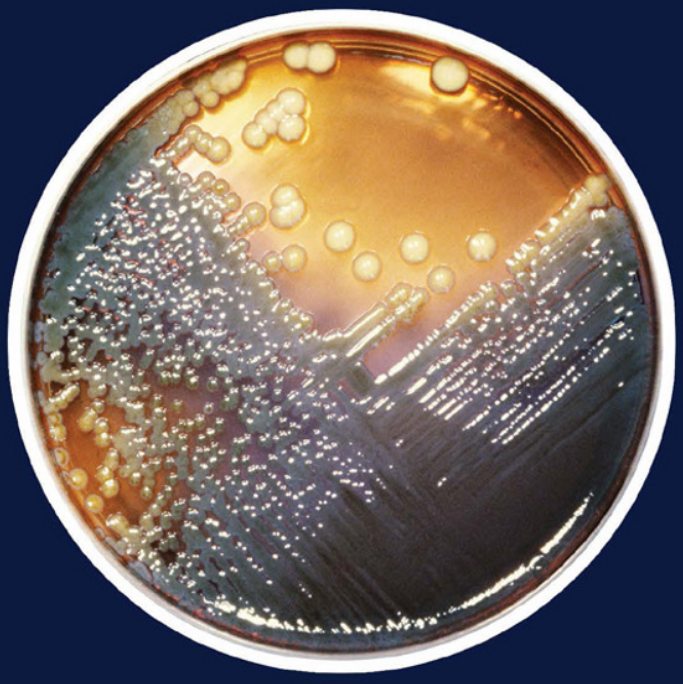
Ninth Edition

Hugo & Russell's Pharmaceutical Microbiology



Edited by
Brendan F. Gilmore & Stephen P. Denyer

with website

WILEY Blackwell

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Preface to the First Edition

When we were first approached by the publishers to write a textbook on pharmaceutical microbiology to appear in the spring of 1977, it was felt that such a task could not be accomplished satisfactorily in the time available.

However, by a process of combined editorship and by invitation to experts to contribute to the various chapters, this task has been accomplished thanks to the cooperation of our collaborators.

Pharmaceutical microbiology may be defined as that part of microbiology which has a special bearing on pharmacy in all its aspects. This will range from the manufacture and quality control of pharmaceutical products to an understanding of the mode of action of antibiotics. The full extent of microbiology on the pharmaceutical area may be judged from the chapter contents.

As this book is aimed at undergraduate pharmacy students (as well as microbiologists entering the pharmaceutical industry), we were under constraint to limit the length of the book to retain it in a defined price range. The result is to be found in the following pages. The editors must bear responsibility for any omissions, a point which has most concerned us. Length and depth of treatment were determined by the dictate of our publishers. It is hoped that the book will provide a concise reading for pharmacy students (who, at the moment, lack a textbook in this subject) and help to highlight those parts of a general microbiological training which impinge on the pharmaceutical industry.

In conclusion, the editors thank most sincerely the contributors to this book, both for complying with our strictures as to the length of their contribution and for providing their material on time, and our publishers for their friendly courtesy and efficiency during the production of this book. We also wish to thank Dr. H. J. Smith for his advice on various chemical aspects, Dr. M. I. Barnett for useful comments on reverse osmosis, and Mr. A. Keall who helped with the table on sterilisation methods.

W. B. Hugo
A. D. Russell

Preface to the Ninth Edition

When we first started planning for this edition in 2017, we could not have foreseen how many aspects of microbiology, particularly pharmaceutical microbiology, would come to prominence in the following pandemic years. Now, as we are slowly emerging from COVID-19, we have first-hand experience of the vital importance of antisepsis, infection prevention and control measures, epidemiology, immunology, vaccine development and an understanding of viral pathogenicity. The power of the pandemic to mobilise individual, national, international and commercial effort to combat infection and research new measures of treatment and immunisation has been inspiring. It is against this backdrop that many of our authors have been preparing their chapters, sometimes in the most demanding of circumstances; we thank them for their willing contribution and for their perseverance. Throughout this time, the understanding and patience of our publishers have been much appreciated.

While the structure of this edition draws much from the previous one, all chapters are revised, some with new authors, and they incorporate many significant developments in the discipline. These include: the gathering momentum of antibiotic resistance; the risk of microbicide cross-resistance; emerging pathogens; healthcare-associated infection; new vaccine technologies; advances in pharmaceutical production; and alternative strategies to antibiosis. Inevitably COVID-19 features, and while our understandings and conclusions may change with long-term analysis, our authors have attempted to draw as many reliable insights as possible while the pandemic progresses.

For this edition, we would like to acknowledge the contribution of past editors, Sean Gorman and Norman Hodges (who both remain as authors), and Barry Hugo and Denver Russell who were instrumental in recognising and building the discipline of pharmaceutical microbiology. They would not have been surprised at the contribution it now makes to safeguarding society.

S. P. Denyer
B. F. Gilmore

About the Companion Website

This book is accompanied by a companion website.

https://www.wiley.com/go/HugoandRussells9e

This website includes:

- Figures from the book available to download in PowerPoint.

Part 1

Introducing Pharmaceutical Microbiology

1

Introduction to Pharmaceutical Microbiology

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CONTENTS

1.1 Pharmaceutical Microbiology: Microorganisms and Medicines

1.1.1 The Discipline of Pharmaceutical Microbiology

In its most literal sense, pharmaceutical microbiology is the study of microorganisms relevant to pharmacy and the pharmaceutical sciences. As a branch of the much wider discipline of applied microbiology, however, it is also concerned with understanding the fundamental importance of microorganisms in global health and disease. Pharmaceutical microbiology therefore includes an understanding of: the fundamentals of microbial physiology and pathogenicity, including host interactions; immunological products; the design, manufacture and appropriate use of antibiotics and other antimicrobial agents; strategies to prevent the emergence of resistance; practices in public health designed to control infectious disease; environmental design for the manufacture of medicinal products and medical devices; microbiological control in the preparation of pharmaceutical products and their preservation during use; the beneficial exploitation of microorganisms, including pharmaceutical biotechnology; and advances in molecular microbiology.

