MICROBIOLOGY and IMMUNOLOGY

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MICROBIOLOGY and IMMUNOLOGY

What Causes Diseases?

Brainstorm what causes diseases:

Introduction

Definition

Microbiology

- ✓ Is the study of living organisms/microbes of microscopic size that cause infection.
- ✓ It is the study of the structure, body functions and physiological processes of microorganism

The process of microbial inversion of the body is called infection.

Historical Development of Microbiology

- ✓ Microbiology had its beginning in the ancient times with an awareness in sanitation (Hippocrates)
- ✓ Some of the pioneers who are credited for the development of microbiology (microB) include: Antony, Edward, Louis, Semmelweis, Joseph, Robert Koch

1. Antony Van Leeuwenhock (1632 - 1723)
   - ❖ He was a Dutch Scientist who was the first person to see micro organism using a microscope.
   - ❖ He was the first man to build a microscope. He is credited for coming up with a bacteria drawing that we see today.
   - ❖ He discovered microorganisms by making magnifying lenses as a profession and later invented microscopes
   - ❖ He reported findings to the medical

2. Edward Jenner (1796)
   - ❖ He discovered that there was a relationship between bacteria and diseases
   - ❖ Edward Jenner also discovered and successfully tried the first smallpox vaccines and the whole concept of immunity

3. Louis Pasteur (1822 - 1895)
   - ❖ Is credited for the development of modern microbiology
   - ❖ He is considered the father of bacteriology.
   - ❖ He demonstrated that fermentation was caused by growth of microorganisms.
   - ❖ He introduced autoclaves in the laboratory
   - ❖ He was pioneer in the development of vaccines (chickenpox, rabies and anthrax vaccines)
   - ❖ He introduced the practice of sterilization of glassware by dry heat at 170ºC.
   - ❖ He showed that sterilization by boiling renders fluid sterile.
   - ❖ He was a French chemist who disapproved the theory of spontaneous generation.
   - ❖ He proved that lactic fermentation was due to living organisms and based on experiments proved that fermentation of wine was caused by bacteria.
   - ❖ He taught farmers about prevention of diseases in animals- silk worms.
   - ❖ He demonstrated that fermentation was caused by growth of microorganisms.

4. Semmelweis (1818-1865)
   - ❖ He showed that man can be a carrier of disease
   - ❖ He later died of an infection by bacteria

5. Joseph Lister (1827-1912)
   - ❖ He heard of Pasteur's work and realized that if bacteria can cause fermentation
   - ❖ Then they would cause suppuration of wounds
   - ❖ He therefore realized that there were ways to stop these " little bodies"
   - ❖ From multiplying by cleaning wounds regularly, soaking instruments and dressings and beginning to use antiseptic technique

6. Robert Koch (1877 - 1882)
   - ❖ Koch was a German general practitioner.
   - ❖ In 1881 he described the method of preparing cultures on solid media which made it possible for the isolation of pure strains of bacteria from single colonies
   - ❖ In 1882 he discovered the Tubercle bacillus and in 1883 the *Vibrio cholera*.
   - ❖ He modified Henre's criteria of disease causation and came up with Koch's postulates.
   - ❖ Described easy methods for bacteriological exams and also devised simple methods for isolating bacteria.
   - ❖ Pasteur's work led to an effective sterilization method
   - ❖ He developed vaccines for diseases;
     - o anthrax, fowl cholera and rabies
   - ❖ Beginning with Pasteur's work, discoveries included the relationship between microbes and disease, immunity, and antimicrobial drugs

What Koch said about Microbiology

According to Koch's postulates a micro-organism can be accepted as a causative agent of infectious disease only if the following conditions are met:

1) The microorganism should be found in plenty in all organisms suffering from the disease but should not be found in healthy animals.
2) The microorganism must be isolated from a diseased organism and grown in pure culture
3) He postulated the Germ Theory of Disease which states that microorganisms are the causes of infectious diseases.

For an organism to be said to have caused a particular disease then;

a) The micro-organism must be found in all cases of disease
b) The micro-organism must be able to be cultured outside the body for several generations
c) The micro-organism must reproduce a disease on inoculation into susceptible animals
d) The antibody to the organism develops during the course of infection

Importance of micro-organisms to man

- ❖ They cause decaying which is essential for the organic cycle
- ❖ Causes disease to both plant and animals
- ❖ Used in fermentation

Why is Microbiology Important in Nursing?

- ❖ Helps the nurse to know pathogens, their sources and composition
- ❖ Helps to know how these microorganisms are transmitted and how they spread diseases
- ❖ It is a step in prevention and cure of many diseases
- ❖ Helps the nurse to take universal precautions when in the hospital environment
- ❖ It is an important aspect in infection control
- ❖ Forms a rich source in health education, immunization and surveillance

Terminologies

- Binary fission
- Capsule
- Aerobe
- Facultative anaerobe
- Genus
- Saprophytic
- Spores
- Opportunistic

Definition of Terminologies

- ❖ **Binary fission** – splitting of one cell into two
- ❖ **Capsule** - Capsules are the outmost structures of bacterial and fungal cells.
- ❖ **Aerobe** - a microorganism which grows in the presence of air or requires oxygen for growth.
- ❖ **Anaerobic** - a microorganism that grows in the absence of oxygen.
- ❖ **Facultative anaerobe** - is an organism that is capable of producing energy through aerobic respiration and then switching back to anaerobic respiration depending on the amounts of oxygen and fermentable material in the environment.
- ❖ **Genus** - a class, kind, or group marked by common characteristics or by one common characteristic
- ❖ **Saprophytic** - any organism that lives on dead organic matter, as certain fungi and bacteria.
- ❖ **Spores** – a minute, typically one-celled, reproductive unit capable of giving rise to a new individual without sexual fusion, characteristic of lower plants, fungi, and protozoans.
- ❖ **Opportunistic** – taking advantage of a situation as they arise.

Assignment

- ✓ Explain Anthony van Leuweenhoeks contribution to the discovery of microorganisms.
- ✓ How did Louis Pasteur defeat the theory of spontaneous generation
- ✓ Draw the bacterial cell
- ✓ Draw the simple cell

Reference
- A.K Chemtai (2012) Immunology 3rd edition, Nairobi: Jomo Kenyatta Foundation
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Categories of Infectious Agents and the Bacterial Cell

Broad Objective

By the end of the lesson, the learner shall be able to demonstrate understanding of the categories of infectious agents and the Bacteria Cell structure and functions.

Specific Objectives

By the end of the lesson the learner shall be able to:

- ✓ Explain the categories of infectious agents
- ✓ Explain the structure of the bacterial cell
- ✓ Explain the functions of the parts of the bacterial cell

Categories of infectious agents – from slide 8

- ✓ Bacteria
- ✓ Viruses
- ✓ Fungi
- ✓ Parasites (protozoa)

1. **Bacteria**
   - ✓ Are unicellular/single celled organisms
   - ✓ They lack membrane bound nucleus and organelles.
   - ✓ Double stranded DNA lies loose in the cytoplasm with a rigid peptidoglycan cell wall
   - ✓ Divide by binary fission
   - ✓ Some are harmful while others are beneficial to man
   - ✓ Bacteria are very small, 0.5 to 1.0 NM in diameter.
   - ✓ Because of their small size they have high surface area/volume ratio which results in a high growth and metabolism rate.

2. **Viruses**
   - ✓ Smaller than bacteria
   - ✓ Contain either DNA or RNA as agenome

3. **Fungi**

✓ Are eukaryotic with rigid cell wall
✓ May replicate asexually or sexually
They form spore like structure

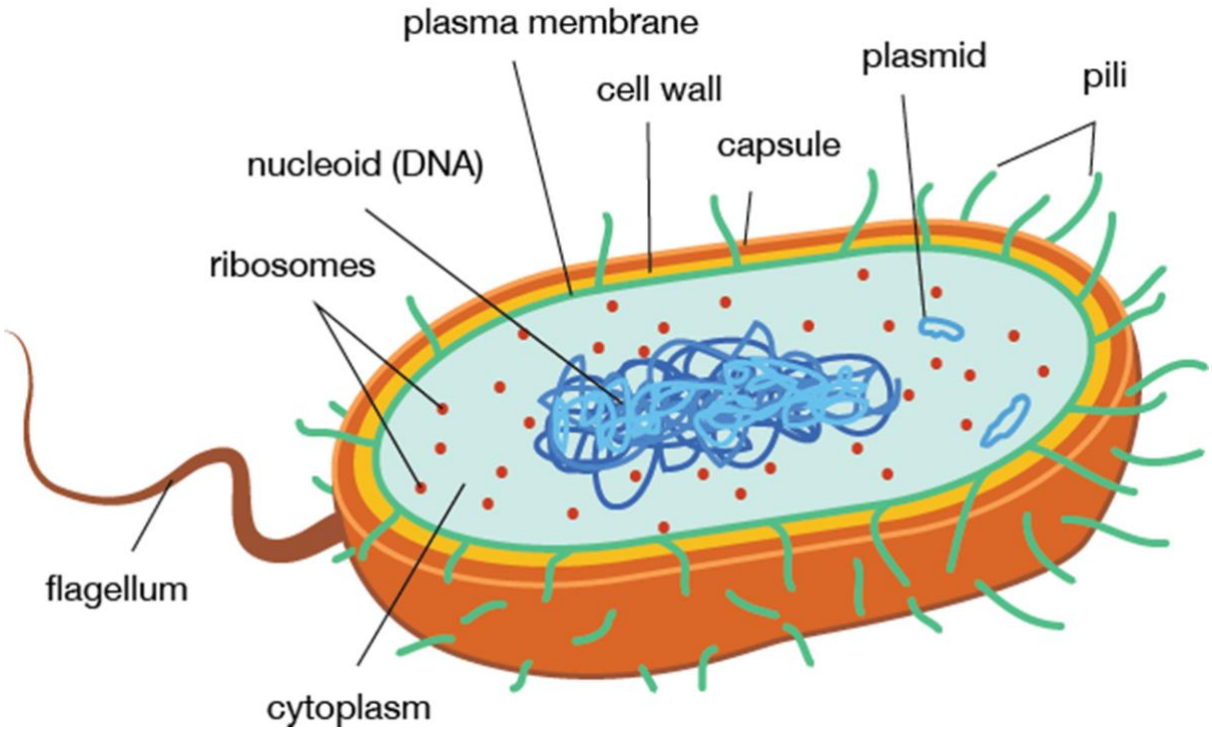
4. Parasite
✓ Parasite comprise of
    ✓ Helminths (worms),
    ✓ Protozoa (malaria parasites)
    ✓ Arthropods

