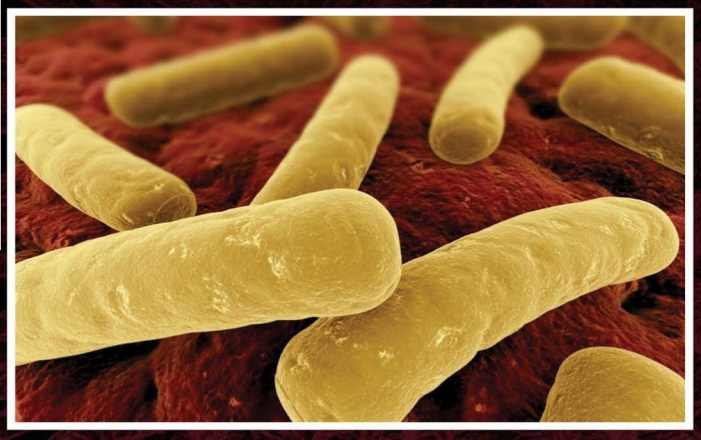
VAYU EDUCATION OF INDIA

MICROBIOLOGY



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MICROBIOLOGY

(FOR PARAMEDICAL STUDENTS)

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2/25, Ansari Road, Darya Ganj, New Delhi-110 002

Microbiology

ISBN: 978-93-83137-81-7

First Edition: 2014

Price: ₹ 250

Printed & bound in India

Published by:

AN ISO 9001:2008 CERTIFIED COMPANY

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PREFACE

Text book of Microbiology for Paramedical students has been thoroughly updated with recent advances and includes extensive revision of all chapters. Constructive suggestions of paramedical students and teachers were taken into consideration for better presentation of this book. We are very optimistic that the book will meet the needs of paramedical students not only in their academic pursuits but also in their day to day professional working. Many of the health problems in developing countries like India are different from those of developed countries. Bacterial diseases still play a considerable role in diseases of our country. In the past few years importance of certain subjects, particularly related to biosafety and nosocomial infections has tremendously increased. In the light of this changing scenario and constructive suggestions this Microbiology book for paramedical students has been thoroughly revised, updated and made more user-friendly. The book of microbiology is specially aims at the ever demanding thoughtful need of absolutely well documented complication of factual details related to theoretical, principles, classifications, diagrammatic profiles, graphic presentations and critical explanation for the exclusive paramedical students throughout the Indian Universities and abroad. Authors are thankful to M/s Vayu Education of India Delhi, for their keen interest and help in publishing the book We would, as usual, keenly look forward to the constructive suggestions from our esteemed readers for improvement of the future editions of the book. Authors are thankful to Dr. R. S. Gupta, Director (Academics) and Former Professor and Head (Vety Microbiology) for his invaluable suggestions from time to time in bringing the book in final shape.

Poonam Bachheti
Aruna Singh
Authors

SYLLABUS
MICROBIOLOGY

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1. GENERAL MICROBIOLOGY
- Introduction, Definition, Scope, History of Microbiology, Fermentation, Germ Theroy of Disease, Immunization, Significance.

2. LABORATORY EQUIPMENTS
- Autoclave, Laminar Air Flow, Incubator, Centrifuge (Principle, use and Maintenance). Microscope, PH Meter, Analytical balance.

3. LABORATORY GLASSWARE
- Introduction, Types of glassware, cleaning of glassware. Care and maintenance of glassware.

4. SAFETY MEASURES IN LABORATORY
- Introduction, precautions taken while handling toxic, hazardous chemicals. Storage of chemicals, reagents, and handling of infectious blood / stool / urine / sputum samples etc.

