseminated candidiasis has a high mortality rate and this opportunistic infection occurs not only in immunocompromised patients but particularly in patients who present rupture of anatomical barriers after catheterisation or surgery in addition to broad-spectrum antibacterial therapy.

Through evolution, fungi have lost their flagella and use other means to invade a host. The hyphae (long tube-like structure branching from the main body of the fungus) in *Candida albicans* for instance is a highly polarised structure that helps the fungus to propel itself within the human body by penetrating epithelial and endothelial cells, causing damage by releasing hydrolytic enzymes, allowing access to the bloodstream and being responsible for systemic infections such as candidiasis. *Candida* spp. and more particularly pathogenic *C. albicans* and *C. glabrata* possess a wide range of adhesins on their surface that allow them to firmly attach to host cells (endothelium, epithelium) via molecules such as fibronectin and glycans, and abiotic surfaces such as glass or plastic, helping them to form biofilms. Some of these adhesins are also found in *Aspergillus fumigatus* and *Cryptococcus neoformans.* Airborne spores produced by fungi such as *Aspergillus* spp. are transported passively into the host and then germinate as hyphae, where they can produce large mycelial lesions in the lungs. In *Cryptococcus* spp. desiccated yeasts are an infectious form that can propagate the infection. After adhesion, to allow invasion, these fungi secrete various digestive enzymes able to dissolve host tissues and provide substrate for survival. Some invasive *Aspergillus* spp. secrete elastases that degrade elastin in the lung alveoli. *Cryptococci* secrete multiple enzymes such as ureases that facilitate the passage through the blood–brain barrier or a metalloprotease also required for central nervous system invasion. *C. albicans*, *A fumigatus* and *Cryptococcus* spp. secrete phospholipases that enhance their virulence by increasing their adhesion to respiratory epithelial cells.

3.5.3 Parasitic pathogens

Intestinal protozoan parasites: Intestinal protozoan parasites are some of the main causes of foodborne infections following the ingestion of contaminated water or food. The most common and relevant to human infections are *Giardia lamblia*, *Cryptosporidium parvum* and *Entamoeba histolytica*. The clinical presentation of the infection caused by these parasites can vary from persistent diarrhoea with loose stools, fatigue, abdominal cramps (*G. lamblia*); mild to acute diarrhoea with nausea, vomiting, abdominal pain and low-grade fever (*C. parvum*); to bloody mucoid diarrhoea with abdominal pain, fever, liver abscesses and life-threatening infection with dissemination to organs other than the gastrointestinal tract (*E. histolytica*).

The overall biological cycle of *Giardia* spp. and *Entamoeba* spp. starts with the ingestion of an infectious cyst via the faecal-oral route (e.g. contaminated water or food). Due to the changes in their environment when reaching the stomach, the parasites emerge from the cyst in a stage known as excystation and transform into trophozoites. These trophozoites, considered as the disease-causing stage, attach to the intestinal epithelial cells where they proliferate. Interaction with the intestinal epithelial cells triggers multiple changes, including surface and attachment-associated proteins such as adhesins and lectins as well as metabolic enzymes, that help source energy and fight host immunity. As the parasite is transported further into the gastrointestinal tract towards the small intestine, it converts back into a cyst (encystation stage), enabling it to survive outside the host for weeks once it is excreted in the faeces. In the case of *Entamoeba histolytica*, the trophozoites colonise the colon. In a large majority of cases the colon is not invaded and individuals remain asymptomatic or present mild symptoms. However, if the colon barrier is breached, the trophozoites may spread to surrounding

tissues and cause local tissue damage such as necrosis and ulcers through the production of cytolytic molecules. If the parasites reach the liver and the lungs via the bloodstream, life-threatening amebiasis may result.

Cryptosporidium hominis is the main cause of cryptosporidiosis in humans in Australia, and in the 1980s it was an AIDS-defining illness. *Cryptosporidium* spp., unlike other apicomplexan parasites such as *Plasmodium* and *Toxoplasma*, completes its life cycle within the gastrointestinal tract of its single host. When the host ingests oocysts, they undergo excystation and release sporozoites, and as for *Giardia* spp., excystation is triggered by environmental factors such as temperature, pancreatic enzymes and bile salts. The sporozoites move across the epithelial cells, invading them. While moving over the host cells, the parasite secretes various proteins involved in attachment and later invasion, leading to its encapsulation in a host-membrane derived vacuole called the parasitophorous vacuole (PV). The cycle continues by a sexual stage multiplication and the formation of cysts that are shed into the environment, allowing propagation of the infectious cycle. Although not fully invasive and confined at the level of the host cell plasma membrane, *Cryptosporidium* spp. are able to disturb the function of the intestinal epithelium.

Also transmitted by ingestion of cysts, *Toxoplasma* parasites, mainly *Toxoplasma gondii*, cause few gastrointestinal symptoms but are responsible for severe pathologies, both acute and chronic, ranging from flu-like to lymphadenopathy, encephalopathy, abortion, stillbirth or congenital abnormalities. Parasites contained in the ingested cysts are released and invade the intestinal epithelial cells where they transform and multiply intracellularly within a PV. *Toxoplasma* uses what has been described as an 'invasion machinery'. This machinery contains proteins needed for cell invasion and manipulation of the host cell. Within the PV, the rapid multiplication leads to the rupture of the cell and invasion of the neighbouring cells. At this stage, depending on the response of the host, parasites can either be eliminated (most common case in immunocompetent hosts) or form a cyst. Many cells can be invaded by the parasite which likely uses this advantage to disseminate to other tissues, notably via infiltrating immune cells but also endothelial cells, through what can be described as a 'Trojan horse' type of invasion. These cysts can therefore be found in areas of low immune surveillance such as the eye or muscles. Parasites can also use this means of invasion to cross the blood–brain barrier and reach the brain.

Protozoan parasites acquired from insect bites:

Whether the insect vector is present in Australia or not, cases of insect-transmitted protozoan infections are frequently diagnosed. Two of the major ones are classified as imported, with malaria caused by *Plasmodium*, and American trypanosomiasis or Chagas disease caused by *Trypanosoma cruzi*. The latter is now considered an emerging disease in Australia. Malaria is caused by the *Plasmodium* species and five strains are known to infect humans—*P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*—with the first being the most virulent and the last being defined as a zoonosis as it historically infected monkeys.

The life cycles of *Trypanosoma cruzi* and *Plasmodium* spp. are complex and involve several hosts, vectors, life cycles and parasite stages specific to each part of the overall cycle. In both cases the parasite is taken up from the infected host through the bite of the vector during a blood meal, i.e., the Anopheles mosquito (malaria) or the triatome bug (trypanosomiasis). In malaria, the parasite is directly injected into a new host during the vector's next blood meal, while in trypanosomiasis the parasite is deposited in the vector's faeces during a blood meal and enters the host via neighbouring mucous membranes or directly via the bite site. From there the parasites penetrate various tissues and multiply in target organs.

Uncomplicated malaria is defined by the occurrence of non-specific flu-like symptoms (fever, chills, body aches) and the presence of parasites in the blood but can evolve into severe or complicated malaria. This occurs mainly in children under the age of five or non-immune individuals, i.e., individuals who have never encountered the parasite or have lost their immunity against the parasite. Severe malaria is mostly associated with *P. falciparum* infection and can clinically present as a neurological syndrome with coma and seizures, a severe anaemia or multi-organ failure. During malaria infection, after being injected into the dermis of the host, the parasites which are motile need to reach the liver by traversing capillaries and being transported to hepatic sinusoids, cross the endothelial cells and enter the hepatic parenchyma. Very much like *Toxoplasma*, *Plasmodium* sporozoites release the contents of invasive organelles to allow for hepatocyte invasion and folding of the plasma membrane to form a PV where the parasite will live and perform its cycle. From the liver, the parasites then migrate to the blood where they start the erythrocytic cycle which is associated with malaria symptomology. The cycle starts when the parasites are

released from the ruptured hepatocytes and then invade erythrocytes. The erythrocytes will be used as a protective vehicle and source of energy and will undergo significant remodelling, including export to the erythrocyte membrane of hundreds of proteins from the PV via a network of conduits. One family of these exported proteins is the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family. Each PfEMP1 protein has a defined adhesive domain that allows it to bind to specific receptors within different vascular beds including the brain and the placenta. This binding to endothelial cells (blood vessels) or syncytiotrophoblasts (placenta) allows infected erythrocytes to sequester into the tissues, escape splenic clearance and be an integral part of the pathogenesis of severe malaria forms such as cerebral and placental malaria.