The Bacterial Cell

Bacteriology
- ✓ This is the study of bacteria.
- ✓ Bacteria is a minute unicellular organism.
- ✓ Has rigid peptidoglycan cell-wall and divides by binary fission

- **Bacteria are characterized based on**
    - ✓ Structure cell arrangement
    - ✓ Size
    - ✓ The cell shape
    - ✓ Staining reactions
    - ✓ Motility
    - ✓ Flagella arrangement.

<u>Bacteria structure</u>



Characteristics of Bacteria (video

https://youtu.be/cI4rUY9S4JU

Differences between Prokaryotes and Eukaryotes

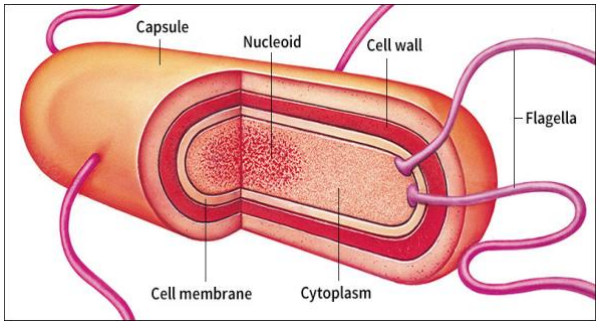
Prokaryotes	Eukaryotes
Belongs to the Monera kingdom (bacteria)	Belongs to Prostista (fungi, plantae, and animalia)
No distinct nuleaus	Have a distinct nucleus
DNA in the form of a singular chromosomes and additional DNA carried in plasmids	DNA carried on several chromosomes in the nucleus
Transcrition and translation – are carried out simultaneously	Transcription requires messengers and translation takes place in cytoplasm

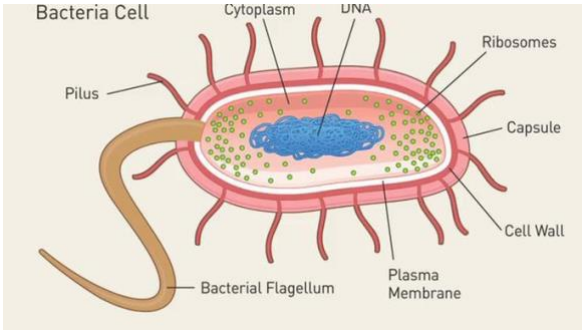
Transcription and translation can be carried out simultaneously in prokaryotes while in eukaryotes, Transcription requires messengers and translation takes place in the cytoplasm

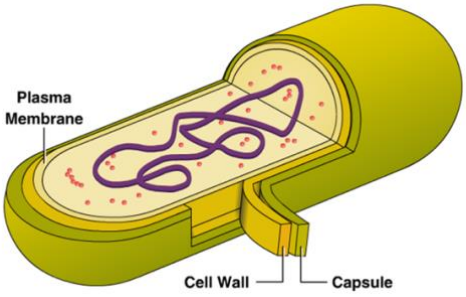
Similarities - Prokaryotes and Eukaryotes

Both cells have ribosomes that influence the process of protein synthesis
Both have DNAs that relate to genes
Cell wall is present
Have four types of major molecules
The process of DNA copying is similar
Some major metabolic pathways are common in both
The process of photosynthesis
Have twenty standard amino acids
Genetic code is identical
Adenosine triphoshate (ATP) is the source of energy

*Adenosine triphosphate (ATP) is **the source of energy for use and storage at the cellular level**. The structure of ATP is a nucleoside triphosphate, consisting of a nitrogenous base (adenine), a ribose sugar, and three serially bonded phosphate groups.*







✓ Capsule

- Is amorphous material which forms the outermost layer of bacteria
- Composed of polysaccharide and proteins
- It inhibits phagocytosis and hence
- Correlates with bacterial virulence

Phagocytosis is a fundamental process of cells to capture and ingest foreign particles. Cells use phagocytosis to capture and clear all particles larger than 0.5 µm, including pathogenic microorganisms and cellular debris. Phagocytosis involves a series of steps from recognition of the target particle, ingestion of it in a phagosome (phagocytic vacuole), maturation of this phagosome into a phagolysosome, to the final destruction of the ingested particle in the robust antimicrobial environment of the phagolysosome.
For the most part, phagocytosis is an efficient process that eliminates invading pathogens and helps maintaining homeostasis.

✓ Cell wall

- Tough rigid structure strengthened by peptidoglycan layers.
- Protects the bacteria against osmotic changes.
- Differences in composition of bacterial cell wall leads to differences in the staining of bacteria, thus Gram +Ve and Gram –Ve bacteria.
    - Gram +ve thick wall, large amount of peptidoglycan (purple)
    - Gram –ve thin wall, small amount of peptidoglycan (red/pink)

✓ Cytoplasmic membrane

- Is a selective barrier, semipermeable membrane controlling water, nutrients, ions, electrolytes movement in and out of bacteria.
- Also site for enzymes involved in active transport of nutrients and other metabolic processes

✓ Mesosome

- Are the areas in the cytoplasmic membrane of prokaryotic cell which fold inwards
- Site for septum formation and involved in DNA segregation during cell division.

Mesosomes are the areas in the cell membrane of prokaryotic cell which fold inwards. It helps in cell wall formation, DNA replication, respiration and increases the surface area of the plasma membrane.

- ✓ **Nuclear material/nucleuos-** for replication of DNA and RNA
- ✓ **Ribosomes**- site for protein synthesis
- ✓ **Flagella**- long filaments structures for bacterial motility

