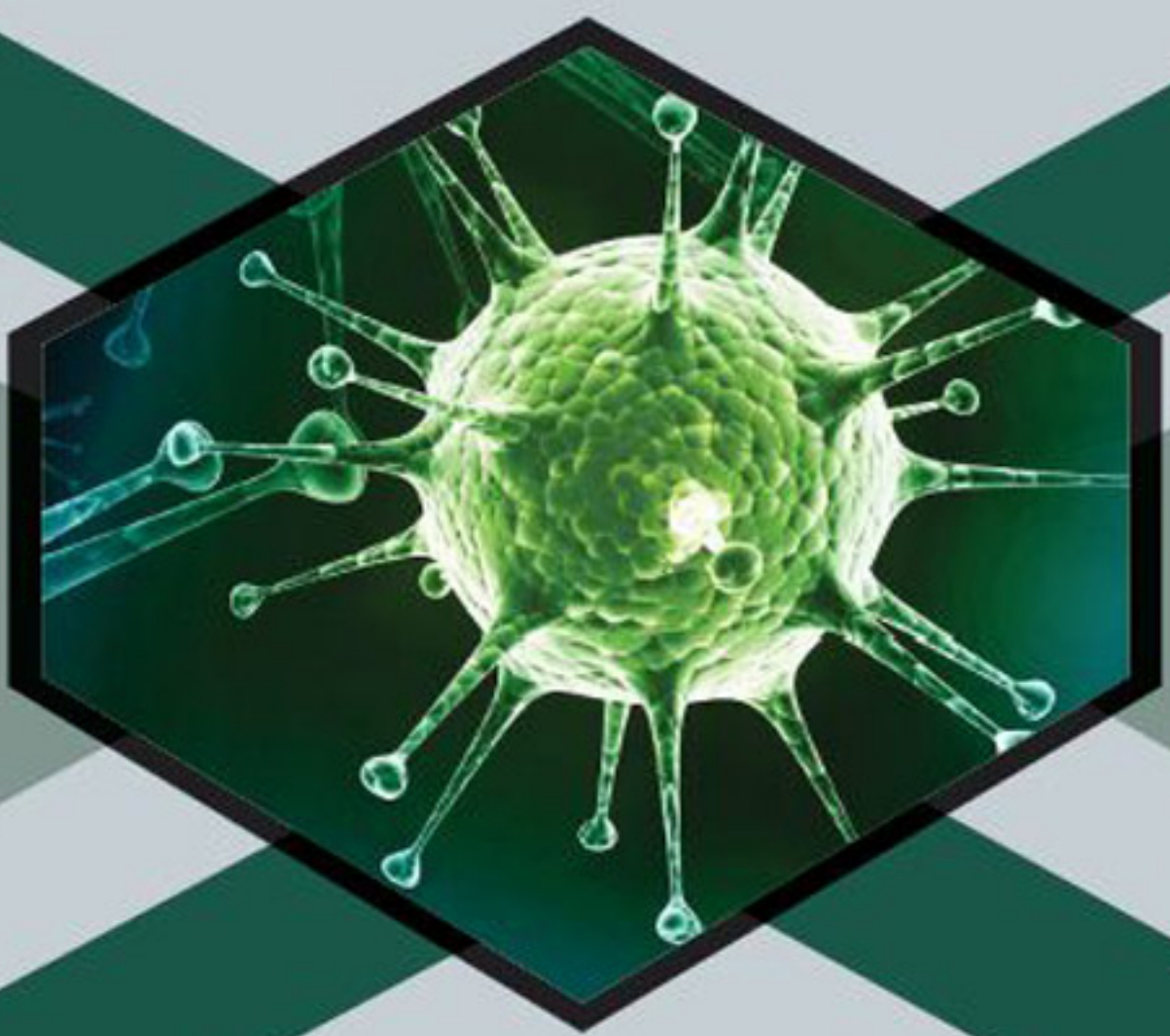
AS PER PCI REGULATIONS
SECOND YEAR B. PHARM. | SEMESTER-III

PHARMACEUTICAL MICROBIOLOGY

Dr. KUNTAL DAS



NIRALI PRAKASHAN
ADVANCEMENT OF KNOWLEDGE

A Text Book of

PHARMACEUTICAL MICROBIOLOGY

As Per PCI Regulations

SECOND YEAR B. PHARM., SEMESTER III

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Dedicated to

My beloved
Parents, Wife and Son

Preface

This is the first edition and first time the attempt has been made to write a Pharmaceutical microbiology text book as per the new syllabus prescribed by Pharmacy Council of India for Semester - III. There are many text books on microbiology already available in market by famous authors. Drug safety is a major focus of pharmaceutical microbiology. Pathogenic bacteria, fungi (yeasts and moulds) and toxins produced by microorganisms are all possible contaminants hence it is require for Quality Assurance and Quality Control on protecting pharmaceutical and healthcare products from spoilage by microorganisms. Most of the information in this book has presented in a very simple manner, with tables, figures and wherever necessary with diagrams. The aim of this book is made easy for understandable to the student. This book is as per the revised syllabus prescribed by the Pharmacy Council of India under Regulations 6, 7 and 8 of the Bachelor of Pharmacy (B. Pharm.) course regulations 2014 in 2016 and amended from 2017 onwards throughout India under same uniform syllabus. As per the norms, the book is compiled with semester wise syllabus in which Pharmaceutical microbiology is under Semester - III.

Semester - III, Pharmaceutical Microbiology subject is divided into five sub-units.

Unit–I, deals with the history of microbiology world, its general introduction and importance, description on Prokaryotes and Eukaryotes. Further detail study on bacteria world with their culture techniques for growth and their identification by various modern microscopic techniques.

Unit-II, deals with various staining techniques and biochemical tests for identification of various types of bacteria. Further most importantly, sterilization techniques are discussed in details with respect to equipments details, evaluation of efficiency and various sterility indicators.

Unit-III, describes about Fungi and Viruses in details. Disinfectants are also discussed in details for identification of bacteriostatic and bactericidal actions. Standard IP, BP and USP methods for sterility testing of products are also equally given importance in this sub-unit.

Unit IV deals with various conditions for culturing microbes under aseptic conditions. Principles and methods of different microbiological assay are highlighted with various methods for standardization of antibiotics, vitamins and amino acids. Assessment of new antibiotics is also added in this important sub-unit.

Unit V is most important where most care and precaution taken for storage of pharmaceutical products from the microbial spoilage and their stability. Microbial contaminants and their contamination processes are also revealed in this unit. Furthermore some methods are described on animal cell culture and their importance in pharmaceutical industry and research.