1.1.2 Microorganisms and Medicines

The observed steady improvement in global public health and the increasing trajectory of human life expectancy owes much to improved sanitation and healthcare, alongside better nutrition and a wider availability and access to effective medicines for the treatment and control of human and animal diseases. Indeed, the opening comments in previous editions of *Hugo & Russell's Pharmaceutical Microbiology* have reflected positively on global trends in controlling infectious disease, whilst recognising that still microbial infections and diarrhoeal diseases remain the leading causes of death in low- and low-middle-income countries (LMICs). Two infectious diseases, smallpox and rinderpest, the high-mortality cattle disease, have been declared eradicated by the World Health Organisation (WHO). At the time of writing, polio, once a global epidemic, remains endemic in only two countries, Afghanistan and Pakistan, with Africa having been declared free of wild-type polio in 2020. It is now expected that polio, and parasitic infection by the guinea worm *Dracunculus medinensis*, will be eradicated in the next few years. These and other significant advances in public health are major achievements, but a new threat has emerged, antibiotic

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resistance, which has been referred to as the silent pandemic. Antibiotics have been estimated to extend human lifespan by 23 years on average, yet the emergence of antibiotic resistance to all current classes of antibiotics has been reported, and no major new class of antibiotic drug has been brought to the market in over 35 years. In 2019, the Institute and Faculty of Actuaries (UK) published their antibiotic resistance working party report on the impact of antimicrobial resistance (AMR) on mortality and morbidity, which predicted for the first time a reduction in UK life expectancy attributable to AMR. A recent analysis of the global burden of antimicrobial resistance estimated that in 2019, 1.27 million deaths were attributable to antibiotic-resistant bacteria, greater than from either human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) or malaria. Antibiotic resistance is a problem not of the future, but of the present.

The development of the many vaccines and medicines that have been crucial to the improvement in world health has been the result of the large investment in research and development made by the major international pharmaceutical companies and also by national governments investing in research infrastructure and training. As a result, the research, development and production of pharmaceuticals has become one of the most successful, profitable and important industries in many countries worldwide. The global pharmaceutical market has experienced unprecedented growth in the past two decades, from $390 billion in 2001 to $1.27 trillion by the end of 2020. In the UK, the pharmaceutical industry is a major contributor to the national economy, with 2 of the top 15 global pharmaceutical companies headquartered there, representing a 2.5% share of the global pharmaceutical sector. The UK industry generates a turnover of £36.7 billion, with an export market value of £23.4 billion and an import value of £21.4 billion. It employs more than 63,000 people, and whilst 41% of pharmaceutical products are exported, 30% are for the domestic UK market and the remainder are substances used in the manufacture of other pharmaceutical products. Overall, the UK pharmaceutical industry contributes £14 billion (gross value added, GVA) to the UK economy. During the coronavirus disease (COVID)-19 global pandemic, the UK pharmaceutical industry supported 68 commercial COVID-19 clinical trials in 2020 alone.

The growth in the pharmaceutical industry in recent decades has been paralleled by the development and implementation of increasingly rigorous and stringent standards and regulations for pharmaceutical product manufacture and quality. Manufacture or assembly of a pharmaceutical product must be conducted under license from the appropriate authority (e.g., the Medicines and Healthcare Products Regulatory Agency [MHRA] in the UK, or the Food and Drug Administration [FDA] in the USA), and manufacturers must demonstrate compliance with current good manufacturing practice (GMP) guidelines, the minimum standards that a medicines manufacturer must meet in their production process. The final product must meet all current standards for quality, safety and efficacy; many of these standards are harmonised across national bodies. Most medicines or pharmaceutical products are complex formulations containing the active ingredient formulated with inactive excipients which ensure the stability, safety and efficacy of the final product. Whilst the efficacy and safety of the active agent or drug fall within the domain of the pharmacologist and the toxicologist, respectively, ensuring the quality of the final pharmaceutical product requires a multidisciplinary approach. Analytical chemists and pharmacists/pharmaceutical scientists take lead responsibility for ensuring that the components of the final formulation are present in the correct physical form and concentration; however, quality is not established solely on the physicochemical properties of the product but also through stringent microbiological quality control to ensure formulation efficacy and safety.

Whilst not yet a feature of this book, the appearance of three-dimensional (3D) printed pharmaceuticals will be a matter for consideration in the future. This technology signals a potential shift from the traditional centralised mass production of medicines to the point-of-care manufacture of discrete batches of highly personalised dosage forms for particular patient and clinical circumstances. Thus, GMP approaches towards quality and safety that have been designed around conventional medicine manufacture will need to be adjusted to include real-time quality-assurance mechanisms, GMP-compliant 3D printer validation and new requirements to accommodate multiple manufacturing sites. The first 3D printed oral antiepileptic drug, levetiracetam, was approved for human use by the FDA in 2015.