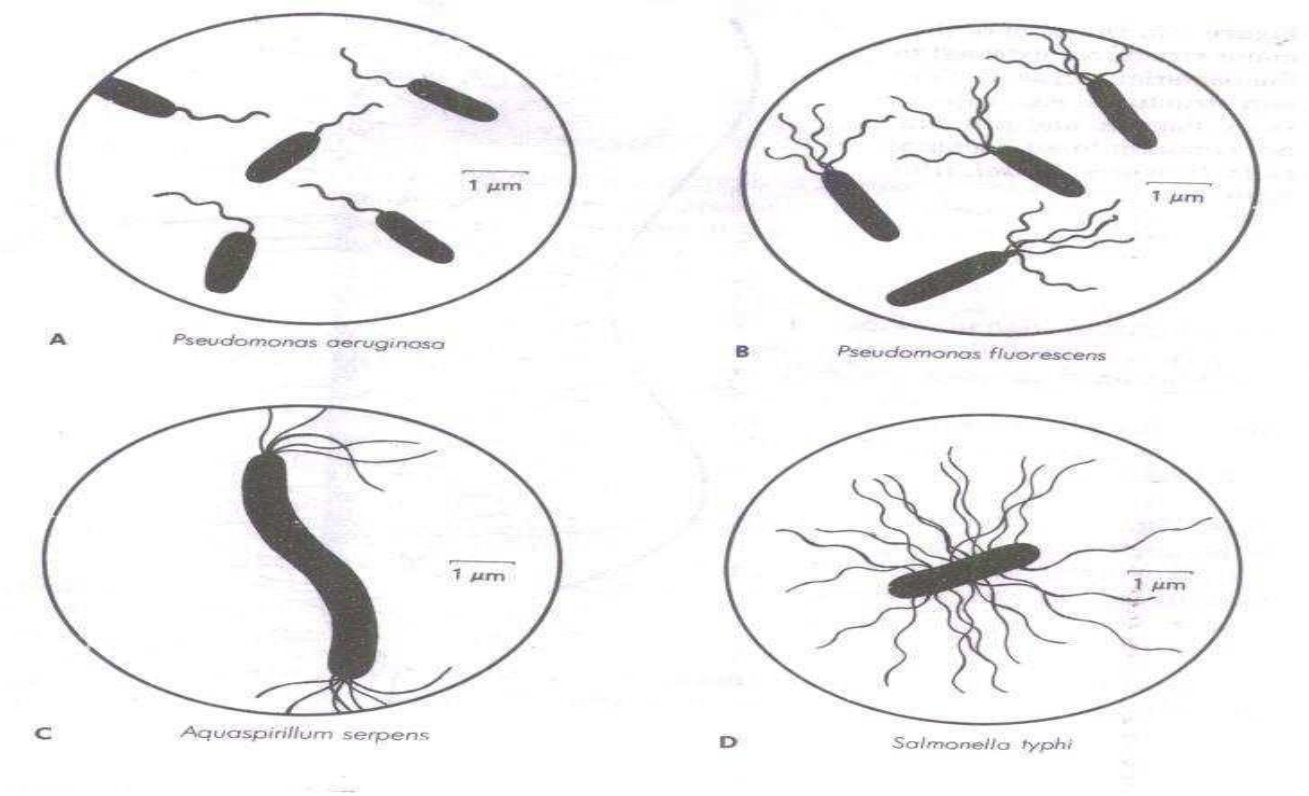
Function of Flagella

- Bacterial propel themselves by rotating their helical flagella.
- Based on their location on the cell, flagella may be polar or lateral.
    (i) Polar: At one or both ends of bacterium.
    (ii) Lateral: Along the sides of the bacterium.

Types of flagella

- **Monotrichous:** A single polar flagellum.

  Many that appears and function as monopolar or bipolar flagella consists of bundles of 2 to 50 single units (polytrichous).
- **Lophotrichous:** A cluster of polar flagella.
- **Amphitrichous:** Flagella, either single or clusters at both cell poles.
- **Peritrichous:** Cell surrounded by lateral flagella.



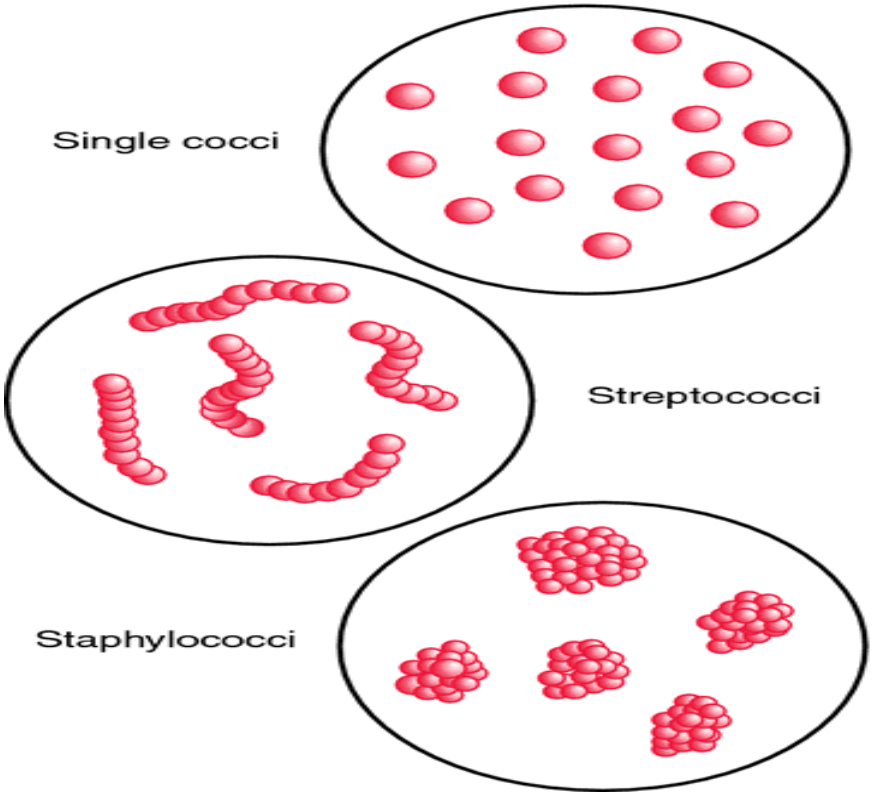
Classification of bacteria

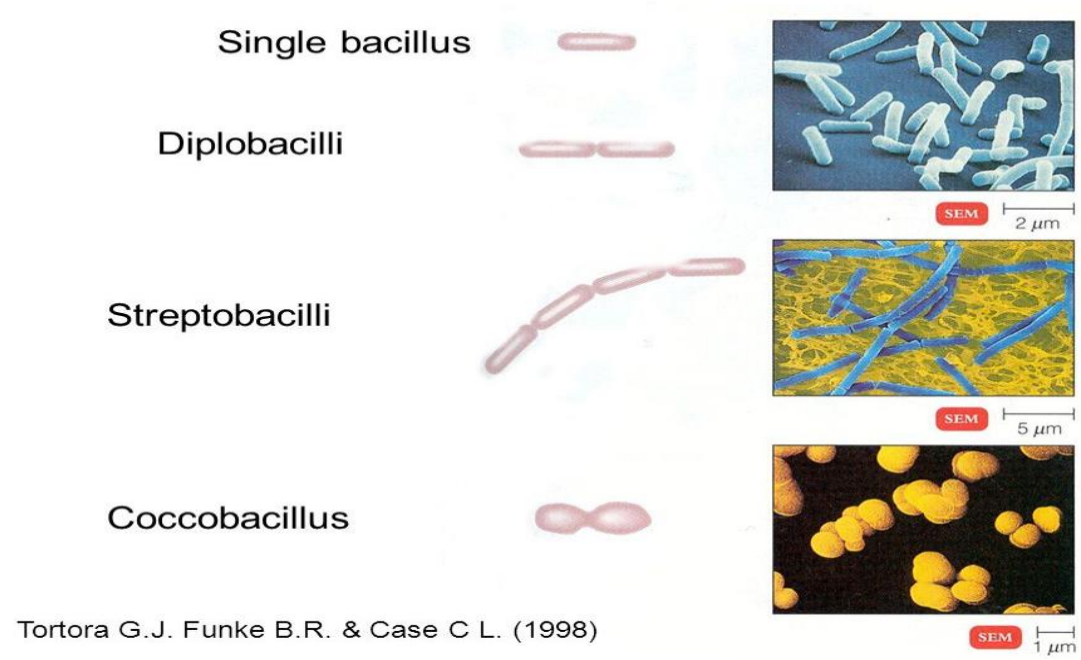
Bacteria classification is based on:

- Morphology – size and shape
- Biochemical characteristics
- Lab identification
- Growth requirement/cultural characteristics

1. Morphological

- ✓ Spherical-cocci eg S. pneumonia
- ✓ Cylindrical-bacilli or rods
- ✓ Helical- spirochaetes, flexible, coiled and motile e.g Trepanoma pallidum
- ✓ Vibrios-coma like

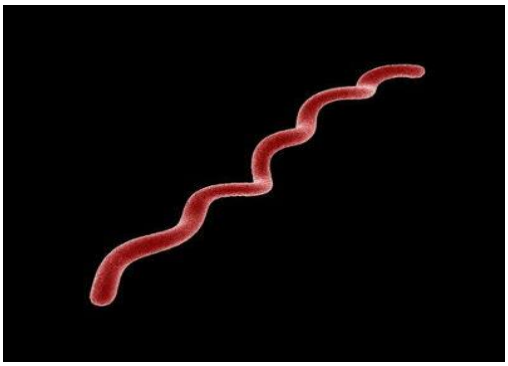




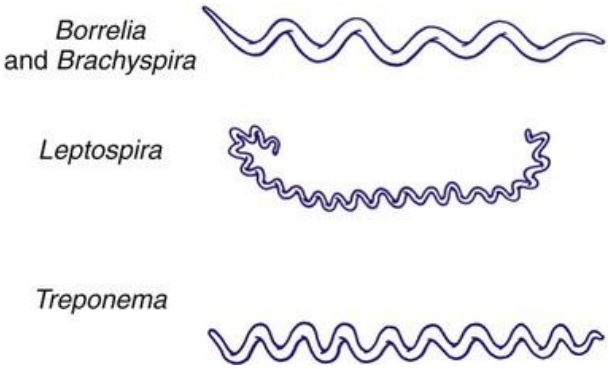
Tortora G.J. Funke B.R. & Case C L. (1998)

Vibros

Spirilla



Spirochets



2. Biochemical

- Coagulase-the enzyme that clots/coagulates plasma and fibrin
- Hyalurodinase-breaks hyaluronic acid the cement of connective tissue, causing increased permeability
- Production of toxin-exotoxins and endotoxins