It is hoped that all the units will provide up to date knowledge to all the students with the detail information by systemic manners described in this book.

My attempt to publish this first edition of the pharmaceutical microbiology book is with better hope to gain the popularity by the students and readers throughout the country. Any criticism and suggestions from the readers are always welcome. In the future editions, such suggestions will be incorporated and other mistakes will be rectified.

It is my great privilege to acknowledge the help from all the published books and websites from the internet for completing this book. My sincere and heartiest gramercy to Dr. Raman Dang, Registrar of DPSRU New Delhi, for encouragement and positive motivation. My sincere thanks and respect to chairman sir, Prof Suresh Nagpal, vice chairperson madam, directors, present principal Dr. Amit Kumar Das, all my teaching, non-teaching staffs of my college for their active co-operation and encouragement.

I felt no word to express sense of indebtedness to my parents, Mrs. Kalyani Das and Dr. Dilip Kumar Das (Emeritus Fellow UGC), whose silent blessings, encouragement, and helping me to put my best foot forward in all my endeavors of chasing my dreams in life.

My special and sincere thanks to my wife Mrs. Sangita Das and son Master Niladri Das for their inspiration to pursue the work in all the situations.

Lastly but not the least, my sincere thank to M/S Nirali Prakashan publishers for kind publication of the book with much care.

Prof. Kuntal Das

M. Pharm, Ph.D

Syllabus

Unit I **10 Hours**

Introduction, History of microbiology, Its branches, Scope and its importance. Introduction to Prokaryotes and Eukaryotes. Study of ultra-structure and morphological classification of bacteria, Nutritional requirements, Raw materials used for culture media and physical parameters for growth, Growth curve, Isolation and preservation methods for pure cultures, Cultivation of anaerobes, Quantitative measurement of bacterial growth (total and viable count). Study of different types of phase constrast microscopy, dark field microscopy and electron microscopy.

Unit II **10 Hours**

Identification of bacteria using staining techniques (Simple, Gram's and Acid fast staining) and biochemical tests (IMViC).

Study of principle, Procedure, Merits, Demerits and applications of physical, Chemical, Gaseous, Radiation and mechanical method of sterilization. Evaluation of the efficiency of sterilization methods. Equipments employed in large scale sterilization. Sterility indicators.

Unit III **10 Hours**

Study of morphology, Classification, Reproduction/replication and cultivation of Fungi and Viruses. Classification and mode of action of disinfectants. Factors influencing disinfection, Antiseptics and their evaluation. For bacteriostatic and bactericidal actions. Evaluation of bactericidal and bacteriostatic. Sterility testing of products (solids, liquids, ophthalmic and other sterile products) according to IP, BP and USP.