5. FIRST AID MEASURES

6. RESPONSIBILITIES OF TECHNICIAN

7. DISPOSAL OF LABORATORY WASTES
- Biological waste, Hazardous wastes. Sharp objects wastes, Anatomical wastes. (Body, Fluid, Urine, Stool, Sputum, Swabs)

8. COLLECTION OF SPECIMEN
- Introduction, collection of specimen, (Blood culture, urine culture, stool culture, swab (Nasal, pus swabs, wound culture, throat culture)
- Transportation, processing of sample. Storage of Specimen (samples)

17. LABORATORY INVESTIGATION OF BACTERIA

- Introduction, lab identification of infectious agents. Lab diagnosis of tuberculosis, lab diagnosis of leprosy, staining method (gram stain, ZN stain, Albert stain etc.) (Collection of specimen, requirement, principle of technique, procedure, results, clinical significance)
- Motility Preparation, Hanging Drop preparation, Gram Negative bacilli and gram positive bacilli.

18. IDENTIFYING CHARACTERISTICS OF COMMON PATHOGENIC BACTERIA

- Introduction (Actionomyces, Bacillus anthracis, Bacteroides, Bordetella, Pertussis, Borrelia) Corynebacterium diphtheria, brucella, clostridium)
- Specimen collection, lab identification method.

19. LABORATORY DIAGNOSIS OF MYCOTIC INFECTIONS

- Introduction of parasitic fungi, classification of mycotic agents, Specimen collection (skin scrapping, nail, hair, pus, sputum, exudates)
- Lab investigations (Macroscopic examination, wet mount in water, wet mount in alkali)
- India ink preparation laboratory culture, microscopic of culture.
- Diagnosis of mycotic infection (Dermatomycosis, Subcutaneous mycosis, systemic mycosis)

20. BIOCHEMICAL REACTIONS

- Introduction, acid production from fermentable substance coagulase, catalase, oxidase, bile salt optochin sensitivity, Indole production, urease activity etc.

CONTENTS

1 CHAPTER

Introduction and Brief History of Microbiology

INTRODUCTION

The study of organisms, invisible by the naked eyes is called as Microbiology. It is concerned with the study of morphology, physiology and other activities of micro-organisms

Medical Microbiology is

1. The study of microbes that infect humans.
2. About the diseases they Cause.
3. Diagnosis of the diseases.
4. Prevention and treatment of diseases.
5. It also deals with the response of the human host to microbes and other antigens

   Definition: Microbiology is the close study of living organisms of microscopic size, which includes bacteria, fungi, algae. It is concerned with their form, structure, physiology and classification.
6. They are closely associated with the health and welfare of human beings. Some are beneficial and some are pathogenic for man. They are involved in making of yogurt, cheese, paneer, wine etc. and they can also cause diseases.
7. Most micro organism are unicellular i.e all life processes are performed by a single cell. and some other are multicelluar.
8. Some microorganism called anaerobes and are capable of carrying out their vital functions in the absence of free Oxygen called aerobes.
9. The introduction of microbiology is made sure with the discovery of the microorganism. Those have some beneficial and harmful interaction with human being. The credit of discovery of simple microscope goes to Leeuwenhoek he gave them a name Micro animalcules.

ANCIENT MICROBIOLOGICAL HISTORY

Ancient man recognized many of the factors involved in disease. The fact that people who recovered from a particular disease were immune to that disease was probably recognized many different times in many places. Often these survivors were expected to nurse the ill. Greek and Roman physicians routinely prescribed diet and exercise as a treatment for ills.

The first person to report seeing microbes under the microscope was an Englishman, **Robert Hooke**. Working with a crude compound microscope he saw the cellular structure of plants around 1665. He also saw fungi which he drew. However, because his lens were of poor quality he was apparently unable to "see" bacteria.

Anton van Leeuwenhoek was a man born before his time. Although not the FIRST TO DISCOVER THE MICROSCOPE or to use magnifying lens, he was the first to see and describe bacteria.