3. Lab Identification

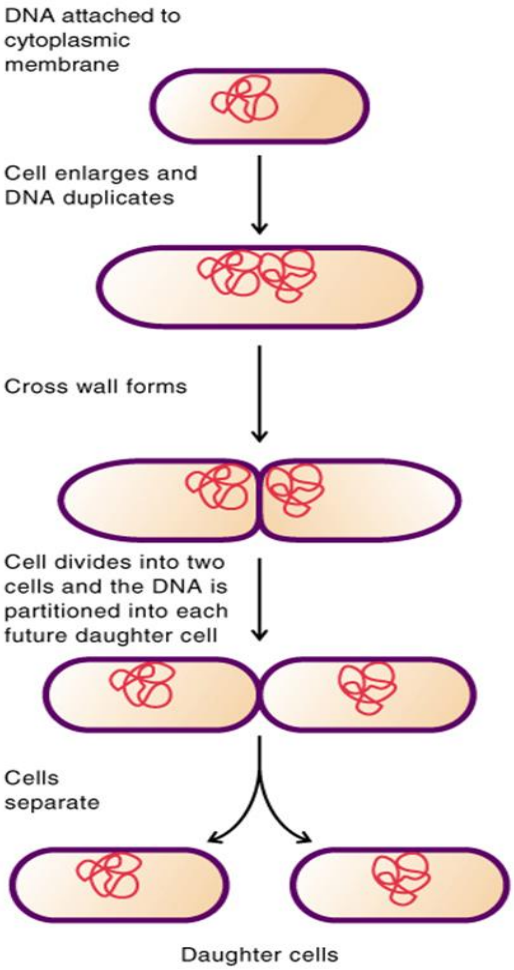
- Gram staining
- Giemsa staining-histopathological diagnosis of malaria and other parasites
- Elisa (HIV) Ziehl Neelsen test- for acid fast bacilli eg mycobacteria AFB
- Urinalysis- E. coli
- CSF analysis
- Field staining for malaria parasite

4. Growth Requirement/Cultural Characteristics

- Binary fission
- Generation time
- Phases of growth

Binary Fission

1. Prokaryote cells grow by increasing in cell\number (as opposed to increasing in size).
2. Replication is by binary fission, the splitting of one cell into two
3. Therefore, bacterial populations increase by a factor of two (double) every generation time.



Generation Time

- The time required for a population to double (doubling time) in number. eg
  - Ex. *Escherichia coli* (*E. coli*) double every 20 minutes
  - Ex. *Mycobacterium tuberculosis* double every 12 to 24 hours

Assignment
Find out other bacterias generation time (how long it takes them to double)

References

- A.K Chemtai (2012) Immunology 3rd edition, Nairobi: Jomo Kenyatta Foundation
- Arora D.R. & BrijBala A. (2011). Microbiology for Nursing and Allied Sciences, 2nd edition India: BS Publishers and Distributors PVT LtD.
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Classification of bacteria continued

Bacteria classification is based on;

- Morphology – size and shape
- Biochemical characteristics
- Lab identification
- Growth requirement/cultural characteristics

Growth requirement/cultural characteristics

- Binary fission
- Generation time
- Phases of growth

Growth in Batch Culture

- Bacteria growing in batch culture produce a growth curve with up to four distinct phases.
- Batch cultures are grown in tubes or flasks and are closed systems where no fresh nutrients are added or waste products removed

Phases of Growth

1. Lag phase occurs when bacteria are adjusting to them medium. For example, with a nutritionally poor medium, several anabolic pathways need to be turned on, resulting in a lag before active growth begins.

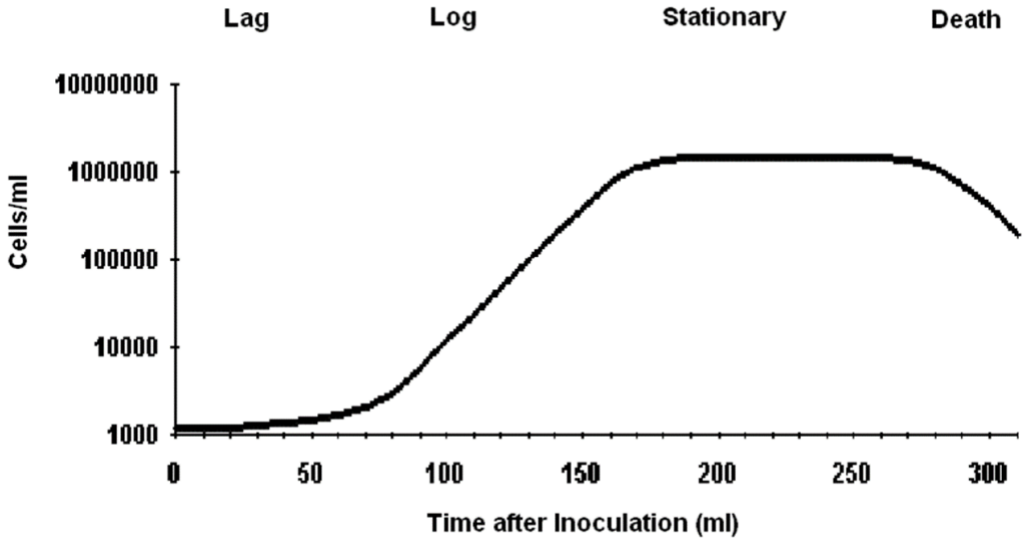
During lag phase, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs. During the lag phase cells change very little because the cells do not immediately reproduce in a new medium. This period of little to no cell division is called the lag phase and can last for 1 hour to several days. During this phase cells are not dormant

2. **Log or Exponential phase,** the cells are growing as fast as they can, limited only by growth conditions and genetic potential. During this phase, almost all cells are alive, they are most nearly identical, and they are most affected by outside influences like disinfectants.
3. Due to nutrient depletion and/or accumulation of toxic end products, replication stops and cells enter a **Stationary phase** where there is no net change in cell number.

The stationary phase is often due to a growth-limiting factor such as the depletion of an essential nutrient, and/or the formation of an inhibitory product such as an organic acid. Stationary phase results from a situation in which growth rate and death rate are equal. The number of new cells created is limited by the growth factor and as a result the rate of cell growth matches the rate of cell death. The result is a "smooth," horizontal linear part of the curve during the stationary phase. Mutationscan occur during stationary phase. Bridges et al. (2001) presented evidence that DNA damage is responsible for many of the mutations arising in the genomes of stationary phase or starving bacteria. Endogenously generated reactive oxygen species appear to be a major source of such damages.

4. **Death phase** occurs when cells can no longer maintain viability and numbers decrease as a proportion.

Growth Curve



Techniques to Study Morphology of Bacteria

1. Microscopy and staining techniques - basic tools to visualize and study morphology of bacteria
   Various techniques of microscopy that have been used in bacteriology include:
   - Light microscopy
   - Phase contrast microscopy
   - Fluoresence microscopy
   - Darkfield microscopy
   - Electron microscopy

Microscopic Examination

- The analysis of a sample taken for diagnostic purposes is generally a cytological and bacteriological analysis. Thus, microscopic examination is a key step in the diagnostic process for bacterial infections.
- Microscopic examination in bacteriology can be carried out without staining the sample by direct observation between slide and coverslip, or after staining

Light Microscopy

- The ordinary light microscopy gives a resolving power of about 200 nm under optimal conditions.
- Objects smaller than this cannot be delineated to reveal their true size or structure

Phase Constrast Microscopy

- The phase contrast microscopy enhances the refractive index differences of the cell components
- This microscopy can be used to reveal details of the internal structures as well as capsules, endospores and motility

Fluorescence Microscopy

- In fluorescence microscopy ultraviolet rays are used to examine cells after treatment with fluorescence dyes.

Dark Field Microscopy

- Dark field microscopy is a technique use where objects are lit at a very low angle from the side so that the background appears dark and the objects show up against this dark background.

Electron Microscopy

- Electron microscopy is a technique used to obtain ultrahigh resolution images of individual atoms of materials and internal structures of cells. The resulting atomic-level or micro images can be used to investigate the sample properties and behaviour of that bacteria.

2. Fresh Living Preparation

- **Live samples of micro organisms are placed in wet or hanging drop amount so that they can be observed as much as possible in their natural state.**
- **Cells are suspended in a suitable fluid (water, broth, saline) that temporarily maintain viability and provide space and medium for locomotion.**
- **A wet amount consists of a drop of two of the culture placed on a slide and over laid with a cover glass.**
- **Coverslide can damage the large cells and the slide is susceptible to drying and can contaminate the slide handler.**

3. Fixed Stain Smear

i. Smear
ii. Staining
iii. Fixation

i. Smear

- The bacterial smear is a thin layer of bacteria placed on a slide for staining.
- To heat-fix a sample a thin layer of the specimen is spread on the slide and the slide is then briefly heated over a heat source

ii. Staining:

- It is technique that is rapid and effective way of preparing a bacterial smear for viewing.
- It is a one-step procedure in which the smear is covered with stain and allowed to sit undisturbed for a minute or so during which the bacterial cytoplasm chemically unites with the basic stain.
- The remaining stain is washed away
- Stains or dyes contains contain salts made up of a positive ion and a negative ion.
- Some staining techniques involve the application of only one dye to the sample while others require more than one dye
- Simple staining is where a single dye is used to emphasize particular structures in the specimen.
- In contrast, differential staining distinguishes organisms based on their interactions with multiple stains

iii. Fixation

- Fixation, this is a method of preparing a specimen for light microscopy.
- The fixing – refers to the process of attaching cells to a slide.
- Fixation is achieved by heating (heat fixing) or chemically treating the specimen
- Fixation kills microorganisms in the specimen

Assignment:

List and explain the nutrition and growth requirement for the optimal growth of bacteria.