Unit IV **08 Hours**

Designing of aseptic area, Laminar flow equipments; Study of different sources of contamination in an aseptic area and methods of prevention, Clean area classification. Principles and methods of different microbiological assay. Methods for standardization of antibiotics, vitamins and amino acids. Assessment of a new antibiotic.

Unit V **07 Hours**

Types of spoilage, Factors affecting the microbial spoilage of pharmaceutical products, Sources and types of microbial contaminants, Assessment of microbial contamination and spoilage. Preservation of pharmaceutical products using antimicrobial agents, Evaluation of microbial stability of formulations. Growth of animal cells in culture, General procedure for cell culture, Primary, established and transformed cell cultures. Application of cell cultures in pharmaceutical industry and research.

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Contents

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Unit ... 1

BASICS OF MICROBIOLOGY

♦ LEARNING OBJECTIVES ♦

After completing this chapter, reader should be able to understand:

- About introduction, history, scope and importance of microbiology.
- About Introduction to Prokaryotes and Eukaryotes organisms.
- About detail study of Bacteria with respect to structures, their classification, growth requirements, their isolation techniques and quantitative measurements.
- About study of different types of microscopy for detection of microorganisms.

1.1 INTRODUCTION

Microbiology is the branch of science that deals with microorganisms. Microorganisms are the small living things that include unicellular, multicellular or acellular. Unicellular are single cells organisms like cocci, bacilli, virio and spirillae (Fig. 1.1). Multicellular are filaments and sheaths to form cell colonies like blue green algae (cyanobacteria), fungi, protozoans and bacteria (Fig. 1.2) whereas acellular are organism without cells, like viruses, prions (Fig. 1.3). These microorganisms are not visible by naked eyes, only observed under microscope. Like other organisms, microorganisms survive, grow and are also require a source of energy and nourishment to survive and to growth. Many microorganisms are beneficial to human and some are pathogenic in nature.

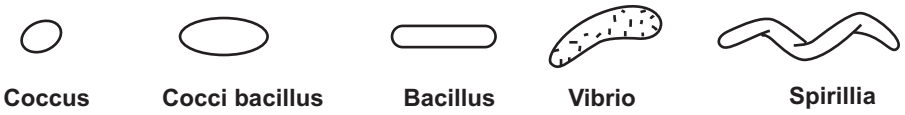


Fig. 1.1: Unicellular single cell organisms

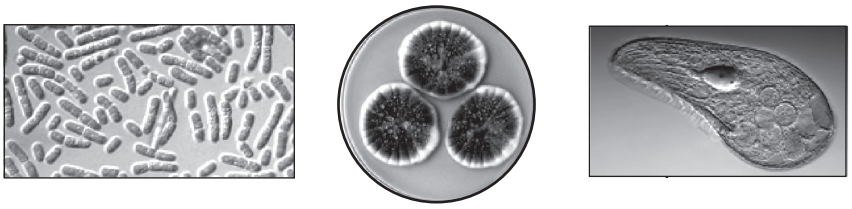
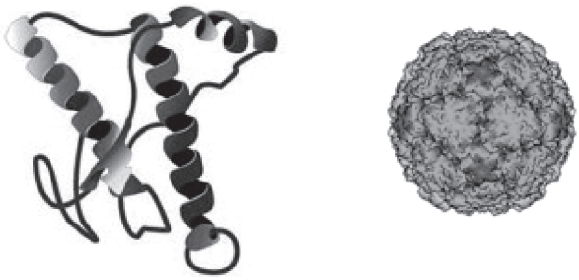


Fig. 1.2: Multicellular organisms



Prion Virus

Fig. 1.3: Acellular Organisms

1.2 HISTORY

History of microbiology is divided in to three stages namely **Discovery stage**, **Transition stage** and **Modern microbiology**.

1.2.1 Discovery Stage

Aristotle (384-322) described living and non-living organisms and their differentiation. **Roger Bacon** (13th Century) described that diseases are caused by living creatures (Fig. 1.4). Thereafter in 1546, **Fracastorius** described that communicable diseases were caused by living agents known as germs. In 1665, the first report on cell structure was described by **Robert Hooke**. Later, during mid of 1600's, **Antony van Leeuwenhoek (1632-1723)** was the first person who used a microscope of his own design to direct observations of microbes (Fig. 1.5). He discovered microorganisms in 1675 and named bacteria and protozoa as "Animalcules". He has provided full description of bacteria. From that time he was known as one of the founders of microbiology.

Fig. 1.4: Antonie van Leeuwenhoek (24 October 1632 – 26 August 1723)

Fig. 1.5: Roger Bacon (13th Century) (c. 1219/20 – c. 1292)

In 1659, **Kircher** was reported minute worms in the blood during plague attack to human.