American trypanosomiasis presents in two phases—acute (up to 2 months) and chronic (up to several years/decades). The acute phase is associated with the presence of parasites in the blood together with non-specific symptoms such as fever, headache, enlarged lymph glands, muscle pain or no symptoms. During that time the parasite multiplies in organs such as the heart and the gastrointestinal tract. Patients can stay asymptomatic (~70%) but others will develop organ-associated symptoms such as chest pain, cardiomyopathy, neurological alterations or enlarged oesophagus or colon. When the parasite enters the host it will invade cells, usually of the mucous membrane of the nose, conjunctiva or any fragile surface, and will transform into amastigotes (intracellular stage of the parasite that has lost its flagella). These forms will then multiply before they burst out of the cells and enter the bloodstream to invade new cells and propagate the infection to tissues such as the heart and the gastrointestinal tract. The parasite uses various virulence factors while interacting with the host. These factors not only allow the parasite to resist host defence mechanisms and evade the immune response but also promote cell adhesion and invasion. The parasites express enzymes that can inhibit the defence mechanism from macrophages. The parasite is also able to avoid lysis by blocking the complement pathways using complement-regulating factors. To allow adhesion and invasion of the host cells, *T. cruzi* expresses at its surface molecules such as trans-sialidases (specific to the parasite), mucins, mucin-associated surface glycoproteins and phospholipases.

Multicellular parasites: Humans and helminth parasites have coexisted and co-evolved for millennia. In Australia, infections caused by roundworm (strongyloidiasis caused by *Strongyloides stercoralis*), hookworm (*Ancylostoma duodenale*), tapeworm (echinococcosis) and soil-transmitted helminthiases can disproportionately affect Indigenous populations. Although helminth infections can be associated to severe comorbidities, they rarely cause lethal infections, suggesting that the hosts have learnt to tolerate the parasites and develop a disease tolerance leading to limited damage to both host and parasite. Some of these parasites' life cycles are simple, involving humans as the definitive host (i.e. host where the parasite completes its sexual stage). In the case of taeniasis, humans become infected by ingesting raw or undercooked meat from infected cattle (*Taenia saginata*) or pigs (*Taenia solium*). Once in the animal, the ingested parasite eggs hatch and invade the intestinal wall and migrate to the striated muscles to form cysts. In the case of *Ancylostoma duodenale*, larvae penetrate the skin, are carried via blood vessels to the heart and lungs, penetrate the pulmonary alveoli, ascend the pharynx and are either coughed up or swallowed by the host. To penetrate the skin, the parasite secretes enzymes that facilitate migration through tissue. While migrating through the body the parasites not only trigger direct tissue damage but also release inflammatory mediators, typically through the shedding of their outer chitin layer during moulting and also via the release of excretory–secretory products including proteases, protease inhibitors, lectins, allergens and glycolytic enzymes. In the case of strongyloidiasis, one of the severe forms is the dissemination of the infection outside the gastrointestinal tract, which can occur in immunocompromised patients where the parasite is able to infiltrate organs such as the lungs, kidneys, liver or central nervous system.

3.5.4 Viral pathogens

Viral infections all follow a generalised cycle that is summarised in Figure 3.16.

1. *Attachment:* Adhesion is the first step and is a very specific interaction between the virus and host cell. The spike proteins facilitate adhesion by binding to host cell receptors. If the receptor is common then the virus may infect a range of host cells. For example, the Ebolavirus is capable of infecting multiple cell types due to the commonality of its host receptor. On the other hand, HIV adheres only to receptors on human immunity $CD4^+$ cells. Some viruses like rabies can

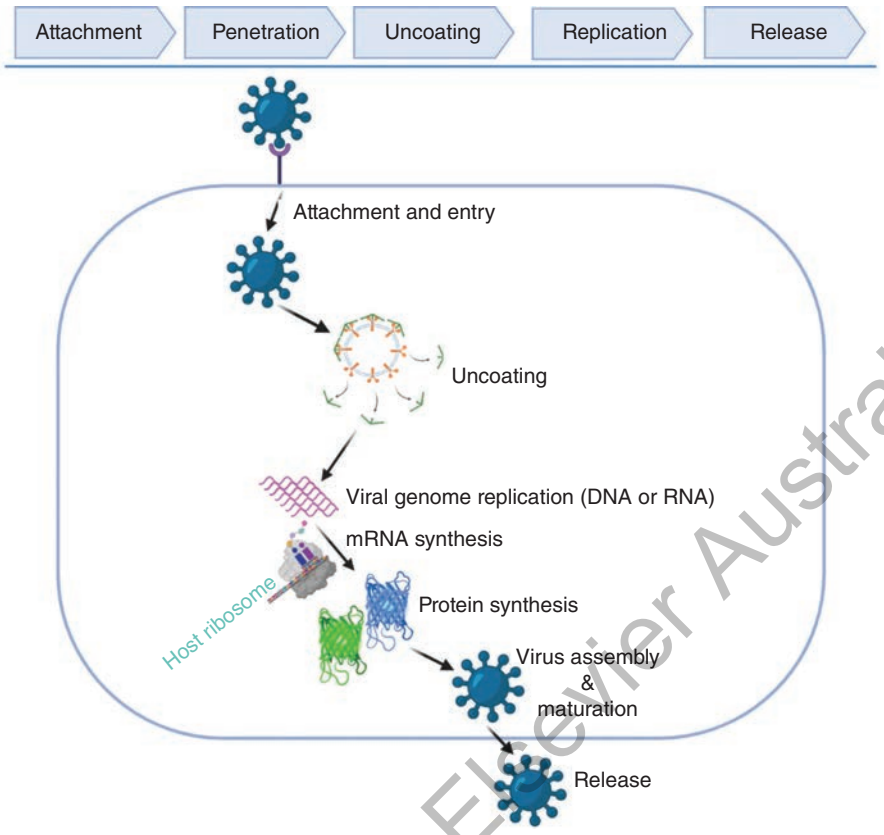


FIGURE 3.16 The general viral replication strategy. A eukaryotic virus will attach to a host cell and enter via endocytosis or membrane fusion. Upon entry, the virion is uncoated allowing release of the nucleic acid. The nucleic acid is replicated and mRNA is produced using host ribosomes to make viral proteins required for viral assembly and maturation. The viral particle then exits the host cell via lysis or budding. Modified from ElBagoury M, et al 2021

Source: Modified from ElBagoury, M., Tolba, M. M., Nasser, H. A., Jabbar, A., Elagouz, A. M., Aktham, Y., & Hutchinson, A. (2021). The find of COVID-19 vaccine: Challenges and opportunities. Journal of infection and public health, 14(3), 389-416.

infect multiple animal species due to the presence of an interspecies receptor.