It is obvious that medicines contaminated with potentially pathogenic microorganisms pose a significant risk of harm to the end-user, especially in vulnerable patients. Indeed, medicines to be administered to patients parenterally, that is, other than via the gastrointestinal tract, which include intravenous, intramuscular, subcutaneous and intraspinal injections, and ocular, otic and intranasal products, must be sterile and are marketed as sterile products. They must also be free from microbially derived toxins and pyrogens, including lipopolysaccharides, which can lead to anaphylactic reactions. Perhaps less predictable, although still self-evident, the presence of microorganisms in pharmaceutical products can cause physical and chemical spoilage, which may lead to changes in the formulation itself or to degradation or decomposition of the active drug and/or excipients. This can result in sub-therapeutic concentrations of the active drug in the contaminated formulation or impaired delivery characteristics, both leading to

therapeutic failure, or the presence of toxic drug metabolites. Physical and chemical changes to the formulation may be obvious, such as changes to odour, flavour or elegance of the product, which would lead to lack of patient acceptance. Thus, it is clear that pharmaceutical microbiology must encompass the practices of sterilisation and preservation of often complex formulations capable of supporting the growth or survival of contaminating microorganisms. The pharmacist or pharmaceutical scientist with responsibility for the safe manufacture of medicines must appreciate the factors which predispose to product spoilage and how these might be managed by effective product design, and also the sources of potential contaminants and their control by good manufacturing practices. In these respects, the pharmaceutical microbiologist has much in common with microbiologists in the food and cosmetic industries, and many practices have been successfully shared.

The properties of antimicrobial chemicals used as disinfectants, preservatives and antiseptics (often termed microbicides) have direct relevance to the pharmacist and pharmaceutical scientist responsible for preserving formulated products and for managing microbiological risks in the manufacturing environment, and also because antiseptics and disinfectants are pharmaceutical products in their own right. However, they are not the only antimicrobial agents with which the pharmaceutical microbiologist must be familiar; antibiotic medicines are one of the most important and frequently prescribed pharmaceutical products, and are a major focus of several chapters of this book. The term antibiotic was originally used to refer to a naturally occurring substance, produced by one microorganism which inhibits the growth of, or kills, another microorganism. This strict definition of an antibiotic as a microbial metabolite did not, however, allow for the many generations of semi-synthetic compounds based on naturally occurring antibiotic templates and wholly synthetic agents (although significantly fewer in number) arising from high-throughput synthetic compound library screening efforts. The manufacture, quality control, formulation and, of particular relevance given the global crisis of antibiotic resistance, the appropriate use of antibiotics are important areas of knowledge which contribute significantly to the discipline of pharmaceutical microbiology.

The study of microorganisms continues to be important in both drug discovery and pharmaceutical manufacture. Screening of microorganisms for the production of bioactive agents has led not only to the discovery of almost all of the classes of antibiotics which are clinically used, but also to a number of important antifungal (such as nystatin, griseofulvin and amphotericin B), anti-cancer (including actinomycin, bleomycin, taxol and doxorubicin) and immunosuppressant (sirolimus and tacrolimus) agents in current clinical use. Following the discovery of penicillin in 1928, commercial antibiotic production began in the 1940s with the large-scale fermentation of *Penicillium chrysogenum* for production of benzylpenicillin (penicillin G). For many years, antibiotics were the only significant example of an active drug that was manufactured using microorganisms. However, exploitation of microorganisms for the manufacture and modification of steroids in the 1950s, and the development of recombinant DNA technologies in the last three decades of the twentieth century, has given significant momentum to the use of microorganisms in the production of pharmaceuticals and has supported a burgeoning biotechnology sector. The global market for recombinant DNA technology is forecast to reach $844.6 billion by 2025, with medical products dominating the market in terms of revenue generation. Biopharmaceuticals, or biological medical products (sometimes shortened to 'biologics' or 'biologicals'), broadly defined as pharmaceuticals inherently biological in nature and manufactured using biotechnology or bioprocesses (including fermentation and recombinant DNA technology/heterologous expression in *Escherichia coli*), represent a major proportion of all drugs under clinical development with an anticipation that biologics will account for 50% of drugs under development in the coming decade. Whilst a traditional focus for pharmaceutical interest in microorganisms has been their control, the more recent exploitation of microbial metabolism for the manufacture of drugs and the particular regulatory and quality control challenges of products arising through biotechnological and bioprocessing pipelines is now an area of knowledge which is of central importance to the discipline of pharmaceutical microbiology. This will be of increasing significance not only in the pharmacy and pharmaceutical sciences curricula, but also those of disciplines employed in the pharmaceutical industry. Table 1.1 summarises the benefits and uses of microorganisms in pharmaceutical manufacture, alongside more widely recognised hazards and problems they present.

Looking ahead, an understanding of microbial physiology and genetics will be increasingly important within the discipline of pharmaceutical microbiology, both in terms of the production of new therapeutic agents and in the understanding of infection microbiology, where host–pathogen interactions and the impact of the human microbiome on drug metabolism and its role in health and disease are becoming increasingly clear. The accessibility of pharmacists to the public often leads to them being called upon to explain the terminology and concepts of genetics and the biological sciences to patients. This has been evident during the global COVID-19 pandemic, which has been characterised by multiple genetic variants of the severe acute respiratory syndrome (SARS)-CoV-2 virus, rapid development of novel mRNA vaccine

Table 1.1 Microorganisms in pharmacy: benefits and problems.