Fig. 1.1: Father of micro biology "Anton van Leeuwenhoek"

Being meticulous, he developed his lens grinding to an art and in the process tested them by seeing how much detail he could observe with a given lens. One can guess that he chanced to look at a sample of pond water or other source rich in microbes and was amazed to see distinct, uniquely shaped organisms going, **apparently purposefully**, about their lives in a tiny microcosm. He made numerous microscopes from silver and gold and viewed everything. His best lens could magnify **~300-500 fold** which allowed him to see microscopic algae and protozoa and larger bacteria. He clearly had excellent eyesight because he accurately drew pictures of microbes that were at the limit of the magnification of his lens. He used only SINGLE LENS and not the compound lens of the true microscopes we employ today; which makes his observations all the more amazing. He wrote of his observations to the Royal Society of London in 1676 and included numerous drawings. He astonished everyone by claiming that many of the tiny things he saw with his lens were ALIVE because he saw them swimming purposefully about.

Robert Hooke was the first person to propose the CELLULAR forms of life.

Many scientists and *trendy* high society people visited him to view his "little **animalcules**". He was a superior observer and an excellent scientist except for the **CRUCIAL FLAW** of not allowing others to copy his techniques and **VERIFY** his results. Because of this and the failure of people to relate these tiny microbes with disease, it was another 200 years before the science of Microbiology really took off.

THE CLEAN NUT AND THE REVENGE OF THE BACTERIA

In the 1800s people (mainly the poor) began to use hospitals. Hospitals also became centers of physician training. In 1841 (30 years before the GERM THEORY of disease was established) young doctor **IGNAZ SEMMELWEIS** was hired to run a maternity ward in a Vienna hospital. There were two birthing wards in his preview, one run by midwives and the other by doctors. Semmelweiss noticed that the death rate among mothers in the doctor's ward ran as high as 18% from the blood infection (of a streptococcus or STREP) known as CHILD BED FEVER or **PUERPERAL SEPSIS**, whereas in the midwife ward the death rate was much lower. He saw a friend die from PUERPERAL SEPSIS after cutting himself during an autopsy of a patient who had died of PUERPERAL SEPSIS. He reasoned that there was an **INVISIBLE AGENT** that caused both deaths and that one could transfer it from the autopsy room to the birthing rooms and thus infect the mothers during birthing. Acting on this assumption, Semmelweis instituted sanitary measures which included having the doctors wash their hands in disinfectant and change from lab coats ***dripping with pus and blood*** from the autopsy room to ***clean lab coats*** before examining patients or assisting in a birth. The death rate of the mothers dropped by 2/3 in his ward.

SPONTANEOUS GENERATION

The mystery of life has puzzled and confounded humans since the first human began to contemplate his world. The religions of ancient societies were built around the seasons, the sun and the renewal of life as these were so clearly tied to survival; both of the human species through birth and death, and of the individual in the attainment of sustenance. **SPONTANEOUS GENERATION** or the idea that life routinely arises from non-life was a **COMMON SENSE** explanation of the miracle of life. As science and the scientific method grew with the slow accumulation of knowledge, observant individuals began to consider the origin of life more deeply. Simple observations convinced many people that all the larger animals and plants produced life from previous life. Despite this, the mass of humans clung to the comfortable idea of SPONTANEOUS GENERATION Further, religions saw it as a convenient way to demonstrate the hand of God operating continuously in the WORLD. Some individuals, such as J.B. van Helment even described how one could make mice from grain, a jar and dirty rags by putting them together in a dark place for a few weeks and soon mice would appear in the jar. Other, more perceptive individuals, like **F. Redi** tested the common idea that maggots arise via SPONTANEOUS GENERATION on rotting meat. He placed a piece of meat in three jars, one he left open, one he corked tightly and the third

he covered with a fine mesh gauze. Maggots only appeared in the open container, no matter how long he left the jars.