2. *Penetration:* The virus penetrates the cell and this occurs by one of two processes, endocytosis or fusion. Endocytosis is a natural process that eukaryotic cells use to bring in substances, and examples of both enveloped and non-enveloped viruses using this method of entry exist. Some enveloped viruses fuse their membrane with the host cell membrane allowing entry of the nucleocapsid into the host cell.
3. *Uncoating:* Depending on the virus, different mechanisms exist for uncoating that facilitate the entry of the nucleic acid into the cytoplasm.
4. *Replication:* The viral nucleic acid dictates the nucleic acid replication strategy. The virus often brings in or produces its own enzyme for nucleic acid replication. Production of viral mRNA is key and host ribosomes drive synthesis of viral proteins that take over the host cell and then synthesise the virion. Viral DNA locates to the nucleus where mRNA is synthesised and transported out into the cytoplasm for protein synthesis. Synthesised proteins are transported into the nucleus for virion assembly. Viral RNA remains in the cytoplasm where synthesised mRNA directs protein synthesis and assembly proceeds. HIV is an RNA retrovirus that converts its RNA genome into DNA and inserts it into the host cell genome in the nucleus using a specialist viral enzyme called reverse transcriptase. From this position, it produces mRNA, which also acts as the viral genome, to drive synthesis of proteins allowing assembly to proceed in the cytoplasm.

5. *Release:* Release of non-enveloped virions usually results in host cell lysis and death whereas enveloped virions bud out through the membrane, acquiring their envelope in the process. During the replication stage, viral proteins are embedded into the host cell membrane, such that the viral envelope contains virus-specific proteins.

Different animal virus infections have different outcomes that are defined as acute, latent, persistent or oncogenic. Acute infections such as influenza or COVID-19 are rapid (days to weeks) resulting in death of infected cells. Latent infections such as lip or genital sores caused by herpes simplex viruses initiate as an acute infection but enter into a latent stage that may revert into an acute phase months or years after the original infection. Some viruses produce persistent infections that progress slowly over weeks and months, slowly replicating and budding from host cells without necessarily destroying them. These infections, which include the glandular fever Epstein-Barr virus, deprive their host of energy, sometimes resulting in chronic fatigue. Finally, oncogenic viruses are those that are capable of transforming host cells into cancerous cells such as the human papilloma virus linked to cancer of the cervix, vulva, vagina, penis and anus.

3.6 Immunological responses

3.6.1 Generality on the role of the immune system during infection

The first and foremost role of the immune system is the defence of the host against pathogens but it is also involved in the elimination of toxic or allergenic substances that can come in contact with or enter the body. During its development, the immune system has learnt through tolerance mechanisms to differentiate the self and the non-self and therefore to not eliminate host cells or to avoid responses that would destroy beneficial, commensal microorganisms. This ability allows it to respond to an invading pathogen through the innate or the adaptive immune responses.

The immune system is composed of a large variety of cells (Fig 3.17 and Table 3.6) which have specific roles in the different phases of the immune response. Innate immunity can be defined as an immediate and non-specific response to infection that involves barriers such as epithelial cells (e.g. skin, mucosa, mucous layers and ciliated epithelial cells) that are all able to physically prevent entry of pathogens. If a pathogen breaks through these barriers, other protective mechanisms involving soluble molecules such as complement system

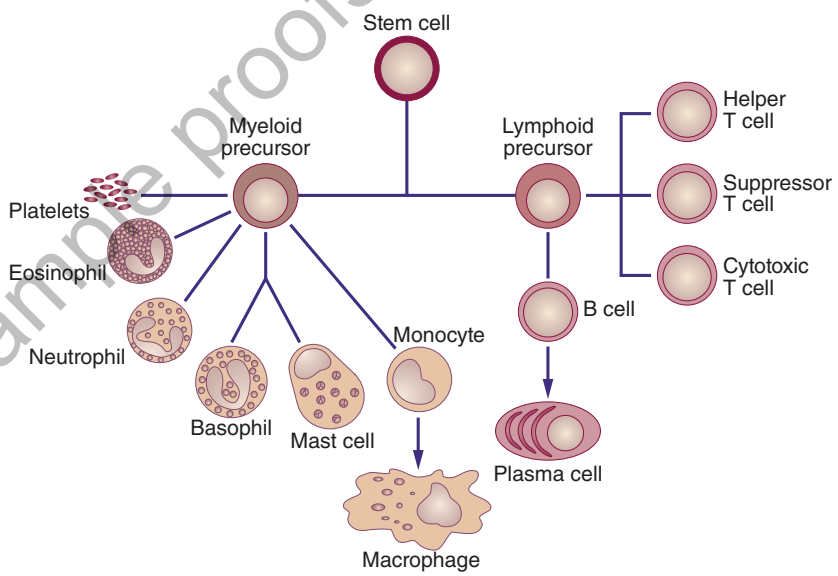


FIGURE 3.17 Cells of the immune system arise from a common haematopoietic stem cell in the bone marrow that will differentiate and mature into two different cell lineages, myeloid and lymphoid. From these precursors, the major cells of the immune response will mature, themselves being able to further differentiate into more specialised cells, such as plasma cells. Table 3.6 describes these cells and their roles

Source: McCuistion, L. E., Yeager, J. J., Winton, M. B., & DiMaggio, K. V. (2021). Pharmacology. Elsevier.

TABLE 3.6 Main cells of the immune system, their role and location

Cell type	Main role	Location
Polymorphonuclear eosinophils	Release toxins that can kill bacteria and parasites	Blood vessels and can migrate to tissue during infection
Polymorphonuclear neutrophils	Ingest and kill microorganisms (mainly bacteria and fungi). First responders at the site of infection. Release molecules that attract other immune cells at the site of infection	Blood vessels and can migrate to tissue during infection
Polymorphonuclear basophils	Defence against parasites. Release molecules that can participate to the expulsion of an extracellular parasite	Blood vessels and can migrate to tissue during infection
Mast cells	Release molecules involved into the destruction of pathogens	Connective tissue, mucous membranes
Monocytes	Phagocytic cells that can engulf pathogens and cells and later differentiate into macrophages once they have left the blood circulation and entered a tissue	Blood vessels and can migrate to tissue during infection
Macrophages	Phagocytic cells that can engulf pathogens and release molecules stimulating other immune cells	Migrate from blood vessels into infected tissues
T-lymphocytes (Helper)/ CD4 T helper cells	Specialised T-lymphocytes helping other T and B-cells perform their functions by interacting directly with them or releasing soluble molecules. Express CD4 antigen	Blood vessels and can migrate to tissue during infection
T-lymphocytes (Suppressor)	Specialised T-lymphocytes (also called regulatory T-cells) that suppress various cellular and humoral mechanisms of the immune response. Express CD4 antigen	Blood vessels and can migrate to tissue during infection
Cytotoxic T-lymphocytes/ CD8 Cytotoxic T-cells	Kill infected cells. Express CD8 antigen	Blood vessels and can migrate to tissue during infection
B-lymphocyte	Sub-type of lymphocyte that can present foreign antigens to other immune cells, secrete soluble molecules and produce antibodies when differentiated	Blood vessels and can migrate to tissue during infection
Plasma cell	Differentiated B-lymphocytes that produce antibodies	Blood vessels and can migrate to tissue during infection
Natural killer cells	Kill mainly virus-infected cells	Blood vessels and can migrate to tissue during infection
Dendritic cells	Initiate antigen-specific immune responses	Resident cells of the skin, lung and digestive tract. Migrate to lymph nodes after activation by a pathogen

proteins, defensins, mediators of inflammation, reactive free radical species and cytokines and chemokines can take over (the humoral part of innate immunity). Innate immunity is able to recognise general classes of pathogens such as bacteria, fungi, viruses and parasites but will not be able to make fine distinctions within each of these. The innate immunity will attempt to immediately destroy the pathogens and if unable, will contain the infection until the more powerful adaptive immune system acts (Fig 3.18).