Benefits or uses	Related study topics	Harmful effects	Related study topics
The manufacture of: Antibiotics Steroids Therapeutic enzymes Polysaccharides Products of recombinant DNA technology	Good manufacturing practice Industrial 'fermentation' technology Microbial genetics	May contaminate non-sterile and sterile medicines with a risk of infection	Non-sterile medicines: Enumeration of microorganisms in the manufacturing environment (environmental monitoring) and in raw materials and manufactured products Identification and detection of specific organisms
Use in the production of vaccines	Quality control of immunological products		Sterile medicines: Sterilisation methods Sterilisation monitoring and validation procedures Sterility testing Assessment and calculation of sterility assurance Aseptic manufacture
As assay organisms to determine antibiotic, vitamin and amino acid concentrations	Assay methods		
To detect mutagenic or carcinogenic activity	Ames mutagenicity test		
As adjuncts or alternatives to antibiotics	Bacteriophage, lysins and probiotic therapy		
		May contaminate non-sterile and sterile medicines with a risk of product deterioration	Enumeration, identification and detection as above, plus: Characteristics, selection and testing of antimicrobial preservatives
		Cause infectious and other diseases	Immunology and infectious diseases Microbial biofilms Microbiome Characteristics, selection and use of vaccines and antibiotics Infection and contamination control Control of antibiotic resistance Alternative strategies for antimicrobial chemotherapy
		Cause pyrogenic reactions (fever) when introduced into the body even in the absence of infection	Bacterial structure Pyrogen and endotoxin testing
		Provide a reservoir of antibiotic resistance genes	Microbial genetics

technologies and delivery of a vaccination programme in part through community pharmacies. Unfortunately, at the time of writing, the UK measles, mumps and rubella (MMR) vaccination rates are at their lowest in 10 years, which has been attributed to lack of parental knowledge of the significant risks associated with infections such as measles in children, and misinformation regarding potential adverse effects. The pharmacist's scientific training in

pharmaceutical microbiology is critically important in advancing public health through patient understanding of the underpinning scientific concepts. The re-emergence of bacterial infections which were once associated with high mortality rates, such as tuberculosis and diphtheria, as antibiotic-resistant infections is posing an additional threat to public health, alongside threats from new pathogens; this latter has been illustrated in particular by SARS-CoV-2 and the global COVID-19 pandemic, estimated in October 2022 to have caused more than 634 million infections and over 6.62 million deaths globally.

The ability of microorganisms to adapt to new environments and exploit changes in modern clinical practices are also considerations within the discipline of pharmaceutical microbiology. The ability to conduct a wider range of routine and life-saving surgeries, and the demographic trend towards ageing populations, has led to an increased reliance on implantable medical devices, made from a variety of materials and used to support normal physiological functions. These include urinary catheters, ureteral stents, endotracheal tubes, central venous catheters, intraocular lenses, prosthetic joints and heart valves. Undoubtedly, such devices have saved countless lives and improved the quality of life for many millions of patients, but they come with the inherent risk of infectious complications. Many bacteria, including commensal bacteria, and fungi are capable of adhering to implantable medical device surfaces and, through production of extensive extracellular polymeric substances, can form biofilms which are often characterised by a uniquely high tolerance to antimicrobial challenge and resistance to clearance by the host's immune system. Biofilms are a source of chronic, recurrent infection which are typically only resolved on removal and replacement of the colonised device, which may lead to discomfort or extended morbidity for the patient, increased risk of mortality and increased attendant care costs to healthcare providers. The development of strategies for the accurate assessment of antimicrobial susceptibility in, and antimicrobial selection for, device-associated biofilm infections and strategies for the prevention and elimination of these infections is a challenge for pharmacy practitioners and other healthcare professionals.

The participation of microorganisms in human disease other than by clear-cut infection is becoming increasingly recognised. This is no more obvious than in the role of a healthy microbiome, where the dysbiosis caused by antibiotic therapy can have a dramatic effect on chronic diseases, with growing evidence that perturbations in the gut microbiome are not only associated with intestinal disorders (inflammatory bowel and coeliac disease) but also extra-intestinal disorders including cardiovascular disease, type-2 diabetes, allergy, asthma, obesity and central nervous system disorders (the impact of the so-called 'gut–brain axis'). Further examples include: the finding that *Helicobacter pylori* is implicated not only in peptic ulceration but also stomach cancers; the oncogenic nature of certain viruses (e.g., the association of human papilloma virus [HPV] and cervical cancer); and recent findings that suggest the Epstein–Barr virus may cause multiple sclerosis. Such discoveries offer the possibility that in situations where microbial infection has a clear link to the development of chronic disease, vaccination programmes could have a preventative role to play. This is best evidenced in the recent widespread school-based immunisation programme against HPV aimed at the prevention of cervical cancer, rather than the infection itself. Whilst not all chronic diseases will be found to have an association with infection, advances in genomic sequencing technologies now permit further examination of the interplay between infectious agents, microbiota, and chronic disease, and it is likely that more relationships will be discovered in the future.

Clearly, a knowledge of the mechanisms whereby microorganisms are able to resist antibiotics, colonise medical devices and cause or predispose humans to other disease states is essential in the development not only of new antibiotics, but of other medicines and healthcare practices, including protection of the host biome, which will minimise the risks of these adverse situations developing.

1.2 Scope and Content of the Book

In the manufacture of medicines, the criteria and standards for microbiological quality are governed primarily by the intended route of administration. The vast majority of medicines intended for oral administration or application to the skin are not required to be sterile. Non-sterile pharmaceuticals, such as creams, ointments, oral suspensions and so on, may contain some microorganisms, within strict acceptance criteria as to the number and type, whereas all parenteral formulations, for example, injections and ophthalmic preparations, must be sterile, that is, free from all living microorganisms. Products for administration at various other sites (nose, ear, vagina and bladder, for instance) are usually sterile formulations but not invariably so (Chapter 22). The microbiological quality of non-sterile pharmaceuticals is controlled by specifications (typically defined in the relevant pharmacopoeial standards) defining the number of organisms that may be present, and requiring the absence of specific, potentially pathogenic microorganisms (also called *objectionable* organisms). To fulfil these stringent requirements, the ability to isolate and identify the microorganisms present, to detect those that are prohibited from particular

categories of pharmaceutical product and to enumerate microbial contaminants in the manufacturing environment, raw materials and the finished product are essential for the pharmaceutical microbiologist (Chapters 2–6). Knowledge of the characteristics of antimicrobial preservatives, included in formulations to restrain growth of microorganisms and prevent product spoilage during storage and use by the patient, and the means of assessment of preservative efficacy within complex formulations are also critical for demonstration that a product conforms to the relevant microbiological quality standards (Chapters 17–19).