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1. Parija S.C. (2012). Textbook of Microbiology & Immunology.(2 ed.). India: Elsevier India.
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Factors that influence Growth and Nutrition of Bacteria

- Bacteria growth – refers to quantitative increase in bacteria numbers over a given period of time.
- Nutrition - this is the process by which chemical substances called nutrients are acquired from the surrounding environment and used in cellular activities example:
  - Metabolism
  - Growth

Factors affecting Growth and Nutrition of Bacteria

- ✓ Nutrients
- ✓ Temperature
- ✓ Oxygen requirement
- ✓ pH
- ✓ Moisture and Desiccants
- ✓ Light and Radiation

Nutrition in Bacteria

Some bacteria require a single source of organic materials for their nutrients (non-fastidious) normal range. While others are nutritionally extracting (fastidious) and require complex media where several organic molecules are available

- ➢ Nutrition based on source of carbon
  - Autotrophs – depends solely on carbon dioxide as the main source of carbon
  - Heterotrophs – depend on complex performed organic molecules as their main source of carbon e.g glucose from carbohydrates.
- ➢ Nutrition based on source of energy:

- Phototrophs – uses light as a source of energy
- Chemotrophs – uses either inorganic or organic chemicals as their energy source
- Saprobes – satisfy their nutritional needs by feeding upon dead decaying organisms

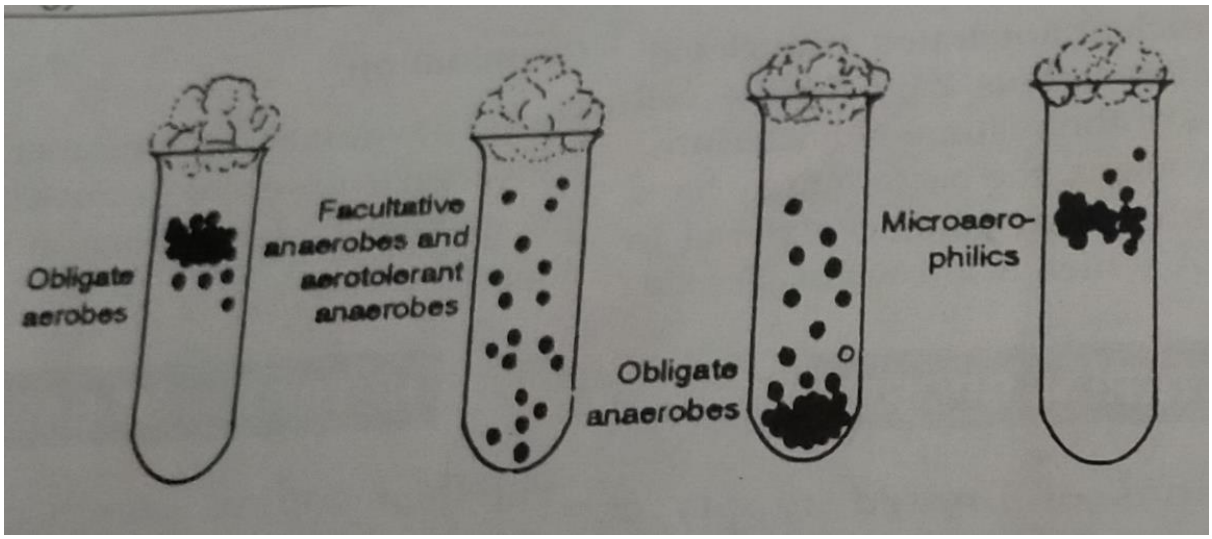
Nutrients

- Macro-nutrients required in large amounts they play principle role in structure and metabolism example:
    - Carbon
    - Nitrogen
    - Potassium
    - Calcium
    - Sodium
    - Magnesium
- Micro-elements (trace elements) required in relatively much smaller amount for enzymes and pigment structure and functions example:
    - Cobalt
    - Zinc
    - Copper
    - Nickel

Oxygen

1. Aeration – aeration is attributed to the need or effect of oxygen and carbon dioxide. This normally reflects the mechanism used by the organism to meet the energy requirement based on aeration they are categorized as:
    - Aerobes – organisms grow only in the presence of oxygen
        - Pseudomonas
        - Bacillus

Oxygen Requirement of Bacteria



Rattan and Rajesh, 2007

- **Obligate aerobes –** cannot grow in absence of oxygen
    - Bacterials
    - Clostridium

- **Facultative anaerobes –** can grow with or without oxygen
    - Vibrio
    - E-coli
    - Salmonella

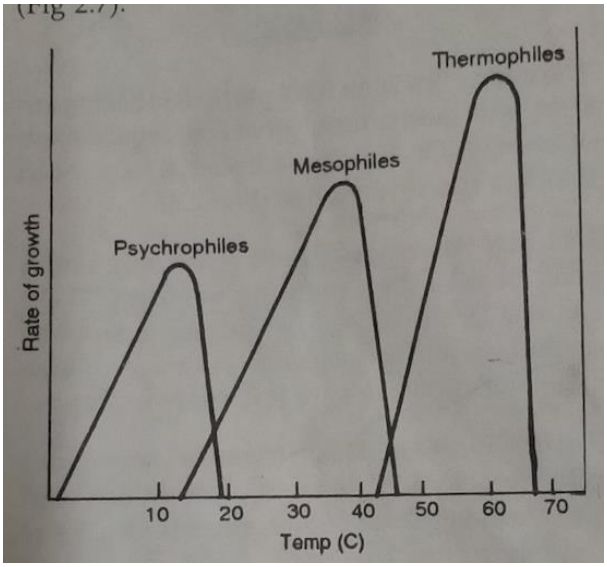
- **Micro erophiles –** bacteria that require reduced oxygen concentration for optimal growth.
    - Shigella
    - Staphylococcus
    - Most cells live on water, soil or human body when oxygen is limited e.g. Haemophilus

- **Anaerobes –** they grow optimally in the absence of oxygen. They are killed by oxygen since they lack enzymes e.g
    - Catalase
    - Peroxidase
    - Superoxide
    - Hydroxyl radical
- **Aero tolerant anaerobes –** they don't utilize oxygen but it tolerates its presence. Have an alternative way of breaking the toxins e.g
    - Lactobacillus
    - Anaerobic streptococci

- **Capnophiles –** grow best in higher concentration of carbon dioxide than normal presence of atmospheric

Temperature Requirement

- Most bacteria have a narrow range of temperature requirement for their optimal growth.
- On the basis of temperature requirement three groups of bacteria are recognised:
    - Psychrophilic bacteria – 5 to 30°C
    - Mesophilic bacteria – 20 to 40°C
    - Thermophilic bacteria – 25 to 80°C



Psychrophilic bacteria

- These bacteria grow between 5-30^{o} C with an optimal growth of 10 to 20^{o} C. Room temperature is lethal to them.
- Refrigeration incubates rather than inhibits. Most of them are not pathogenic to man.

Mesophilic bacteria

- These bacteria grow best at 20 to 40^{o}C with a range of 10 to 45^{o} C.
- All the medically important bacteria belong to this group
- Some of the bacteria in this group have an affinity for a specific temperature
- Mycobacterium leprae (causes leprosy) is one such important example and it can grow only at reduced temperature
- Most of them are pathogenic to human

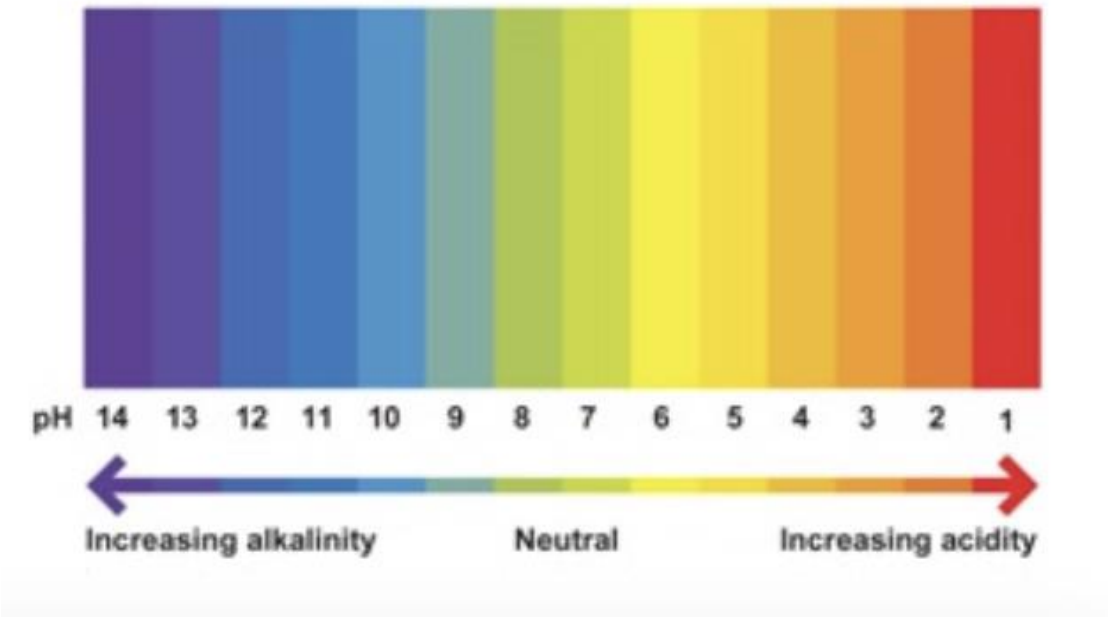
Thermophilic bacteria

- These bacteria prefer high temperatures for their growth - 25-80^{o} C and yield maximum growth at 50-60^{o} C
- They are heat loving
- Mose of them are spore forming eg. bacillus species, clostridium species.
- A small number is pathogenic to man