Adaptive immunity can be defined as a delayed and very specific response that is able to recognise each unique type of foreign antigen. It is slower to respond, with effector cells produced within a week and the whole response occurring within two weeks. Contrary to innate immunity which will mount the same response upon repeated exposure to the same pathogen, adaptive immunity develops a memory of a specific pathogen and will respond upon re-exposure in a faster or more potent manner. Both B- and T-lymphocytes are major cells of the adaptive immunity and are responsible for the specific immune recognition of pathogens. The adaptive immunity combines a humoral immunity defined by the production of antibodies (Fig 3.19) by differentiated B-cell lymphocytes (called plasma cells) and a cell-mediated immunity involving T-lymphocytes that will mature into

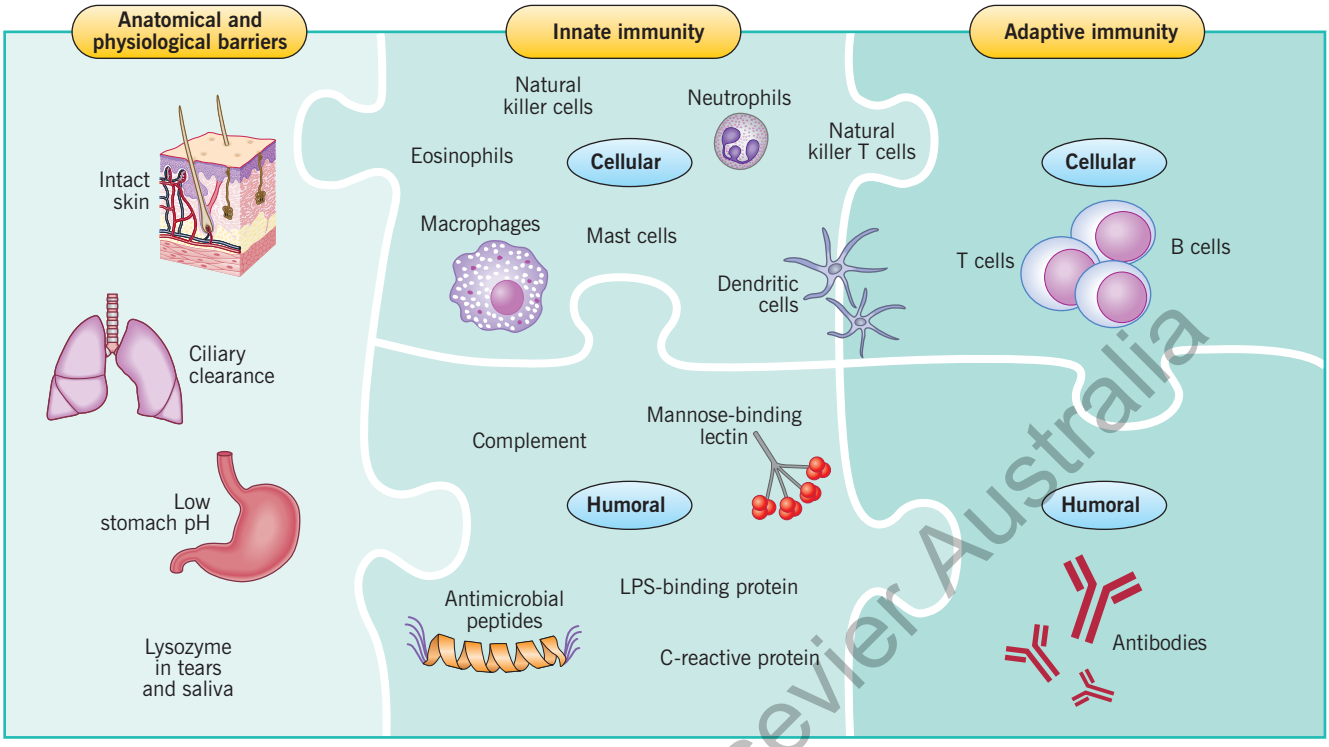


FIGURE 3.18 Innate and adaptive immune responses. The human microbial defence system can be simplistically viewed as consisting of three levels: 1) anatomical and physiological barriers; 2) innate immunity; and 3) adaptive immunity. Both innate and adaptive immunity can be further subdivided into cellular and humoral. The innate cellular immunity involves specific cell types such as macrophages or polymorphonuclear cells (eosinophils and neutrophils) that have non-specific antimicrobial responses while adaptive cellular immunity involves highly specialised T- and B- lymphocytes. Cells such as natural killer cells or dendritic cells are part of both types of cellular responses and are considered as the interface between the two responses. Humoral immunity will involve soluble molecules, proteins or peptides; in the case of innate humoral response these proteins, peptides or other molecules will non-specifically target pathogens while antibodies, part of the adaptive humoral response, will recognise specific epitopes on pathogens

Source: Malamed, S. F. (2022). Medical Emergencies in the Dental Office. Elsevier.

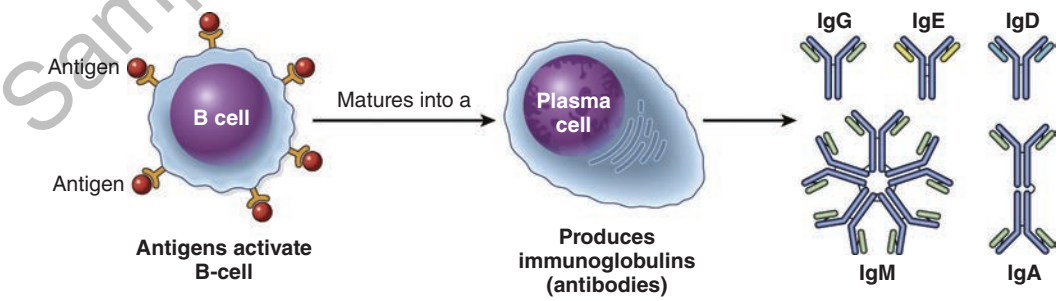


FIGURE 3.19 Production of immunoglobulin by B-cells. When an antigen activates a B-cell, they mature into a plasma cell and that then produces immunoglobulins (antibodies). The first classes of immunoglobulins produced are the IgM and IgD produced by mature B-cells. Following activation by specific signal, the plasma cells switch the production of immunoglobulins to classes IgE, IgA and IgG. See Table 3.7 for main roles of immunoglobulin classes

Source: Chabner, D. E. (2022). Medical Terminology: A Short Course. Elsevier.

effector helper cells (CD4 T-lymphocytes) and cytotoxic T-cells (CD8 T-lymphocytes) (Table 3.6). Adaptive immunity can be summarised by the following sequence of events: a CD4 T-cell encounters an antigen-presenting cell (dendritic cell) and gets activated via the engagement of receptors on its surface. The CD4 T-cell then releases molecules (cytokines) that will either activate B-cells that will in turn produce antibodies and engage the humoral response, or stimulate cytotoxic CD8 T-cells that will engage the cell-mediated response. Plasma cells and cytotoxic T-cells are called effector cells as their involvement ends up with the destruction of the pathogen or the infected host cell.

A critical link between innate and adaptive immunity is the dendritic cells which phagocytose pathogens that have entered tissues, process them and present antigens to the cells of the adaptive system (CD4 and CD8)—hence their name of professional antigen-presenting cells. Dendritic cells express at their surface pattern recognition receptors (PRR) such as toll-like receptors that recognise pathogen associated molecular patterns (PAMPs) on pathogens.