For sterile products, quality criteria are simple: there should be no detectable microorganisms whatsoever. The product must be able to pass a sterility test, and a knowledge of the experimental design and procedures, and interpretation of results of these tests alongside understanding concepts of sterility assurance levels and process validation are important aspects of pharmaceutical microbiology (Chapter 21). In addition to stringent requirements for sterility, parenteral products for injection are also required to be free from pyrogens; these are substances which cause a rise in body temperature when administered to the patient. Strictly, any compound capable of causing fever following administration is classified as a pyrogen; however, with respect to pharmaceutical formulation, the vast majority of pyrogens are of bacterial origin, with thermally stable lipopolysaccharides from Gram-negative bacteria a particular issue (Chapter 3). Therefore, the detection, assay and removal of bacterial pyrogens (endotoxins) fall within the realm of pharmaceutical microbiology (Chapter 22).

There are two main strategies for the manufacture of sterile medicines. The first, and most straightforward, is to make the product, package it in its final market container and sterilise it by heat, radiation or other means. This approach, known as terminal sterilisation (Chapter 21), is the preferred option, as samples of the batch may be assessed for sterility to provide an assurance of sterility of the population of sterile items. The alternative is to formulate the product using sterile ingredients under conditions that do not permit entry of contaminating microorganisms (aseptic manufacture, Chapters 17 and 22). This strategy is usually adopted when the ingredients or physical form of the product render it unstable, or heat- or radiation-sensitive. It is a suitable approach for the manufacture of sterile products which have a short shelf- or half-life. Those responsible for the manufacture of sterile products must be familiar with both aseptic manufacturing techniques and sterilisation procedures for different product types, and assessment of the microbiological quality of those formulations. Those who have caused to open, use or dispense sterile products should be aware of the aseptic manipulation procedures to be adopted to minimise the risk of product contamination.

The microbial spoilage of medicines has as its main consequence financial loss rather than harm to patients, since microbial spoilage is often detected, through change of odour, appearance and so on, before the formulation is used. However, the other major problem associated with microbial contamination of medicines, that of patient harm through initiation of infection, although uncommon, is far more important in terms of risk of morbidity and mortality in the patient (Chapters 7 and 17).

The range of antimicrobial drugs to treat infections is large, despite being restricted to a relatively small number of mechanistic classes, thanks to the discovery of antibiotic scaffolds which are amenable to modification and optimisation using medicinal chemistry approaches. This has given rise to multiple generations of antibiotics from the same class, for example, penicillins and cephalosporins. Whilst approval of new antibiotics used to depend on the demonstration of superiority to currently available drugs, the crisis in the antibiotic discovery pipeline means that the need to find new antibiotics to treat infections that were once manageable with available agents has become critical. Therefore, there has been some focus not only on developing narrow-spectrum antibiotics for specific difficult-to-treat infections caused by antibiotic-resistant pathogens, including the ESKAPE pathogens (Chapter 13), but also away from finding superior drugs with the intention instead of identifying safe and effective antibiotics which are 'non-inferior' rather than 'superior' to treat those infections where therapeutic options are limited. As a result of this range and diversity of drugs, and the emergence of antibiotic resistance to current antibiotics, pharmacists are required to advise on the relative merits and appropriateness of certain antibiotics, and on the development of formularies and prescribing guidelines to ensure compliance with the principles of good antimicrobial stewardship (Chapters 11, 12, 14, and 15). In addition to knowledge of the drugs in question, this also requires an understanding of the factors which might influence the success of a given antibiotic therapy, including the likelihood of microbial growth as a biofilm, where antibiotic tolerance is significantly elevated (Chapters 7 and 8).

Whilst cardiovascular, pulmonary and malignant diseases are more frequent causes of death in the most developed high-income countries, infectious diseases still remain of paramount importance in countries of low to middle income, with lower respiratory infections and diarrhoeal diseases ranking above malaria, tuberculosis and HIV/AIDS. In 2019, over 12% of the 55.4 million deaths worldwide were caused by these five communicable diseases; the WHO estimates that overall 1 in 5 global deaths can be attributed to sepsis, the life-threatening reaction to infection caused by disease or injury. Infectious diseases,