Redi's experiment was important because of its eloquent simplicity. Anyone could repeat it and obtain the same clear results. Nevertheless, many people clung fiercely to the idea of SPONTANEOUS GENERATION, while others designed experiments to test it. In every case the results of the majority of these experiments indicated that SPONTANEOUS GENERATION did not occur. The intellectual ferment this controversy stirred up gradually evolved into the SCIENTIFIC METHOD as the various antagonists questioned each other's assumptions and, more importantly, their **experimental design**. These arguments forced the designing of **BETTER EXPERIMENTS** and eventually persuaded all but the most recalcitrant believers to discard SPONTANEOUS GENERATION as an explanation for all higher life forms. Then, in the 1800's the refinement of the microscope, through which people could see tiny life forms that they assumed were SIMPLE, gave SPONTANEOUS GENERATION proponents new life.

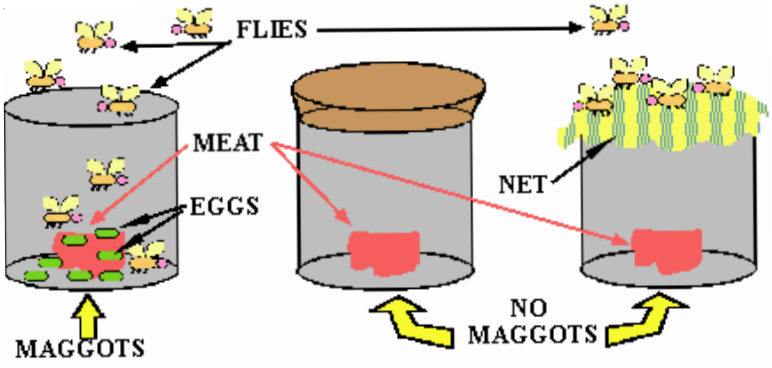


Fig. 1.2: "Redi's experiment proving maggots are not spontaneously produced in rotten" meat.

Again, flawed COMMON SENSE led reasonable people astray. As the existence of microscopic life was accepted, the assumption was that such life must be **SIMPLE** compared to higher, more **COMPLEX** life. The reasoning that followed this erroneous assumption was that since the microbes were ***small*** they must be ***simple*** & it followed that they were formed by SPONTANEOUS GENERATION, hence God was still at work creating micro-life.. A number of scientists performed elementary experiments in which they treated soups and broth's, which left unheated would team with microbes after a few days, with heat to destroy any life present in them and asked the question: "***Would new life arise in these sterile soups***"? Spallanzani boiled "soup" in glass containers and melted the glass closed. The observation that nothing subsequently grew in this "heated" soup suggested that SPONTANEOUS GENERATION didn't work. His detractors, rightly criticized his experiments, proposing that since air is necessary for life and since he had sealed the flask to air, obviously NO LIFE could develop. Others boiled soups and microbes grew, thus

apparently supporting SPONTANEOUS GENERATION. But again the preponderance of data suggested that SPONTANEOUS GENERATION did not even apply to the "***simple***" microbes.

In 1859 one of the fathers of modern microbiology, **L. Pasteur** decided to settle the question of SPONTANEOUS GENERATION once and for all. A genius at devising definitive experiments, Pasteur first drew the necks of glass flasks out so that they remained open to the air, but were bent so that air could only enter by a ***curved path.*** He then added broth and boiled it to destroy contaminating microbes. These flasks were then incubated and observed for months. He reasoned that the microbes in the air that could contaminate the sterile broth would be trapped on the sides of the thin glass necks ***before they reached the sterile broth***. If SPONTANEOUS GENERATION didn't occur no growth should take place. This is exactly what happened, the flasks remained sterile indefinitely, until Pasteur tipped the sterile broth up into the curved neck where he predicted the airborne organisms would have settled. After doing this the broths ***ALWAYS GREW MICROBES***. These experiments ended the SPONTANEOUS GENERATION controversy because these experiment was so elegant and simple, and the results so clear, that anyone could repeat them.