The immune system therefore responds to different microbes in specialised ways that are best to eliminate them. Broadly, the adaptive immune response can be separated into a pro-inflammatory or an anti-inflammatory response depending on the type of pathogen involved and the type of soluble molecules (cytokines and chemokines) secreted to regulate the recruitment, differentiation and activation or inhibition of immune cells. A T-helper cell type 1 (Th1) response is characterised by the overall production of pro-inflammatory molecules while a T-helper cell type 2 (Th2) is associated with the presence of anti-inflammatory molecules (Fig 3.20). To eliminate pathogens that require internalisation to survive, such as intracellular parasites, viruses or some intracellular bacteria,

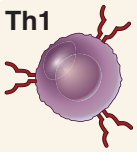
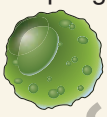
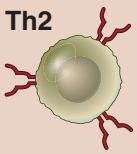
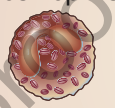
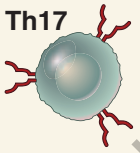
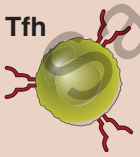
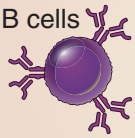
Effector T-cells	Defining cytokines	Principal target cells	Major immune reactions	Host defense	Role in disease
Th1 	IFN-γ	Macrophages 	Macrophage activation	Intracellular pathogens	Autoimmunity; chronic inflammation
Th2 	IL-4 IL-5 IL-13	Eosinophils 	Eosinophil and mast cell activation; alternative macrophage activation	Helminths	Allergy
Th17 	IL-17 IL-22	Neutrophils 	Neutrophil recruitment and activation	Extracellular bacteria and fungi	Autoimmunity; inflammation
Tfh 	IL-21 (and IFN-γ or IL-4)	B cells 	Antibody production	Extracellular pathogens	Autoimmunity (autoantibodies)

FIGURE 3.20 T-cell subsets and Th responses. Naïve CD4+ T-cells (have never been exposed to the foreign antigen) can differentiate into subsets of effector cells following interaction with an antigen or in response to cytokines. These Th responses are associated with a panel of cytokines that will in turn activate target cells that will have a specific role in the response to different pathogens. Th1 cells produce interferon-γ (IFN-γ), which activates macrophages to kill intracellular microbes. Th2 cells produce cytokines (interleukins, IL) that stimulate immunoglobulin E production and activate eosinophils in response to parasitic infection. Th17 cells secrete IL-17 and IL-22 that play an important role in responses to fungi. Tfh cells produce IL-21 and provide help to B-cells for antibody production

Source: Townsend, C. M. (2016). Sabiston textbook of surgery. Elsevier.

a Th1 polarised response is usually necessary. Conversely, for large extracellular parasites such as helminths, a Th2 response is usually present and is considered protective.

3.6.2 Immune response to extracellular pathogens (bacteria, yeasts, parasites)

The immune response to various types of pathogens will vary depending on how accessible they are to immune cells and molecules. The innate immunity is effective on pathogens that are not inside cells, whether they are strict extracellular pathogens or have an extracellular stage in their life cycle. This includes extracellular bacteria, worms, fungi, protozoa or viruses that can be found in interstitial spaces, blood, lymph or at the surface of epithelial cells. In these sites, pathogens can be targeted by antibodies (Table 3.7) such as immunoglobulin type A (IgA) that can block viral adherence and cell entry, immunoglobulin type M and G (IgM, IgG) that can activate the complement system and directly lyse pathogens and enveloped viruses, or they can be engulfed by phagocytic cells such as macrophages (increased phagocytosis is triggered, for instance, by the recognition of lipopolysaccharide on Gram-negative bacteria or the flagellin of motile bacteria such as *Listeria monocytogenes*).

Extracellular parasites such as helminths are too large to be phagocytosed and the parasite tegument coat cannot be penetrated by the complement proteins. Chronic exposure to worm antigens triggers a Th2-like response which results in activation of a sub-type of polymorphonuclear cells that play a major role in the innate immune response, the eosinophils and release of immunoglobulin type E (IgE) that all participate in the expulsion of the parasite. However, it can also lead to delayed type hypersensitivity (DTH) from a Th1-like response and activated macrophages, leading to the formation of tissue granulomas as seen around eggs during schistosomiasis.

3.6.3 Immune response to intracellular pathogens (bacteria, viruses, parasites)

The immune response to intracellular pathogens is a Th1-like response associated with the release of pro-inflammatory molecules that will activate macrophages and other antigen presenting cells. When the pathogen is digested, peptides are presented via the Major Histocompatibility Complex I and CD8 cytotoxic T-cells are activated in order to kill the infected cell. Natural killer cells may also be involved. This is the common response to most viruses. In addition, for pathogens such as *Legionella* spp., mycobacteria or *Cryptococcus neoformans* that have developed ways to survive inside vesicular structures of macrophages (legionella containing vacuoles [LCV], phagolysosomes) the immune system will need to activate further macrophages to reverse the changes triggered by the pathogen. In the case of tuberculosis for instance, the mycobacterium has developed a way to inhibit the antimicrobial responses of macrophages and survive within the phagolysosome.

3.6.4 Vaccines

Vaccination is a way to provide long-term protection against specific pathogens. A vaccine trains the cells of the immune system to specifically recognise and mount a protective immune response against a pathogen. As the response is remembered by specialised cells of the immune system (both memory T- and B-cells) the next time an individual is exposed to this same pathogen, this protective response can be effective before the pathogen has time to cause harm. During vaccination, an individual is usually injected (the vaccine can also be given orally or nasally) with part (peptide, toxin, surface protein, synthetic) or whole pathogen (weakened or inactivated) together with an adjuvant. This adjuvant usually contains molecules that will trigger a strong activation of the immune system, therefore priming it to better respond against the pathogen.

The importance of vaccine development has never been more realised than during the COVID-19 pandemic where several highly efficient vaccines were developed and released in record time. Vaccines generated using different approaches were widely discussed and mediatised in a way that was never seen before and scientific terms such as mRNA or PCR are now part of everyday language. However, the development of

TABLE 3.7 Main classes of immunoglobulins and their roles

Immunoglobulin class	IgM	IgG	IgE	IgA
Main function	Complement activation	Bind to pathogen and increase phagocytosis by recognition of its receptor by the phagocytic cell; complement activation	Immunity against helminths	Mucosal immunity: transport of IgA through epithelia to form a barrier and take up foreign antigens

vaccines is not always this successful. Looking at the different vaccines available to prevent human infections it is easy to see that they cover mostly viruses and bacteria. Although a vaccine against the HIV virus has been in development for decades and many candidates are in the pipeline, so far none has proved successful. There is also no known vaccine against human parasites. Only very recently, an anti-malaria vaccine, decades in the making, was released (RTS,S/AS01) and offered only to a very select population of children at risk of severe malaria in Africa. The hope, in the vaccine developers' community, is that the progress made to develop the COVID-19 vaccine will be able to be transferred to other infections.

There are several types of vaccines, from inactivated vaccines, live-attenuated vaccines, messenger RNA (mRNA) vaccines and subunit/recombinant/polysaccharide/conjugate vaccines to toxoid vaccines. Each type of vaccine uses a different approach to trigger a protective immune response:

- Inactivated vaccines use a killed form of the pathogen and are used to protect against hepatitis A, the flu, polio and rabies.
- Live-attenuated vaccines use the same pathogen that causes the disease but in a weakened form. They are used against measles, mumps, rubella (combined into the MMR vaccine), rotavirus, smallpox, chicken pox and yellow fever and often necessitate regular booster shots.
- Subunit/recombinant/polysaccharide/conjugate vaccines are designed to create a response against a specific protein or sugars from the pathogen. They are used against *Haemophilus influenzae* type b, hepatitis B, human papilloma virus, whooping cough, pneumococcal disease, meningococcal disease and shingles.
- Toxoid vaccines trigger a response that is directed against a toxin released by the pathogen instead of the pathogen itself. It is used against diphtheria and tetanus.
- Messenger-RNA vaccines (mRNA) are the most recent type of vaccine to be used in human health. These vaccines contain mRNA encoding a pathogen protein that is encapsulated in a lipid bubble that when delivered onto the host, is translated to produce the protein that will trigger an immune response. This technique bypasses the need to manufacture and purify pathogen proteins and considerably shortens the development time. Although studied for decades, and with several pathogens in the pipeline (HIV, flu, cytomegalovirus), the vaccine against COVID-19 is the only one that has been commercialised for humans.