although showing significant reductions in mortality over the last 20 years, still rank amongst the top 10 leading causes of death globally, despite the significant advances in their treatment through the introduction of safe and effective antibiotics and vaccines. Worryingly too, the reservoir of antibiotic resistance is growing and the WHO estimates that 1.27 million people died worldwide in 2019 from antibiotic-resistant infections. Confidence that the antibiotic pipeline would continue to deliver antibiotics for the treatment of the vast majority of bacterial infectious diseases indefinitely has now been replaced by the realisation that the widespread emergence of resistance, and diminishing returns in antibiotic discovery programmes, has left the antibiotic pipeline critically depleted and unable to keep pace with antibiotic resistance (Chapter 13). Resistance to antibiotics has been reported for all major classes of antibiotics, and across virtually all pathogenic bacteria. As mentioned above, antibiotic-resistant infections have imposed a significant, and increasing, burden of mortality globally. It is clear that the number of infections, particularly healthcare-acquired infections, for which there is no effective antibiotic (and none in development), is on the rise. This worrying scenario leads to an increasing reliance on effective infection control measures designed to minimise the risk of transmission of infection from one patient to another within the healthcare setting (Chapter 16). The importance of such infection-control measures in reducing the transmission of SARS-CoV-2 during the global COVID-19 pandemic has brought this important aspect of global public health microbiology squarely into the public arena. As a key component of infection-control measures, the properties of microbicides (disinfectants and antiseptics), the assessment of their antimicrobial activity in real-world scenarios and the factors which influence their selection for use in infection-control strategies, or for contamination control in pharmaceutical manufacturing, are topics with which pharmacists, pharmaceutical scientists and industrial microbiologists should be familiar (Chapters 18–20). Furthermore, as antibiotic resistance threatens to curtail the 'antibiotic era', significant interest is now focused on potential alternatives, such as bacteriophage therapy, endolysins, novel vaccines and biological drugs, and antimicrobial peptides, bringing diversification to the available therapeutic options for treatment of infection (Chapter 26).

The beneficial biotechnological applications of microorganisms in the production of antibiotics, steroids and other medicines, including recombinant proteins from heterologous expression systems, have revolutionised the pharmaceutical world, giving rise to a burgeoning biopharmaceuticals and biological pharmaceuticals industry. The application of microorganisms and their enzymes (biocatalysts) has driven down the manufacturing cost of active pharmaceutical ingredients by improving yield, streamlining synthetic pathways by circumvention of multiple synthetic steps and by providing cheap and accessible starting materials for semi-synthetic compounds. Recombinant DNA technologies have supported the growth of a pharmaceutical sector worth hundreds of billions of pounds, and recombinant proteins (such as insulin and growth hormone), recombinant monoclonal antibodies and recombinant enzymes and vaccines are available for the treatment of a diverse range of diseases, including infections (Chapters 10, 23, and 24).

All of these developments, alongside miscellaneous applications in the detection of mutagenic and carcinogenic activity in drugs and chemicals, and in the assay of vitamins, amino acids and antibiotics (Chapter 25), have cemented the role of microorganisms in the production of human and animal medicines, and a basic knowledge of immunology (Chapter 9), gene cloning and expression and other biotechnological approaches (Chapter 24) form an integral part of pharmaceutical microbiology.

Part 2

Biology of Microorganisms

2

Fundamental Features of Microbiology

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2.1 Introduction

Microorganisms differ enormously in terms of their shape, size and appearance and in their genetic and metabolic characteristics. All these properties are used in classifying microorganisms into the major groups with which many people are familiar, for example, bacteria, fungi, protozoa and viruses, and into the less well-known categories such as chlamydia, rickettsia and mycoplasmas. The major groups are the subject of individual chapters immediately following this, so the purpose here is not to describe any of them in great detail but to summarise their features so that the reader may better understand the distinctions between them. A further aim of this chapter is to avoid undue repetition of information in the early part of the book by considering such aspects of microbiology as cultivation, enumeration and genetics that are common to some, and sometimes all, of the various types of microorganism.

2.1.1 Viruses, Viroids and Prions

Viruses do not have a cellular structure. They are particles composed of nucleic acid surrounded by protein; some possess a lipid envelope and associated glycoproteins, but recognisable chromosomes, cytoplasm and cell membranes are invariably absent. Viruses are incapable of independent replication, as they do not contain the enzymes necessary to copy their own nucleic acids; as a consequence, all viruses are intracellular parasites and are

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reproduced using the metabolic capabilities of the host cell. Viruses, and virus-like entities such as viroids, are sometimes known as mobile genetic elements; these are considered selfish genetic elements that move between hosts and/or change their integration in host genes. A great deal of variation is observed in the shape of viruses (helical, linear or spherical), size (20–1500 nm) and nucleic-acid composition (single- or double-stranded, linear or circular RNA or DNA), but almost all are smaller than bacteria and they cannot be seen with a normal light microscope; instead they may be viewed using an electron microscope which affords much greater magnification.

Viroids (virusoids) are even simpler than viruses, being infectious particles comprising single-stranded RNA without any associated protein. Those that have been described are plant pathogens, sometimes of considerable economic importance; so far, there are no known human pathogens in this category, although human hepatitis D virus shares some features in common with viroids, and may have originated from them.

Prions are unique as infectious agents in that they contain no nucleic acid. A prion is an atypical form of a mammalian protein that can interact with a normal protein molecule and cause it to undergo a conformational change so that it, in turn, becomes a prion and ceases its normal function. Prions are the agents responsible for transmissible spongiform encephalopathies, for example, Creutzfeldt–Jakob disease (CJD) and bovine spongiform encephalopathy (BSE). They are the simplest and most recently recognised agents of infectious disease, and are important in a pharmaceutical context owing to their extreme resistance to conventional sterilising agents such as steam, gamma radiation and disinfectants (see Chapter 21).