1.2.2 Transition Stage

In this era, **Francesco Redi (1626-1697)** showed that maggots would not arise from decaying covered meat. Further **John Needham (1713-1781)** proposed that tiny organisms arise spontaneously on the mutton gravy and he supported the spontaneous generation theory. There after **Lazzaro Spallanzani (1729-1799)** demonstrated that air carried germs to

the culture medium and also revealed that boiled broth would not give growth of microorganisms (Fig. 1.6). In Next, **John Tyndall (1820-1893)** in 1877 proved the need for prolonged heating for elimination of microbial life from infusions, which are recently termed as tyndallization in which heat stable as well as heat sensitive bacteria both are killed (Fig. 1.7). In 1835, **Augustino Bassi** demonstrated that a silk worm disease called muscardine was due to fungal infection.

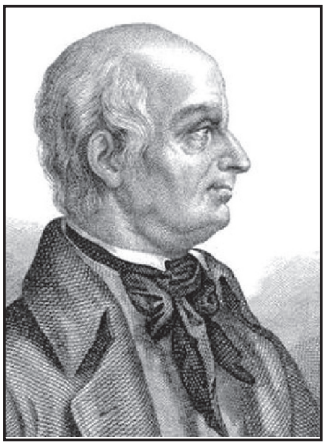


Fig. 1.6: Lazzaro Spallanzani (10 January 1729 – 12 February 1799)

Fig. 1.7: John Tyndall (2 August 1820 – 4 December 1893)

1.2.3 Modern Microbiology

The actual development of microbiology came with Louis Pasteur, Robert Koch, Lord Lister, Alexander Flemming and Paul Ehrlich.

Louis Pasteur is known as the father of medical microbiology because he has coined the terms microbiology, aerobic and anaerobic. In 1897, he suggested that mild heating at 62.8°C for 30 minutes was more effective than boiling to destroy the pathogenic organisms without change of taste of the product. This method was known as **Pasteurization**. Hence, he was known as the inventor of the Pasteurization. Thereafter he also invented fermentation process and developed effective live attenuated vaccines against rabies and anthrax. He also demonstrated disease of silkworm was due to protozoan parasite (Fig. 1.8).

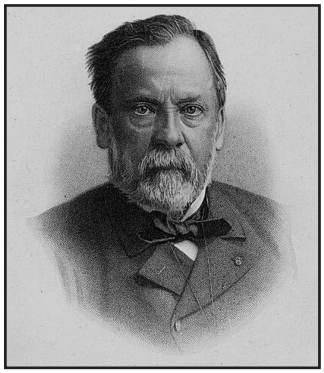


Fig. 1.8: Louis Pasteur (December 27, 1822 – September 28, 1895)

Lord Joseph Lister: He is known as father of antiseptic surgery. He also revealed that wound infections were due to microorganisms and discovered the method of destroying microorganisms in the operation theatre by spraying a fine mist of carbolic acid in the air (Fig. 1.9).

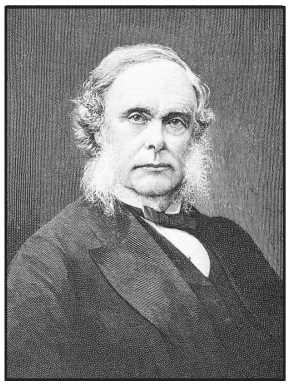


Fig. 1.9: Lord Joseph Lister

(5 April 1827 – 10 February 1912)

Robert Koch (Fig. 1.10): He demonstrated the role of bacteria in causing diseases and also invented technique for bacteria isolated from pure culture. He only explained first the germ theory of diseases in 1876. He prepared gelatin for solid media but was not ideal because gelatin is a protein which is digested by the bacteria and produce a proteolytic exo-enzyme gelatinase that hydrolyses protein into amino acids; thereafter gelatin also melted in temperature more than 25°C.

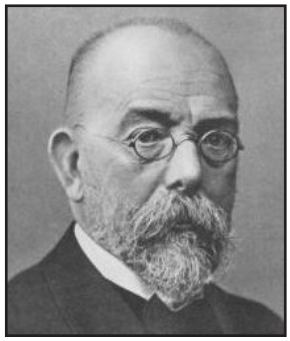


Fig. 1.10: Robert Koch

(11 December 1843 – 27 May 1910)

There are **four Koch's Postulates** viz.**:** (a) The causative agents are surely present in every individual with the disease. (b) They must be isolated and grown in pure culture, (c) When the pure culture inoculated into an experimental animal it surely cause the disease and (d) The causative agent must be re-isolated and re-identified from the experimental animal as well as in pure culture respectively.

Further, **Fanne Eilshemius Hesse (1850-1934)** used agar as solid culture media and proved the media was not attacked by most of the bacteria. Thereafter it can sustain at

Pharmaceutical Microbiology

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