3.6.5 Strategies used by pathogens to evade the immune system

Unsurprisingly, some microorganisms have evolved mechanisms to resist and evade host immunity; these mechanisms are important virulence determinants of various infectious agents.

Anatomical seclusion: Some pathogens live intracellularly, therefore avoiding both the innate and adaptive immune responses. Infected cells are not recognised by the immune cells unless they present foreign proteins on their surface. Viruses such as *Varicella zoster* (chicken pox) can hide from the immune system by invading neurons where they can remain for years, re-emerging if the defences of the host are lowered. Bacteria such as *Borrelia burgdorferi* (Lyme disease) and *Burkholderia pseudomallei* (meliodosis) can also remain hidden, with patients presenting symptoms years after the initial infection. During their life cycle, *P. vivax* and *P. ovale* produce a specific stage of the parasite (hypnozoite) that remains hidden in the liver and can start a new parasite cycle months or years after the initial mosquito bite and exposure to the parasite. The radical cure used to treat *P. vivax* infections must kill both the blood stages of the parasite as well as liver hypnozoites. By residing in immunologically privileged sites, i.e., where little immunosurveillance by immune cells occurs, such as the eye or the brain, helminths can also avoid the immune response.

Antigenic variation: This strategy has been adopted by many pathogens and allows them to change the molecules expressed at their surface, making it difficult for the immune system to mount specific responses that adapt to the changes. Some key examples are given below:

- *Plasmodium* spp. expresses different antigens (hundreds of variants) at different stages of its life cycle and the switch between each variant is very fast.
- *Giardia* spp. expresses one variant surface protein (VSP) at a time but has a repertoire of 190 VSPs and can spontaneously switch to a different variant.
- African trypanosomes have one glycoprotein (VSG) that covers the entire surface of the parasite and is immunodominant for antibody responses, but gene cassettes of VSGs (2000 genes involved) allow regular switching between the genes.
- Bacteria such as *Neisseria meningitidis* or *Streptococcus pneumoniae* are capable of capsule switching (there are 84 known *S. pneumoniae*, each with a different polysaccharide capsule, i.e.,

serotype). *Neisseria gonorrhoeae* is also capable of antigenic variation at the level of its pilin protein pilE by altering the sequence of the *pilE* gene, generating variability affecting the pilus function and allowing it to evade immune surveillance.

Molecular mimicry: Bacteria and parasites such as helminths can express surface proteins that are similar to host proteins, therefore avoiding being recognised as 'non-self' by the immune system. *Neisseria meningitidis* or some strains of *E. coli* have a capsule containing polysaccharides that are structurally similar to polysaccharides on the surface of mammalian cells. Although this pathway provides the pathogen with a survival advantage, the host immune system still has other ways to recognise the presence of the pathogen. In addition, molecular mimicry is not all advantageous for the pathogen as firstly, maintaining the production of the mimic protein can be energetically costly for the pathogen, and secondly, molecular mimicry has been associated with autoimmunity. This occurs because the immune response mounted against the pathogen can also target molecules from the host that have a similar structure therefore triggering a response against the self, also called autoimmunity. On the other hand, according to the 'hygiene hypothesis', there is an inverse association between exposure to pathogens such as parasites and the onset of autoimmune diseases, which means that the increase in occurrence of autoimmune diseases may be associated with less infections.

Manipulation of immune responses: A classic example of this strategy is the ability of helminth parasites, such as *Schistosoma* spp. or *Ascaris* spp., to inhibit the activation of innate immune cells, induce the production of T- and B-regulatory T-cells (characteristic of an immunotolerant phenotype) instead of effector T-cells (CD4 helper T-cells and CD8 cytotoxic T-cells) and antibody-producing plasma cells. One particularity of these helminths is the release of secretory molecules or helminth-derived products that are powerful modulators of inflammation. They can modulate toll-like receptor signals and trigger the production of anti-inflammatory mediators that prevent the expulsion of the parasite and allow its survival.

3.7 Diagnosis of pathogens

Accurate disease diagnosis and therefore pathogen identification are important as they help determine the course of treatment and patient outcome. In general, a sample is required and this is usually taken from where in the body the pathogen is causing disease. For example, nasal or throat swabs are often taken for respiratory infections, urine for urinary tract infections, faecal samples for gastrointestinal infections, cerebrospinal fluid for meningitis, pus for wounds or a blood sample for systemic infections. Diagnosis can be made directly on the appropriate specimen using a variety of techniques but in some cases may require culture of the pathogen to allow identification and test for susceptibility to antimicrobial drugs. Depending on whether the disease is in an acute or a chronic phase, different approaches can be taken to either identify the pathogen itself or the level of the immune response through the titration of specific immunoglobulins/antibodies. The latter approach, however, may not be indicative of current infection. The type of immunoglobulin detected can provide some information regarding the time of the infection, as immunoglobulins type M appear two to four weeks after infection while immunoglobins type G takes about four to six weeks to be detectable in the blood.

Direct microscopy of a specimen, whether it is a blood smear, a wet mount of urine, stools or a cerebrospinal fluid, remains the definitive diagnostic criteria for many infections, notably parasitic (malaria, trypanosomiasis, schistosomiasis, giardiasis) but also bacterial or fungal infections. Microscopic examination of a specimen can confirm the presence, quantity and identity of the type of organism and, in some cases, differentiate between species and therefore inform the appropriate treatment (e.g. *P. falciparum* vs *P. vivax*). However, notably in the case of parasitic infections, it is time consuming, and an experienced microscopist is essential in order to obtain the right diagnosis.

Microscopy is therefore often paired with faster and easier approaches, including the use of molecular techniques such as Quantitative Polymerase Chain Reaction (qPCR). This method amplifies a specific pathogen nucleic acid sequence (typically <500-bp) in a sample and is sensitive enough to detect very low levels. It is routinely used to detect and quantify viral, bacterial and parasitic pathogens. The method can target the DNA, or RNA in the case of many viruses, using Reverse Transcriptase qPCR (RT-qPCR) that allows conversion of RNA to DNA before qPCR. The use of qPCR and RT-qPCR to identify a pathogen in CSF can dramatically improve the outcome for patients by allowing identification within a few hours rather than the 24–48 hours needed to culture a bacterial pathogen, for instance. In the case of parasitic infections such as malaria, qPCR is the only definitive diagnostic method accepted outside direct microscopy.

Advanced methods such as matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) mass spectrometry (MS) are routinely used in diagnostic laboratories to identify bacteria or fungi (Bruker or Biomerieux). This simple and rapid method uses a protein fingerprint to identify pathogens down to the strain level if the instrument contains the appropriate library. Its sensitivity and specificity also allows identification of pathogens within a specimen such as blood or urine, therefore removing the need to culture the pathogen and significantly shortening the identification time and consequently the start of an appropriate treatment.