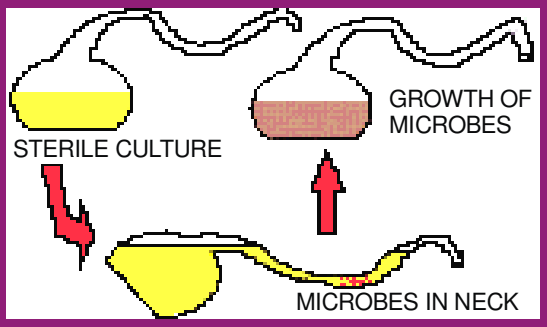


Fig. 1.3: Pasteur's swan-neck flask experiment. Pasteur's SWAN NECK flasks put the nails in the coffin of spontaneous generation. The experiment was simple, and easily repeatable by anyone with modest means & the result unequivocal. However, it is important to note that few scientific experiments are this straight-forward.

Later, an earlier problem, in which occasional heated-broths did not remain sterile, was explained with the discovery of the heat resistant bacterial SPORES , some of which could can survive several hours of boiling without being killed.

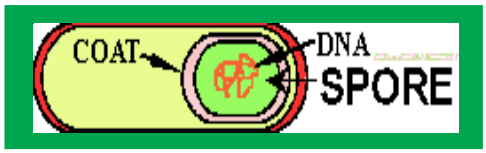


Fig. 1.4: Spore structure

Pasteur discovered many of the basic principles of microbiology and, along with R. Koch, laid the foundation for the science of microbiology. In 1857 Napoleon III was having trouble with his sailors mutinying because their wine was spoiling after only a few weeks at sea. He could distinguish between the contaminants that caused the spoilage and even predict the taste of the wine solely from his microscopic observations. He then reasoned that if one were to heat the wine to a point where its flavor was unaffected, but the harmful microbes were killed it wouldn't spoil. As we are aware this process, today known as **PASTEURIZATION**, worked exactly the way

he predicted and is the foundation of the modern treatment of bottled liquids to prevent their spoilage. It is important to realize that pasteurization is NOT the same as sterilization. Pasteurization only kills organisms that may spoil the product, but it allows many microbes to survive, whereas **STERILIZATION** kills all the living organisms in the treated material. Pasteur also realized that the **yeast** that was present in all the wine produced the alcohol in wine.

ROBERT KOCH

In the late 1870s a country physician, **R. Koch** became interested in anthrax, a common disease of both the farmers and their animals in his rural practice. Using a microscope, Koch saw a large bacterium in the blood of anthrax victims. He reasoned that it might be the agent of the disease. Using a closet at home as his lab and developing basic microbiological techniques as he proceeded, Koch painstakingly teased out the anthrax bacterium and purified it. He then inoculated the purified bacteria into healthy animals and produced the classical clinical disease. When he examined the blood of the inoculated animals he was able to re-isolate the same bacterium. He repeated the isolation, infection and disease cycle until he was certain he had found the agent of anthrax. Because it was such an important commercial disease and because his techniques could be easily duplicated, others quickly verified his findings and Koch became famous. He soon had his own institute (like Pasteur) and other discoveries soon followed. Koch attracted other bright scientists and together they (along with Pasteur's group) developed the basic techniques of microbiology labs we still use today. These include the sterile culture techniques, pure culture techniques, the use of petri plates, inoculation needles, solid medium, the use of agar and gelatin to produce a solid surface, the Gram stain and other staining procedures. In addition Koch discovered the etiological agents of cholera, and tuberculosis. His studies, in combination of those of Pasteur's, established the **GERM THEORY** of disease. His procedure for defining the agent of any disease, called **KOCH'S POSTULATES**, consists of the following 4 steps.

- FIRST, **isolate** the suspected agent from a disease victim.
- SECOND, grow the agent in **pure culture**.
- THIRD, infect a healthy host and show that the organism produces the **CLASSICAL CLINICAL DISEASE**.
- FOURTH, **ISOLATE** the "**same**" organism from the new victim.