pH Requirement

- The pH (potential hydrogen) of the medium of growth of bacteria has profound effect upon the multiplication of organisms.
- The pH range tolerated by most bacteria extends over 3-4 units but rapid growth is confined to one unit or less.
- Most pathogenic bacteria require a pH of 7.2 to 7.6 for their optimal growth
- The pH of the growth medium keeps on changing depending upon the reaction performed by the bacterium with the nutrients.
- An optimal initial pH may not last long especially if the organism exhibits a fermentative type of metabolism
- Most bacteria are of three kinds:
    - Acidophiles – grow well in acidic
    - Neutralphiles – grow well in neutral
    - Alkalophiles – grow best in alkalines

pH Scale



Acidophiles examples

Examples of acidophiles include

- Thiobacillus acidophilus (a type of bacteria),
- Vorticella (a type of eukaryote), and
- Crenarchaeota (a type of archaea).

Neutrophiles examples

There are many examples of encapsulated bacteria that have been described as inhibiting neutrophil phagocytosis including

- Streptococcus spp.,
- Neisseria meningitidis
- Klebsiella spp.,
- Escherichia coli
- Pseudomonas aeruginosa
- Haemophilus influenzae

Alkaliphiles examples

Many different kinds of alkaliphilic microorganisms, including bacteria belonging to the genera

- Bacillus,
- Micrococcus,
- Pseudomonas, and
- Streptomyces and
- Eukaryotes such as yeasts and filamentous fungi, have been isolated from a variety of environments.

Moisture and Desiccants

- Moisture and desiccants – moisture is very imperative for bacteria growth, example
- Gonococcus and
- Treponema pallidum are very delicate and die quickly in dry conditions.
- A desiccant is a hygroscopic material that absorbs or adsorbs water molecules from the surrounding air, decreasing the levels of moisture.
- Desiccants decrease the amount of shipped goods that are wasted due to moisture damage

Light and Radiation

- Light - growth and viability of bacteria is favored by darkness
- Radiation - while ultraviolet (UV) and ionizing radiation are lethal to bacteria.

Assignment

Read about Normal flora of the human body

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Normal Microbial Flora of the Human Body

What is Normal Flora?

- Normal flora are microorganisms (bacteria, fungi, protozoa, and viruses), mostly bacteria that continuously inhabited the human body (Resident Normal Flora).
- Under normal conditions in a healthy human they are harmless and may even be beneficial.
- Also called Commensals i.e. organisms that dine together or Microflora.

About Normal Flora

- A fetus is sterile when born (No Normal Flora), then newborn start having the normal flora from its mother, air, food and the environment.
- Our internal organs are sterile like the spleen, liver, pancreas, bladder, CSF, and blood unless during infection.
- Normal flora differ from one human to another depending on age, diet, and geographic habitat.
- When the number of resident normal flora is greatly reduced, opportunistic microbes can easily cause infections in these areas e.g. Candida albicans that cause candidiasis.

Why Should We Know About Normal Flora?

Knowledge of types and distribution of normal flora in our bodies because:

1. It gives us better understanding of the possible infections that result from injury to a specific body site.
2. As well as the possible sources and significance of microorganisms isolated from the site of an infection.

Normal Microbial Flora

- The term normal microbial flora refers to the population of microorganisms that inhabit the skin and mucous membranes of a healthy human being.
- Normal body flora are of two types:
  - ✓ Transient flora
  - ✓ Resident flora

Transient Normal Flora

- Normal flora that are temporarily living on and within humans.
- The transient microbes living in the external environment are attracted to moist, warm body areas.
- They consist of non-pathogenic or potentially pathogenic microorganisms that inhabit a particular body area for a limited period.
- If transient flora are intact there is very little significance.
- If transient flora are disturbed they may take over, multiply and produce disease.

Why are Transient microbes temporary?

1. They may be washed from external areas by bathing.
2. They may not be able to compete with resident normal flora.
3. They may be killed by substances produced by the resident normal flora.
4. They may not survive in the acidic or alkaline pH of the site.
5. They may be flushed away by bodily secretions like tears, sweat, oil, urine, feces,..).

Resident Normal Flora

The resident flora consists of fixed types of microorganisms regularly found in a given area at a given age and if disturbed it promptly re-establishes itself.

Why is Resident Flora important

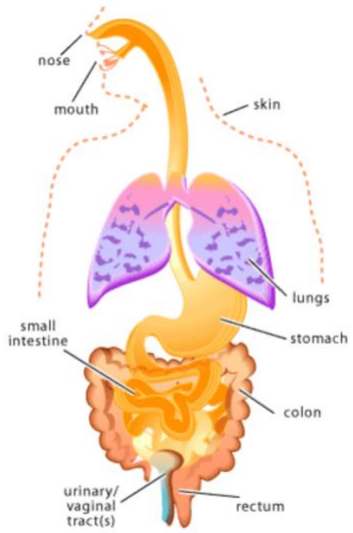
- They maintain proper health by performing various functions.
- They can also act as a source of spread of bacterial pathogens from one part of the world to another.
- Various serotypes of **Neisseria meningitidis** are normally caried in the nasopharynx – spread in crowds like in pilgrims, and those pilgrims take back the infection to their countries and hence leads to disease outbreaks.

Sites of Normal flora and Microorganisms

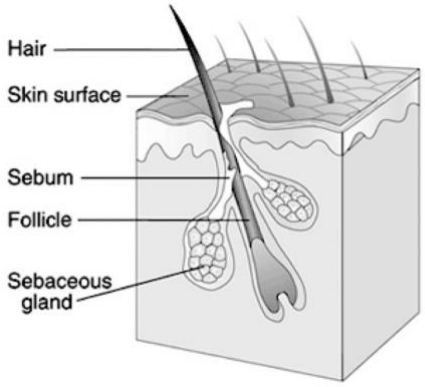
Site of Normal Flora	Microorganism present
Skin (head, leg, feet and other places)	Staphlococcus epidermidis Propionibacterium acnes Pityrosporum ovale
Nose	Staphlococcus aureus Staphlococcus epidermidis Corynebacterium species
Mouth	Streptococcus species Fusobacterium species Actinomyces species Leptotricia species Veilonella speciesi
Throat	Streptococcus species Branhamella catarrhalis Corynebacterium species Haemophilus species Neisseria species Mycoplasma species
Lung	Pneumocystis carinii

Intestine	Bacteroides fragilis Escherichia coli Proteus mirabillis Klepbsiella species Lactobacillus species Streptococcus species Candida albicans Clostridium species Pseudomonas species Enterococcus species
Urethra and vagina	Streptococcus species Lactobacillus species Candida Albicans Gardnerella vaginalis Mycobacterium species Escherichia coli Bacteroides specieis