Immunological methods relying on the detection of antigens by ELISA (enzyme-linked immunosorbent assay), rapid detection tests (RDT, also called rapid antigen test, RAT) or card agglutination test (CAT) can be used to identify the presence of pathogen antigens in the bodily fluid tested (blood, serum, saliva, urine, CSF). They use the same principle where a major antigen from the pathogen is captured by a specific antibody immobilised on a plastic plate (ELISA), a nitrocellulose membrane (RDT) or on latex beads (CAT). The reaction can then be revealed by a colorimetric enzymatic method or the development of aggregates visible to the naked eye (Figs 3.21 and 3.22). ELISA are

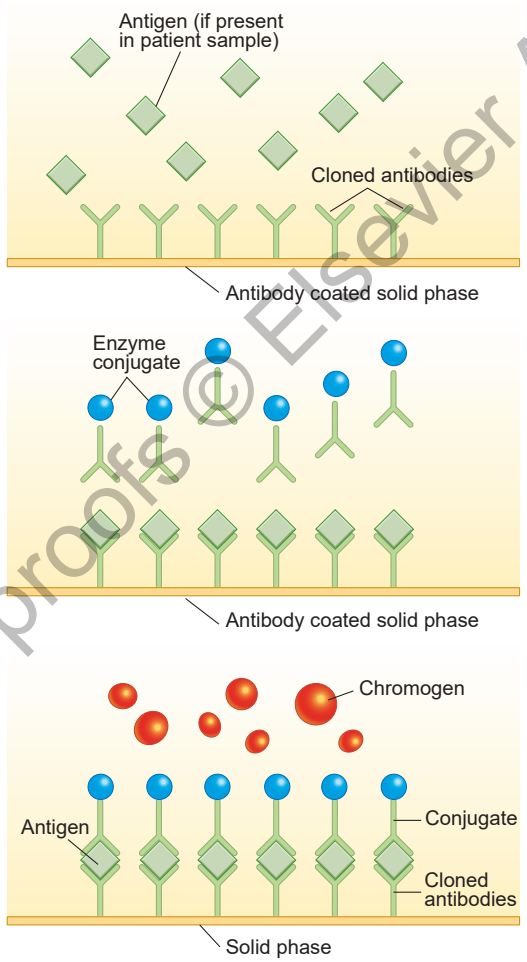


FIGURE 3.21 ELISA principle (a). An antibody specific for a pathogen antigen is coated on a surface. A suspension (patient plasma, serum, other bodily fluid or tissue lysate) containing (or not) the pathogen antigen is incubated with the coated antigen (top image). A second antibody (identical to the coated one or targeting another epitope of the pathogen antigen) is then added. This secondary antibody is conjugated with a signal molecule (middle image). This signal molecule is then recognised by a chromogen that allows the visualisation of the reaction (bottom image). A typical conjugate/chromogen is the enzyme-linked antibody to which is added a substrate, for instance horseradish peroxidase-conjugated antibody + hydrogen peroxide which will result in a brown colour. A standard curve performed with a synthetic form of the antigen allows the quantification of levels of antigen in the patient plasma.

Continued

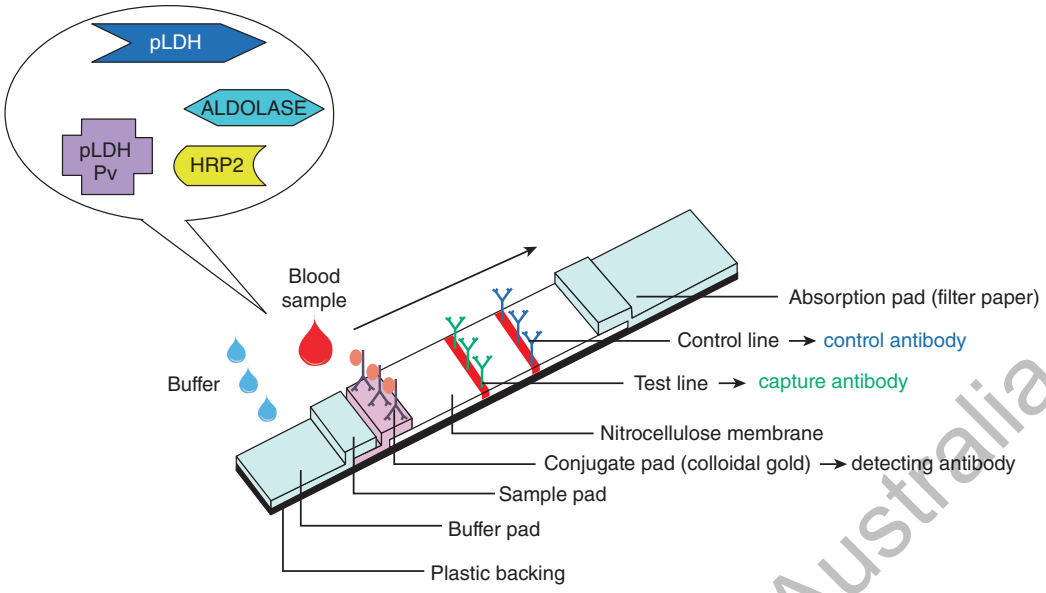


FIGURE 3.21 (cont'd) Schematic drawing of a malaria rapid diagnostic test (b). Blood and buffer are applied, respectively, to the sample and buffer pad. The content migrates via capillary action through the pad where it first encounters the conjugate pad, which contains a detection antibody against a Plasmodium antigen, such as PfHRP2, Pf-pLDH, Pv-pLDH, pan-pLDH or aldolase. This detection antibody is a mouse-antibody conjugated to colloidal gold. If present in the sample, the Plasmodium antigen is bound to this detection antibody-conjugate. Next, the antigen-antibody-conjugate complex migrates further until it is bound to the capture antibody, which binds to another site of the Plasmodium target antigen. As the capture antibody is applied on a narrow section of the strip, the complex with the conjugated signal is concentrated and the colloidal gold becomes visible as a coloured line. The excess of detection antibody-conjugate that was not bound by the antigen and the capture antibody moves further until it is bound to an anti-mouse antibody (control antibody), thereby generating a control line *PfHRP2: Plasmodium falciparum* histidine-rich proteins 2*; Pv-pLDH:* P. vivax-specific parasite lactate dehydrogenase*; pan-pLDH:* pan-parasite lactate dehydrogenase

Source: Maltha, J., Gillet, P., & Jacobs, J. (2013). Malaria rapid diagnostic tests in travel medicine. Clinical Microbiology and Infection, 19(5), 408-415.

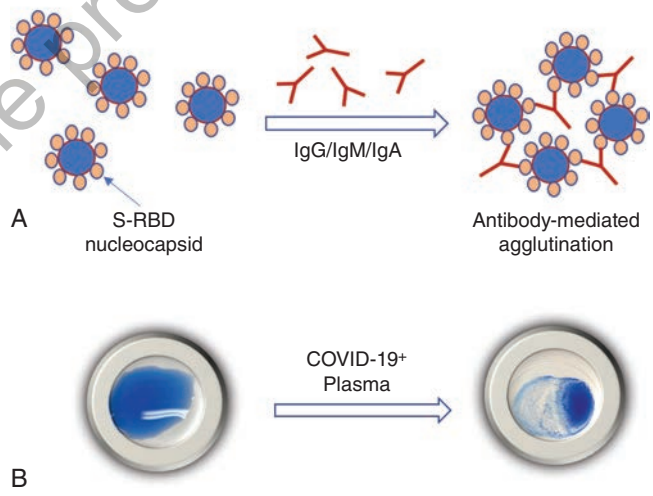


FIGURE 3.22 Illustration of the principle of agglutination assay for SARS-CoV-2 antibody testing. (A) Latex particles or red blood cells (RBCs) are coated on their surface with a SARS-CoV-2 antigen, S-RBD, or nucleocapsid. Incubation with plasma containing antibodies against the antigen will induce agglutination of the latex particles or RBCs. (B) Representative image of a positive agglutination assay using latex beads coated with S-RBD

S-RBD: SARS-CoV-2 receptor binding domain

Source: Esmail, S., Knauer, M. J., Abdoh, H., Voss, C., Chin-Yee, B., Stogios, P., ... & Li, S. S. C. (2021). Rapid and accurate agglutination-based testing for SARS-CoV-2 antibodies. Cell reports methods, 1(2), 100011.

widely employed in epidemiology where they can be used for high throughput testing of samples, while RDTs are useful for suggestive diagnostic criteria giving rapid results (usually 15–30 min). Due to their capacity to give false positives or negatives in a minority of cases, they usually need verification by qPCR. The COVID-19 pandemic has seen the use of RDTs increase significantly to allow the general public to punctually self-test, or to perform large screening of populations as seen prior to HSC exams in Australia. RDTs also exist for malaria where they can detect antigens common to all *Plasmodium* species or specific to either *P. falciparum* or *P. vivax*. CAT is often used in low-income countries to perform mass screening of populations for trypanosomiasis, for instance. However, agglutination tests are widely used in microbiology to discriminate between different pathogens to discriminate between pathogens of the same genus (beta-haemolytic *Streptococci* via Lancefield grouping, *Staphylococci* via coagulase test), to determine the serotype of pathogens (*Staphyloccoci*, *Salmonella*) or to identify a pathogen within a CSF among the main causative agents of meningitis (CSF test).