2.1.2 Prokaryotes and Eukaryotes

The most fundamental distinction between the various microorganisms having a cellular structure (i.e., all except those described in Section 2.1.1 above) is their classification into two groups – the prokaryotes and eukaryotes – based primarily on their structural characteristics and mode of reproduction. Expressed in the simplest possible terms, prokaryotes are the bacteria and archaea (see Section 2.1.2.1), and eukaryotes are all other cellular microorganisms, for example, fungi, protozoa and algae. The crucial difference between these two types of cell is the possession by the eukaryotes of a true cell nucleus in which the chromosomes are separated from the cytoplasm by a nuclear membrane. The prokaryotes have no true nucleus; they normally possess just a single chromosome that is not separated from the other cell contents by a membrane. Other major distinguishing features of the two groups are that prokaryotes are normally haploid (possess only one copy of the set of genes in the cell) and reproduce asexually; eukaryotes, by contrast, are usually diploid (possess two copies of their genes) and normally have the potential to reproduce sexually. The capacity for sexual reproduction confers the major advantage of creating new combinations of genes, which increases the scope for selection and evolutionary development. The restriction to an asexual mode of reproduction means that the organism in question is heavily reliant on mutation as a means of creating genetic variety and new strains with advantageous characteristics, although many bacteria are able to receive new genes from other strains or species (see Section 2.6.1 and Chapter 3). Table 2.1 lists some distinguishing features of the prokaryotes and eukaryotes.

2.1.2.1 Bacteria and Archaea

Bacteria are essentially unicellular, although some species arise as sheathed chains of cells. They possess the properties listed under prokaryotes in Table 2.1, but, like viruses and other categories of microorganisms, exhibit great diversity of form, habitat, metabolism, pathogenicity and other characteristics. The bacteria of interest in pharmacy and medicine belong to the group known as the eubacteria. The other subdivision of prokaryotes, the archaea, while formerly considered largely to comprise organisms capable of living in extreme environments (e.g., high temperatures, extreme salinity or pH) or organisms exhibiting specialised modes of metabolism (e.g., by deriving energy from sulphur or iron oxidation or the production of methane), are now known to occur in a wide variety of habitats. This recognised tolerance to extremes has led to consideration of the archaea as biocatalysts for industrial processes; as a source of extremely stable enzymes, they are well suited to biotechnological applications, some of which are of potential pharmaceutical importance.

The eubacteria are typically rod-shaped (bacillus, see Figure 2.1a), spherical (cocci), curved or spiral cells of approximately 0.5–5.0 μm (longest dimension) and are divided into two groups designated Gram-positive and Gram-negative according to their reaction to a staining procedure developed in 1884 by Christian Gram (see Chapter 3). Although all the pathogenic species are included within this category, there are very many other eubacteria that are harmless or positively beneficial. Some of the bacteria that contaminate or cause spoilage of pharmaceutical materials are saprophytes, that is, they obtain their energy by decomposition of animal and vegetable material, while many could also be described as parasites (benefiting from growth on or in other living organisms without causing detrimental effects) or pathogens (parasites damaging the host). Rickettsia and chlamydia are

Table 2.1 Distinguishing features of prokaryotes and eukaryotes.

Characteristic	Eukaryotes	Prokaryotes
Size	Normally >10 μm	Typically, 1–5 μm
Location of chromosomes	Within a true nucleus separated from the cytoplasm by a nuclear membrane	In the cytoplasm, usually attached to the cell membrane
Nuclear division	Exhibit mitosis and meiosis	Mitosis and meiosis are absent
Nucleolus	Present	Absent
Reproduction	Asexual or sexual reproduction	Normally asexual reproduction
Chromosome number	>1	1
Mitochondria and chloroplasts	May be present	Absent
Cell membrane composition	Sterols present	Sterols absent
Cell wall composition	Cell walls (when present) usually contain cellulose or chitin but not peptidoglycan	Walls usually contain peptidoglycan
Ribosomes	Cytoplasmic ribosomes are 80S	Ribosomes are smaller, usually 70S
Endoplasmic reticulum	Present	Absent
Extracellular capsule/slime	Absent	Often present
Flagella	Structurally complex	Structurally simple
Pili	Absent	Present
Fimbriae	Cilia	Present
Storage compounds	Poly-β-hydroxybutyrate absent	Poly-β-hydroxybutyrate often present

types of bacteria that are obligate intracellular parasites, that is, they are incapable of growing outside a host cell and so cannot easily be cultivated in the laboratory. Most bacteria of pharmaceutical and medical importance possess rigid cell walls (and are therefore relatively resistant to osmotic stress), grow well at temperatures between ambient and human body temperature and exhibit wide variations in their requirement for, or tolerance of, oxygen. Strict aerobes require atmospheric oxygen, but for strict anaerobes oxygen is toxic. Many other bacteria would be described as facultative anaerobes (normally growing best in air but can grow without it) or microaerophiles (preferring oxygen concentrations lower than those in normal air).

2.1.2.2 Fungi

Fungi are structurally more complex and varied in appearance than bacteria, and, being eukaryotes, differ from them in the ways described in Table 2.1. Fungi are considered to be non-photosynthesising plants, and the term *fungus* covers both yeasts and moulds, although the distinction between these two groups is not always clear. Yeasts are normally unicellular organisms that are larger than bacteria (typically 5–10 μm) and divide either by a process of binary fission (see Section 2.4.2) or budding (whereby a daughter cell arises as a swelling or protrusion from the parent that eventually separates to lead an independent existence, Figure 2.1b). *Mould* is an imprecise term used to describe fungi that do not form fruiting bodies visible to the naked eye, thus excluding toadstools and mushrooms. Most moulds consist of a tangled mass (mycelium) of filaments or threads (hyphae) which vary between 1 and over 50 μm wide (Figure 2.1c); they may be differentiated for specialised functions, for example, absorption of nutrients or reproduction. Some fungi may exhibit a unicellular (yeast-like) or mycelial (mould-like) appearance depending upon cultivation conditions. Although fungi are eukaryotes that should, in theory, be capable of sexual reproduction, there are some species in which this has never been observed. Most fungi are saprophytes with relatively few having pathogenic potential, although this view is changing in the case of immunocompromised patients. The ability of fungi to form spores that are resistant to drying makes them important as contaminants of pharmaceutical raw materials, particularly materials of vegetable origin.