Sites of Norml Flora



Skin



The Skin

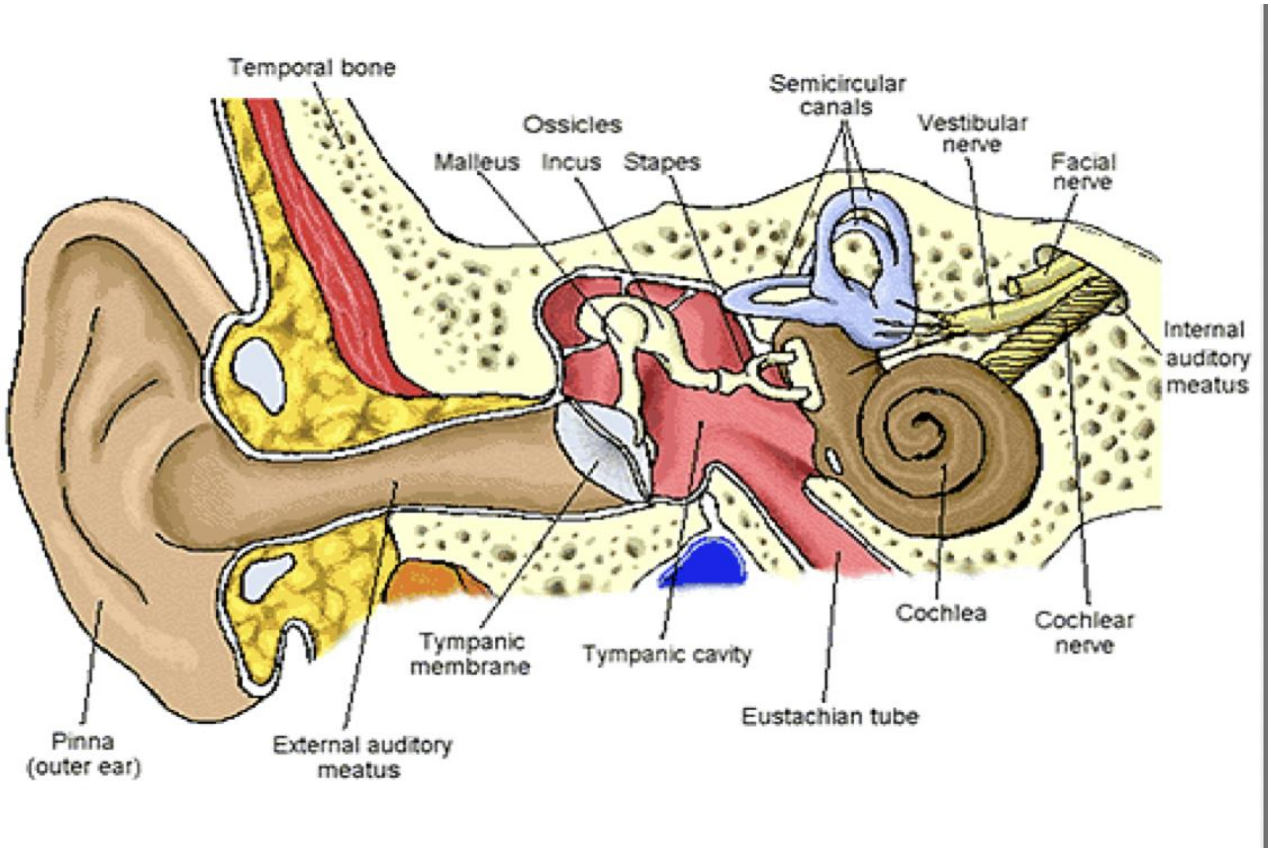
- Aerobic Bacteria:
  - Present in the outer layer of skin.
  - Staphylococcus epidermidis (accounts 90%) +Staphylococcus aureus.

- Anaerobic Bacteria:
  - Present in the deeper skin layers, hair follicles, and sweat & sebaceous glands.
  - Propionibacterium acnes.

- Skin normal flora are generally harmless but it might cause bloodstream infections if skin was penetrated.

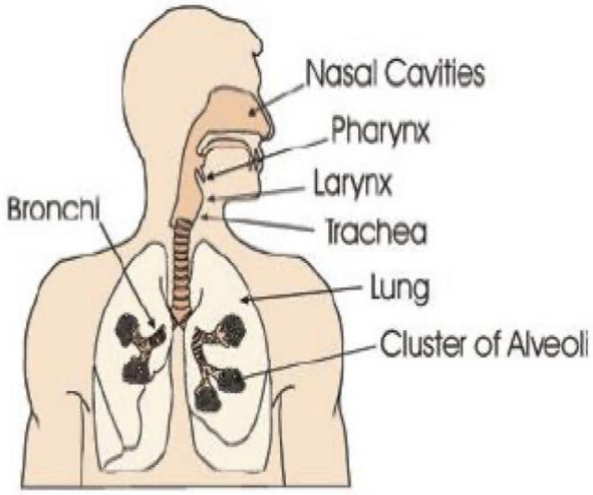
Health Care Workers must be particularly careful to keep their skin and clothing free of transient microbes as possible to prevent personal infections and to avoid transferring pathogen to patient.

Microflora of the EARS



- **The middle ear and inner ear: are usually sterile.**
- **The outer ear and the auditory canal: contain the same normal flora of the skin.**
- **When the person coughs, sneezes, or blows his nose, these microbes may move into the middle ear where they cause infection.**

Respiratory Tract Flora



Upper Respiratory Tract: - Nose and throat have many microorganisms. Some are normal flora, some are opportunistic, and others are carried like Corynebacterium diphtheriae (C. diphtheroids)

Nasopharynx: Streptococcus pneumoniae
In immune compromised or elderly it might cause acute bacterial pneumonia

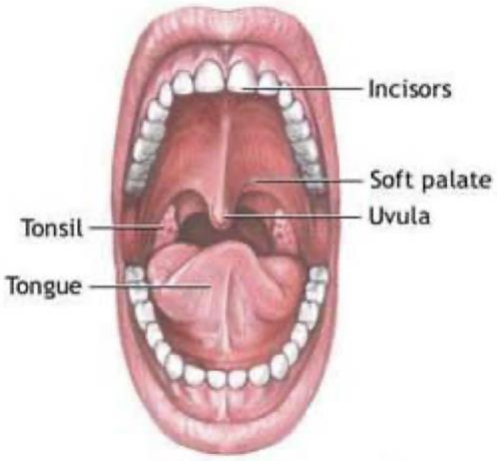
Lower Respiratory Tract:

- Is usually sterile because the mucous membranes of the lungs remove any microbes

Oral Cavity (Mouth)

They have both aerobic and anaerobic bacteria.
The most common ones are:

- Corynebacterium diphtheroides
- Staphylococcus aureus
- Staphylococcus epidermidis.
- Also yeasts, protozoa, and viruses can be living in the mouth.



Teeth and Gengiva: Streptococcus mutans

- Poor dental hygiene help bacteria to grow and cause dental caries, gingivitis,...
- After dental surgeries, there might be a risk of bloodstream infection that might cause endocarditis.

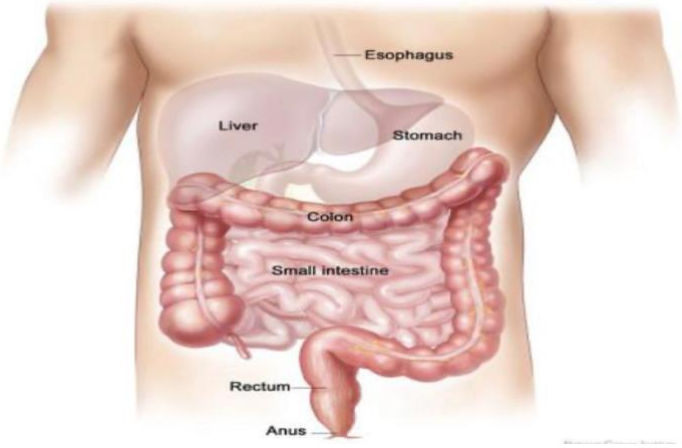
Microflora of the Gastrointestinal Tract

Stomach:

- Only few bacteria are present in the stomach due to the gastric enzymes and acidic pH.

Small intestines:

- Only few normal flora are present in the upper part of small intestines because bile kills them.
- Lower parts have more number of normal flora



Large Intestines:

- Has more bacteria than any other part of the body
- 99% of normal flora in the large intestine are anaerobic Bacteroides species.
- Also many fungi, protozoa, and virus can live there.
- Many of th enormal flora are opportunistic – i.e. if they move to to other areas e.g. E.Coli cause urinary infection

Microflora of the Urogenital Tract

Urinary Tract

- Kidneys, Ureters and Urinary Bladder: are sterile.
- Lower Urethra and external opening: bacteria, yeast, and viruses. Has the same bacteria present on the skin.

Genital Organs

1. Male and female genitals: are sterile except vagina.
2. Vagina: Lactobacillus spp. keeps the pH acidic to protect the vagina from opportunistic infections e.g. fungal vaginitis (Candida albicans) or bacterial vaginosis (Bacteroides spp., Gardnerella vaginalis).

Factors influencing normal flora

- Local environment – pH, temperarure, oxygen, water and nutrient levels
- Diet
- Age
- Health condition (immunity activity)
- Antibiotics

Significance of normal flora

Normal flora may aid the host in several ways:

- Aid in digestion of food
- Help the development of micosal immunity
- Protect the host from colonization with pathogenic microbes.

Benefits of normal flora

1. Protect our organs and systems that are in direct contact with the external environment from invading pathogens. Some normal flora produce substances that kills pathogens and others compete with them for nutrients.
2. In newborns, normal flora stimulates the development of immune system.
3. Normal flora of the gut provides important nutrients such as Vitamin K which aid in digestion and absorption of nutrients**.**

Harmful effects of normal flora

1. When the normal flora are displaced from their normal site of the body e.g. bloodstream infections by Staphlococcus epidermidis.
2. When potential pathogens gain a competitive advantage due to diminished populations of harmless competitors e.g. Clostridioides difficile (C. difficile) growing in the gut after antibiotic therapy.

3. When harmless, commonly ingested food substances are converted into carcinogenic derivatives by bacteria in the colon e.g. sweetener cyclamate.
4. When individuals are immunocompromised, normal flora can overgrow and become pathogenic.