As mentioned above, accurate pathogen identification and disease diagnosis will inform the appropriate treatment; however, another parameter needs to be entered into the equation due to antimicrobial resistance (AMR): antimicrobial susceptibility. This often requires culturing the pathogen and can be performed manually using techniques such as the EUCAST (European Committee for Antimicrobial Susceptibility Testing) method that is widely used in Australia. These methods are time consuming, detect sensitivity to selected antimicrobials and cannot identify all resistant strains. Instruments such as the VITEK 2 (Biomerieux) combines high throughput identification and antimicrobial sensitivity testing. The system records simultaneously fluorescence, turbidity and colorimetric signals and can provide minimum inhibitory concentrations (MICs) for all antibiotics tested. A possible future alternative is next generation sequencing (NGS) such as Illumina, PacBio and Nanopore sequencing technologies that are fast becoming cheap.[10] These allow samples to be whole genome sequenced and the sequence interrogated for pathogens and any AMR genes they harbour using bioinformatics (the science of analysing complex biological data such as nucleic acid sequences). Importantly, the presence of an antimicrobial resistance gene does not tell you anything about the MIC like culture-based methods possibly excluding an antimicrobial that may be effective.

Beyond AMR, NGS techniques are becoming highly valuable in investigating infectious disease outbreaks. Comparison of pathogen genomes is highly useful in identifying possible sources and reservoirs. Tied with databases comprising of clinical and environmental samples from around the globe, the movement of pathogens and specific genetic elements (e.g. plasmids) can be tracked. During the COVID-19 outbreak, genome sequences identified multiple variants and was useful in tracking their movement. At a fine scale, genome sequencing could be used to determine if a viral infection was locally acquired or acquired from another geographic area, thus helping to identify transmissive pathways (e.g. leaks from quarantine facilities).

3.8 Therapies for pathogens

By and large, the bulk of therapies for pathogens are antimicrobial chemotherapeutics that target the pathogen; drugs that target toxins produced by the pathogen; or drugs that modify the immune response the pathogen elicits. Where there are no chemotherapeutics, symptom management is used.

3.8.1 Antimicrobial chemotherapeutics

Simply, these are chemicals used in medicine to kill or inhibit the growth of pathogens. Antimicrobials can be subdivided into categories depending on the microorganism/virus they are active against, such as antibacterials, antivirals, antifungals or antiprotozoals. Often, the term 'antibiotic' is used which, historically, is defined as a chemical produced by a microorganism to kill or inhibit the growth of another microorganism and which, in nature, is used by microorganisms to outcompete each other. For the most part, antimicrobial chemotherapeutics are antibiotics and have simply been repurposed for medical therapy. These chemicals have been chemically modified to create newer antibiotics (semi-synthetic antibiotics) that have altered characteristics allowing oral administration, broadening their spectrum of activity or to overcome resistance. It is important to note that as most antibiotics are antibacterial, the term 'antibiotic' has become synonymous with 'antibacterial'.

Key to the success of chemotherapeutic antimicrobials is selective toxicity, an important feature that requires the drug to selectively kill or inhibit the pathogen at a concentration that does not adversely affect host cells. Additionally, the drug must be capable of reaching a concentration at the site of infection that will have activity. Antimicrobials can be delivered topically, orally or intravenously. Topical or oral delivery are

ideal as they can be self-administered by the patient; however, some antimicrobials cannot be delivered in this manner for reasons such as being destroyed by the acidic pH of the stomach or not reaching the desired concentration at the site of infection and these therefore require intravenous delivery. Some antimicrobial drugs are narrow spectrum, meaning they only attack certain microorganisms, whereas broad spectrum drugs will attack a wider range. Generally, narrow spectrum drugs are less likely to drive antimicrobial resistance due to the lesser selection pressure they impose.

A greater range of antibacterial chemotherapeutics exist than antifungals, antiprotozoals and antivirals, arguably due to the number of sites that are distinct between bacterial and eukaryotic host cells. As fungi and protozoa are eukaryotic, identifying drug targets that are non-toxic to host cells is more challenging, particularly for systemic infections. Superficial mycoses are easier to treat using topical treatments. Being intracellular obligate pathogens, viruses use host cell machinery for viral replication and so antivirals tend to target specific nucleic acid viral replicative enzymes or other processes unique to the viral life cycle, such as blocking adhesion or entry into the host cell or blocking uncoating. In bacteria, antibacterial chemotherapeutics target five main mechanisms: peptidoglycan cell wall synthesis; protein synthesis; nucleic acid synthesis; function of the cell membrane; and metabolic pathways. Bacterial resistance to these drugs is from one or more of the following mechanisms: reduced permeability to the drug; drug inactivation; alteration of drug target; modification of a metabolic pathway; or effective efflux of the drug.

3.8.2 Bacteriophage therapy

Bacteriophage therapy uses viruses that infect bacteria (called bacteriophage) to treat infection. Although bacteriophage therapy is not new, it has only recently been welcomed into Western medicine due to rising rates of antimicrobial resistance.[11] The therapy relies on cultivating and purifying bacteriophages that are active against a bacterial pathogen and administering it to an infected patient. They have the benefit of multiplying as they infect and kill their target, thus not requiring as many doses as antibacterials and, as they are very specific to their target bacteria, they do not disrupt the host microbiome. The disadvantage of this specificity is that it is difficult to isolate single bacteriophage that are active against all strains of a pathogen. Additionally, resistance to the bacteriophage can develop. As a result, multiple bacteriophages are usually delivered to minimise the likelihood of resistance developing and to cover a variety of pathogenic strains.

3.8.3 Monoclonal antibody (MAb) therapy

Monoclonal antibody (MAb) therapy potential has been studied for many years and is increasingly promising for treatment of infectious diseases. Several of these MAbs are currently in clinical trials or approved, notably to target bacterial toxins such as the bezlotoxumab for *C. difficile* TcdB or MEDI4893 to neutralise the haemolysin of *Staphylococcus aureus* and prevent invasion. More work is underway to produce MAbs against outer membrane surface proteins notably involved in bacterial adhesion or immune evasion or against polysaccharides. In 2020, approval was given by the US Food and Drug Administration for a co-administration of casirivimab and imdevimab (Regen-Cov) to treat mild to moderate COVID-19 in adults and paediatric patients. In August 2021, the Australian Therapeutic Goods Administration (TGA) granted provisional approval for the use of sotrovimab that was shown to reduce the risks of COVID-19 disease progression.

3.8.4 Other therapies

In some instances, tissue injury and disease may be caused both by the host response to the microbe and the infectious agent itself. Therefore, eliminating the pathogen is important but limiting or controlling the host response is also primordial. To that effect, adjunctive therapies are administered at the same time as the antimicrobial treatment. For instance, during a septic shock, treatment is given to maintain blood pressure and during meningitis corticosteroids can be administered to prevent excessive brain oedema. In cases of diphtheria or tetanus, anti-toxins are used to block the deleterious effects of the toxins produced by the bacteria.

Useful websites/resources

- Pathology Tests. The Royal College of Pathologists. https://www.rcpa.edu.au/Manuals/RCPA-Manual/
- Pathology-Tests Test Reference Manual. QML Pathology. https://www.qml.com.au/clinicians/testreference-manual/
- The Australian Society of Microbiology. https://www.theasm.org.au/
- The AustralianVirology Society. https://www.avs.org.au/

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