2.1.2.3 Protozoa

Protozoa are eukaryotic, predominantly unicellular microorganisms that have been regarded in the past as animals rather than plants ('protozoa' means 'first animals'), although the distinction between protozoa and fungi is not always clear. Many protozoa are free-living motile organisms that occur in water and soil, although some are

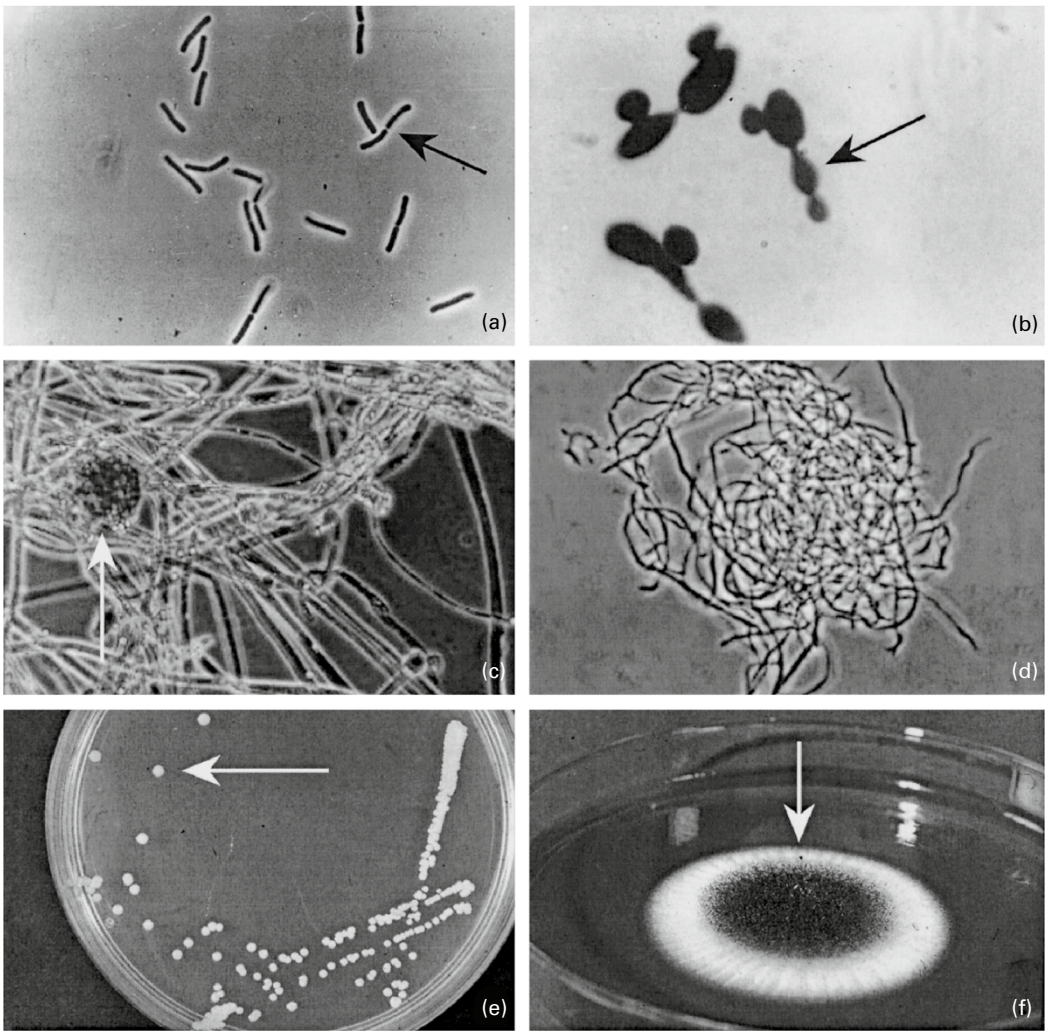

Figure 2.1 (a) A growing culture of *Bacillus megaterium* in which cells about to divide by binary fission display constrictions (arrowed) prior to separation. (b) A growing culture of the yeast *Saccharomyces cerevisiae* displaying budding (arrowed). (c) The mould *Mucor plumbeus* exhibiting the typical appearance of a mycelium in which masses of asexual zygospores (arrowed) are formed on specialised hyphae. (d) The bacterium *Streptomyces rimosus* displaying the branched network of filaments that superficially resembles a mould mycelium. (e) The typical appearance of an overnight agar culture of *Micrococcus luteus* inoculated to produce isolated colonies (arrowed). (f) A single colony of the mould *Aspergillus niger* in which the actively growing periphery of the colony (arrowed) contrasts with the mature central region where pigmented asexual spores have developed.

parasites of plants and animals, including humans, for example, the organisms responsible for malaria and amoebic dysentery. Protozoa are not normally found as contaminants of raw materials or manufactured medicines and the relatively few that are of pharmaceutical interest owe that status primarily to their potential to cause disease.

2.2 Naming of Microorganisms

Microorganisms, just like other organisms, are normally known by two names: that of the genus (plural = genera) and that of the species. The former is normally written with an uppercase initial letter and the latter with a lowercase initial letter, for example, *Staphylococcus aureus* or *Escherichia coli*. These may be abbreviated by shortening the name of the genus provided that the shortened form is unambiguous, for example, *S. aureus* or *E. coli*. Both the full and the shortened names are printed in *italics* to designate their status as proper names (in old books, theses or manuscripts, they might be in roman type but underlined). The species within a genus are sometimes referred to by a collective name, for example, staphylococci or pseudomonads, and neither these names nor names describing groups of organisms from different genera, for example, coliforms, are italicised or spelt with an uppercase initial letter.