MICROBIAL SERENDIPITY: Fanny Angelina Eilshemius used **AGAR-AGAR**, a complex polysaccharide extracted from seaweed, to keep them solid in hot weather. AGAR-AGAR had been used as a ***gelling agent*** in ASIA for centuries. The following characteristics of AGAR-AGAR make it almost perfect for the growth of microbes on solid medium:

a. Non-toxic to most microbes.
b. Only melts at 100°C, but solidifies at about 45°C (a temperature most bacteria can survive).

c. nontoxic to other forms of life.

d. Stable to sterilization temperatures.

· e. physiologically inert as very few bacteria have the enzymes for digesting it.

E. JENNER

Smallpox was one of the greatest scourges of mankind. For thousands of years it swept through human populations, killing up to 40% of its victims and leaving many of the survivors horribly scarred for life; their faces covered with deep red pits. The ancient Chinese recognized that those who recovered from a case of "the pox" were **IMMUNE** to smallpox.

Through a series of serendipitous events, Jenner was led to the discovery of immunization and to the eventual elimination of the scourge of smallpox from the earth. As a young man he had lived in the country and had been told by a milkmaid that ***"She never had to worry about catching smallpox because she had had "cowpox***", a mild chronic disease of cows that milk-maids usually contracted as a rash on their hands ". By 1796 he became convinced that the story was true so he inoculated an 8-yr. old boy with cowpox and 8 weeks later inoculated the same boy with the pus from a smallpox lesion. The boy showed no effects and Jenner repeated the experiment. As word of his results spread, others began to test it and by 1803 it was an established medical procedure in England. Shortly thereafter Ben Franklin encouraged American doctors to adapt this technique in view of the dangers inherent in the older technique.

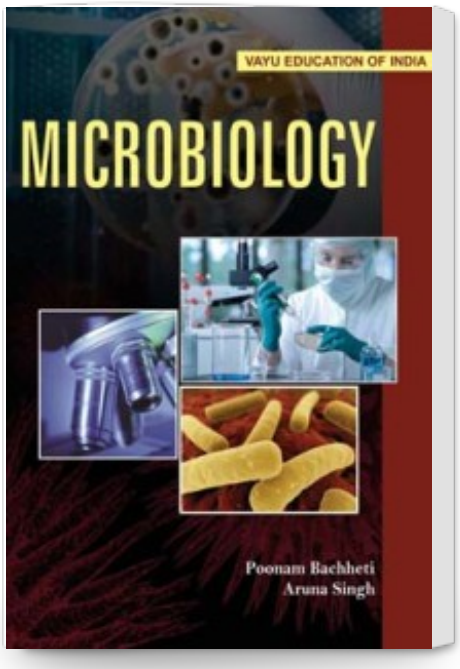
THE MAGIC BULLET

Paul Ehrlich worked in Koch's lab where he learned to study bacteria. While considering the phenomenon of differential staining of different bacteria and of different components of eukaryotic cells, he speculated that if a dye chemical could bind to one cell and not another or to one substance within a cell and not others, perhaps you could find chemicals that would selectively kill certain pathogens without harming the surrounding host cells; this would act like a MAGIC BULLET selectively killing the villain and sparing the innocent victim. He embarked on a search for a magic bullet to cure syphilis, which in the late 19th century was a scourge as terrible as AIDS is today. In the final stages of syphilis, a sexually transmitted disease or STD, its victim suffered horribly and eventually died insane as the brain was destroyed by the infection. Over many years he tested 100s of chemicals and finally in 1910 he found one, he named SALVARSAN or compound 606, that killed the syphilis organisms without killing the host (usually). This discovery laid the ground for the discovery of antibiotics and other chemotherapeutic agents.

History

- **Varo and Columella** is the first century BC postulated that diseases were caused by invisible being, inhaled or ingested.
- **Fracastorius of Verona (1546)** proposed contagious agent as a source of infection.

Microbiology By Poonam Bachheti



Publisher : Vayu Education ISBN : 9789383137817 Author : Poonam Bachheti