Assignment

Find out what diseases do normal comensuls cause if they enter a zone that is not their normal zone

- Respiratory system comensuls
- Digestive system comensuls

Classification of Bacteria

Gram Positive vs Gram Negative

https://youtu.be/Jvo6IGKTvxA

Gram Positive Bacteria

Gram-positive cocci:

1. staphylococcus aureus
2. Staphylococcus epidermidis
3. Streptococcus pneumoniae
4. Streptococcus pyogenes
5. Streptococcus agalactiae
6. Enterococci

Gram-positive rods:

1. Clostridia
2. Bacillus anthraci
3. Corynebacterium diphtheria
4. Listeria monocytogenes

Staphylococcus aureus

- It is a gram-positive, catalase-positive, coagulase-positive cocci in clusters.
- S. aureus can cause inflammatory diseases, including skin infections, pneumonia, endocarditis, septic arthritis, osteomyelitis, and abscesses.
- S. aureus can also cause toxic shock syndrome toxin (TSST-1), scalded skin syndrome

Staphylococcus epidermidis

- **It is a gram-positive, catalase-positive, coagulase-negative cocci in clusters and is novobiocin sensitive.**
- ***S. epidermidis* commonly infects prosthetic devices and IV catheters producing biofilms.**
- ***Staphylococcus saprophyticus* is novobiocin resistant and is a normal flora of the genital tract and perineum.**
- ***S. saprophyticus* accounts for the second most common cause of uncomplicated urinary tract infection (UTI).**

Streptococcus pneumoniae

- It is a gram-positive, encapsulated, lancet-shaped diplococci, most commonly causing:
  - otitis media,
  - pneumonia,
  - sinusitis, and
  - meningitis.
- Streptococcus viridans consist of Strep. mutans and Strep mitis found in the normal flora of the oropharynx commonly cause dental carries and subacute bacterial endocarditis (Strep. sanguinis).

Streptococcus pyogenes

- It is a gram-positive group A cocci that can cause
  - pyogenic infections (pharyngitis, cellulitis, impetigo, erysipelas),
  - toxigenic infections (scarlet fever, necrotizing fasciitis), and
  - immunologic infections (glomerulonephritis and rheumatic fever).
- ASO titer detects S. pyogenes infections.

ASO titer – is a test done in the laboratory
Antistreptolysin O (ASO) titer is a blood test to measure antibodies against streptolysin O, a substance produced by group A streptococcus bacteria. Antibodies are proteins our bodies produce when they detect harmful substances, such as bacteria.
*Erysipelas is **a bacterial skin infection involving the upper dermis that characteristically extends into the superficial cutaneous lymphatics**. It is a tender, intensely erythematous, indurated plaque with a sharply demarcated border*

Streptococcus agalactiae

- It is a gram-positive group B cocci that colonize the vagina and is found mainly in babies. Pregnant women need screening for Group-B Strep (GBS) at 35 to 37 weeks of gestation.

Enterococci

- This is a gram-positive group D cocci found mainly in the colonic flora and can cause
  - Biliary tract infections and UTIs.
  - Vancomycin-resistant enterococci (VRE) are an important cause of nosocomial infections

Gram-positive rods

- Clostridia is a gram-positive spore-forming rod consisting of
  - *C. tetani*,
  - *C. botulinum*,
  - *C. perfringens*, and
  - *C. difficile*. - this is often secondary to antibiotic use (clindamycin/ampicillin), PPI use, and recent hospitalization. Treatment involves primarily with oral vancomycin.

Bacillus anthracis

- It is a gram-positive spore-forming rod that produces anthrax toxin resulting in an ulcer with a black eschar.
- Bacillus cereus is a gram-positive rod that can be acquired from spores surviving under-cooked or reheated rice. Symptoms include
  - nausea, vomiting, and watery non-bloody diarrhea

Corynebacterium diphtheria

- It is a gram-positive club-shaped rod that can cause
  - pseudomembranous pharyngitis,
  - myocarditis, and
  - arrhythmias.
- Toxoid vaccines prevent diphtheria.

Listeria monocytogenes

- It is a gram-positive rod acquired
  - by the ingestion of cold deli meats and unpasteurized dairy products or
  - by vaginal transmission during birth.
- It can cause neonatal meningitis, meningitis in immunocompromised patients, gastroenteritis, and septicemia. Treatment includes ampicillin.

Gram-negative bacteria (GNB)

They are among the world's most significant public health problems due to their high resistance to antibiotics.
These microorganisms have significant clinical importance in hospitals because they put patients in the intensive care unit (ICU) at high risk and lead to high morbidity and mortality.

Two large groups:

- *Enterobacteriaceae* and
- non-fermenters,

are responsible for most clinical isolates; nevertheless, other clinically concerning gram-negative organisms exist, including but not limited to

- Neisseria,
- *Haemophilus spp*.,
- *Helicobacter pylori*, and
- *Chlamydia trachomatis*.

Gram Negative Bacteria.

1	**Pseudomonas**	**6**	**Escherichia**
2	**Klebsiella**	**7**	**Morganella**
3	**Proteus**	**8**	**Aeromonas**
4	**Salmonella**	**9**	**Citrobacter**
5	**Providencia**		

Concept of Infection

Objectives

By the end of this session, the learner will be able to:

1. Describe the stages of development of an infection
2. Define various terms related to infection
3. Describe the phases of an infectious disease

What is infection?

Infection

- Is invasion by and multiplication of pathogenic microorganisms in a body part or tissue
- This invasion may produce subsequent tissue injury and progress to disease through a variety of toxic mechanisms.

Stages in development of infection

1. Acquisition
2. Adhesion to host cells
3. Penetration of cells

} Mostly occur together

4. Multiplication in tissues
5. Damage to tissues
6. Spread to other tissues
7. Resolution or death

1. Acquisition, 2. Adhesion & 3. Penetration

- The microorganism must adhere to the cell, then penetrate it.
- The microbe may use various methods to:
  - Special hairs on their surface,
  - Fimbriae (thin appendages used in attachment) or Pilli (thin appendages used in genetic exchange),
  - Secrete sticky substances (e.g. dextran)
  - Produce slime. (e.g. biofilm)

2. Multiplication in the host

- The microbe multiplies in the host cell & overpowers the host's defenses so as to cause disease.
- The virus has the ability to switch the metabolism of the cell to the production of viral components.

3. Tissue damage

- Pathogenic microorganisms cause disease by damaging the host's tissue.
- Damage occurs due to release of enzymes or substances that destroy cells or tissues in the local area.

4. Spread to other tissues

- Some infections remain at the site of invasion, causing symptoms related to invasion of epithelial tissue at the particular site.(e.g. Shigella invades the GIT)
- Some microorganisms spread once they have been established in a particular site. (e.g. salmonella typhi establishes itself in the GIT, then spreads through blood and causes fever).

5. Resolution or death

- The function of the cell may be disrupted OR the cell may be destroyed when new microbes are released.
- The effects depend on the particular microbe and the location of the infected cells. *(e.g. Polio virus infects motor neuron cells and shuts down protein synthesis causing the death of neurons and paralysis of the muscles that they innervate*)
- The infection may resolve (death of the pathogen) due to;
  - Improved host defenses
  - Antibiotic therapy

Definitions Related to Infection

1. Pathogenicity	7. Systematic infection
2. Primary infection	8. Clinical infection
3. Secondary infection	9. Sub-clinical infection
4. Acute infection	10. Opportunistic infection
5. Chronic infection	11. Nosocomial infection
6. Localized infection	

Pathogenicity

- Pathogens are microbial species that invade and damage tissue to cause disease.
- Pathogenicity is the capacity of microorganism to cause disease.

- Some microorganisms cause a single disease (e.g. clostridium tetani-tetanus)
- Others cause a range of diseases:
    - e.g. Staphylococcus aureus can cause: skin infections, wound infection, pneumonia and Osteomyelitis.
- Some pathogens cause infections that become severe in debilitated people.
- **Opportunistic pathogens** cause disease in individuals with impaired defenses. (e.g. Pneumocystis carinii, a normal flora in healthy people but causes PCP in immune-compromised people)

Primary vs. Secondary Infection

- Primary Infection
    - An infection that develops in an otherwise healthy individual
- Secondary Infection
    - An infection that develops in an individual who is already infected with a different pathogen

Acute vs. Chronic Infection

- Acute Infection
    - An infection characterized by sudden onset, rapid progression, and often with severe symptoms
- Chronic Infection
    - An infection characterized by delayed onset and slow progression

Localized vs. Systemic infection

- Localized Infection
    - An infection that is restricted to a specific location or region within the body of the host
- Systemic Infection
    - An infection that has spread to several regions or areas in the body of the host

Clinical vs. Subclinical infection

- **Clinical Infection**
    - An infection with obvious observable or detectable symptoms
- **Subclinical Infection**
    - An infection with few or no obvious symptoms

Opportunistic infection

- An infection caused by microorganisms that are commonly found in the host's environment (normal flora)

Nosocomial Infections

Also called Hospital Acquired Infections (HAI's)

- Infection which was neither present nor incubating at the time of admission
- Infection that appears between 48 hours & 4 days following admission to a hospital or other healthcare facility.

Assignment

Find out what diseases do normal comensuls cause if they enter a zone that is not their normal zone
Respiratory system comensuls
Digestive system comensuls

Reference

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